RNA Binding Proteins in Breast, Colon, and Rectal Cancer: A Comprehensive Study on Their Influence on Disease Progression and Potential Clinical Applications

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HACEN CONSTAR QUE:

La memoria **"RNA Binding Proteins in Breast, Colon, and Rectal Cancer: A Comprehensive Study on Their Influence on Disease Progression and Potential Clinical Applications"** ha sido realizada por Jennyfer M. García-Cárdenas bajo nuestra dirección y constituye la Tesis que presenta para optar al Título de Doctora por la Universidad de A Coruña en el Programa de Doctorado Biología Celular y Molecular.

A Coruña, julio de 2023.

Fdo. Santiago Guerrero Jijón

Fdo. María Esperanza Cerdán

A mi amada familia

Abstract

Breast cancer (BC) and Colorectal adenocarcinoma (COREAD) are major health problems worldwide. While significant progress has been made in understanding their molecular subtypes and genetics, a cure remains elusive. An emerging area of interest is the role of RNA-binding proteins (RBPs) in the development and progression of these cancers. RBPs are critical regulators of every hallmark of cancer and could serve as sensitive biomarkers for diagnosis, prognosis, and potential targets. In COREAD, a multidata integration strategy identified putative roles of NOP56, RBM12, NAT10, FKBP1A, EMG1, and CSE1L in the progression of colon cancer (COAD) and rectal cancer (READ). FKBP1A, NOP56, and NAT10 mRNA expression may predict poor prognosis in COREAD and COAD patients. In BC, integrated *in silico* analyses of human RBPs in major cancer databases revealed five putative BC RBPs (PUF60, TFRC, KPNB1, NSF, and SF3A3) with robust oncogenic features. PUF60 and SF3A3 were identified as central elements of a spliceosome-related cluster involving RBPs and cancer driver genes (CDGs). RBPs hold significant potential as diagnostic, prognostic, and therapeutic targets in BC, COAD, and READ. Further research on these RBPs is crucial to unveil their molecular mechanisms, validate their clinical potential, and develop novel treatment strategies.

Resumen

El cáncer de mama (BC, por sus siglas en inglés) y el adenocarcinoma colorrectal (COREAD, por sus siglas en inglés) son importantes problemas de salud a nivel mundial. Si bien se ha logrado un progreso significativo en la comprensión de sus subtipos moleculares y su genética, la cura sigue siendo difícil de alcanzar. Un área emergente de interés es el papel de las proteínas de unión al ARN (RBPs, por sus siglas en inglés) en el desarrollo y progresión de estos cánceres. Las RBPs son reguladoras críticas de todas las características del cáncer y podrían servir como biomarcadores sensibles para el diagnóstico, el pronóstico y dianas terapéuticas potenciales. En COREAD, una estrategia de integración de datos múltiples identificó roles putativos de NOP56, RBM12, NAT10, FKBP1A, EMG1 y CSE1L en la progresión del cáncer de colon (COAD, por sus siglas en inglés) y el cáncer de recto (READ, por sus siglas en inglés). La expresión del ARNm de FKBP1A, NOP56 y NAT10 puede predecir un mal pronóstico en pacientes con COREAD y COAD. En BC, los análisis *in silico* integrados de las RBPs en las principales bases de datos de cáncer revelaron cinco RBPs implicadas en BC (PUF60, TFRC, KPNB1, NSF y SF3A3) con características oncogénicas sólidas. PUF60 y SF3A3 se identificaron como elementos centrales de un grupo relacionado con espliceosoma que involucra RBPs y genes conductores del cáncer (CDG, por sus siglas en inglés). Las RBPs tienen un potencial significativo como dianas diagnósticas, pronósticas y terapéuticas en BC, COAD y READ. La investigación adicional sobre estas RBPs es crucial para revelar sus mecanismos moleculares, validar su potencial clínico y desarrollar nuevas estrategias de tratamiento.

Resumo

O cancro de mama (BC, Breast Cancer em inglês) e o adenocarcinoma colorrectal (COREAD, Colorectal Adenocarcinoma em inglês) son problemas de saúde importantes a nivel mundial. Aínda que se logrou un progreso significativo na comprensión dos seus subtipos moleculares e a súa xenética, a cura segue sendo difícil de alcanzar. Unha área emerxente de interese é o papel das proteínas de unión ao ARN (RBPs, RNA-Binding Proteins en inglés) no desenvolvemento e progresión destes cancros. As RBPs son reguladoras críticas de todas as características do cancro e poderían servir como biomarcadores sensibles para o diagnóstico, o pronóstico e posibles dianas terapéuticas. En COREAD, unha estratexia de integración de datos múltiples identificou roles putativos de NOP56, RBM12, NAT10, FKBP1A, EMG1 e CSE1L na progresión do cancro de colon (COAD em inglês) e o cancro de recto (READ em inglês). A expresión do ARNm de FKBP1A, NOP56 e NAT10 pode predicir un mal pronóstico en pacientes con COREAD e COAD. En BC, os análises *in silico* integrados das RBPs nas principais bases de datos de cancro revelaron cinco RBPs implicadas en BC (PUF60, TFRC, KPNB1, NSF e SF3A3) con características oncogénicas sólidas. PUF60 e SF3A3 identificáronse como elementos centrais dun grupo relacionado co espliceosoma que involucra RBPs e xenes condutores do cancro (CDG em inglês). As RBPs teñen un potencial significativo como dianas diagnósticas, pronósticos e terapéuticas en BC, COAD e READ. A investigación adicional sobre estas RBPs é crucial para revelar os seus mecanismos moleculares, validar o seu potencial clínico e desenvolver novas estratexias de tratamento.

Index

1 Introduction

Cancer is a complex and devastating group of diseases characterized by the uncontrolled growth of cells, leading to the formation of tumors and potentially metastasizing to other parts of the body [1,2]. Breast cancer (BC) and colorectal adenocarcinoma (COREAD) are two prominent examples, with BC being the leading cause of cancer-associated death and the most commonly diagnosed cancer among women worldwide [3,4]. In 2020, more than 2.2 million cases of BC were registered [5]. COREAD, on the other hand, ranks as the third most common malignant tumor and the second most deadly cancer, affecting 1.8 millions of people worldwide [6,7]. From a clinical standpoint, it is apparent that these malignancies exhibit an absence of distinct biomarkers that can be directly associated with both treatment response and disease progression [2,4,8]. Despite ongoing research efforts, the identification of reliable biomarkers remains elusive, making it difficult to effectively monitor disease status and evaluate the efficacy of treatment options[9–11]. Moreover, according to Paschke *et al.* COREAD should be treated as two separated identities since colon cancer (COAD) and rectal cancer (READ) possess differences in molecular carcinogenesis, pathology, surgical topography and procedures, and multimodal treatment. This separation could improve the identification of new biomarkers and therapeutic targets for both types of cancer [12].

RNA biology represents an under-investigated aspect of cancer [13,14]. RNA-binding proteins (RBPs) are emerging as critical modulators of every hallmark of cancer, playing significant roles in post-transcriptional RNA regulons and controlling every aspect of RNA metabolism, including capping, splicing, polyadenylation, nucleocytoplasmic transport, stability, translation, and degradation of mRNA [15–17]. RBPs can modulate the expression levels of oncogenes and tumor suppressors, thereby impacting various aspects of cancer progression, such as angiogenesis, metastasis, or chemotherapy resistance [17,18]. Despite the importance of RBPs in cancer, only a small fraction of the 1,393 identified human RBPs have been implicated in the carcinogenic process, and even fewer in BC and COREAD [19].

Efforts to better understand the role of RBPs in cancer have led to the analysis and integration of large-scale datasets, such as the Cancer Genome Atlas (TCGA) [20–25], the Cancer Dependency Map (DepMap) [26–28], and the Human Protein Atlas (HPA) [29–31], which have provided invaluable insights into the molecular processes of cancer and redefined cancer drug development, diagnosis, and treatment. However, additional fundamental features of oncogenesis, tumor growth, and dissemination remain to be discovered, particularly in the field of post-transcriptional regulation of tumorigenesis [17].

The study of RBPs and their role in cancers such as breast, colon, and rectal adenocarcinoma holds significant potential for advancing our understanding of tumor biology and identifying novel therapeutic targets and prognostic biomarkers [3,4,6,7]. By integrating large-scale datasets and employing multidata integration strategies, researchers can explore the complex interactions between RBPs and various aspects of cancer development and progression [19,32,33]. Separating colon and rectal cancer into distinct tumor identities may further improve the specificity and effectiveness of individualized treatment [12].

As the field of RNA biology and the study of RBPs continue to evolve [19], it is crucial to maintain an interdisciplinary approach, combining cutting-edge computational methods,

experimental techniques, and clinical data to fully harness the potential of RBPs in cancer research [34–38]. Thus, as is depicted in Figure 1, we have integrated several bioinformatic tools to prioritize RBPs that are involved in various aspects of cancer progression.

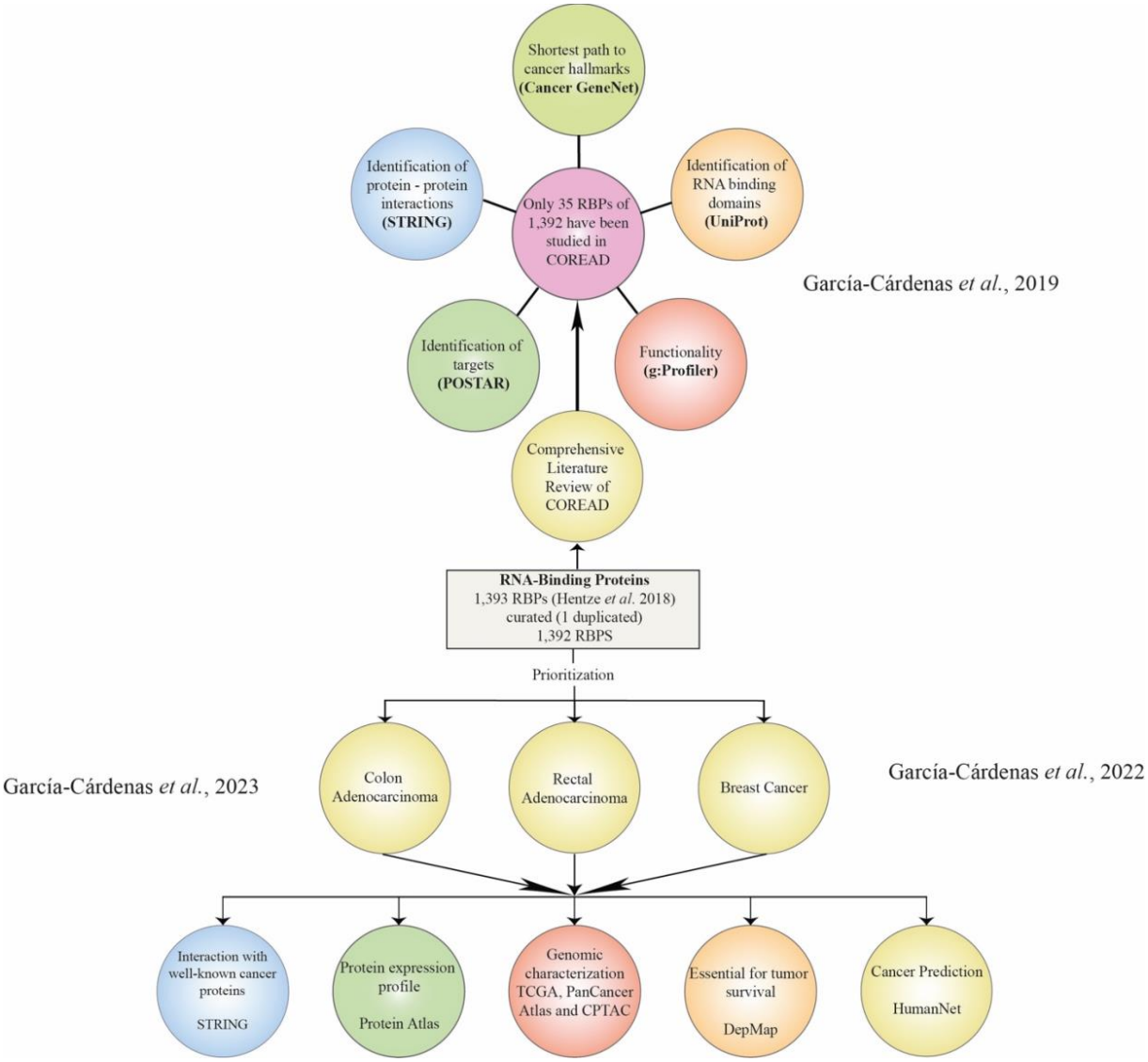

Figure 1. Schematic representation of the data mining strategy used in this thesis. All bioinformatic tools and databases interrogated for prioritization: UniProt [39], Cancer GeneNet [40], POSTAR [41], g:Profiler [42], The Cancer Genome Atlas [34], The Human Protein Atlas [35], STRING [36], and DepMap [37], and further cancer association analysis was performed in HumanNet [38].

Introduction References

1. Hanahan, D. Hallmarks of Cancer: New Dimensions. *Cancer Discov.* **12**, 31–46 (2022).
2. Guerrero, S. *et al.* Analysis of Racial/Ethnic Representation in Select Basic and Applied Cancer Research Studies. *Sci. Rep.* **8**, 13978 (2018).
3. Sung, H. *et al.* Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA. Cancer J. Clin.* **71**, 209–249 (2021).
4. Harbeck, N. *et al.* Breast cancer. *Nat. Rev. Dis. Prim.* **5**, 66 (2019).
5. Globocan. All cancers. *World Health Organization. International Agency for Research on Cancer* https://gco.iarc.fr/today (2020).
6. Gao, C. *et al.* Downregulation of Msi1 suppresses the growth of human colon cancer by targeting p21cip1. *Int. J. Oncol.* **46**, 732–740 (2015).
7. Xi, Y. & Xu, P. Global colorectal cancer burden in 2020 and projections to 2040. *Transl. Oncol.* **14**, 101174 (2021).
8. Hiatt, R. A. & Brody, J. G. Environmental Determinants of Breast Cancer. *Annu. Rev. Public Health* **39**, 113–133 (2018).
9. Assis, J. V. de, Coutinho, L. A., Oyeyemi, I. T., Oyeyemi, O. T. & Grenfell, R. F. e Q. Diagnostic and therapeutic biomarkers in colorectal cancer: a review. *Am. J. Cancer Res.* **12**, 661 (2022).
10. Cohen, R., Pudlarz, T., Delattre, J. F., Colle, R. & André, T. Molecular Targets for the Treatment of Metastatic Colorectal Cancer. *Cancers (Basel).* **12**, 1–18 (2020).
11. Liu, Z. *et al.* Genomic Alteration Characterization in Colorectal Cancer Identifies a Prognostic and Metastasis Biomarker: FAM83A|IDO1. *Front. Oncol.* **11**, 607 (2021).
12. Paschke, S. *et al.* Are Colon and Rectal Cancer Two Different Tumor Entities? A Proposal to Abandon the Term Colorectal Cancer. *Int. J. Mol. Sci.* **19**, (2018).
13. Lukong, K. E., Chang, K.-W., Khandjian, E. W. & Phane Richard, S. RNA-binding proteins in human genetic disease. *Cell* **24**, 416–425 (2008).
14. Morris, A. R. *et al.* Alternative Cleavage and Polyadenylation during Colorectal Cancer Development. *Clin. Cancer Res.* **18**, 5256–5266 (2012).
15. Wurth, L. *et al.* UNR/CSDE1 Drives a Post-transcriptional Program to Promote Melanoma Invasion and Metastasis. *Cancer Cell* **30**, 694–707 (2016).
16. Martinez-Useros, J. *et al.* UNR/CSDE1 Expression Is Critical to Maintain Invasive Phenotype of Colorectal Cancer through Regulation of c-MYC and Epithelial-to-Mesenchymal Transition. *J. Clin. Med.* **8**, 560 (2019).
17. Abdel-Wahab, O. & Gebauer, F. Editorial overview: Cancer genomics: RNA metabolism and translation in cancer pathogenesis and therapy. *Curr. Opin. Genet. Dev.* **48**, iv–vi (2018).
18. Wurth, L. & Gebauer, F. RNA-binding proteins, multifaceted translational regulators in cancer. *Biochim. Biophys. Acta - Gene Regul. Mech.* **1849**, 881–886 (2015).
19. Hentze, M. W., Castello, A., Schwarzl, T. & Preiss, T. A brave new world of RNA-binding proteins. *Nat. Rev. Mol. Cell Biol.* **19**, 327–341 (2018).
20. Ellrott, K. *et al.* Scalable Open Science Approach for Mutation Calling of Tumor Exomes Using Multiple Genomic Pipelines. *Cell Syst.* (2018) doi:10.1016/j.cels.2018.03.002.
21. Taylor, A. M. *et al.* Genomic and Functional Approaches to Understanding Cancer Aneuploidy. *Cancer Cell* **33**, 676-689.e3 (2018).

22. Liu, J. *et al.* An Integrated TCGA Pan-Cancer Clinical Data Resource to Drive High-Quality Survival Outcome Analytics. *Cell* (2018) doi:10.1016/j.cell.2018.02.052.
23. Sanchez-Vega, F. *et al.* Oncogenic Signaling Pathways in The Cancer Genome Atlas. *Cell* **173**, 321-337.e10 (2018).
24. Gao, Q. *et al.* Driver Fusions and Their Implications in the Development and Treatment of Human Cancers. *Cell Rep.* **23**, 227-238.e3 (2018).
25. Hoadley, K. A. *et al.* Cell-of-Origin Patterns Dominate the Molecular Classification of 10,000 Tumors from 33 Types of Cancer. *Cell* **173**, 291-304.e6 (2018).
26. Meyers, R. M. *et al.* Computational correction of copy number effect improves specificity of CRISPR–Cas9 essentiality screens in cancer cells. *Nat. Genet.* **49**, 1779–1784 (2017).
27. Tsherniak, A. *et al.* Defining a Cancer Dependency Map. *Cell* **170**, 564-576.e16 (2017).
28. McFarland, J. M. *et al.* Improved estimation of cancer dependencies from large-scale RNAi screens using model-based normalization and data integration. *Nat. Commun.* **9**, 4610 (2018).
29. Uhlén, M. *et al.* Proteomics. Tissue-based map of the human proteome. *Science* **347**, 1260419 (2015).
30. Thul, P. J. *et al.* A subcellular map of the human proteome. *Science* **356**, eaal3321 (2017).
31. Uhlen, M. *et al.* A pathology atlas of the human cancer transcriptome. *Science* **357**, eaan2507 (2017).
32. García-Cárdenas, J. M. *et al.* Post-transcriptional Regulation of Colorectal Cancer: A Focus on RNA-Binding Proteins. *Front. Mol. Biosci.* **6**, (2019).
33. Kang, D., Lee, Y. & Lee, J. S. RNA-Binding Proteins in Cancer: Functional and Therapeutic Perspectives. *Cancers (Basel).* **12**, 1–33 (2020).
34. Tomczak, K., Czerwińska, P. & Wiznerowicz, M. The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge. *Contemp Oncol (Pozn)* **19**, 68–77 (2015).
35. Pontén, F., Jirström, K. & Uhlen, M. The Human Protein Atlas—a tool for pathology. *J. Pathol.* **216**, 387–393 (2008).
36. Szklarczyk, D. *et al.* STRING v11: Protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res.* **47**, D607–D613 (2019).
37. Yu, C. *et al.* High-throughput identification of genotype-specific cancer vulnerabilities in mixtures of barcoded tumor cell lines. *Nat. Biotechnol.* **34**, 419–423 (2016).
38. Kim, C. Y. *et al.* HumanNet v3: an improved database of human gene networks for disease research. *Nucleic Acids Res.* **50**, D632–D639 (2022).
39. The UniProt Consortium. UniProt: a worldwide hub of protein knowledge. *Nucleic Acids Res.* **47**, D506–D515 (2019).
40. Repana, D. *et al.* The Network of Cancer Genes (NCG): a comprehensive catalogue of known and candidate cancer genes from cancer sequencing screens. *Genome Biol.* **20**, 1–12 (2019).
41. Zhu, Y. *et al.* POSTAR2: deciphering the post-transcriptional regulatory logics. *Nucleic Acids Res.* **47**, 203–211 (2018).
42. Raudvere, U. *et al.* g:Profiler: a web server for functional enrichment analysis and conversions of gene lists (2019 update). *Nucleic Acids Res.* **47**, W191–W198 (2019).

2 Objectives

2.1 Main Objective

Discover novel RNA-binding proteins implicated in the progression of breast, colon, and rectal cancer.

2.2 Specific objectives

- To comprehensively examine the involvement of RNA-binding proteins in colorectal cancer, including previous information about their regulation by microRNAs, xenograft studies, and their potential clinical implications.
- To investigate the common features of RNA-binding proteins in colorectal cancer, specifically focusing on their protein domains, protein-protein interactions, RNA targets and oncogenic capabilities
- Detect *in silico* potential RBPs that can be used as prognosis and diagnosis biomarkers of breast, colon, and rectal cancer and possibly as new therapeutic targets.

3 Results (compendium of articles)

frontiers in Molecular Biosciences

REVIEW
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Post-transcriptional Regulation of Colorectal Cancer: A Focus on RNA-Binding Proteins

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Colorectal cancer (CRC) is a major health problem with an estimated 1. 8 million new cases worldwide. To date, most CRC studies have focused on DNA-related aberrations, leaving post-transcriptional processes under-studied. However, post-transcriptional alterations have been shown to play a significant part in the maintenance of cancer features. RNA binding proteins (RBPs) are uprising as critical regulators of every cancer hallmark, yet little is known regarding the underlying mechanisms and key downstream oncogenic targets. Currently, more than a thousand RBPs have been discovered in humans and only a few have been implicated in the carcinogenic process and even much less in CRC. Identification of cancer-related RBPs is of great interest to better understand CRC biology and potentially unveil new targets for cancer therapy and prognostic biomarkers. In this work, we reviewed all RBPs which have a role in CRC, including their control by microRNAs, xenograft studies and their clinical implications.

Keywords: colorectal cancer, RBPs, post-transcriptional regulation, oncogene, tumor suppressor

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INTRODUCTION

Worldwide, every year an estimated 1.8 million new cases of colorectal cancer (CRC) are diagnosed, setting it in the third place of the most common malignant tumor, and consequently a major health care problem (Gao et al., 2015; Bray et al., 2018). Heretofore, most studies in CRC biology have been focused on DNA-related aberrations (e.g., mutation, methylation changes, DNA copy number alterations, loss of genomic stability, etc.), leaving the post-transcriptional processes under-studied. However, post-transcriptional alterations play a significant role in the preservation of tumor cells by modulating every hallmark in cancer (Lukong et al., 2008; Morris et al., 2012; Paz-Y-Mino et al., 2015; Wurth et al., 2016; Martinez-Useros et al., 2017).

RNA biology represents an under-investigated aspect of cancer; this is puzzling considering that pleiotropic changes in the transcriptome are a key feature of cancer cells (Wurth and Gebauer, 2015). RNA binding proteins (RBPs) are relevant because they are part of post-transcriptional RNA regulons. These RNA regulons are formed by Ribonucleoproteins (RNP) which interact with other trans-elements, non-coding RNAs, metabolites and untranslated sequence elements found within the mRNAs (USER). These RNP complexes control the expression of hundreds to thousands mRNAs of functionally related proteins from the transcription to translation process, allowing the cell to respond to several stimuli with such a great agility ensuring cellular homeostasis (Keene, 2007; Wurth, 2012; Iadevaia and Gerber, 2015; Wurth and Gebauer, 2015). The complex interaction

of RBPs and their RNA partners (e.g., mRNAs or miRNAs) are achieved through RNA-recognition domains which increases the specificity and affinity of these interactions. While there is much progress still to understand such interactions, some of these domains have been ully characterized: the RNA-recognition motif (RRM), the zinc finger motif, and the K-homology domain (Iadevaia and Gerber, 2015). RBPs are able to control every aspect of RNA metabolism: capping, splicing, polyadenylation, nucleocytoplasmic transport, stability, translation, and degradation of mRNA (Burd and Dreyfuss, 1994; Lukong et al., 2008; Kechavarzi and Janga, 2014). As a result, when any RBP is altered this affect either its mRNA affinity or its subcellular localization, disturbing cellular homeostasis (Iadevaia and Gerber, 2015). In this regard, RBPs are emerging as critical modulators of every hallmark of cancer, and still very little is known about their cancer related molecular functions and targets (Wurth and Gebauer, 2015; Hentze et al., 2018).

Hentze et al. compiled all published RNA interactomes into RBP supersets, they stringently curated and updated the annotations of RBPs identified from several sources. Finally, a list of 1,393 RBPs was retrieved in humans and only a few have been implicated in the carcinogenic process and even much less in CRC (Hentze et al., 2018). The identification of RBPs will provide a better understanding of tumor biology and potentially unveil new targets for cancer therapy and prognostic biomarkers. In this work, we reviewed all RBPs having a role in CRC, including their control by microRNAs (miRNAs), xenograft studies and their clinical implications.

LIN28

General Features

LIN28 is an evolutionarily conserved RBP and an emerging oncogenic driver (Zhang et al., 2018). Mammals produce two LIN28 paralogs, *LIN28A* and *LIN28B* which are separately or jointly involved in various biological functions; including metabolism development, tissue regeneration, and oncogenesis (Tu et al., 2015; Wang T. et al., 2015; Jiang and Baltimore, 2016; Wang et al., 2016; Pereira et al., 2017). Human *Lin28A* is located on chromosome 1p36 and encodes a protein of 209 amino acids whereas *Lin28B* is on chromosome 6q16.3 and its protein is composed of 250 amino acids. In addition, LIN28A is predominantly localized in the cytoplasm, whereas LIN28B resides exclusively in the nucleus. Interestingly, both proteins are expressed mainly in the cytoplasm in CRC (Guo et al., 2006; Wang et al., 2016).

LIN28 proteins have two cold shock domains and retroviral-type Cys-Cys-His-Cys (CCHC) zinc fingers that confer RNA-binding ability. These proteins also modulate the let-7 family of miRNAs, which consists of 12 members frequently deleted in human cancers and considered as tumor suppressors (Zhou et al., 2013; Triboulet et al., 2015; Wang S. et al., 2015; Jiang and Baltimore, 2016; Jiang et al., 2017). Both proteins inhibit biogenesis and induce the degradation of the let-7 family. LIN28B interacts with pri-let-7 (primary-miRNAs) and inhibits its processing by the Microprocessor complex, whereas LIN28A blocks the pre-let-7 (the hairpin structure formed by the cleavage of DROSHA/DGCR8 enzymes) processing by DICER1 via TUT4 recruitment (Kim et al., 2014; Wang et al., 2016). Conversely, there is a double-negative feedback loop, where the 3′UTR of LIN28A/B is recognized by let-7 miRNA. Thus, once let-7 miRNA binds to the 3′UTR the expression of these proteins is inhibited (Wang T. et al., 2015). Indicating that when *LIN28A/B* are expressed let-7 is not (Piskounova et al., 2011; Wang T. et al., 2015).

Both LIN28A and LIN28B could enhance colon cancer cells proliferation but mechanically their mode of action is different. *LIN28A* overexpression promotes the transition from S to G2/M phase, whereas constitutive expression of *LIN28B* enables the shift of cell cycle phases (from G1 to S phase and from S to G2/M phase) (Wang et al., 2016). Activation of *LIN28* in different primary tumors leads to translational enhancement or suppression of cancer-related mRNAs (e.g., IGF2 and MYOD1 mRNAs) (Viswanathan and Daley, 2010; Rappaport et al., 2017). Both *LIN28A* and *LIN28B* are expressed in about 30% of colorectal tumors, but the expression level of *LIN28B* is higher compared to *LIN28A* (King et al., 2011a,b; Wang et al., 2016).

Xenograft Studies

LIN28B knockdown in cancer cells reduces their proliferative and invasive abilities *in vitro* and inhibits both primary and metastatic tumor growth *in vivo* (Jiang et al., 2017). LIN28B increases cancer cell invasion in intestinal and colorectal adenocarcinomas in murine models (Jiang et al., 2017). Also, *LIN28* overexpressed tumors exhibited augmented areas of moderate differentiation and increased glandular formation and mucin production; in contrast to wild-type tumors that are poorly differentiated and rarely exhibit mucinous (King et al., 2011a). In addition, Tu et al. experimentally demonstrated that LIN28 cooperates with APC in accelerating neoplastic lesions formation in $Apc^{Min/+}$ mice. APC alterations (or other changes that target WNT signaling) occur in most colon tumors, which could be upregulating the expression of *LIN28B*. This may be mediated by MYC, a transcriptional target of the canonical WNT signaling (Mayr et al., 2007; King et al., 2011a; Zhou et al., 2013; Wang et al., 2016; Pereira et al., 2017). It has also been demonstrated that LIN28/let-7 axis promotes invasive intestinal adenocarcinoma in murine models by interacting with the WNT pathway (Piskounova et al., 2008; Tu et al., 2015; Voutsadakis, 2018).

miRNAs Control

To date, besides let-7, several miRNAs have been reported to repress LIN28A/LIN28B translation once they bind to their 3′UTR, such as miR-9, miR-26a, miR-27, miR-30, miR-125, miR-181, and miR-212. In cancer cells, these miRNAs are under-expressed due to *LIN28A/B* overexpression (Wang T. et al., 2015).

Clinical Relevance

LIN28A/LIN28B influence the clinical outcome in patients by enhancing tumor aggressiveness and early metastasis (Mayr et al., 2007; Viswanathan et al., 2009; King et al., 2011a; Wang et al., 2016; Pereira et al., 2017). Irregular *LIN28A/B* expression is usually correlated with poor survival. Several studies have

demonstrated that high levels of *LIN28* in colon tumors are associated with advanced tumor stages and increased probability of tumor recurrence (King et al., 2011a; Madison et al., 2013; Jiang and Baltimore, 2016; Zhang et al., 2018). King et al. have found that LIN28B protein levels are increased in CRC patients promoting cancer progression and metastasis (King et al., 2011a). In addition, over-expression of *LIN28A/LIN28B* could enhance chemotherapy sensitivity of HCT116 cells to 5-Fu via different mechanisms (Wang et al., 2016). LIN28 may also serve as a predictive biomarker for chemotherapy in patients with colon cancer (King et al., 2011a; Pang et al., 2014; Wang T. et al., 2015; Wang et al., 2016; Jiang et al., 2017). All RBPs and their CRC-related features are listed in **Table 1**.

MSI

General Features

In humans, Musashi RNA binding protein (MSI) is composed of two isoforms: MSI1 and MSI2 (Voutsadakis, 2018). *MSI1* and *MSI2* genes are evolutionarily conserved given their 75% amino acid sequence. These two proteins are placed on chromosome 12q24 (*MSI1*) and 17q22 (*MSI2*). They comprise two RNA recognition motif (RRMs) domains which bind to (G/A)U1-3(AGU) motifs in the 3′-UTR of their target mRNAs (Sakakibara et al., 2001; Okano et al., 2005; Glazer et al., 2012). They can be regulated by ELAV1 by maintaining the stabilization of their mRNAs, as well as by tumor suppressor miRNAs (Gao et al., 2015). Musashi emerges as a critical player in controlling multiple targets that form networks from where MSI1 and MSI2 are able to regulate cell death, differentiation, and cell cycle (de Sousa Abreu et al., 2009; Guinney et al., 2015; Kharas and Lengner, 2017). They are also key oncogenic players in promoting intestinal transformation (Wang S. et al., 2015; Kharas and Lengner, 2017). Musashi may also co-operate with LIN28 by binding and inhibiting some mRNAs. Besides, Musashi represses translation of Numb (an inhibitor of the NOTCH pathway), APC, PTEN, and P21, but upregulates WNT pathway at transcriptional level when Numb is inhibited (Qiao and Wong, 2009; Lan et al., 2015; Wang S. et al., 2015; Voutsadakis, 2018).

Xenograft Studies

Several studies have shown the potential therapeutic target of MSI1 given that when *MSI1* is knockdown tumor growth is delayed as well as cell proliferation, migration, and invasion (Sureban et al., 2008; Gao et al., 2015; Smith, 2015; Kharas and Lengner, 2017). In addition, murine models upregulated for *MSI1* and *K-RasG12D* are highly resistant to oxaliplatin and 5-fluorouracil (Todaro et al., 2014).

miRNA Control

miR-137 is a tumor suppressor, which negatively regulates MSI1 and Notch/WNT signaling pathway (Smith, 2015). There is an inverse correlation between miR-137 and MSI1 expression; thus, the overexpression of miR-137 decreases *MSl1* expression reducing cell growth, colony formation, and tumor sphere growth (Liang et al., 2013; Smith et al., 2015).

Clinical Relevance

MSI1/2 are highly expressed in colon primary tumors and metastatic lesions in the lymph nodes; this correlates to an increased metastatic risk and poorer survival (Fan et al., 2010; Li et al., 2011). Therefore, it has been suggested that inhibition of both MSIs' RNA binding activity could fully abrogate tumor growth in CRC (Potten et al., 2003; Cheng et al., 2015; Gao et al., 2015; Lan et al., 2015; Kharas and Lengner, 2017). Besides, MSI increases colorectal cancer stem cells (CSCs) survival, migration, and resistance to 5-FU, the chemotherapy drug that constitutes the backbone of the most currently used one in colon cancer treatment (Yuqi et al., 2008; Voutsadakis, 2018; López-Cortés et al., 2019).

ELAVL1

General Features

Embryonic Lethal, Abnormal Vision Drosophila-Like 1 (*ELAVL1*) or Hu Antigen R (*HuR*) was the first factor to be identified for its ability to cooperate and compete with miRNA activity (Franceschini et al., 2012; Ciafrè and Galardi, 2013; Iadevaia and Gerber, 2015). ELAVL1 consists of 326 amino acids harboring three RRMs, which bind to specific mRNAs in their AU- or U-rich elements (AREs) in their 3′UTRs (López de Silanes et al., 2004b). *ELAVL1* expression changes were found to occur early during tumorigenesis (Fan and Steitz, 1998), upregulating key survival or growth-related genes by increasing both their mRNA stability and/or their protein translation (Fan and Steitz, 1998; López de Silanes et al., 2004b; Franceschini et al., 2012). For example, ELAVL1 promotes the stability and translation of COX-2 mRNA by binding to its ARE sequences located within the 3′UTR in an advanced tumor stage of CRC tissues (Fan and Steitz, 1998; Dixon et al., 2001; Denkert et al., 2006; Badawi et al., 2017). COX-2 is a major facilitator of several cellular activities (e.g., proliferation, cell death resistance, angiogenesis, and metastasis) (Fan and Steitz, 1998; Denkert et al., 2006; Jang et al., 2017; López-Cortés et al., 2019). ELAVL1 is normally found in the nucleus, where it participates in splicing and polyadenylation, but in CRC cells ELAVL1 is localized in the cytoplasm promoting mRNA stabilization of its targets (Fan and Steitz, 1998; Brennan and Steitz, 2001; López de Silanes et al., 2011; Akaike et al., 2014; Liu et al., 2018). ELAVL1 regulates numerous mRNAs that encode proteins related to proliferation, cell cycle (cyclins A2, B1, D1, p21, and p27), tumor suppressors (p53 and Von Hippel-Lindau tumor suppressor), proto-oncogene products (c-Fos and c-Myc), growth factors (IGF-1R VEGF, EGF, TGF, GM-CSF), inhibitors (p21 and p27) and signaling molecules, which are crucial (β-catenin, cyclin D1, and c-Myc) for the CRC WNT-activated pathway (Lin et al., 2017).

Xenograft Studies

ELAVL1 enhances pathogenic gene expression necessary for cancer development (Blanco et al., 2016). This was corroborated when *ELAVL1* overexpression increased colon cancer cells growth in a nude mouse xenograft model (López de Silanes et al., 2004b; Liu et al., 2018). Subcutaneous injection of ELAVL1-overexpressing RKO cells into nude mice produced

TABLE 1 | A summary of all CRC-related RBPs reviewed in this work: miRNAs control and their clinical relevance.

RBP	Control by miRNAs	Clinical relevance
LIN28	miR-let-7, miR-26a, miR-181, miR-9, miR-30, miR-125, miR-212 and miR-27	- Aberrant expression correlates with reduced patient survival. - Predictive biomarker for chemotherapy.
MSI	miR-137	- High expression correlates with increased metastatic risk and poorer survival. - Promotes resistance to 5-FU.
ELAVL1	miR-519 and miR-22	- High expression correlates with malignancy and multidrug resistance.
QKI	miR-574-5p and miR-155	- Low expression correlates with poorer prognosis. - Could predict recurrence and prognosis.
RBM3		- Promotes resistance to chemotherapy
CELF1	miR-503	
IGF2BPs		- Overexpression correlates to unfavorable clinical outcomes: early dissemination, poor response to the therapy, increased tumor aggressiveness, and short survival.
ESRP1		- Overexpression associates with a favorable overall survival outcome.
TTP	miR-29a	- Downregulation correlates with poor prognosis, tumor aggressiveness, and necrosis.
hnRNPs		- Poor prognosis marker.
TIA1	miR-19a	- Increased numbers of TIA-1 positive TILs is associated with an improved clinical outcome. - TIA1 can also be used to supplement prognostic information related to TNM stage and adjuvant therapy.
KHDRBS1		- KHDRBS1 nuclear localization and overexpression is correlated with poor tumor differentiation, advanced T stage, lymph node involvement, and distant metastasis.
CPEB4	miR-203	- Overexpression correlates with tumor progression and poor overall survival.
CSDE1		- Overexpression is associated to poor prognosis.

significantly larger tumors; conversely, RKO cells expressing low ELAVL1 levels significantly reduced tumor growth (López de Silanes et al., 2003). *ELAVL1* deletion in adult normal mice was lethal and several critical defects were observed: defective intestinal stem cell dynamics, villus atrophy, and defects in hematopoietic progenitor cell production (Ghosh et al., 2009). Mice lacking *ELAVL1* in myeloid-lineage cells, which include many of the innate immune system cells, showed a rapid progression of chemical-induced colitis and increased susceptibility to endotoxemia and colitis-associated cancer (Yiakouvaki et al., 2012).

miRNAs Control

ELAVL1 levels are downregulated by miR-519, a tumor-suppressive miRNA. miR-519 promotes anti-proliferative properties in CRC cell lines by targeting and reducing ELAVL1 transcripts. This, in turn, decreases the expression of several ELAVL1 target mRNAs and markedly reduces cell proliferation (Abdelmohsen et al., 2008). ELAVL1 levels are also downregulated by miR-22, which has a more profound tumor-suppressive effect. Expression of miR-22 is inversely correlated with ELAVL1 in both CRC tissues and CRC cell lines. miR-22 directly binds to the 3′UTR of ELAVL1 leading to its inhibition, which, in turn, represses CRC proliferation and migration *in vitro* and decelerates CRC xenografted tumor growth *in vivo* (5). Conversely, Al-Haidari et al. have found that when miR-155-5p expression was reduced in serum-starved CRC cells, it decreased the expression of ELAVL1 (Al-Haidari et al., 2018).

Clinical Relevance

Increased expression and cytoplasmic abundance of ELAVL1 is correlated with malignancy in colon cancer tissues (López de Silanes et al., 2004a; Denkert et al., 2006). Numerous studies have indicated that cytoplasmic accumulation of ELAVL1 has a link to multidrug resistance (MDR) acquired after chemotherapy and therefore causing poor prognosis in various cancer types. Accordingly, suppression of ELAVL1's cytoplasmic accumulation could increase chemotherapeutic agent accumulation and induced apoptosis, leading to increased cytotoxic effect and reversing drug resistance (Blanco et al., 2016; Lin et al., 2017). *ELAVL1* inhibition could, therefore, improve the efficacy of current therapy regimes (Badawi et al., 2017; Lin et al., 2017).

QKI

General Features

Quaking (QKI) is a human RBP placed on chromosome 6q26. QKI is part of STAR (signal transduction and activation of RNA) protein family and presents two specific regions (QUA1 and QUA2) and a KH domain. So far, four mRNA splice variants have been recognized: QKI-5, QKI-6, QKI-7, and QKI-7b (Kondo et al., 1999; Yang et al., 2010). The 3 well-studied isoforms (QKI5, 6, and 7) appear to have different roles in development. QKI isoforms are constructed with the same 311 amino acid body (share exons 1–6), however, their C-terminal differs from the rest (35 amino acids) (Yang et al., 2010). From all human isoforms, QKI-5 is the most abundant in colon tissues where its maximum expression is seen in the

nucleus, while QKI-7 is mainly a cytoplasmic protein. QKI-6 can be found in both nuclear and cytoplasmic compartments (Yang et al., 2010; Ji et al., 2013). The cellular localization of QKI7b remains unknown due to the lack of specific antibodies (Liu Q. et al., 2013).

QKI affects several RNA-related processes (pre-mRNA splicing, mRNA stabilization and turnover, nuclear retention, miRNA processing, and circular RNA biogenesis), regulating cell cycle and differentiation, programmed cell death, development, new blood vessels formation, and cell fate determination (Nilsen and Graveley, 2010; Ji et al., 2013). To date, altered expression of the STAR proteins has been seen in several developmental defects and diseases. Concerning to CRC, Yang et al. discovered that QKI5 and QKI6 are very little expressed or even absent, acting as tumor suppressor proteins. This was associated with unusual regulation of β-catenin and p27Kip1 signaling (Yang et al., 2010). This reduction in *QKI* expression has been for an anomalous dropping of the histone variant macroH2A1.1 (Ji et al., 2013). QKI is a critical regulator of colon epithelial differentiation, whose aberrant reduction (hypermethylation) might contribute to gastrointestinal cancer initiation and facilitate colon carcinogenesis (Yang et al., 2010; Iwata et al., 2017).

Xenograft Studies

QKI null mice phenotype presented several abnormalities in the vascular remodeling or vitelline vessels that make them impossible to survive later of the day 10.5. *QKI* conditional knockout mice died by the third week after birth, displaying severe hypomyelination in the central nervous system (Darbelli et al., 2016). The lethal phenotype in *QKI* knockout mice highlights the importance of this gene in the regulation of normal cellular functions (Yang et al., 2010).

miRNAs Control

miR-574-5p negatively controls the expression of QKI6/7/7b through binding to QKI's 3'UTRs. This negatively regulation has been seen in mice and humans colorectal tissues where β-catenin and p27Kip1 signaling is affected once miR-574-5p is significant upregulated (Ji et al., 2013). Another regulator is miR-155 which downregulates *QKI* and thus promotes proliferation and invasion of CRC cells (He et al., 2015).

Clinical Relevance

Low *QKI* expression is a risk factor for tumor recurrence after surgery. Thus, patients with low *QKI* expression had significantly poorer prognosis. Furthermore, the relapse-free survival (RFS) and overall survival of patients with stage I, II, and III CRC with low *QKI* expression was significantly shorter than those with high QKI expression. QKI could be therefore a useful clinical biomarker for predicting recurrence and prognosis (Iwata et al., 2017). In addition, if methylation-related mechanisms contribute to the inactivation of *QKI*, demethylation could be an appropriate therapeutic strategy (Yang et al., 2010; Iwata et al., 2017).

RBM3

General Features

RNA-binding motif protein 3 (RBM3) has been identified as a cold-shock protein. RBM is activated in cellular distress (e.g., hypothermia, hypoxia, and oxidative stress), but it is necessary for cell proliferation (Melling et al., 2016; Siesing et al., 2017). RBM3 is part of the glycine-rich RNA-binding protein family and has one RRM domain. Currently, two isoforms have been identified where the longest comprehends 157 amino acids with a molecular mass of 17 kD (Derry et al., 1995; Melling et al., 2016; Jang et al., 2017; Rappaport et al., 2017). Cold-shock proteins have been suggested to be important mediators of the caspase-independent mitotic death (CIMD) (Jang et al., 2017). RBM3 interferes the access of mRNA initiation factors to the 60S ribosome which modulates the potential activity of kinases in tumors (Chappell and Mauro, 2003; Dresios et al., 2005).

RBM3 plays a key role in carcinogenesis and proto-oncogene function. RBM3 augments mRNA stability and translation of rapidly degraded transcripts by binding to their AREs; for instance, RBM3 stabilizes COX-2, IL-8, and VEGF (Sureban et al., 2008; Venugopal et al., 2016). These cells also exhibit augmented stem cell markers via an increase in β-catenin activity. Therefore, the β-catenin signaling pathway may be regulated through alterations in the expression of *RBM3*. Interestingly, RBM3 is also regulated by hypoxia in a HIF1α independent mechanism; this provides a novel target to further examine RBM3-mediated hypoxia induced stem cell signaling (Venugopal et al., 2016).

Xenograft Studies

RBM3 overexpression enhanced the development of multicellular tumor spheroids in NIH3T3 mouse fibroblasts. This suggests that *RBM3* could malignantly transform cells by inducing anchorage-independent growth. However, in xenografts models *RBM3* downregulation reduces tumor growth and angiogenesis. Given the reduction in the expression of *IL-8* and the proangiogenic factors *COX-2* and *VEGF* (Sureban et al., 2008).

miRNAs Control

It has been reported that RBM3 alters miRNA levels which in turn will modify global protein expression and thus tumor progression (Jang et al., 2017).

Clinical Relevance

RBM3 overexpression in HCT116 and DLD1 colon cancer cells increases proliferation and engenders hypoxia, serum deprivation and resistance to classical chemotherapeutic agents (e.g., cisplatin, doxorubicin, and paclitaxel) (Venugopal et al., 2016). It has been suggested that RBM3 is capable of increasing chemoresistance by inducing cells with high xenobiotic efflux capacity and through the induction of ATP-binding cassette (ABC) transporters (Venugopal et al., 2016). In contrast, RBM3 downregulation decreases HCT116 colon adenocarcinoma cell proliferation (Sureban et al., 2008). There is an association between RBM3 and more favorable clinic

pathological parameters, the higher *RBM3* expression, the higher the disease-free survival (DFS) rate is, particularly in patients who received first line oxaliplatin-based chemotherapy (Jones et al., 2014; Venugopal et al., 2016; Jang et al., 2017; Liu Y. et al., 2017; Siesing et al., 2017; Ye et al., 2017).

Besides, a positive relationship between microsatellite instability with high expression of *RBM3* was observed (Venugopal et al., 2016; Jang et al., 2017). High microsatellite instability is commonly associated with good prognosis and right-sided colon cancer development. Melling and colleagues found that the higher the expression of *RBM3* the higher the overall survival is in CRC (stages I-III). This may be clinically relevant for the selection of patients with a likely adverse clinical course for adjuvant chemotherapy. Although, no difference in survival was seen for rectal carcinomas (Melling et al., 2016). Noteworthy, Wang and colleagues found that *RBM3* positive expression correlates with an improved prognosis in young CRC patients (Wang M. J. et al., 2015).

CELF1

General Features

CUGBP Elav-like family member 1 (CELF1), is a multifunctional RBP that generally binds mRNAs through GU-rich elements in the 3′-UTRs or coding regions of its targets. CELF1 forms part of a family named CELF (CELF1, CELF2, CELF3, CELF4, CELF5, and CELF6). All the family members possess a divergent domain loaded with alanine and glutamine residues and three RRMs, two near the N-terminal region and one located at the C-terminal domain. CELF1 promotes and represses RNA splicing and mRNA translation (Kim and Gorospe, 2008; Vlasova et al., 2008; Vlasova-St. Louis and Bohjanen, 2011; Yang et al., 2014; Liu et al., 2015). The three of them recognize different motifs and arrangements, which gives specificity and a wide range of binding partners. CELF1 regulates protein expression implicated in the tight junction (TJ) and gut barrier function. For instance, CELF1 represses occludin translation by increasing occludin mRNA recruitment to processing bodies, resulting in dysfunction of the epithelial barrier. Interestingly, CELF1 and ELAVL1 compete for the same occludin 3′UTR binding element, competitively regulating occludin translation and in opposite directions (Yang et al., 2014; Liu et al., 2015). CELF1 also regulates intestinal epithelial homeostasis by modulating intestinal epithelial cells (IECs) proliferation, apoptosis and cell-to-cell interaction (Cui et al., 2012; Liu et al., 2015). Increased levels of cellular *CELF1* desensitize IECs to apoptosis, whereas *CELF1* silencing increases the sensitivity of IECs to apoptosis (Cui et al., 2012; Tu et al., 2015).

Xenograft Studies

In a mouse fasting model, a reduction in the proliferating crypt cell population and a decrease in the lengths of villi and crypts were correlated with a significant increase in the levels of *CELF1*. This suggests the involvement of *CELF1* in the pathogenesis of intestinal mucosal atrophy (Madison et al., 2013, 2015; Liu et al., 2015).

miRNA Control

CELF1 is repressed by the tumor suppressor miR-503 in IECs. CELF1 abundance is regulated by miRNA-503 mostly by binding to sites located in CELF1 coding region (Ciafrè and Galardi, 2013; Yang et al., 2014; Liu et al., 2015).

CELF2

General Features

CUGBP Elav-like family member 2 (CELF2) is a ubiquitously expressed protein of 490 amino acids located on chromosome 10p13–p14 (Choi et al., 1999; Lichtner et al., 2002; Ramalingam et al., 2012). CELF2 regulates several RNAs at different posttranscriptional levels: alternative splicing (e.g., Tau and troponin T), RNA editing (e.g., apolipoprotein B), RNA stability, and mRNA translation (e.g., cyclooxygenase-2 and Mcl1) (Ramalingam et al., 2012). CELF2 is expressed in the nucleus of intestinal epithelial cells acting as a tumor suppressor protein (Natarajan et al., 2008). CELF2 has at least three identified isoforms, each of them with differential expression levels in human colon cancer cells (Ramalingam et al., 2008). *CELF2* overexpression results in reduced colony formation in CRC cells. CELF2 attaches to AREs of COX-2 3′UTR increasing COX-2 mRNA stability but inhibiting its translation. Reduction of COX-2, in turn, decreases PGE2 known to modulate cell proliferation and tumor invasion in many cancer types (Sureban et al., 2008; Ramalingam et al., 2012). *COX-2* is upregulated in colorectal adenomas, thereby suggesting that CELF2 might prevent cancer development by inhibiting COX-2 and PGE2. These data suggest that *CELF2* expression may be deleterious to cancer cells (Ramalingam et al., 2012).

IGF2BP1-3

General Features

The mammalian IGF2 mRNA-binding protein family (IGF2BP) comprises three RNA-binding proteins (IGF2BP1-3) with a conserved domain structure including four K homology (KH) domains and two RRMs (Ross et al., 2001; Dimitriadis et al., 2007; Lederer et al., 2014). IGF2BPs exhibit different expression patterns despite their high degree of likeness and show distinct RNA-binding properties and are associated with variable target transcripts. IGF2BP1 stabilizes the MYC mRNA by shielding it from ribonuclease cleavage when binding to the coding region instability determinant (Lederer et al., 2014). Thereby, it prolongs the half-life of MYC mRNA up to 8 fold, promoting tumor cell proliferation and survival (Ross et al., 2001; Dimitriadis et al., 2007; Hamilton et al., 2013; Lederer et al., 2014). IGF2BP1 also regulates CD44, ALCAM, AMIGO2, MCAM, CD24, dysadherin, and MMP1 mRNAs that encode proteins of cell adhesion and invasiveness (Dimitriadis et al., 2007; Vainer et al., 2008). In addition, IGF2BP1 binds to and stabilizes F-box protein βTrCP1 whose continued activation in CRC is well established by suppressing apoptosis via NF-κB activation (Dimitriadis et al., 2007; Hamilton et al., 2013).

Concerning IGF2BP2, it has been shown that this RBP controls NRAS, PINCH2, and MURF-3 expression which are

responsible for carcinogenesis and cellular mobility (Lederer et al., 2014; Ye et al., 2016). In addition, IGF2BP2 targets RAF1 mRNA which is an essential component of MAPK pathway activation upon (MEK)1/2 phosphorylation. MEK1/2, in turn, phosphorylate and activate extracellular-related kinase (ERK)1/2. ERK1/2 regulate downstream pathways involved in survival and cell proliferation (Ye et al., 2016).

Regarding IGF2BP3, this protein contributes to RNA trafficking and stabilization, cell development and division, migration and adhesion (Lochhead et al., 2012; Lin et al., 2013; Lederer et al., 2014; Kumara et al., 2015). Also, *in vitro* studies have shown that IGF2BP3 promotes tumor cell survival, proliferation, anchorage-independent growth, chemoresistance migration and invasiveness (Lederer et al., 2014). It has been demonstrated that IGF2BP3 along with HNRNPM controls the fate of cyclin D1, D3, and G1 encoding transcripts in the nucleus (Li et al., 2009; Lederer et al., 2014). Cyclins are key components of the cell cycle and disorders of their function can lead to carcinogenesis. IGF2BP3 also regulates the gene expression of IGF-II, which binds to and activates IGF-I. Thus, IGF-I induces a cell to begin cell division in an alter manner which in turn causes excessive cell proliferation and cancer (Lin et al., 2013).

Xenograft Studies

IGF2BP1 plays an essential role for normal intestinal morphogenesis since deficient mice exhibit dwarfism and severe histological abnormalities in small (villous hypoplasia) and large intestine (short and irregular crypts in the colon) (Dimitriadis et al., 2007). On the contrary, *IGF2BP1* overexpression promotes tumor-cell growth in CRC (Hamilton et al., 2013). *IGF2BP2* knock-out mice possess a higher frequency of autoantibody response to IGF2BP2/p62 in colon cancer, although the mechanisms and its role in CRC carcinogenesis are still unknown (Ye et al., 2016). Ectopic expression of *IGF2BP3* enhances tumor cell aggressiveness in transgenic animals (Dimitriadis et al., 2007; Lederer et al., 2014).

miRNAs Control

No miRNA has been reported to inhibit any member of the IGF2BP family. However, these RBPs protect some mRNAs from miRNA attack. For instance, IGF2BP1 protects Beta-transducin repeats-containing protein 1 (βTrCP1) mRNA, an important player in signal transduction, from miR-183-directed turnover (Elcheva et al., 2009; Ciafrè and Galardi, 2013). Also, IGF2BP2 regulates RAF1 (proto-oncogene) expression by blocking its degradation by miR-195 (Ye et al., 2016).

Clinical Relevance

The suppression of apoptosis via NF-κB activation originated by IGF2BP1 in tumors are linked to unfavorable clinical outcomes in CRC patients. These patients present propensity toward early dissemination, poor response to therapy and increased tumor aggressiveness. On the contrary, the absence of IGF2BP1 expression is an independent favorable prognostic factor for survival (Dimitriadis et al., 2007; Vainer et al., 2008; Hamilton et al., 2013). CRC patients also present a high antibody response to IGF2BP2, making this protein a possible biomarker for diagnosis and prognosis (Liu W. et al., 2013). Furthermore, IGF2BP2 may be important for chemoresistance and recurrence of the disease, given its participation in the maintenance of CSCs (Degrauwe et al., 2016; Jang et al., 2017). Concerning IGF2BP3 clinical relevance, it has been shown that this protein is a marker for aggressiveness, poor differentiation and tumor progression and it is related with an unfavorable prognostic and short survival times (Lochhead et al., 2012; Lederer et al., 2014; Chen et al., 2017). Also, IGF2BP3 positive patients have a nearly 11-fold increased risk of distant metastases (Li et al., 2009; Lin et al., 2013; Wei et al., 2015; Chen et al., 2017). This strong correlation suggests, that IGF2BP3 plays an important role in epithelial-mesenchymal transition (EMT) (Li et al., 2009; Lin et al., 2013; Wei et al., 2015; Chen et al., 2017).

ESRP1

General Features

Also named RBM35A, epithelial splicing regulatory protein 1 (ESRP1) contains three putative RRMs, which are mutational hotspots of primary colon tumors with microsatellite instability (MSI) causing rapid degradation of the mutated transcripts (Leontieva and Ionov, 2009; Deloria et al., 2016; Mager et al., 2017). ESRP1 controls alternative splicing and regulates mRNA stability and translation of several mRNAs (Fagoonee et al., 2017). For example, ESRP1 has been identified as a key regulator for Ig-like III domain variant splicing of the fibroblast growth factor receptor 2 (FGFR2). Also, ESRP1 regulates transcript variants from genes associated with EMT such as *CD44*, *ENAH*, and *CTNND1* (p120-catenin) (Deloria et al., 2016). ESRP1 is a tumor suppressor in CRC due to their ability to regulate translation of several cancer-related genes by binding to their mRNA 5′UTRs. In addition, ESRP1 suppresses cancer cell motility through distinct mechanisms during EMT (Leontieva and Ionov, 2009; Deloria et al., 2016; Fagoonee et al., 2017). Ectopic expression of ESRP1 protein resulted in suppression of tumorigenic potential of LS180 colon cancer cells (Leontieva and Ionov, 2009). *ESRP1* is negatively regulated by mesenchymal transcription factors such as SNAIL, ZEB1, and ZEB2 (Mager et al., 2017).

Despite its role as a tumor suppressor, Fagoonee et al. recently demonstrated a pro-metastatic function of ESRP1 (Fagoonee et al., 2017). ESRP1 contributes to anchorage-independent growth of CRC cells, when Caco-2 cells are grown in suspension, enhances FGFR1/2 signaling, supports constant *Akt* phosphorylation and *Snail* upregulation. FGFR or PI3K/Akt inhibition reverted the pro-oncogenic phenotype of ESRP1 upregulation. High *ESRP1* expression may stimulate cancer epithelial cell growth in the colon, as well as, at distant sites promoting CRC progression (Fagoonee et al., 2017).

Xenografts Studies

ESRP1 has a key role in intestinal homeostasis and disease in mice (Mager et al., 2017). Partial loss of *ESRP1* function impairs intestinal epithelial barrier integrity, increases susceptibility to colitis and alters CRC development. In addition, *ESRP1* overexpression has been correlated with liver macrometastasis in

murine models, probably due to its ability to promote cancer cell growth at distant sites (Fagoonee et al., 2017).

Clinical Relevance

ESRP1 expression is associated with a favorable overall survival outcome in CRC patients. On the contrary, loss of *ESRP1* expression negatively correlates with CRC patient survival (Mager et al., 2017). Decreased *ESRP1* expression might also indicate the presence of EMT and thus disease progression and metastasis (Deloria et al., 2016). In addition, by upregulating Snail expression, ESRP1 has been associated with poor prognosis and shortened relapse-free survival (Fagoonee et al., 2017).

TTP

General Features

Tristetraprolin (TTP) also called ZFP36 or TIS11 forms part of a family of tandem Cys3His zinc finger proteins (Lai et al., 1999; Sharma et al., 2013; Sobolewski et al., 2015; Lee et al., 2018). TPP is mainly cytoplasmic, where interacts with stress granules (SGs), regulates mRNA stability and promotes degradation of inflammatory cytokines, proto-oncogenes and growth regulatory genes (Carrick and Blackshear, 2007; Cha et al., 2011; Lee et al., 2013). TTP functions as a tumor suppressor by inhibiting expression of cancer-related genes that encode AREs in their mRNA 3′UTRs. TTP target mRNAs encode inflammatory cytokines, cell growth factors, angiogenesis, apoptosis, and differentiation-related factors. For instance, TTP downregulates VEGF levels by reducing VEGF mRNA accumulation; this, in turn, decreases angiogenesis and reduces CRC growth (López de Silanes et al., 2003; Lee et al., 2010, 2018). TTP also regulates the expression of cancer-related proteins (Fos, Myc, COX-2, cIAP2, E2F1, Bcl-2, Mcl-1, LATS2, Lin28, and Cyclin D1), which contribute to inflammation, apoptosis, and angiogenesis in CRC (Lee et al., 2010, 2018; Sobolewski et al., 2015). Accordingly, *TTP* downregulation occurs at early stages of tumorigenesis and ectopic expression of *TTP* in CRC attenuates cell proliferation (Sobolewski et al., 2015).

Xenografts Studies

The inverse correlation between the expression levels of *TTP* and *VEGF* has been seen in nude mice, where tumor growth and angiogenesis are inhibited by TTP-mediated VEGF downregulation (Lee et al., 2010). Besides, *TTP* knockout mouse model develops multiple inflammatory syndromes due to the increased expression of tumor necrosis factor, *COX-2* and other pro-inflammatory proteins (Sobolewski et al., 2015).

miRNA Control

miR-29a downregulates TTP in a breast cancer model and is known to be upregulated in colon cancer (Sobolewski et al., 2015).

Clinical Relevance

Fallahi et al. have shown that *TTP* reduction is associated with poor prognosis, tumor aggressiveness and necrosis (Fallahi et al., 2014). The pharmacologic activation of *TTP* may limit colon cancer growth when patients present resistance to anti-VEGF therapies (Lee et al., 2010). In this regard, some therapies have been developed to activate *TTP*. For instance, Resveratrol, a natural anti-cancer compound, induces cellular apoptosis and decreases migration and invasion by activating *TTP* and regulating other cancer pathways (MYC, KRAS, and FOS) (Lee et al., 2018). Another agent aiming to restore *TTP* expression in cancer cells is Vorinostat® (SAHA), already in phase 1 clinical trial (Sobolewski et al., 2015). Another option to increase *TTP* expression is the use of histone deacetylase inhibitors (HDAC inhibitors), which can restore *TTP* expression at the transcriptional level (Sobolewski et al., 2015).

HNRNPS

General Features

Heterogeneous nuclear ribonucleoproteins (hnRNPs) are normally localized in the nucleus; however, some may shuttle between the nucleus and cytoplasm due to their nuclear export signals. They are known as pre-mRNA/mRNA binding proteins that participate in important cellular mechanisms, such as DNA repair, response to hypoxia, splicing, nucleocytoplasmic transport, apoptosis and transcriptional and translational regulation (Ushigome et al., 2005; Hope and Murray, 2011; Lai et al., 2016). Quantitative and qualitative alterations of hnRNPs have shown to disturb cellular functions and facilitate malignant transformation (Ushigome et al., 2005).

To date, at least 20 major hnRNP proteins, from hnRNP Al to U, have been identified in human cells (Ushigome et al., 2005). All members of the hnRNP family share a similar protein structure, consisting of at least one RRM combined with other auxiliary domain: RGG box or the acidic domain responsible for protein-protein interactions (Lai et al., 2016). Given that all hnRNPs belong to the same family, their phenotypic impact is likely similar (Hope and Murray, 2011; Budak et al., 2017). Despite their great importance, few studies have been focused on cancer and much less on CRC. **Table 2** summarizes their function and effect in CRC.

TIA1

General Features

T-cell intracellular Antigen-1 (TIA1) is a cytoplasmic granule-associated RBP which contains three RRMs (Zlobec et al., 2010; Hamdollah Zadeh et al., 2014; Yang et al., 2014). TIA1 is linked to multiple biological processes associated with RNA metabolism and plays an important role in the regulation of gene expression, predominantly under conditions of cellular stress (Liu et al., 2014; Yang et al., 2014). TIA1 is alternatively spliced in exon 5 to form two isoforms (short and long), both of them reported to be expressed in cytolytic cells. TIA1 inhibits both transcriptional and posttranscriptional events of many transcripts involved in cancer cell proliferation, apoptosis, angiogenesis, invasiveness, and metastasis as well as in immune evasion (Hamdollah Zadeh et al., 2014; Liu Z. P. et al., 2017). For example, TIA1 can promote cell apoptosis by regulating Fas alternative splicing, while also enhancing NK cell

TABLE 2 | Function and effect of hnRNPs in CRC.

hnRNP	Function	Effect in CRC	Reference
AI	- Unwinds intramolecular folded-back quadruplex structures of telomere repeats and G-rich short tandem repeats (STRs). - Abrogates DNA synthesis arrest. - Promotes a protective effect against apoptosis.	- A potential biomarker. It has a significant cytoplasmic immunoreaction in tumor cells.	Ushigome et al., 2005; Zhang et al., 2006; Hope and Murray, 2011
A18	- Promotes inflammatory responses when present extracellularly.	- Higher hnRNPA18 expression in CRC cells could be used as an independent prognostic marker.	Sakurai et al., 2014; Chang et al., 2016; Jang et al., 2017
D	- Destabilizes RNA and regulates expression of pro-inflammatory Cytokines, proto-oncogenes, and regulators of apoptosis, and the cell cycle. - Enhances mRNA stability and translation.	- Indirectly regulates cancer-related mRNAs by inhibiting Dicer-mediated mature miRNA formation. HnRNPD binds to Dicer mRNA reducing its stability. An inverse correlation between Dicer and hnRNPD expression has been observed in CRC tissues.	Dixon, 2004; Zucconi and Wilson, 2011; Ciafrè and Galardi, 2013; Dai et al., 2019
DL	- Acts as a transcriptional regulator. - Promotes transcription repression. - Stimulates transcription activation in differentiated myotubes.	- Confers growth advantage through its ability to promote cell cycle progression.	Balasubramani et al., 2006; Rappaport et al., 2017
F	- Plays a role in the regulation of alternative splicing events. - Binds G-rich sequences in pre-mRNAs and keeps target RNA in an unfolded state.	- Involved in early CRC genesis.	Balasubramani et al., 2006; Rappaport et al., 2017
H	- Mediates pre-mRNA alternative splicing regulation.	- hnRNPH is associated with good prognosis, especially in left-sided (distal) colonic tumors and rectal tumors.	Hope and Murray, 2011; Rappaport et al., 2017
I	- Activates exon skipping of its own pre-mRNA during muscle cell differentiation.	- Silences Notch signaling pathway, which is a critical mediator of stem cell proliferation and differentiation of colonic epithelium.	Hope and Murray, 2011; Jin et al., 2017; Rappaport et al., 2017
K	- Plays an important role in TP53 response to DNA damage, acting at both transcription activation, and repression.	- Could be used as a poor prognosis marker. Altered expression and cellular localization correlates with CRC tumor stage.	Hope and Murray, 2011; Guo et al., 2012; Sugimasa et al., 2015; Zhang et al., 2016; Budak et al., 2017; Rappaport et al., 2017
M	- Acts as a receptor for carcinoembryonic antigen in Kupffer cells - Initiates a series of signaling events leading to tyrosine phosphorylation of proteins and induction of IL-1 alpha, IL-6, IL-10, and tumor necrosis factor alpha.	- Positively correlates with proliferation, invasion and metastasis of CRC cells.	Chen et al., 2013
L	- Involved in the synthesis of new blood vessels.	- Promotes angiogenesis in CRC cells.	Hope and Murray, 2011
Q	- Promotes MYC mRNA stability - Modulates the posttranscriptional C to U RNA-editing of the APOB mRNA.	- Increases cell proliferation and contribute to tumorigenesis.	Lai et al., 2016; Rappaport et al., 2017
U	- Repairs double-strand DNA.	- Aberrantly found in the nucleus of CRC cells, compared with normal colonic epithelium.	Hope and Murray, 2011

cytotoxic activity (Zlobec et al., 2010; Liu Z. P. et al., 2017). TIA1 is downregulated at the protein level in CRC, which is therefore considered as a tumor suppressor RBP (Liu Y. et al., 2017).

Xenograft Studies and miRNA Control

TIA1 is a direct target of miR-19a in CRC, where is highly expressed (Liu Y. et al., 2017). miR-19a is part of a family known as mir17-92, which possess several cellular functions as survival, proliferation, differentiation, and formation of new blood vessels (Olive et al., 2009). This miRNA promotes cell proliferation and migration *in vitro* and accelerates tumor growth in xenografed mice. miR-19a binds directly to the 3′-UTR of TIA1 mRNA inhibiting TIA1 cancer suppressive features. Suppression of miR-19a activity could increase cellular levels of TIA1, therefore impairing cancer-related cellular processes (Liu Y. et al., 2017).

Clinical Relevance

TIA1 is a robust prognostic immunological biomarker in CRC and particularly in tumors with marked cytotoxic CD8+ tumor-infiltrating lymphocytes (TILs). Increased numbers of

TIA1 positive TILs is associated with an improved clinical outcome representing an independent prognostic factor (Zlobec et al., 2010). TIA1 can also be used to supplement prognostic information related to TNM (tumor, node, and metastases) stage and adjuvant therapy in mismatch repair-proficient colorectal cancer patients (Liu Y. et al., 2017).

KHDRBS1

General Features

KH RNA binding domain containing signal transduction associated 1 (KHDRBS1) protein or Src-associated in mitosis 68 kDa protein is part of STAR family KH domain-containing RBPs (Sánchez-Jiménez and Sánchez-Margalet, 2013). It is a substrate for Src kinases, which are often activated in human cancers. KHDRBS1 is usually a nuclear protein, which is a mitogene and is also involved in transformation and tumorigenesis. This RBP plays a major protagonist in the life cycle of RNA molecules. Besides, KHDRBS1 regulates the alternative splicing of several genes, most of them involved in human cancer, such as *CD44, Bcl-xl, Sgce, SMN2, SF2/ASF,* and *Cyclin D1*. KHDRBS1 also participates in early cellular responses to DNA damage by controlling the signaling cascade that links DNA damage recognition in the nucleus to NF-κB liberation and activation in the cytoplasm. Accordingly, *KHDRBS1* downregulation promotes self-destruction of colon cancer cells under exposure to DNA-damaging agents. KHDRBS1 is therefore important for CRC development and survival (Fu et al., 2016).

Xenograft Studies

KHDRBS1 null mice produced delays in colon tumor growth, metastasis, cell migration, and extremely sensitiveness to agents that cause DNA damage (Lukong and Richard, 2007; Fu et al., 2016).

Clinical Relevance

Several studies have reported *KHDRBS1* to be overexpressed in CRC tissues. KHDRBS1 nuclear localization and overexpression is correlated with poorly differentiated cancer cells, advanced T, N, and M1 stage. Poor prognosis and a higher risk of recurrence have been seen in patients with high levels of this protein or nuclear localization (Liao et al., 2013; Fu et al., 2016).

CPEB4

General Features

Cytoplasmic Polyadenylation Element Binding Protein 4 (CPEB4) is a ubiquitous cytoplasmic zinc-finger RBP (Zhong et al., 2015; He et al., 2017; Rappaport et al., 2017). *CPB4* gene is localized on chromosome 5q35 and encodes a protein composed of 729 amino acids (He et al., 2017). CPEB4 can modulate the cellular epigenetic profile and influence several biological activities such as cell proliferation and differentiation, chromatin-remodeling, and chromosome segregation (Zhong et al., 2015; He et al., 2017). Furthermore, CPEB4 recruits translational repression or polyadenylation machinery, which targets mRNAs that regulate mitotic and meiotic cell cycle and senescence (Cortés-Guiral et al., 2017; He et al., 2017). Importantly, *CPEB4* is highly expressed in a variety of malignant tumors, including CRC, promoting tumor proliferation, invasion, migration, and vascularization (Zhong et al., 2015; Cortés-Guiral et al., 2017; He et al., 2017). CPEB4 can also influence apoptosis of tumoral cells by modulating the expressions of B-cell lymphoma extra-large (Bcl-XL) and B-cell lymphoma-2-associated X (Bax) proteins. *CPEB4* knockdown increases Bax expression but decreases Bcl-XL expression. Changes in the homeostatic balance of Bax and Bcl-XL lead to a deregulation of apoptosis during tumor development. In addition, three parameters are considered as prognostic markers in CRC: (i) age, (ii) body tumor location, and (iii) Bax/Bcl-2 ratio (Zhong et al., 2015).

miRNA Control

Recent studies have shown miR-203, a tumor suppressive miRNA, significantly decreased in colorectal cancers. miR-203 inhibits cancer growth and enhances cell apoptosis by suppressing CPEB4 expression post-transcriptionally (Zhong et al., 2015; Cortés-Guiral et al., 2017). Hence, miR-203-mediated CPEB4 degradation might be a novel strategy in CRC treatment (Zhong et al., 2015).

Clinical Relevance

CPEB4 is aberrantly expressed in CRC tissues and correlates with tumor progression and poor overall survival in CRC patients. Thus, detecting CPEB4 expression in CRC tissues or peripheral blood might be used as an additional parameter to identify patients with a high risk of tumor invasiveness and/or metastasis. Patients with these characteristics could be considered for more personalized and aggressive treatment (Zhong et al., 2015; He et al., 2017).

AGO

General Features

The Argonaute (AGO) proteins are fundamental components of RNA-induced silencing complexes (RISC) and RNA interference (RNAi) machinery, which induce endonuclease cleavage of mRNA and miRNA passenger strand. Currently, two subfamilies with 4 Argonaute-like proteins within each have been described: (i) eIF2C/AGO subfamily with AGO1, AGO2, AGO3, and AGO4 and (ii) PIWI subfamily with PIWIL1, PIWIL2, PIWIL3, and PIWIL4. AGO subfamily genes are ubiquitously expressed and are regulated in a cell-context-dependent manner (Li et al., 2010; Rüdel et al., 2011).

Small interfering RNAs (siRNAs) and/or miRNAs are used by Ago proteins as silencing mechanisms in both transcriptional and posttranscriptional processes. Overexpression of AGO members has been associated with excessive growth and programmed cell death inhibition of cancer stem cells. Specifically, increased expression of *AGO2-4* and *PIWIL4* has been associated with colon cancer occurrence in advanced tumors with distant metastasis (Li et al., 2010).

CSDE1

General Features

Cold shock domain containing E1 (*CSDE1*), formerly named Upstream of the *NRAS* (UNR), is an RBP composed of 798 amino acids with 5 cold shock domains. *CSDE1* gene is located on chromosome 1p13.2 upstream of the *NRAS* locus. CSDE1 has been shown to regulate mRNA stability and translation of several oncogenes, such as *c-MYC, c-FOS, VIM, PTEN*, among many others, in melanoma, breast, pancreatic and prostate cancer (Grosset et al., 2000; Evans et al., 2003; Chang et al., 2004; Wurth et al., 2016). Recently, Martinez-Useros et al. have reported key oncogenic features of CSDE1 in CRC. According to this study, *CSDE1* is overexpressed in several CRC-derived cell lines, paired tumor samples, colonospheres and cell cultures originated from metastatic lesions. In contrast, *CSDE1* downregulation increases sensitivity to apoptosis and decrease invasiveness, cell viability and migration by an EMT regulation process. In addition, *CSDE1* expression positively correlates with *c-MYC* expression in CRC samples and cell lines, supporting its role as a CRC oncogene (Martinez-Useros et al., 2019).

Clinical Relevance

Martinez-Useros et al. also demonstrated a possible role of *CSDE1* as a clinical marker, predicting poor outcome of CRC patients. Patients with high *CSDE1* expression presented shorter mean survival than patients with low expression. Although the sample size to achieved

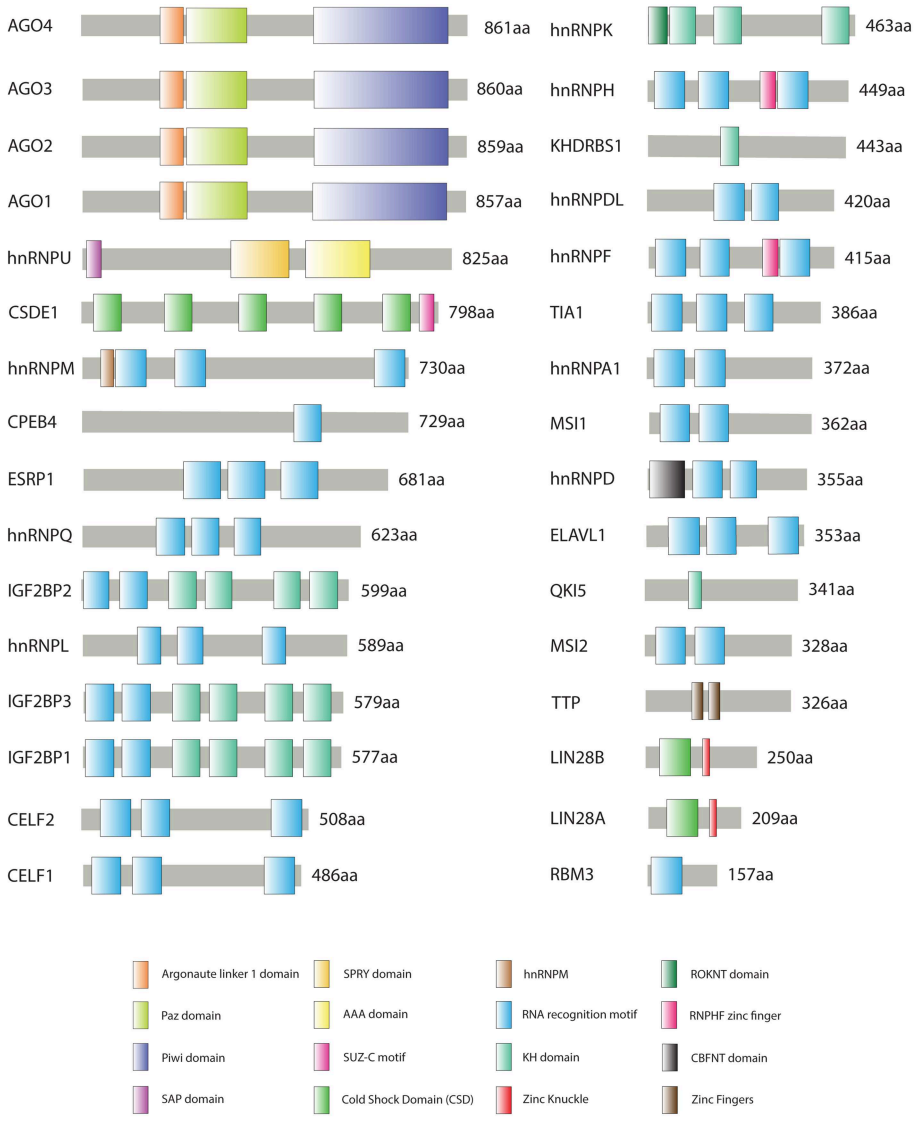

FIGURE 1 | Schematic representation of colorectal cancer (CRC)-associated RNA-Binding Proteins (RBPs) structural domains according to UniProt database (https://www.uniprot.org). Sixteen structural domains represented by colored boxes, protein names and scaled lengths are shown.

this conclusion was small, an *in silico* analysis using The Cancer Genome Atlas (TCGA)-Colorectal Cancer Dataset showed similar results: patients with high *CSDE1* expression presented shorter mean progression-free survival than patients with low *CSDE1* expression (Martinez-Useros et al., 2019).

COMMON FEATURES OF ALL COLON CANCER RELATED RBPS

RBPs are pivotal members of the posttranscriptional process and key players of RNA regulons. Within this notion, RBPs regulate mRNAs that encode functionally related proteins through a RNP-driven mechanism. Genomic alterations of RBPs could therefore produce erroneous conformation of these RNP complexes leading to atypical protein expression and cancer development (Castello et al., 2016; Wurth et al., 2016; Pereira et al., 2017). To shed light on CRC RNA regulons, we studied CRC RBPs common features related to their protein domains, protein-protein interactions, and common RNA targets. In addition, we also organized CRC RBPs oncogenic capabilities into the hallmarks of cancer (Hanahan and Weinberg, 2011).

Figure 1 shows all CRC-associated RBPs and their protein domains according to UniProt database (https://www.uniprot.org/) (The UniProt Consortium, 2019); we detected 16 protein domains, of which 13 are RNA-binding domains: RRM, CSD, KH domain, Zinc finger, etc. RRM is the most prevalent domain of the CRC-related RBPs, followed by KH domain and CSD. To detect protein-protein interactions, we generated an interaction network using STRING database (Szklarczyk et al., 2017) with experiment scores > 0.9. Seventeen out Thirty-Two RBPs form a complex interaction network (**Figure 2A**); we also observed interactions between all AGO members.

Over the past decade, high-throughput technologies have been developed to identify RBP biding sites *in vivo* (ultraviolet crosslinking followed by immunoprecipitation and sequencing—CLIP) and mRNA translation performance (Ribosome profiling—Ribo-seq) (Ingolia et al., 2009). Recently, Zhu et al. integrated several datasets from these high-throughput technologies to investigate post-transcriptional regulatory processes mediated by RBPs through a database

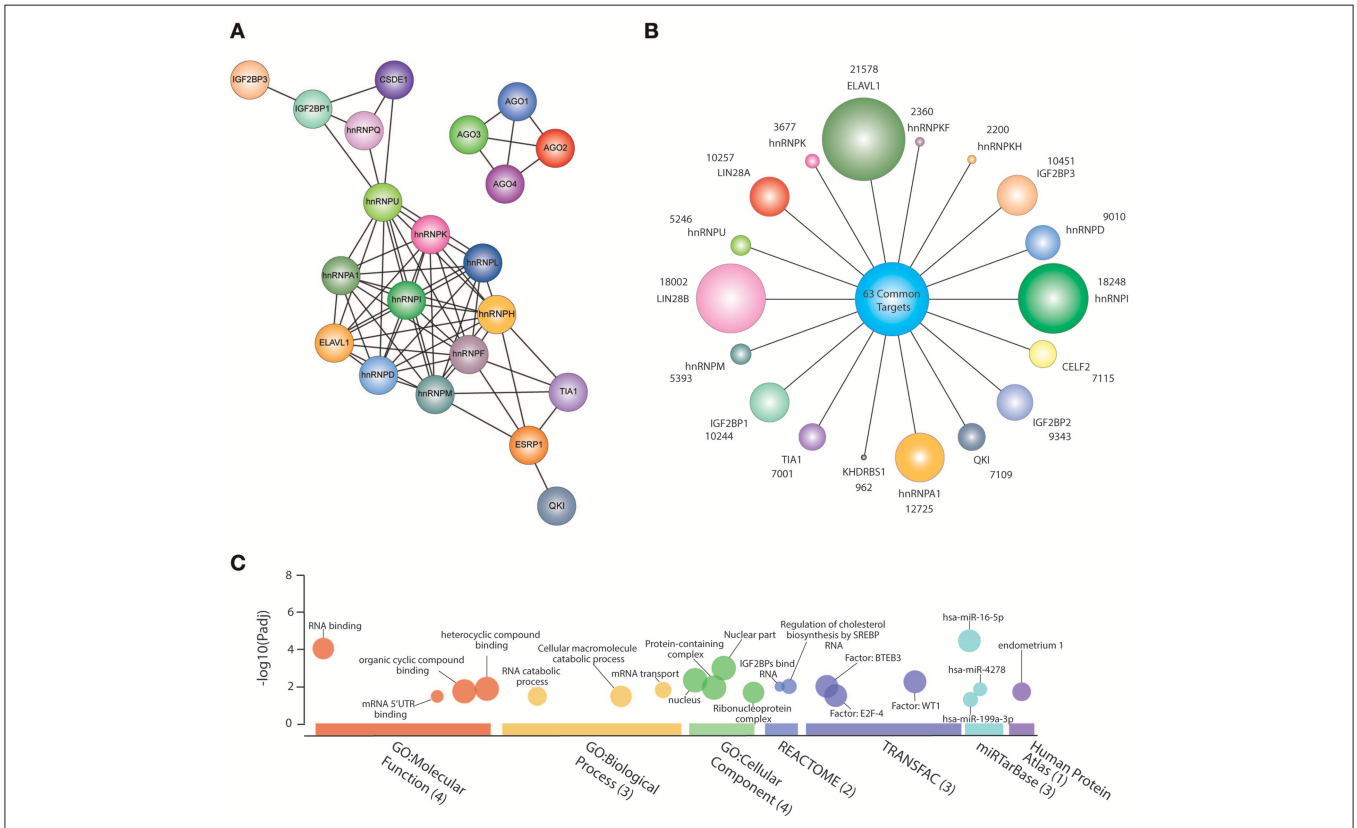

FIGURE 2 | Common features of colorectal cancer (CRC)-related RNA-Binding Proteins (RBPs). **(A)** CRC RBPs RNA targets according to POSTAR2 database. Circle sizes are correlated with the number of targets of each protein; shared RNA targets are shown in the middle. **(B)** A network showing CRC RBPs protein-protein interactions from experimental data and databases (interaction score: > 0.9). **(C)** Gene set enrichment analysis showing all significantly enriched terms concerning Gene Ontology (Molecular Function, Biological Process, and Cellular Component), Reactome, Transfac, miRTasBase, and Human Protein Atlas through g:Profiler (https://biit.cs.ut.ee/gprofiler/gost). The size of the circle is correlated with the number of genes overrepresented in association with certain type of molecular function or biological process. *P*-value adjusted (Padj) for multiple testing using Benjamini-Hochberg method.

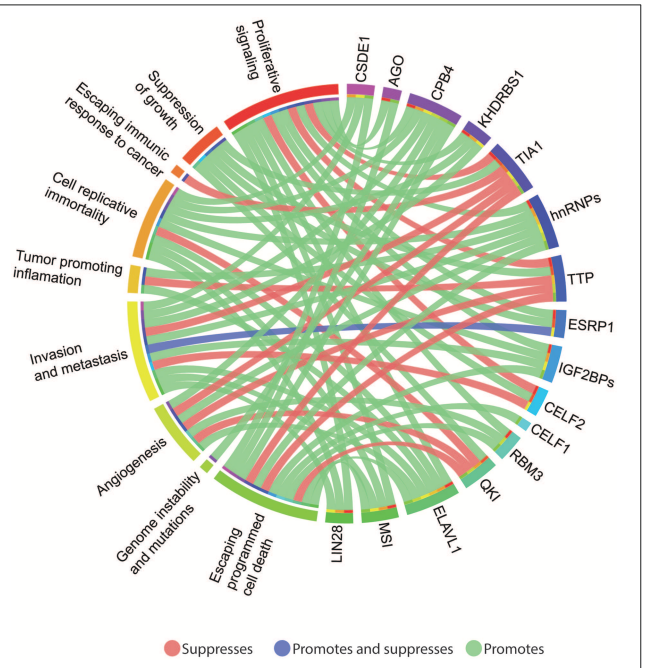

FIGURE 3 | Circos plot (http://circos.ca/intro/features/) depicting the relationships between colorectal cancer (CRC) RNA-binding proteins (RBPs) and the hallmarks of cancer. Outer circle: right, CRC RBPs and left, hallmarks of cancer associated with each studied protein. Green-colored links indicate a cancer promoting activity, while red links, suppression, and the blue-colored link denotes both, promotion and suppression activity.

named POSTAR2 (http://lulab.life.tsinghua.edu.cn/postar/). This allowed us to identify all RNA targets of 18 out 32 CRC-related RBPs (**Figure 2B**). For example, ELAVL1 could bind to 21578 RNAs, while KHDRBS1 interacts with 962. A *post-hoc* analysis revealed 63 common targets (**Supplementary Table 1**). Interestingly, these RNAs encode proteins or participate in RNA-related processes, such as RNA binding or mRNA transport (**Figure 2C**), adding another layer to the RNA regulon model: interacting RBPs that regulate RNAs implicated in RNA-associated processes. In fact, 30% of these common targets ($n = 19$) are also RBPs (**Figure 2C**) (Culjkovic-Kraljacic and Borden, 2018). However, some well-known cancer driver genes, such as *MYC* and *COX-2* are common targets of 11 and 7, respectively out of 18 CRC RBPs.

Finally, to better understand the oncogenic potential of the aforementioned RBPs, their capabilities were organized according to the hallmarks of cancer. As shown in **Figure 3**, most RBPs (11 out of 16) act as oncogenes and only 5 (TIA1, TTP, QKI, ESRP1, CELF2, and QKI) present tumor suppressive abilities. Worthy of note, TIA1 is the only RBP that suppresses the ability of cancer cells to escape immune response by enhancing NK cell cytotoxic activity (Zlobec et al., 2010; Liu Z. P. et al., 2017); also, CPEB4 is the only RBP that promotes genome instability by influencing chromatin-remodeling (Negrini et al., 2010; Zhong et al., 2015; He et al., 2017).

CONCLUDING REMARKS AND PERSPECTIVES

Several efforts have been devoted to decipher the molecular basis behind the carcinogenesis process. Most of this knowledge was achieved by studying DNA and protein function, leaving post-transcription under-investigated. In this regard, RBPs play a significant role in controlling gene expression through complex interconnected networks named RNA regulons. Consequently, RBPs alterations could greatly disrupt cellular homeostasis promoting cancer development.

We believe this work offers a comprehensive list of all CRC-associated RBPs, including their individual features to common interactions and targets. However, we only found in the literature 35 RBPs out of 1,393 having oncogenic roles in CRC; a comprehensive characterization of RBPs is therefore still missing. To shed light on this matter, not only the identification of the CRC-RBPome should be prioritized, but also its dynamics concerning CRC RNA regulons implicated in cancer progression. As we shown in **Figure 2**, these interactions are highly complex and more research is needed to identify key therapeutic interactions. To date, no RBP-based drug has been developed to treat CRC, according to the Open Target Platform (https://www.targetvalidation.org).

RNA-based research is generating large datasets from high-throughput technologies, such CLIP, Ribo-seq or interactome capture. To successfully understand this complexity, all this data should be compiled and analyzed by bioinformatics and systems biology approaches, such as POSTAR2 or the ones developed by the RNA Bioinformatics Center (Backofen et al., 2017). In addition, large datasets, such as The Cancer Genome Atlas (Tomczak et al., 2015), the Human Protein Atlas (Pontén et al., 2008) or Depmap (Yu et al., 2016) could be exploited to identify key RBPs that, with further research, could be used as CRC biomarkers or new therapeutic targets.

AUTHOR CONTRIBUTIONS

JG-C and SG conceived the subject and wrote the manuscript. CP supervised the project and provided conceptual advice and valuable scientific input. AL-C, IA-C, PG-R, AP-V, VY, AZ, and PL made a substantial contribution to the structure and design of the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmolb.2019.00065/full#supplementary-material

REFERENCES

Abdelmohsen, K., Srikantan, S., Kuwano, Y., and Gorospe, M. (2008). miR-519 reduces cell proliferation by lowering RNA-binding protein HuR levels. *Proc. Natl. Acad. Sci. U.S.A.* 105, 20297–20302. doi: 10.1073/pnas.0809376106

Akaike, Y., Masuda, K., Kuwano, Y., Nishida, K., Kajita, K., Kurokawa, K., et al. (2014). HuR regulates alternative splicing of the TRA2 gene in human colon cancer cells under oxidative stress. *Mol. Cell. Biol.* 34, 2857–2873. doi: 10.1128/MCB.00333-14

Al-Haidari, A., Algaber, A., Madhi, R., Syk, I., and Thorlacius, H. (2018). MiR-155-5p controls colon cancer cell migration via post-transcriptional regulation of Human Antigen R (HuR). *Cancer Lett.* 421, 145–151. doi: 10.1016/j.canlet.2018.02.026

Backofen, R., Engelhardt, J., Erxleben, A., Fallmann, J., Grüning, B., Ohler, U., et al. (2017). RNA-bioinformatics: tools, services and databases for the analysis of RNA-based regulation. *J. Biotechnol.* 261, 76–84. doi: 10.1016/j.jbiotec.2017.05.019

Badawi, A., Hehlgans, S., Pfeilschifter, J., Rödel, F., and Eberhardt, W. (2017). Silencing of the mRNA-binding protein HuR increases the sensitivity of colorectal cancer cells to ionizing radiation through upregulation of caspase-2. *Cancer Lett.* 393, 103–112. doi: 10.1016/j.canlet.2017.02.010

Balasubramani, M., Day, B. W., Schoen, R. E., and Getzenberg, R. H. (2006). Altered expression and localization of creatine kinase B, heterogeneous nuclear ribonucleoprotein F, and high mobility group box 1 protein in the nuclear matrix associated with colon cancer. *Cancer Res.* 66, 763–769. doi: 10.1158/0008-5472.CAN-05-3771

Blanco, F. F., Preet, R., Aguado, A., Vishwakarma, V., Stevens, L. E., Vyas, A., et al. (2016). Impact of HuR inhibition by the small molecule MS-444 on colorectal cancer cell tumorigenesis. *Oncotarget* 7, 74043–74058. doi: 10.18632/oncotarget.12189

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., and Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* 68, 394–424. doi: 10.3322/caac.21492

Brennan, C. M., and Steitz, J. A. (2001). HuR and mRNA stability. *Cell. Mol. Life Sci.* 58, 266–277. doi: 10.1007/PL00000854

Budak, G., Srivastava, R., and Janga, S. C. (2017). Seten: a tool for systematic identification and comparison of processes, phenotypes, and diseases associated with RNA-binding proteins from condition-specific CLIP-seq profiles. *RNA* 23, 836–846. doi: 10.1261/rna.059089.116

Burd, C., and Dreyfuss, G. (1994). Conserved structures and diversity of functions of RNA-binding proteins. *Science* 265, 615–621. doi: 10.1126/science.8036511

Carrick, D. M., and Blackshear, P. J. (2007). Comparative expression of tristetraprolin (TTP) family member transcripts in normal human tissues and cancer cell lines. *Arch. Biochem. Biophys.* 462, 278–285. doi: 10.1016/j.abb.2007.04.011

Castello, A., Fischer, B., Frese, C. K., Horos, R., Alleaume, A. M., Foehr, S., et al. (2016). Comprehensive identification of RNA-binding domains in human cells. *Mol. Cell* 63, 696–710. doi: 10.1016/j.molcel.2016.06.029

Cha, H. J., Lee, H. H., Chae, S. W., Cho, W. J., Kim, Y. M., Choi, H. J., et al. (2011). Tristetraprolin downregulates the expression of both VEGF and COX-2 in human colon cancer. *Hepatogastroenterology* 58, 790–795.

Chang, E. T., Parekh, P. R., Yang, Q., Nguyen, D. M., and Carrier, F. (2016). Heterogenous ribonucleoprotein A18 (hnRNP A18) promotes tumor growth by increasing protein translation of selected transcripts in cancer cells. *Oncotarget* 7, 10578–10593. doi: 10.18632/oncotarget.7020

Chang, T. C., Yamashita, A., Chen, C. Y. A., Yamashita, Y., Zhu, W., Durdan, S., et al. (2004). UNR, a new partner of poly(A)-binding protein, plays a key role in translationally coupled mRNA turnover mediated by the c-fos major coding-region determinant. *Genes Dev.* 18, 2010–2023. doi: 10.1101/gad.1219104

Chappell, S. A., and Mauro, V. P. (2003). The internal ribosome entry site (IRES) contained within the RNA-binding motif protein 3 (Rbm3) mRNA is composed of functionally distinct elements. *J. Biol. Chem.* 278, 33793–33800. doi: 10.1074/jbc.M303495200

Chen, L., Xie, Y., Li, X., Gu, L., Gao, Y., Tang, L., et al. (2017). Prognostic value of high iMP3 expression in solid tumors: a meta-analysis. *Oncol. Targets Ther.* 10, 2849–2863. doi: 10.2147/OTT.S128810

Chen, S., Zhang, J., Duan, L., Zhang, Y., Li, C., Liu, D., et al. (2013). Identification of HnRNP M as a novel biomarker for colorectal carcinoma by quantitative proteomics. *Am. J. Physiol. Gastrointest. Liver Physiol.* 306, G394–G403. doi: 10.1152/ajpgi.00328.2013

Cheng, D. L., Xiang, Y. Y., Ji, L. J., and Lu, X. J. (2015). Competing endogenous RNA interplay in cancer: mechanism, methodology, and perspectives. *Tumor Biol.* 36, 479–488. doi: 10.1007/s13277-015-3093-z

Choi, D. K., Ito, T., Tsukahara, F., Hirai, M., and Sakaki, Y. (1999). Developmentally-regulated expression of mNapor encoding an apoptosis-induced ELAV-type RNA binding protein. *Gene* 237, 135–142. doi: 10.1016/S0378-1119(99)00312-1

Ciafrè, S. A., and Galardi, S. (2013). microRNAs and RNA-binding proteins: a complex network of interactions and reciprocal regulations in cancer. *RNA Biol.* 10, 927–935. doi: 10.4161/rna.24641

Cortés-Guiral, D., Pastor-Iodate, C., Díaz del Arco, C., del Puerto-Nevado, L., and Fernández-Aceñero, M. J. (2017). CPEB4 immunohistochemical expression is associated to prognosis in stage IV colorectal carcinoma. *Pathol. Res. Pract.* 213, 639–642. doi: 10.1016/j.prp.2017.04.020

Cui, Y. H., Xiao, L., Rao, J. N., Zou, T., Liu, L., Chen, Y., et al. (2012). miR-503 represses CUG-binding protein 1 translation by recruiting CUGBP1 mRNA to processing bodies. *Mol. Biol. Cell.* 23, 151–162. doi: 10.1091/mbc.e11-05-0456

Culjkovic-Kraljacic, B., and Borden, K. L. B. (2018). The impact of post-transcriptional control: better living through RNA regulons. *Front. Genet.* 9:512. doi: 10.3389/fgene.2018.00512

Dai, W., Mu, L., Cui, Y., Li, Y., Chen, P., Xie, H., et al. (2019). Berberine promotes apoptosis of colorectal cancer via regulation of the long non-coding RNA (lncRNA) cancer susceptibility candidate 2 (CASC2)/AU-binding factor 1 (AUF1)/B-Cell CLL/ lymphoma 2 (Bcl-2) axis. *Med. Sci. Monit.* 25, 730–738. doi: 10.12659/MSM.912082

Darbelli, L., Vogel, G., Almazan, G., and Richard, S. (2016). Quaking regulates neurofascin 155 expression for myelin and axoglial junction maintenance. *J. Neurosci.* 36, 4106–4120. doi: 10.1523/JNEUROSCI.3529-15.2016

de Sousa Abreu, R., Sanchez-Diaz, P. C., Vogel, C., Burns, S. C., Ko, D., Burton, T. L., et al. (2009). Genomic analyses of musashi1 downstream targets show a strong association with cancer-related processes. *J. Biol. Chem.* 284, 12125–12135. doi: 10.1074/jbc.M809605200

Degrauwe, N., Schlumpf, T. B., Janiszewska, M., Martin, P., Cauderay, A., Provero, P., et al. (2016). The RNA binding protein IMP2 preserves glioblastoma stem cells by preventing let-7 target gene silencing. *Cell Rep.* 15, 1634–1647. doi: 10.1016/j.celrep.2016.04.086

Deloria, A. J., Höflmayer, D., Kienzl, P., Łopatecka, J., Sampl, S., Klimpfinger, M., et al. (2016). Epithelial splicing regulatory protein 1 and 2 paralogues correlate with splice signatures and favorable outcome in human colorectal cancer. *Oncotarget* 7, 73800–73816. doi: 10.18632/oncotarget.12070

Denkert, C., Koch, I., Von Keyserlingk, N., Noske, A., Niesporek, S., Dietel, M., et al. (2006). Expression of the ELAV-like protein HuR in human colon cancer: association with tumor stage and cyclooxygenase-2. *Mod. Pathol.* 19, 1261–1269. doi: 10.1038/modpathol.3800645

Derry, J. M. J., Kerns, J. A., and Francke, U. (1995). RBM3, a novel human gene in Xp11.23 with a putative RNA-binding domain. *Hum. Mol. Genet.* 4, 2307–2311. doi: 10.1093/hmg/4.12.2307

Dimitriadis, E., Trangas, T., Milatos, S., Foukas, P. G., Gioulbasanis, I., Courtis, N., et al. (2007). Expression of oncofetal RNA-binding protein CRD-BP/IMP1 predicts clinical outcome in colon cancer. *Int. J. Cancer* 121, 486–494. doi: 10.1002/ijc.22716

Dixon, D. (2004). Dysregulated post-transcriptional control of COX-2 gene expression in cancer. *Curr. Pharm. Des.* 10, 635–646. doi: 10.2174/1381612043453171

Dixon, D. A., Tolley, N. D., King, P. H., Nabors, L. B., McIntyre, T. M., Zimmerman, G. A., et al. (2001). Altered expression of the mRNA stability factor HuR promotes cyclooxygenase-2 expression in colon cancer cells. *J. Clin. Invest.* 108, 1657–1665. doi: 10.1172/JCI12973

Dresios, J., Aschrafi, A., Owens, G. C., Vanderklish, P. W., Edelman, G. M., and Mauro, V. P. (2005). Cold stress-induced protein Rbm3 binds 60S ribosomal subunits, alters microRNA levels, and enhances global protein synthesis. *Proc. Natl. Acad. Sci. U.S.A.* 102, 1865–1870. doi: 10.1073/pnas.0409764102

Elcheva, I., Goswami, S., Noubissi, F. K., and Spiegelman, V. S. (2009). CRD-BP protects the coding region of betaTrCP1 mRNA from miR-183-mediated degradation. *Mol. Cell* 35, 240–246. doi: 10.1016/j.molcel.2009.06.007

Evans, J. R., Mitchell, S. A., Spriggs, K. A., Ostrowski, J., Bomsztyk, K., Ostarek, D., et al. (2003). Members of the poly (rC) binding protein family stimulate the activity of the c-myc internal ribosome entry segment *in vitro* and *in vivo*. *Oncogene* 22, 8012–8020. doi: 10.1038/sj.onc.1206645

Fagoonee, S., Picco, G., Orso, F., Arrigoni, A., Longo, D. L., Forni, M., et al. (2017). The RNA-binding protein ESRP1 promotes human colorectal cancer progression. *Oncotarget* 8, 10007–10024. doi: 10.18632/oncotarget.14318

Fallahi, M., Amelio, A. L., Cleveland, J. L., and Rounbehler, R. J. (2014). CREB targets define the gene expression signature of malignancies having reduced levels of the tumor suppressor tristetraprolin. *PLoS ONE* 9:e115517. doi: 10.1371/journal.pone.0115517

Fan, L. F., Dong, W. G., Jiang, C. Q., Xia, D., Liao, F., and Yu, Q. F. (2010). Expression of putative stem cell genes Musashi-1 and β1-integrin in human colorectal adenomas and adenocarcinomas. *Int. J. Colorectal Dis.* 25, 17–23. doi: 10.1007/s00384-009-0791-2

Fan, X. C., and Steitz, J. A. (1998). Overexpression of HuR, a nuclear-cytoplasmic shuttling protein, increases the *in vivo* stability of ARE-containing mRNAs. *EMBO J.* 17, 3448–3460. doi: 10.1093/emboj/17.12.3448

Franceschini, A., Szklarczyk, D., Frankild, S., Kuhn, M., Simonovic, M., Roth, A., et al. (2012). STRING v9.1: protein-protein interaction networks, with increased coverage and integration. *Nucleic Acids Res.* 41, D808–D815. doi: 10.1093/nar/gks1094

Fu, K., Sun, X., Wier, E. M., Hodgson, A., Liu, Y., Sears, C. L., et al. (2016). Sam68/KHDRBS1 is critical for colon tumorigenesis by regulating genotoxic stress-induced NF-κB activation. *Elife* 5:e15018. doi: 10.7554/eLife.15018

Gao, C., Han, C., Yu, Q., Guan, Y., Li, N., Zhou, J., et al. (2015). Downregulation of Msi1 suppresses the growth of human colon cancer by targeting p21cip1. *Int. J. Oncol.* 46, 732–740. doi: 10.3892/ijo.2014.2749

Ghosh, M., Aguila, H. L., Michaud, J., Ai, Y., Wu, M. T., Hemmes, A., et al. (2009). Essential role of the RNA-binding protein HuR in progenitor cell survival in mice. *J. Clin. Invest.* 119, 3530–3543. doi: 10.1172/JCI38263

Glazer, R. I., Vo, D. T., and Penalva, L. O. (2012). Musashi1: an RBP with versatile functions in normal and cancer stem cells. *Front. Biosci.* 17, 54–64. doi: 10.2741/3915

Grosset, C., Chen, C. Y., Xu, N., Sonenberg, N., Jacquemin-Sablon, H., and Shyu, A. B. (2000). A mechanism for translationally coupled mRNA turnover: interaction between the poly(A) tail and a c-fos RNA coding determinant via a protein complex. *Cell* 103, 29–40. doi: 10.1016/S0092-8674(00)00102-1

Guinney, J., Dienstmann, R., Wang, X., de Reyniès, A., Schlicker, A., Soneson, C., et al. (2015). The consensus molecular subtypes of colorectal cancer. *Nat. Med.* 21, 1350–1356. doi: 10.1038/nm.3967

Guo, Y., Chen, Y., Ito, H., Watanabe, A., Ge, X., Kodama, T., et al. (2006). Identification and characterization of lin-28 homolog B (LIN28B) in human hepatocellular carcinoma. *Gene* 384, 51–61. doi: 10.1016/j.gene.2006.07.011

Guo, Y., Zhao, J., Bi, J., Wu, Q., Wang, X., and Lai, Q. (2012). Heterogeneous nuclear ribonucleoprotein K (hnRNP K) is a tissue biomarker for detection of early Hepatocellular carcinoma in patients with cirrhosis. *J. Hematol. Oncol.* 5:37. doi: 10.1186/1756-8722-5-37

Hamdollah Zadeh, M. A., Amin, E., Hoareau-Aveilla, C., Domingo, E., Symonds, K. E., Ye, X., et al. (2014). Alternative splicing of TIA-1 in human colon cancer regulates VEGF isoform expression, angiogenesis, tumour growth and bevacizumab resistance. *Mol. Oncol.* 9, 167–178. doi: 10.1016/j.molonc.2014.07.017

Hamilton, K. E., Noubissi, F. K., Katti, P. S., Hahn, C. M., Davey, S. R., Lundsmith, E. T., et al. (2013). IMP1 promotes tumor growth, dissemination and a tumor-initiating cell phenotype in colorectal cancer cell xenografts. *Carcinogenesis* 34, 2647–2654. doi: 10.1093/carcin/bgt217

Hanahan, D., and Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell* 144, 646–674. doi: 10.1016/j.cell.2011.02.013

He, B., Gao, S. Q., Huang, L. D., Huang, Y. H., Zhang, Q. Y., Zhou, M. T., et al. (2015). MicroRNA-155 promotes the proliferation and invasion abilities of colon cancer cells by targeting quaking. *Mol. Med. Rep.* 11, 2355–2359. doi: 10.3892/mmr.2014.2994

He, X., Lin, X., Cai, M., Fan, D., Chen, X., Wang, L., et al. (2017). High expression of cytoplasmic polyadenylation element-binding protein 4 correlates with poor prognosis of patients with colorectal cancer. *Virchows Arch.* 470, 37–45. doi: 10.1007/s00428-016-2037-3

Hentze, M. W., Castello, A., Schwarzl, T., and Preiss, T. (2018). A brave new world of RNA-binding proteins. *Nat. Rev. Mol. Cell Biol.* 19, 327–341. doi: 10.1038/nrm.2017.130

Hope, N. R., and Murray, G. I. (2011). The expression profile of RNA-binding proteins in primary and metastatic colorectal cancer: relationship of heterogeneous nuclear ribonucleoproteins with prognosis. *Hum. Pathol.* 42, 393–402. doi: 10.1016/j.humpath.2010.08.006

Iadevaia, V., and Gerber, A. P. (2015). Combinatorial control of mRNA fates by RNA-binding proteins and non-coding RNAs. *Biomolecules* 5, 2207–2222. doi: 10.3390/biom5042207

Ingolia, N. T., Ghaemmaghami, S., Newman, J. R. S., and Weissman, J. S. (2009). Genome-wide analysis *in vivo* of translation with nucleotide resolution using ribosome profiling. *Science* 324, 218–223. doi: 10.1126/science.1168978

Iwata, N., Ishikawa, T., Okazaki, S., Mogushi, K., Baba, H., Ishiguro, M., et al. (2017). Clinical significance of methylation and reduced expression of the quaking gene in colorectal cancer. *Anticancer Res.* 37, 489–498. doi: 10.21873/anticanres.11341

Jang, H. H., Lee, H. N., Kim, S. Y., Hong, S., and Lee, W. S. (2017). Expression of RNA-binding motif protein 3 (RBM3) and cold-inducible RNA-binding protein (CIRP) is associated with improved clinical outcome in patients with colon cancer. *Anticancer Res.* 37, 1779–1785. doi: 10.21873/anticanres.11511

Ji, S., Ye, G., Zhang, J., Wang, L., Wang, T., Wang, Z., et al. (2013). miR-574-5p negatively regulates Qki6/7 to impact b-catenin/Wnt signalling and the development of colorectal cancer. *Gut* 62, 716–726. doi: 10.1136/gutjnl-2011-301083

Jiang, Q., Crews, L. A., Holm, F., and Jamieson, C. H. M. (2017). RNA editing-dependent epitranscriptome diversity in cancer stem cells. *Nat. Rev. Cancer* 17, 381–392. doi: 10.1038/nrc.2017.23

Jiang, S., and Baltimore, D. (2016). RNA-binding protein Lin28 in cancer and immunity. *Cancer Lett.* 375, 108–113. doi: 10.1016/j.canlet.2016.02.050

Jin, Z., Liang, F., Yang, J., and Mei, W. (2017). hnRNP I regulates neonatal immune adaptation and prevents colitis and colorectal cancer. *PLoS Genet.* 13:1006672. doi: 10.1371/journal.pgen.1006672

Jones, P., Binns, D., Chang, H. Y., Fraser, M., Li, W., Mcanulla, C., et al. (2014). InterProScan 5: genome-scale protein function classification. *Bioinformatics* 30, 1236–1240. doi: 10.1093/bioinformatics/btu031

Kechavarzi, B., and Janga, S. C. (2014). Dissecting the expression landscape of RNA-binding proteins in human cancers. *Genome Biol.* 15:R14. doi: 10.1186/gb-2014-15-1-r14

Keene, J. D. (2007). RNA regulons: coordination of post-transcriptional events. *Nat. Rev. Genet.* 8, 533–543. doi: 10.1038/nrg2111

Kharas, M. G., and Lengner, C. J. (2017). Stem cells, cancer, and MUSASHI in blood and guts. *Trends Cancer* 3, 347–356. doi: 10.1016/j.trecan.2017.03.007

Kim, H. H., and Gorospe, M. (2008). GU-Rich RNA: expanding CUGBP1 function, broadening mRNA turnover. *Mol. Cell* 29, 151–152. doi: 10.1016/j.molcel.2008.01.005

Kim, S. K., Lee, H., Han, K., Kim, S. C., Choi, Y., Park, S. W., et al. (2014). SET7/9 methylation of the pluripotency factor LIN28A is a nucleolar localization mechanism that blocks let-7 biogenesis in human ESCs. *Cell Stem Cell* 15, 735–749. doi: 10.1016/j.stem.2014.10.016

King, C. E., Cuatrecasas, M., Castells, A., Sepulveda, A. R., Lee, J. S., and Rustgi, A. K. (2011a). Tumor and stem cell biology LIN28B promotes colon cancer progression and metastasis. *Cancer Res.* 71, 4260–4268. doi: 10.1158/0008-5472.CAN-10-4637

King, C. E., Wang, L., Winograd, R., Madison, B. B., Mongroo, P. S., Johnstone, C. N., et al. (2011b). LIN28B fosters colon cancer migration, invasion and transformation through let-7-dependent and -independent mechanisms. *Oncogene* 30, 4185–4193. doi: 10.1038/onc.2011.131

Kondo, T., Furuta, T., Mitsunaga, K., Ebersole, T. A., Shichiri, M., Wu, J., et al. (1999). Genomic organization and expression analysis of the mouse qkI locus. *Mamm. Genome* 10, 662–669.

Kumara, H. S., Kirchoff, D., Caballero, O. L., Su, T., Ahmed, A., Herath, S. A., et al. (2015). Expression of the cancer testis antigen IGF2BP3 in colorectal cancers;

IGF2BP3 holds promise as a specific immunotherapy target. *Oncoscience* 2, 607–614. doi: 10.18632/oncoscience.174

Lai, C. H., Huang, Y. C., Lee, J. C., Ta-Chien Tseng, J., Chang, K. C., Chen, Y. J., et al. (2016). Translational upregulation of Aurora-A by hnRNP Q1 contributes to cell proliferation and tumorigenesis in colorectal cancer. *Cell Death Dis.* 8:e2555. doi: 10.1038/cddis.2016.479

Lai, W. S., Carballo, E., Strum, J. R., Kennington, E. A., Phillips, R. S., and Blackshear, P. J. (1999). Evidence that tristetraprolin binds to AU-rich elements and promotes the deadenylation and destabilization of tumor necrosis factor alpha mRNA. *Mol. Cell. Biol.* 19, 4311–4323. doi: 10.1128/MCB.19.6.4311

Lan, L., Appelman, C., Smith, A. R., Yu, J., Larsen, S., Marquez, R. T., et al. (2015). Natural product (–)-gossypol inhibits colon cancer cell growth by targeting RNA-binding protein Musashi-1. *Mol. Oncol.* 9, 1406–1420. doi: 10.1016/j.molonc.2015.03.014

Lederer, M., Bley, N., Schleifer, C., and Hüttelmaier, S. (2014). The role of the oncofetal IGF2 mRNA-binding protein 3 (IGF2BP3) in cancer. *Semin. Cancer Biol.* 29, 3–12. doi: 10.1016/j.semcancer.2014.07.006

Lee, H. H., Son, Y. J., Lee, W. H., Park, Y. W., Chae, S. W., Cho, W. J., et al. (2010). Tristetraprolin regulates expression of VEGF and tumorigenesis in human colon cancer. *Int. J. Cancer* 126, 1817–1827. doi: 10.1002/ijc.24847

Lee, H. H., Yang, S. S., Vo, M. T., Cho, W. J., Lee, B. J., Leem, S. H., et al. (2013). Tristetraprolin down-regulates IL-23 expression in colon cancer cells. *Mol. Cells* 36, 571–576. doi: 10.1007/s10059-013-0268-6

Lee, S. R., Jin, H., Kim, W. T., Kim, W. J., Kim, S. Z., Leem, S. H., et al. (2018). Tristetraprolin activation by resveratrol inhibits the proliferation and metastasis of colorectal cancer cells. *Int. J. Oncol.* 53, 1269–1278. doi: 10.3892/ijo.2018.4453

Leontieva, O. V., and Ionov, Y. (2009). RNA-binding motif protein 35A is a novel tumor suppressor for colorectal cancer. *Cell Cycle* 8, 490–497. doi: 10.4161/cc.8.3.7679

Li, D., Peng, X., Yan, D., Tang, H., Huang, F., Yang, Y., et al. (2011). Msi-1 is a predictor of survival and a novel therapeutic target in colon cancer. *Ann. Surg. Oncol.* 18, 2074–2083. doi: 10.1245/s10434-011-1567-9

Li, D., Yan, D., Tang, H., Zhou, C., Fan, J., Li, S., et al. (2009). IMP3 Is a novel prognostic marker that correlates with colon cancer progression and pathogenesis. *Ann. Surg. Oncol.* 16, 3499–3506. doi: 10.1245/s10434-009-0648-5

Li, L., Yu, C., Gao, H., and Li, Y. (2010). Argonaute proteins: potential biomarkers for human colon cancer. *BMC Cancer* 10:38. doi: 10.1186/1471-2407-10-38

Liang, L., Li, X., Zhang, X., Lv, Z., He, G., Zhao, W., et al. (2013). MicroRNA-137, an HMGA1 target, suppresses colorectal cancer cell invasion and metastasis in mice by directly targeting FMNL2. *Gastroenterology* 144, 624–635.e4. doi: 10.1053/j.gastro.2012.11.033

Liao, W. T., Liu, J. L., Wang, Z. G., Cui, Y. M., Shi, L., Li, T. T., et al. (2013). High expression level and nuclear localization of Sam68 are associated with progression and poor prognosis in colorectal cancer. *BMC Gastroenterol.* 13:126. doi: 10.1186/1471-230X-13-126

Lichtner, P., Attié-Bitach, T., Schuffenhauer, S., Henwood, J., Bouvagnet, P., Scambler, P., et al. (2002). Expression and mutation analysis of BRUNOL3, a candidate gene for heart and thymus developmental defects associated with partial monosomy 10p. *J. Mol. Med.* 80, 431–442. doi: 10.1007/s00109-002-0331-9

Lin, G. L., Ting, H. J., Tseng, T. C., Juang, V., and Lo, Y. L. (2017). Modulation of the mRNA-binding protein HuR as a novel reversal mechanism of epirubicin-triggered multidrug resistance in colorectal cancer cells. *PLoS ONE* 12:e0185625. doi: 10.1371/journal.pone.0185625

Lin, L., Zhang, J., Wang, Y., Ju, W., Ma, Y., Li, L., et al. (2013). Insulin-like growth factor-II mRNA-binding protein 3 predicts a poor prognosis for colorectal adenocarcinoma. *Oncol. Lett.* 6, 740–744. doi: 10.3892/ol.2013.1458

Liu, L., Christodoulou-Vafeiadou, E., Rao, J. N., Zou, T., Xiao, L., Kyoung Chung, H., et al. (2014). RNA-binding protein HuR promotes growth of small intestinal mucosa by activating the Wnt signaling pathway. *Mol. Biol. Cell* 25, 3308–3318. doi: 10.1091/mbc.e14-03-0853

Liu, L., Ouyang, M., Rao, J. N., Zou, T., Xiao, L., Chung, H. K., et al. (2015). Competition between RNA-binding proteins CELF1 and HuR modulates MYC translation and intestinal epithelium renewal. *Mol. Biol. Cell* 26, 1797–1810. doi: 10.1091/mbc.E14-11-1500

Liu, Q., Luo, Y., Li, X., Yuan, L., Xu, R., and Yang, J. Y. (2013). Preparation and characterizations of polyclonal antibodies against STAR protein QKI7b. *Appl. Biochem. Biotechnol.* 169, 2273–2280. doi: 10.1007/s12010-012-0081-2

Liu, W., Li, Z., Xu, W., Wang, Q., and Yang, S. (2013). Humoral autoimmune response to IGF2 mRNA-binding protein (IMP2/p62) and its tissue-specific expression in colon cancer. *Scand. J. Immunol.* 77, 255–260. doi: 10.1111/sji.12032

Liu, Y., Chen, X., Cheng, R., Yang, F., Yu, M., Wang, C., et al. (2018). The Jun/miR-22/HuR regulatory axis contributes to tumourigenesis in colorectal cancer. *Mol. Cancer* 17:11. doi: 10.1186/s12943-017-0751-3

Liu, Y., Liu, R., Yang, F., Cheng, R., Chen, X., Cui, S., et al. (2017). miR-19a promotes colorectal cancer proliferation and migration by targeting TIA1. *Mol. Cancer* 16:53. doi: 10.1186/s12943-017-0625-8

Liu, Z. P., Liu, S., Chen, R., Huang, X., and Wu, L. Y. (2017). Structure alignment-based classification of RNA-binding pockets reveals regional RNA recognition motifs on protein surfaces. *BMC Bioinformatics* 18:27. doi: 10.1186/s12859-016-1410-1

Lochhead, P., Imamura, Y., Morikawa, T., Kuchiba, A., Yamauchi, M., Liao, X., et al. (2012). Insulin-like growth factor 2 messenger RNA binding protein 3 (IGF2BP3) is a marker of unfavourable prognosis in colorectal cancer. *Eur. J. Cancer* 48, 3405–3413. doi: 10.1016/j.ejca.2012.06.021

López de Silanes, I., Fan, J., Galbán, C. J., Spencer, R. G., Becker, K. G., and Gorospe, M. (2004a). Global analysis of HuR-regulated gene expression in colon cancer systems of reducing complexity. *Gene Expr.* 12, 49–59. doi: 10.3727/000000004783992215

López de Silanes, I., Fan, J., Yang, X., Zonderman, A. B., Potapova, O., Pizer, E. S., et al. (2003). Role of the RNA-binding protein HuR in colon carcinogenesis. *Oncogene* 22, 7146–7154. doi: 10.1038/sj.onc.1206862

López de Silanes, I., Lal, A., and Gorospe, M. (2011). HuR: post-transcriptional paths to malignancy. *RNA Biol.* 2, 11–13. doi: 10.4161/rna.2.1.1552

López de Silanes, I., Zhan, M., Lal, A., Yang, X., and Gorospe, M. (2004b). Identification of a target RNA motif for RNA-binding protein HuR. *Proc. Natl. Acad. Sci. U.S.A.* 101, 2987–2992. doi: 10.1073/pnas.0306453101

López-Cortés, A., Paz-y-Miño, C., Guerrero, S., Jaramillo-Koupermann, G., Intriago-Baldéon, D., García, J., et al. (2019). Pharmacogenomics, biomarker network and allele frequencies in colorectal cancer. *bioRxiv [preprint]. bioRxiv:561316*. doi: 10.1101/561316

Lukong, K. E., Chang, K. W., Khandjian, E. W., and Phane Richard, S. (2008). RNA-binding proteins in human genetic disease. *Cell* 24, 416–425. doi: 10.1016/j.tig.2008.05.004

Lukong, K. E., and Richard, S. (2007). Targeting the RNA-binding protein Sam68 as a treatment for cancer? *Futur. Oncol.* 3, 539–544. doi: 10.2217/14796694.3.5.539

Madison, B. B., Jeganathan, A. N., Mizuno, R., Winslow, M. M., Castells, A., Cuatrecasas, M., et al. (2015). Let-7 represses carcinogenesis and a stem cell phenotype in the intestine via regulation of hmga2. *PLoS Genet.* 11:e1005408. doi: 10.1371/journal.pgen.1005408

Madison, B. B., Liu, Q., Zhong, X., Hahn, C. M., Lin, N., Emmett, M. J., et al. (2013). LIN28B promotes growth and tumorigenesis of the intestinal epithelium via Let-7. *Genes Dev.* 27, 2233–2245. doi: 10.1101/gad.224659.113

Mager, L. F., Koelzer, V. H., Stuber, R., Thoo, L., Keller, I., Koeck, I., et al. (2017). The ESRP1-GPR137 axis contributes to intestinal pathogenesis. *Elife* 6:e28366. doi: 10.7554/eLife.28366

Martinez-Useros, J., Garcia-Carbonero, N., Li, W., Fernandez-Aceñero, M. J., Cristobal, I., Rincon, R., et al. (2019). UNR/CSDE1 expression is critical to maintain invasive phenotype of colorectal cancer through regulation of c-MYC and epithelial-to-mesenchymal transition. *J. Clin. Med.* 8:560. doi: 10.3390/jcm8040560

Martinez-Useros, J., Georgiev-Hristov, T., Fernández-Aceñero, M. J., Borrero-Palacios, A., Indacochea, A., Guerrero, S., et al. (2017). UNR/CDSE1 expression as prognosis biomarker in resectable pancreatic ductal adenocarcinoma patients: a proof-of-concept. *PLoS ONE* 12:e0182044. doi: 10.1371/journal.pone.0182044

Mayr, C., Hemann, M. T., and Bartel, D. P. (2007). Disrupting the pairing between let-7 and Hmga2 enhances oncogenic transformation. *Science* 315, 1576–1579. doi: 10.1126/science.1137999

Melling, N., Simon, R., Mirlacher, M., Izbicki, J. R., Stahl, P., Terracciano, L. M., et al. (2016). Loss of RNA-binding motif protein 3 expression is associated with right-sided localization and poor prognosis in colorectal cancer. *Histopathology* 68, 191–198. doi: 10.1111/his.12726

Morris, A. R., Bos, A., Diosdado, B., Agami, R., Meijer, G. A., Carvalho, B., et al. (2012). Alternative cleavage and polyadenylation during colorectal cancer development. *Clin. Cancer Res.* 18, 5256–5266. doi: 10.1158/1078-0432.CCR-12-0543

Natarajan, G., Ramalingam, S., Ramachandran, I., May, R., Queimado, L., Houchen, C. W., et al. (2008). CUGBP2 downregulation by prostaglandin E 2 protects colon cancer cells from radiation-induced mitotic catastrophe. *Am. J. Physiol. Gastrointest. Liver Physiol.* 294, 1235–1244. doi: 10.1152/ajpgi.00037.2008

Negrini, S., Gorgoulis, V. G., and Halazonetis, T. D. (2010). Genomic instability — an evolving hallmark of cancer. *Nat. Rev. Mol. Cell Biol.* 11, 220–228. doi: 10.1038/nrm2858

Nilsen, T. W., and Graveley, B. R. (2010). Expansion of the eukaryotic proteome by alternative splicing. *Nature* 463, 457–463. doi: 10.1038/nature08909

Okano, H., Kawahara, H., Toriya, M., Nakao, K., Shibata, S., and Imai, T. (2005). Function of RNA-binding protein Musashi-1 in stem cells. *Exp. Cell Res.* 306, 349–356. doi: 10.1016/j.yexcr.2005.02.021

Olive, V., Bennett, M. J., Walker, J. C., Ma, C., Jiang, I., Cordon-Cardo, C., et al. (2009). miR-19 is a key oncogenic component of mir-17-92. *Genes Dev.* 23, 2839–2849. doi: 10.1101/gad.1861409

Pang, M., Wu, G., Hou, X., Hou, N., Liang, L., Jia, G., et al. (2014). LIN28B promotes colon cancer migration and recurrence. *PLoS ONE* 9:e109169. doi: 10.1371/journal.pone.0109169

Paz-Y-Mino, C., Salazar, C. A., López-Cortés, A., and Leone, P. E. (2015). Positive association between the polymorphic variant CCND1 A870G and colorectal cancer in Ecuadorian mestizo population. *J. Can. Res. Updates* 4, 163–170. doi: 10.6000/1929-2279.2015.04.04.4

Pereira, B., Billaud, M., and Almeida, R. (2017). RNA-binding proteins in cancer: old players and new actors. *Trends Cancer* 3, 506–528. doi: 10.1016/j.trecan.2017.05.003

Piskounova, E., Polytarchou, C., Thornton, J. E., Lapierre, R. J., Pothoulakis, C., Hagan, J. P., et al. (2011). Lin28A and Lin28B inhibit let-7 microRNA biogenesis by distinct mechanisms. *Cell* 147, 1066–1079. doi: 10.1016/j.cell.2011.10.039

Piskounova, E., Viswanathan, S. R., Janas, M., LaPierre, R. J., Daley, G. Q., Sliz, P., et al. (2008). Determinants of microRNA processing inhibition by the developmentally regulated RNA-binding protein Lin28. *J. Biol. Chem.* 283, 21310–21314. doi: 10.1074/jbc.C800108200

Pontén, F., Jirström, K., and Uhlen, M. (2008). The human protein atlas—a tool for pathology. *J. Pathol.* 216, 387–393. doi: 10.1002/path.2440

Potten, C. S., Booth, C., Tudor, G. L., Booth, D., Brady, G., Hurley, P., et al. (2003). Identification of a putative intestinal stem cell and early lineage marker; musashi-1. *Differentiation* 71, 28–41. doi: 10.1046/j.1432-0436.2003.700603.x

Qiao, L., and Wong, B. C. Y. (2009). Role of notch signaling in colorectal cancer. *Carcinogenesis* 30, 1979–1986. doi: 10.1093/carcin/bgp236

Ramalingam, S., Natarajan, G., Schafer, C., Subramaniam, D., May, R., Ramachandran, I., et al. (2008). Novel intestinal splice variants of RNA-binding protein CUGBP2: isoform-specific effects on mitotic catastrophe HHS public access. *Am. J. Physiol. Gastrointest. Liver Physiol.* 294, 971–981. doi: 10.1152/ajpgi.00540.2007

Ramalingam, S., Ramamoorthy, P., Subramaniam, D., and Anant, S. (2012). Reduced expression of RNA binding protein CELF2, a putative tumor suppressor gene in colon cancer. *Immunogastroenterology* 1, 27–33. doi: 10.7178/ig.1.1.7

Rappaport, N., Fishilevich, S., Nudel, R., Twik, M., Belinky, F., Plaschkes, I., et al. (2017). Rational confederation of genes and diseases: NGS interpretation via GeneCards, MalaCards and VarElect. *Biomed. Eng. Online* 16(Suppl. 1):72. doi: 10.1186/s12938-017-0359-2

Ross, J., Lemm, I., and Berberet, B. (2001). Overexpression of an mRNA-binding protein in human colorectal cancer. *Oncogene* 20, 6544–6550. doi: 10.1038/sj.onc.1204838

Rüdel, S., Wang, Y., Lenobel, R., Körner, R., Hsiao, H. H., Urlaub, H., et al. (2011). Phosphorylation of human argonaute proteins affects small RNA binding. *Nucleic Acids Res.* 39, 2330–2343. doi: 10.1093/nar/gkq1032

Sakakibara, S., Nakamura, Y., Satoh, H., and Okano, H. (2001). Rna-binding protein Musashi2: developmentally regulated expression in neural precursor cells and subpopulations of neurons in mammalian CNS. *J. Neurosci.* 21, 8091–8107. doi: 10.1523/JNEUROSCI.21-20-08091.2001

Sakurai, T., Kashida, H., Watanabe, T., Hagiwara, S., Mizushima, T., Iijima, H., et al. (2014). Stress response protein cirp links inflammation and tumorigenesis in colitis-associated cancer. *Cancer Res.* 74, 6119–6128. doi: 10.1158/0008-5472.CAN-14-0471

Sánchez-Jiménez, F., and Sánchez-Margalet, V. (2013). Role of Sam68 in post-transcriptional gene regulation. *Int. J. Mol. Sci.* 14, 23402–23419. doi: 10.3390/ijms141223402

Sharma, A., Bhat, A. A., Krishnan, M., Singh, A. B., and Dhawan, P. (2013). Trichostatin-A modulates claudin-1 mRNA stability through the modulation of hu antigen R and tristetraprolin in colon cancer cells. *Carcinogenesis* 34, 2610–2621. doi: 10.1093/carcin/bgt207

Siesing, C., Sorbye, H., Dragomir, A., Qvortrup, C., Pontén, F., Jirströ, K., et al. (2017). High RBM3 expression is associated with an improved survival and oxaliplatin response in patients with metastatic colorectal cancer. *PLoS ONE* 12:e0182512. doi: 10.1371/journal.pone.0182512

Smith, A. R. (2015). *The Regulation of Musashi RNA Binding Proteins and the Implications for Cancer Therapy* (Ph.D. Dissertation). University of Kansas.

Smith, A. R., Marquez, R. T., Tsao, W. C., Pathak, S., Roy, A., Ping, J., et al. (2015). Tumor suppressive microRNA-137 negatively regulates Musashi-1 and colorectal cancer progression. *Oncotarget* 6, 12558–12573. doi: 10.18632/oncotarget.3726

Sobolewski, C., Sanduja, S., Blanco, F. F., Hu, L., and Dixon, D. A. (2015). Histone deacetylase inhibitors activate tristetraprolin expression through induction of early growth response protein 1 (EGR1) in colorectal cancer cells. *Biomolecules* 5, 2035–2055. doi: 10.3390/biom5032035

Sugimasa, H., Taniue, K., Kurimoto, A., Takeda, Y., Kawasaki, Y., and Akiyama, T. (2015). Heterogeneous nuclear ribonucleoprotein K upregulates the kinetochore complex component NUF2 and promotes the tumorigenicity of colon cancer cells. *Biochem. Biophys. Res. Commun.* 459, 29–35. doi: 10.1016/j.bbrc.2015.02.043

Sureban, S. M., Ramalingam, S., Natarajan, G., May, R., Subramaniam, D., Bishnupuri, K. S., et al. (2008). Translation regulatory factor RBM3 is a proto-oncogene that prevents mitotic catastrophe. *Oncogene* 27, 4544–4556. doi: 10.1038/onc.2008.97

Szklarczyk, D., Morris, J. H., Cook, H., Kuhn, M., Wyder, S., Simonovic, M., et al. (2017). The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. *Nucleic Acids Res.* 45, D362–D368. doi: 10.1093/nar/gkw937

The UniProt Consortium (2019). UniProt: a worldwide hub of protein knowledge. *Nucleic Acids Res.* 47, D506–D515. doi: 10.1093/nar/gky1049

Todaro, M., Gaggianesi, M., Catalano, V., Benfante, A., Iovino, F., Biffoni, M., et al. (2014). Cell stem cell article CD44v6 is a marker of constitutive and reprogrammed cancer stem cells driving colon cancer metastasis. *Stem Cell* 14, 342–356. doi: 10.1016/j.stem.2014.01.009

Tomczak, K., Czerwinska, P., and Wiznerowicz, M. (2015). The cancer genome atlas (TCGA): an immeasurable source of knowledge. *Contemp. Oncol.* 19, 68–77. doi: 10.5114/wo.2014.47136

Triboulet, R., Pirouz, M., and Gregory, R. I. (2015). A single Let-7 microRNA bypasses LIN28-mediated repression. *Cell Rep.* 13, 260–266. doi: 10.1016/j.celrep.2015.08.086

Tu, H. C., Schwitalla, S., Qian, Z., LaPier, G. S., Yermalovich, A., Ku, Y. C., et al. (2015). LIN28 cooperates with WNT signaling to drive invasive intestinal and colorectal adenocarcinoma in mice and humans. *Genes Dev.* 29, 1074–1086. doi: 10.1101/gad.256693.114

Ushigome, M., Ubagai, T., Fukuda, H., Tsuchiya, N., Sugimura, T., Takatsuka, J., et al. (2005). Up-regulation of hnRNP A1 gene in sporadic human colorectal cancers. *Int. J. Oncol.* 26, 635–640. doi: 10.3892/ijo.26.3.635

Vainer, G., Vainer-Mosse, E., Pikarsky, A., Shenoy, S., Oberman, F., Yeffet, A., et al. (2008). A role for VICKZ proteins in the progression of colorectal carcinomas: regulating lamellipodia formation. *J. Pathol.* 215, 445–456. doi: 10.1002/path.2376

Venugopal, A., Subramaniam, D., Balmaceda, J., Roy, B., Dixon, D. A., Umar, S., et al. (2016). RNA binding protein RBM3 increases b-catenin signaling to

increase stem cell characteristics in colorectal cancer cells. *Mol. Carcinog.* 55, 1503–1516. doi: 10.1002/mc.22404

Viswanathan, S. R., and Daley, G. Q. (2010). Lin28: a microRNA regulator with a macro role. *Cell* 140, 445–449. doi: 10.1016/j.cell.2010.02.007

Viswanathan, S. R., Powers, J. T., Einhorn, W., Hoshida, Y., Ng, T. L., Toffanin, S., et al. (2009). Lin28 promotes transformation and is associated with advanced human malignancies. *Nat. Genet.* 41, 843–848. doi: 10.1038/ng.392

Vlasova, I. A., Tahoe, N. M., Fan, D., Larsson, O., Rattenbacher, B., SternJohn, J. R., et al. (2008). Conserved GU-rich elements mediate mRNA decay by binding to CUG-binding protein 1. *Mol. Cell* 29, 263–270. doi: 10.1016/j.molcel.2007.11.024

Vlasova-St. Louis, I., and Bohjanen, P. R. (2011). Coordinate regulation of mRNA decay networks by GU-rich elements and CELF1. *Curr. Opin. Genet. Dev.* 21, 444–451. doi: 10.1016/j.gde.2011.03.002

Voutsadakis, I. A. (2018). The pluripotency network in colorectal cancer pathogenesis and prognosis: an update. *Biomark. Med.* 12, 653–665. doi: 10.2217/bmm-2017-0369

Wang, M. J., Ping, J., Li, Y., Adell, G., Arbman, G., Nodin, B., et al. (2015). The prognostic factors and multiple biomarkers in young patients with colorectal cancer. *Sci. Rep.* 5:10645. doi: 10.1038/srep10645

Wang, S., Li, N., Yousefi, M., Nakauka-Ddamba, A., Li, F., Parada, K., et al. (2015). Transformation of the intestinal epithelium by the MSI2 RNA-binding protein. *Nat. Commun.* 6:6517. doi: 10.1038/ncomms7517

Wang, T., He, Y., Zhu, Y., Chen, M., Weng, M., Yang, C., et al. (2016). Comparison of the expression and function of Lin28A and Lin28B in colon cancer. *Oncotarget* 7, 79605–796116. doi: 10.18632/oncotarget.12869

Wang, T., Wang, G., Hao, D., Liu, X., Wang, D., Ning, N., et al. (2015). Aberrant regulation of the LIN28A/LIN28B and let-7 loop in human malignant tumors and its effects on the hallmarks of cancer. *Mol. Cancer* 14, 1–13. doi: 10.1186/s12943-015-0402-5

Wei, Q., Huang, X., Fu, B., Liu, J., Zhong, L., Yang, Q., et al. (2015). IMP3 expression in biopsy specimens of colorectal cancer predicts lymph node metastasis and TNM stage. *Int. J. Clin. Exp. Pathol.* 8, 11024–11032.

Wurth, L. (2012). Versatility of RNA-binding proteins in cancer. *Comp. Funct. Genomics* 2012, 1–11. doi: 10.1155/2012/178525

Wurth, L., and Gebauer, F. (2015). RNA-binding proteins, multifaceted translational regulators in cancer. *Biochim. Biophys. Acta* 1849, 881–886. doi: 10.1016/j.bbagrm.2014.10.001

Wurth, L., Papasaikas, P., Olmeda, D., Bley, N., Calvo, G. T., Guerrero, S., et al. (2016). UNR/CSDE1 drives a post-transcriptional program to promote melanoma invasion and metastasis. *Cancer Cell* 30, 694–707. doi: 10.1016/j.ccell.2016.10.004

Yang, G., Fu, H., Zhang, J., Lu, X., Yu, F., Jin, L., et al. (2010). RNA-binding protein quaking, a critical regulator of colon epithelial differentiation and a suppressor of colon cancer. *Gastroenterology* 138, 231–240.e5. doi: 10.1053/j.gastro.2009.08.001

Yang, H., Rao, J. N., and Wang, J. Y. (2014). Posttranscriptional regulation of intestinal epithelial tight junction barrier by RNA-binding proteins and microRNAs. *Tissue Barriers* 2:e28320. doi: 10.4161/tisb.28320

Ye, F., Jin, P., Cai, X., Cai, P., and Cai, H. (2017). High RNA-binding motif protein 3 (RBM3) expression is independently associated with prolonged overall survival in intestinal-type gastric cancer. *Med. Sci. Monit.* 23, 6033–6041. doi: 10.12659/MSM.905314

Ye, S., Song, W., Xu, X., Zhao, X., and Yang, L. (2016). IGF2BP2 promotes colorectal cancer cell proliferation and survival through interfering with RAF-1 degradation by miR-195. *FEBS Lett.* 590, 1641–1650. doi: 10.1002/1873-3468.12205

Yiakouvaki, A., Dimitriou, M., Karakasiliotis, I., Eftychi, C., Theocharis, S., and Kontoyiannis, D. L. (2012). Myeloid cell expression of the RNA-binding protein HuR protects mice from pathologic inflammation and colorectal carcinogenesis. *J. Clin. Invest.* 122, 48–61. doi: 10.1172/JCI45021

Yu, C., Mannan, A. M., Yvone, G. M., Ross, K. N., Zhang, Y. L., Marton, M. A., et al. (2016). High-throughput identification of genotype-specific cancer vulnerabilities in mixtures of barcoded tumor cell lines. *Nat. Biotechnol.* 34, 419–423. doi: 10.1038/nbt.3460

Yuqi, L., Chengtang, W., Ying, W., Shangtong, L., and Kangxiong, L. (2008). The expression of Msi-1 and its significance in small intestinal mucosa severely damaged by high-dose 5-FU. *Dig. Dis. Sci.* 53, 2436–2442. doi: 10.1007/s10620-007-0155-0

Zhang, H., Zong, Y., Qiu, G., Jia, R., Xu, X., Wang, F., et al. (2018). Silencing Lin28 promotes apoptosis in colorectal cancer cells by upregulating let-7c targeting of antiapoptotic BCL2L1. *Mol. Med. Rep.* 17, 5143–5149. doi: 10.3892/mmr.2018.8483

Zhang, Q. S., Manche, L., Xu, R. M., and Krainer, A. R. (2006). hnRNP A1 associates with telomere ends and stimulates telomerase activity. *RNA* 12, 1116–1128. doi: 10.1261/rna.58806

Zhang, Z., Zhou, C., Chang, Y., Zhang, Z., Hu, Y., Zhang, F., et al. (2016). Long non-coding RNA CASC11 interacts with hnRNP-K and activates the WNT/β-catenin pathway to promote growth and metastasis in colorectal cancer. *Cancer Lett.* 376, 62–73. doi: 10.1016/j.canlet.2016.03.022

Zhong, X., Xiao, Y., Chen, C., Wei, X., Hu, C., Ling, X., et al. (2015). MicroRNA-203-mediated posttranscriptional deregulation of CPEB4 contributes to colorectal cancer progression. *Biochem. Biophys. Res. Commun.* 466, 206–213. doi: 10.1016/j.bbrc.2015.09.008

Zhou, J., Ng, S. B., and Chng, W. J. (2013). LIN28/LIN28B: an emerging oncogenic driver in cancer stem cells. *Int. J. Biochem. Cell Biol.* 45, 973–978. doi: 10.1016/j.biocel.2013.02.006

Zlobec, I., Karamitopoulou, E., Terracciano, L., Piscuoglio, S., Iezzi, G., Muraro, G., et al. (2010). TIA-1 cytotoxic granule-associated RNA binding protein improves the prognostic performance of CD8 in mismatch repair-proficient colorectal cancer. *PLoS ONE* 5:e14282. doi: 10.1371/journal.pone.0014282

Zucconi, B. E., and Wilson, G. M. (2011). Modulation of neoplastic gene regulatory pathways by the RNA-binding factor AUF1. *Front. Biosci.* 16, 2307–2325. doi: 10.2741/3855

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Integrated In Silico Analyses Identify PUF60 and SF3A3 as New Spliceosome-Related Breast Cancer RNA-Binding Proteins

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Simple Summary: Globally, breast cancer (BC) is the most common cancer in women. Although numerous studies have attempted to address this worldwide health problem, it has not yet been possible to understand cancer in its entirety, mainly because most of the investigations have been focused on traditional molecular traits of DNA. Thus, new characteristics of breast tumorigenesis must be tackled, such as RNA-binding proteins (RBPs), which are crucial regulators of important cellular processes. To identify novel breast cancer RNA-binding proteins, we integrated several bioinformatic resources derived from experimentation on BC patient samples and cell lines. Consequently, we identified five putative breast cancer RNA-binding proteins (PUF60, TFRC, KPNB1, NSF, and SF3A3) showing strong tumorigenic characteristics. Supplementary investigation of the molecular and cellular functions of these proteins identified PUF60 and SF3A3 as new spliceosome-related breast cancer RNA-binding proteins. Further experimentation should center on these five RBPs to identify their role in breast tumorigenesis and potentially discover new druggable targets.

Abstract: More women are diagnosed with breast cancer (BC) than any other type of cancer. Although large-scale efforts have completely redefined cancer, a cure remains unattainable. In that respect, new molecular functions of the cell should be investigated, such as post-transcriptional regulation. RNA-binding proteins (RBPs) are emerging as critical post-transcriptional modulators of tumorigenesis, but only a few have clear roles in BC. To recognize new putative breast cancer RNA-binding proteins, we performed integrated in silico analyses of all human RBPs (n = 1392) in three major cancer databases and identified five putative BC RBPs (PUF60, TFRC, KPNB1, NSF, and SF3A3), which showed robust oncogenic features related to their genomic alterations, immunohistochemical changes, high interconnectivity with cancer driver genes (CDGs), and tumor vulnerabilities. Interestingly, some of these RBPs have never been studied in BC, but their oncogenic functions have been described in other cancer types. Subsequent analyses revealed PUF60 and SF3A3 as central elements of a spliceosome-related cluster involving RBPs and CDGs. Further research should focus on the mechanisms by which these proteins could promote breast tumorigenesis, with the potential to reveal new therapeutic pathways along with novel drug-development strategies.

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1. Introduction

Breast cancer (BC) is the leading cause of cancer-associated death (15%: 626,679 cases) and the most commonly diagnosed cancer (24%: 2,088,849 cases) among women worldwide [1]. BC is characterized by a complex interaction between environmental factors and biological traits, such as gene deregulation, hormone disruption, or ethnicity [2–4]. Despite treatment efforts, advanced BC with distant organ metastasis is considered to be incurable [2]. Therefore, a better understanding of BC's molecular processes is still pertinent to identifying new therapeutic targets. Current oncological research generates large-scale datasets that harbor essential aspects of tumor biology. For instance, the Cancer Genome Atlas (TCGA), with over 2.5 petabytes of data, has molecularly characterized over 20,000 patient samples covering 33 cancer types [5–10]. Additionally, the Cancer Dependency Map (DepMap) project, using loss-of-function genetic screens, has identified essential genes for cancer proliferation and survival ex vivo [11–13]. Additionally, the Human Protein Atlas (HPA) constitutes a comprehensive resource to explore the human proteome in healthy and tumoral human tissues [14–16]. Although these datasets have completely redefined cancer drug development, diagnosis, and treatment, additional fundamental features of oncogenesis, tumor growth, and dissemination remain to be discovered. In this respect, post-transcriptional regulation of tumorigenesis represents an understudied trait of cancer research [17].

RNA-binding proteins (RBPs) are particularly relevant due to their implication in every post-transcriptional step of gene expression: RNA splicing, transport, stability, translation, and localization. As a result, genomic alterations of these proteins lead to dysfunctional cellular processes, but only a few have defined functions in BC [18–25]. To date, 1393 RBPs have been experimentally identified in the human RNA interactome [26]. Despite efforts to understand their role in cancer [27,28], an integrated analysis of the aforementioned databases along with other in silico approaches is still missing for BC. To shed light on this matter, we analyzed and integrated RBPs genomic alterations, protein–protein interaction (PPI) networks, immunohistochemical profiles, and loss-of-function experiments to find new putative breast cancer RNA-binding proteins.

2. Materials and Methods

2.1. Gene Sets

A total of 1393 RBPs were extracted from Hentze et al. [26] and checked for new annotations using Ensembl (http://www.ensembl.org (accessed on 5 February 2022)) [29,30]. Only one duplicate was found: ENSG00000100101 and ENSG00000273899, both correspond to *NOL12*, leaving a final list of 1392 RBPs. BC genes (n = 171) were obtained from the Network of Cancer Genes 6.0 (NCG6) [31]. Non-cancer gene list was constructed from Piazza et al. [32], without RBPs and NCG6 [31] genes, and reanalyzed using Piazza's OncoScore algorithm (https://www.galseq.com/next-generation-sequencing/oncoscore-software (accessed on 7 January 2022)), giving a final list of 177 non-cancer genes (Table S1).

2.2. Genomic Analysis

Genomic alterations of RBPs, non-cancer, and BC genes were analyzed through the cBioPortal (https://www.cbioportal.org (accessed on 12 March 2022)) [33,34] using the Breast Invasive Carcinoma (TCGA, PanCancer Atlas) database (n = 994 complete samples) and the Clinical Proteomic Tumor Analysis Consortium (CPTAC) database (n = 122 complete samples) [5–10,35]. To compare the aforementioned gene sets, genomic alterations per protein were corrected by the number of genes or individuals. A Mann–Whitney U test was used to compare genomic alterations between gene sets or clinical characteristics (Table S2).

2.3. Network Construction

Experimental and database interactions (Table S3) between RBPs (n = 1392) and BC proteins (n = 171) [31], having an interaction score of 0.9 (highest confidence), were extracted from the STRING database (Table S6) [36] and visualized using the Cytoscape 3.7.1 (Seattle, USA) platform [37].

2.4. Protein Expression Analysis

Immunohistological levels of 1212 available RBPs in normal and BC tissues were extracted from Protein Atlas version 18.1 (https://www.proteinatlas.org (accessed on 15 June 2021)) [14–16]. Expression levels of normal tissues were taken from glandular cells, while a consensus level was manually generated for BC tissues (Table S7) based on tissue level frequency. Immunohistological images were taken from https://www.proteinatlas.org/ENSG00000182481-KPNA2/tissue/breast#img (accessed on 15 June 2021) (KPNA2 staining of normal tissue), https://www.proteinatlas.org/ENSG00000182481-KPNA2/pathology/tissue/breast+cancer#img (accessed on 15 June 2021) (KPNA2 staining in tumoral tissue), https://www.proteinatlas.org/ENSG00000138757-G3BP2/tissue/breast#img (accessed on 15 June 2021) (G3BP2 staining in normal tissue), https://www.proteinatlas.org/ENSG00000138757-G3BP2/pathology/breast+cancer#img (accessed on 15 June 2021) (G3BP2 staining in tumoral tissue), https://www.proteinatlas.org/ENSG00000109111-SUPT6H/tissue/breast#img (accessed on 15 June 2021) (SUPT6H staining in normal tissue), and https://www.proteinatlas.org/ENSG00000138757-G3BP2/pathology/breast+cancer#img (accessed on 15 June 2021) (SUPT6H staining in tumoral tissue).

2.5. Cancer-Dependency Analysis

RBP cancer-dependency scores from CERES [11] (1288 available RBPs) and DEMETER2 [12,13] (1290 available RBPs) were obtained from the Dependency Map (DepMap) portal (https://depmap.org/portal (accessed on 10 June 2021)). Molecular subtypes of 82 (DEMETER2 [12,13]) and 28 (CERES [11]) BC cell lines were obtained from Smith et al. [38], Dai et al. [39], and Kao et al. [40] (Table S10).

2.6. Cancer-Related Networking Analysis

Previously prioritized RBPs (PUF60, TFRC, KPNB1, NSF, and SF3A3) were integrated into a disease gene network (filtered by RBPs [n = 125] and CDGs ((n = 202 genes)) by using the HumanNet XN (fully extended functional gene network) v2 software (https://www.inetbio.org/humannet (accessed on 9 July 2021)) and visualized through Cytoscape V3.8.2 [37]. We then used MCODE [41] to find complexes within the network according to level-3 parameters: node score cutoff = 0.1, fluff = 0, and no haircut. The resulting network was interpreted through CORUM [42], a database of mammalian protein complexes (https://mips.helmholtz-muenchen.de/corum (accessed on 8 July 2021)).

3. Results

3.1. An Overview of RNA-Binding Protein Genomic Alterations in Breast Cancer

To globally assess the potential role of RBPs in BC, we performed complementary analyses, which are depicted in Figure 1.

Then, to evaluate the potential role of RBPs in BC versus well-known BC genes, we interrogated the Breast Invasive Carcinoma (TCGA, PanCancer Atlas) and Breast Cancer (CPTAC) [5–10,35] database for genomic alterations of RBPs (n = 1392), BC genes (n = 171) [31], and non-cancer genes (n = 170) [32] (Table 1). As shown in Figure 2A, both genomic alteration frequencies of RBPs and BC genes were significantly higher than the ones observed for non-cancer genes. Interestingly, RBPs present a similar degree of genomic alterations as BC genes (Figure 2A), highlighting the putative role of RPBs in BC.

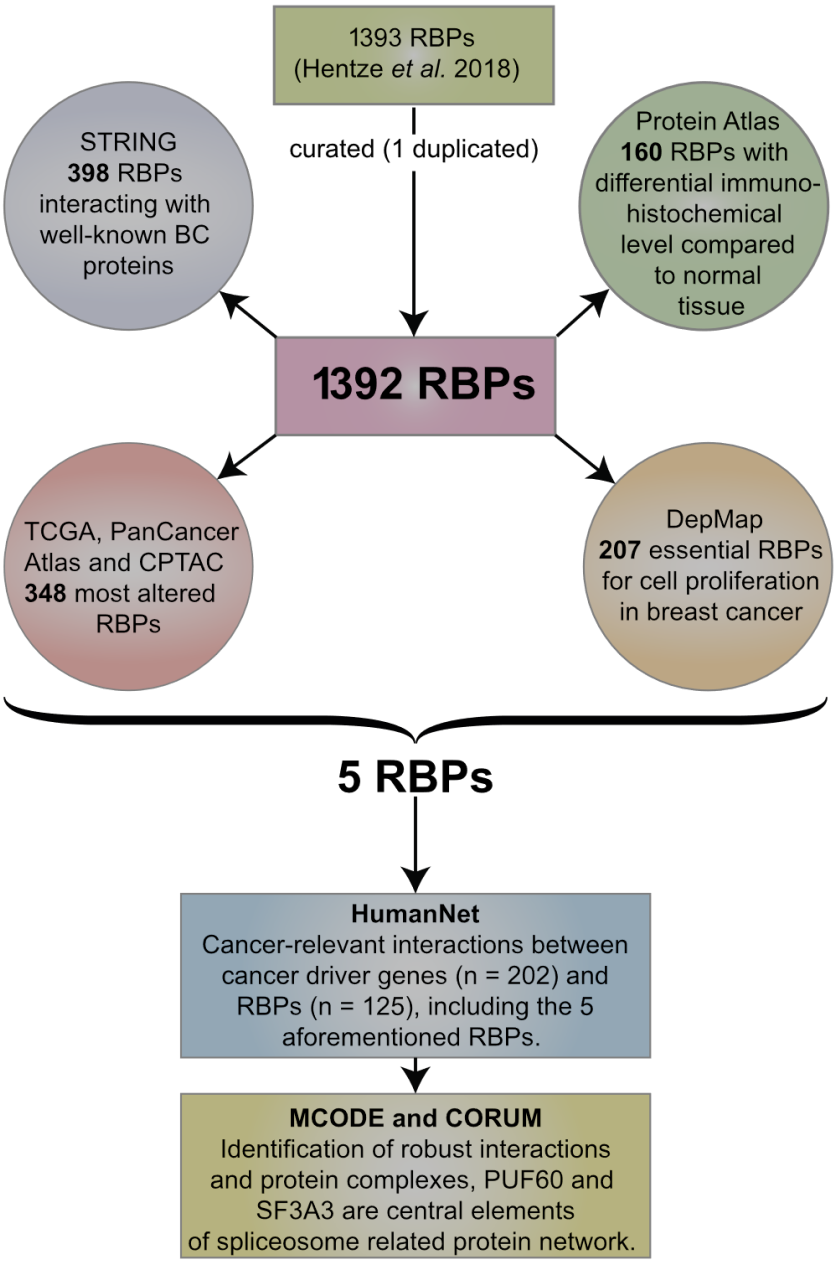

Figure 1. Workflow of the prioritization strategy. This scheme describes all major steps performed to identify PUF60 and SF3A3 as new spliceosome-related breast cancer RNA-binding proteins.

To obtain insights into how these proteins are altered in BC, we cataloged their genomic alteration types. As shown in Figure 2B and (Table S2), most genomic alterations are related to an overrepresentation of the mRNA (68.7%) or gene loci (15.4%).

3.2. Identification of Highly Altered Breast Cancer RNA-Binding Proteins

To identify breast cancer-related RNA-binding proteins, we next interrogated the Network of Cancer Genes 6.0 (NCG6) [31] for RBP having known or predicted cancer driver roles. NCG6 harbors the most recent catalog of cancer driver genes (CDG) [31]. Thus, we identified 225 RBPs, 14 implicated in BC (2 oncogenes, 4 tumor suppressors, and 8 unknown), indicating that these proteins remain poorly studied in breast carcinogenesis, and 211 related to other cancer types (21 oncogenes, 24 tumor suppressors, and 166 unknown) (Figure 3A, Table S3).

Table 1. Top ten most altered RNA-binding proteins in invasive breast carcinoma (TCGA, PanCancer Atlas) and breast cancer (CPTAC).

Genomic Alterations	Protein Name	Number of Alterations	Known BC Molecular and Cellular Functions	Related to Other Cancer Types	Pubmed Citations
Amplification + mRNA upregulation + fusion + mutations	MRPL13	579	No. However, MRPL13 is an ESR2 protein interactor in MCF7 cells [43]	No	34
	DCAF13	574	Yes. It is overexpressed in 171 primary breast tumors [44]	Yes [45]	23
	YWHAZ	532	Yes. Often amplified in BC [46], leading to increased glycolysis [47]. YWHAZ is also an ESR2 protein interactor [43]	Yes	492
	DAP3	491	Yes. DAP3 silencing contributes to breast carcinogenesis [18]	Yes [48]	77
	NUCKS1	490	Yes. NUCKS1 is overexpressed in breast tumors [49]	Yes [50]	58
	TFB2M	488	No	No	36
	MTDH	469	Yes. MTDH promotes cancer proliferation and metastasis [19]	Yes [51]	273
	C1ORF131	463	No	No	13
	PTDSS1	458	No. However, PTDSS1 is an ESR2 protein interactor in MCF7 cells [43]	No	27
	RBM34	452	No. However, RBM34 is an ESR2 protein interactor in MCF7 cells [43]	No	35
Deep deletion + mRNA downregulation + fusion + mutations	CCAR2	378	Yes. CCAR2 functions as a tumor suppressor [20]	Yes [52]	149
	DDX19A	240	No	No	24
	DHX38	180	No. However, DHX38 is an ESR2 protein interactor in MCF7 cells [43]	No	50
	ADD1	165	No. However, ADD1 is an ESR2 protein interactor in MCF7 cells [43]	Yes [53]	223
	KMT2C	135	Yes. KMT2C regulates ERα activity [54]	Yes, it is a tumor suppressor in esophageal squamous cell carcinoma [55]	88
	ZC3H18	135	No. However, ZC3H18 is an ESR2 protein interactor in MCF7 cells [43]	No	39
	NCBP3	130	No. However, NCBP3 is an ESR2 protein interactor in MCF7 cells [43]	No	26
	RARS2	123	No	No	26
	EIF4ENIF1	122	No	No	52
	NMT1	109	No	Yes [56]	92

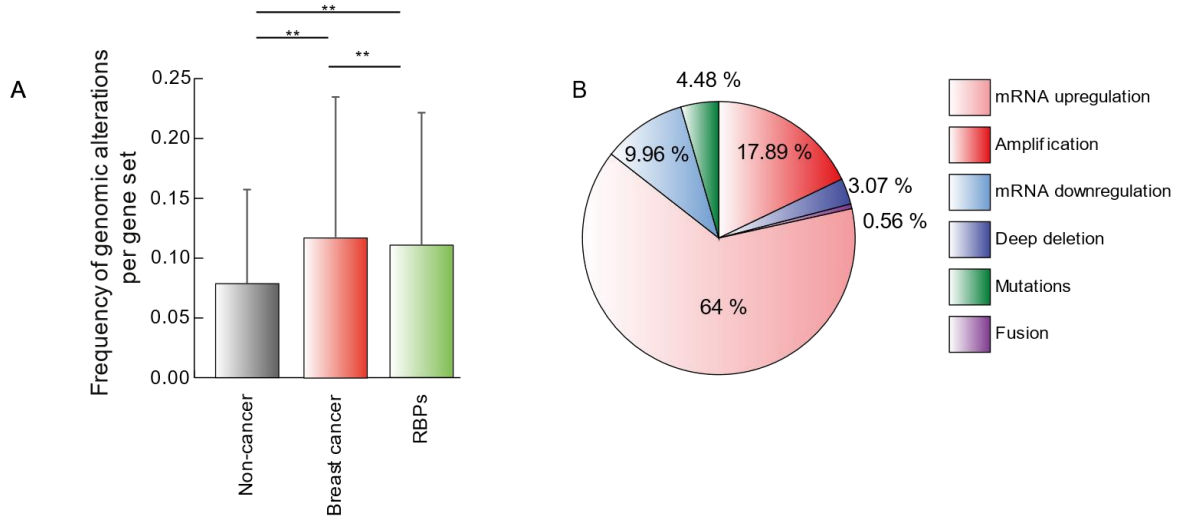

Figure 2. Genomic alterations of RBPs in BC. (**A**) Frequency of genomic alterations per gene set (non-cancer genes (n = 170), BC genes (n = 171), and RBPs [n = 1392]) using the Breast Invasive Carcinoma (TCGA, PanCancer Atlas) and Breast Cancer (CPTAC) database [5–10,35]. Genomic alterations per patient were corrected by the number of genes; a Mann–Whitney U test was used to compare genomic alterations between gene sets. ** = very significant difference; *ns* = not significant. (**B**) A pie chart describing RBPs' genomic alteration types.

To categorize putative RBPs implicated in tumor progression or suppression, we analyzed RBPs' genomic alterations based on their progressor or suppressor profiles. Tumor progressors tend to be overexpressed (mRNA upregulation or genomic amplification), while suppressors are downregulated (mRNA downregulation or genomic deletion) in malignant cells [57]. Gene mutations or fusions have been observed in both tumor progressors and suppressors. On this basis, we identified highly altered breast cancer RNA-binding proteins (Tables 1 and S2). Interestingly, 30% of all human RBPs interact with the tumor suppressor ESR2 (Figure 3B) [43]. We also found known BC progressor and suppressor proteins, such as DAP3 [18], MTDH [19], or CCAR2 [20], which validate our strategy (Tables 1 and S2). This analysis also reveals proteins that have not been related to tumorigenesis, and yet they are highly altered in BC (e.g., TFB2M, C1ORF131, or DDX19A) (Tables 1 and S2).

To further identify important RBPs implicated in BC, we analyzed RBPs' genomic alterations by subtype (Normal, LumA, LumB, Her2, and Basal) (Table S4) or staging (Stage I to IV) (Table S5). As shown in Figure 3C, RBP genomic alterations found in the Basal subtype samples were statistically significant compared to other subtypes ($p < 0.001$). Similarly, RBP genomic alterations of Stage IV samples were statistically significant compared to other stages ($p < 0.001$) (Figure 3D). Individually, some RBPs reached high frequencies of genomic alterations per subtype (Figure 3C, Table S4) or stage (Figure 3D, Table S5). For instance, ARF1, the most altered protein in Stage IV (Figure 3D), has been shown to promote BC metastasis [58]; PARP1 has also been demonstrated to enhance metastasis not only in BC [59] but also in other cancer types [60]. In contrast, SCAMP3 and HEATR6, which present similar degrees of genomic alterations (Figure 3D), have not been studied in BC.

3.3. RNA-Binding Proteins Interact with Well-Known Breast Cancer Proteins

Networking analysis has proved useful in identifying RNA regulons and crucial tumoral proteins [57]. On this basis, we next explored PPIs between RBPs (n = 1392) and well-known BC proteins (n = 171) [31] using the STRING database [61]. The interactions were obtained from experiments and databases; the interaction score was 0.9. This is the highest possible confidence of an interaction to be true based on all the available evidence. Thus, we identified 113 BC proteins interacting with 398 RBPs (Table S6). By narrowing down our analysis to experimental interactions only (Figure 4), we observed

two main networks around SF3B1 and CDC5L proteins. According to the g:Profiler [62], proteins interacting with SF3B1 are implicated in RNA splicing ($P_{adj} = 3.783 \times 10^{-34}$; GO:0000377) (*p*-value adjusted (P_{adj}) for multiple testing using the Benjamin–Hochberg method), while proteins connected to CDC5L are mainly involved in chromatin binding ($P_{adj} = 1.500 \times 10^{-2}$; GO:0003682). We also observed proteins with both BC and RNA-binding features present in the two main networks: SF3B1, CTNNA1, RBMX, and SPEN. Additionally, 18 RBPs interact with at least 1 BC protein. Thus, we identified RBPs that may have a putative role in BC's molecular pathways through PPIs.

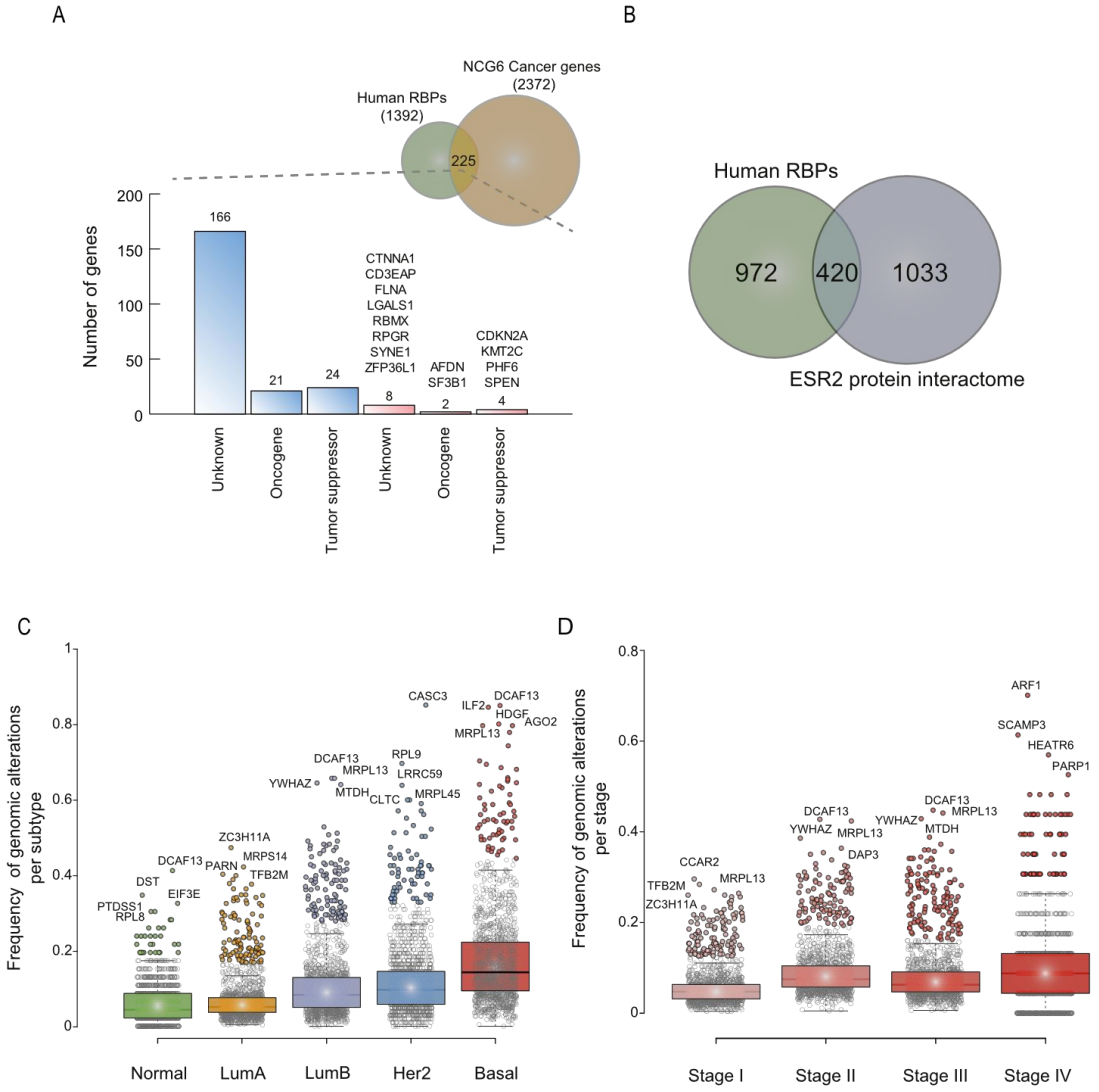

Figure 3. Identification of highly altered breast cancer RNA-binding proteins. (**A**) A histogram describing the status of RBPs in the Network of Cancer Genes 6.0 (NCG6). In blue, RBP status in other cancer types; in red, breast cancer RBPs. (**B**) A Venn diagram depicting the relationship between RBPs and ESR2 protein interactomes. RBPs genomic alterations per subtype (**C**) and stage (**D**), using the Breast Invasive Carcinoma (TCGA, PanCancer Atlas) and Breast Cancer (CPTAC) [5–10,35] database, are displayed. Genomic alterations per subtype and per stage were corrected by the number of patients; a Mann–Whitney U test was used to compare genomic alterations between sets. All possible comparisons between sets present significant differences ($p < 0.001$) except Normal vs. Lum A subtypes; *ns* = not significant.

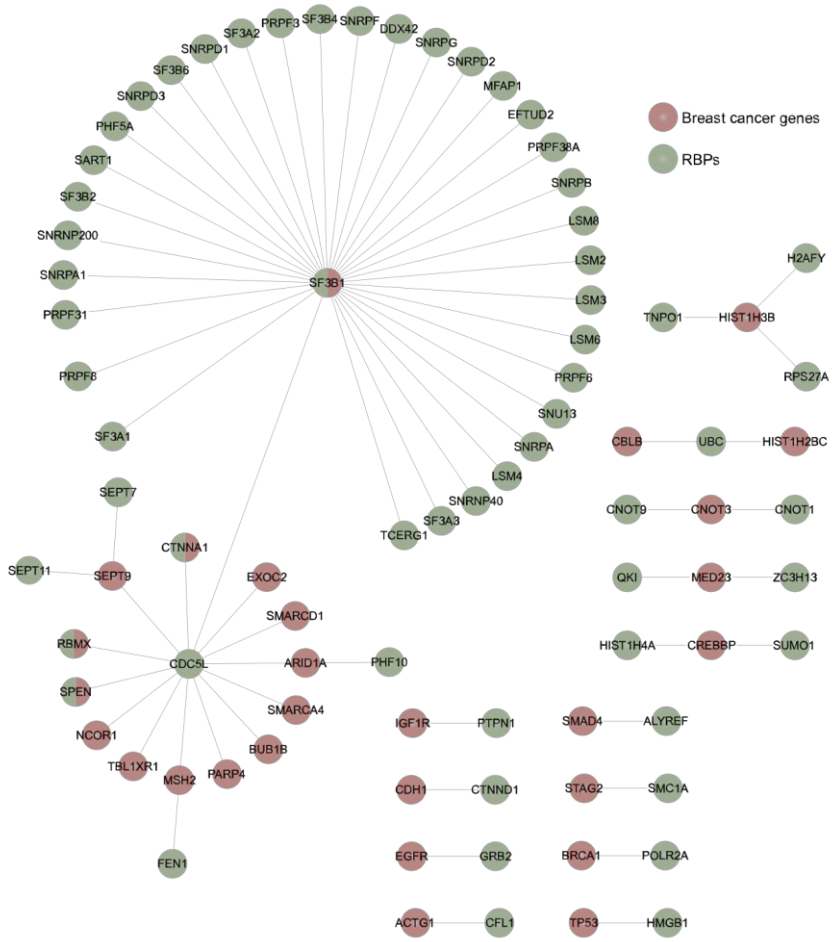

Figure 4. Experimental protein–protein interactions between RNA-binding proteins and well-known breast cancer proteins. An interaction network, constructed using STRING database and the Cytoscape 3.7.1 platform, is presented: red, BC proteins; green, RBPs.

3.4. Identification of Differentially Expressed RNA-Binding Proteins in Breast Tumor Tissues

The Human Protein Atlas (HPA) constitutes [14–16] a major effort to address protein expression in healthy and tumoral human tissues. We, therefore, identified RBPs with a different protein expression profile in tumor breast tissues. To this end, we compared immunohistochemical levels (not detected, low, medium, and high) of 1212 available RBPs between normal and cancerous breast tissues (Figure 5A, Table S6). Most RBPs presented common immunohistochemical levels between both breast tissues: not detected (n = 130), low (n = 52), medium (n = 366), and high (n = 72) (Figure 5A). Moderate protein expression changes, defined by one level variation (e.g., not detected to low or medium to high), were observed in 406 RBPs.

To identify RBPs with highly altered protein expression profiles in tumor tissues, we categorized RBPs with a twofold variation level as upregulated or downregulated compared with normal tissues; thus, we identified 24 upregulated and 62 downregulated RBPs (Figure 5A, Table S6). As expected, our approach revealed well-known BC proteins, such as KPNA2 [21] or G3BP2 [22], which validate our analysis. KPNA2 is highly expressed in BC tissues (7 out of 12 tumor samples are classified as high) (Figure 5B, Table S6). On the contrary, G3BP2 expression is reduced in tumoral breast tissues (Figure 5B, Table S6). We also observed two RBPs that have never been studied in BC, DARS2 (overexpressed) and SUPT6H (downregulated) (Figure 5B, Table S6).

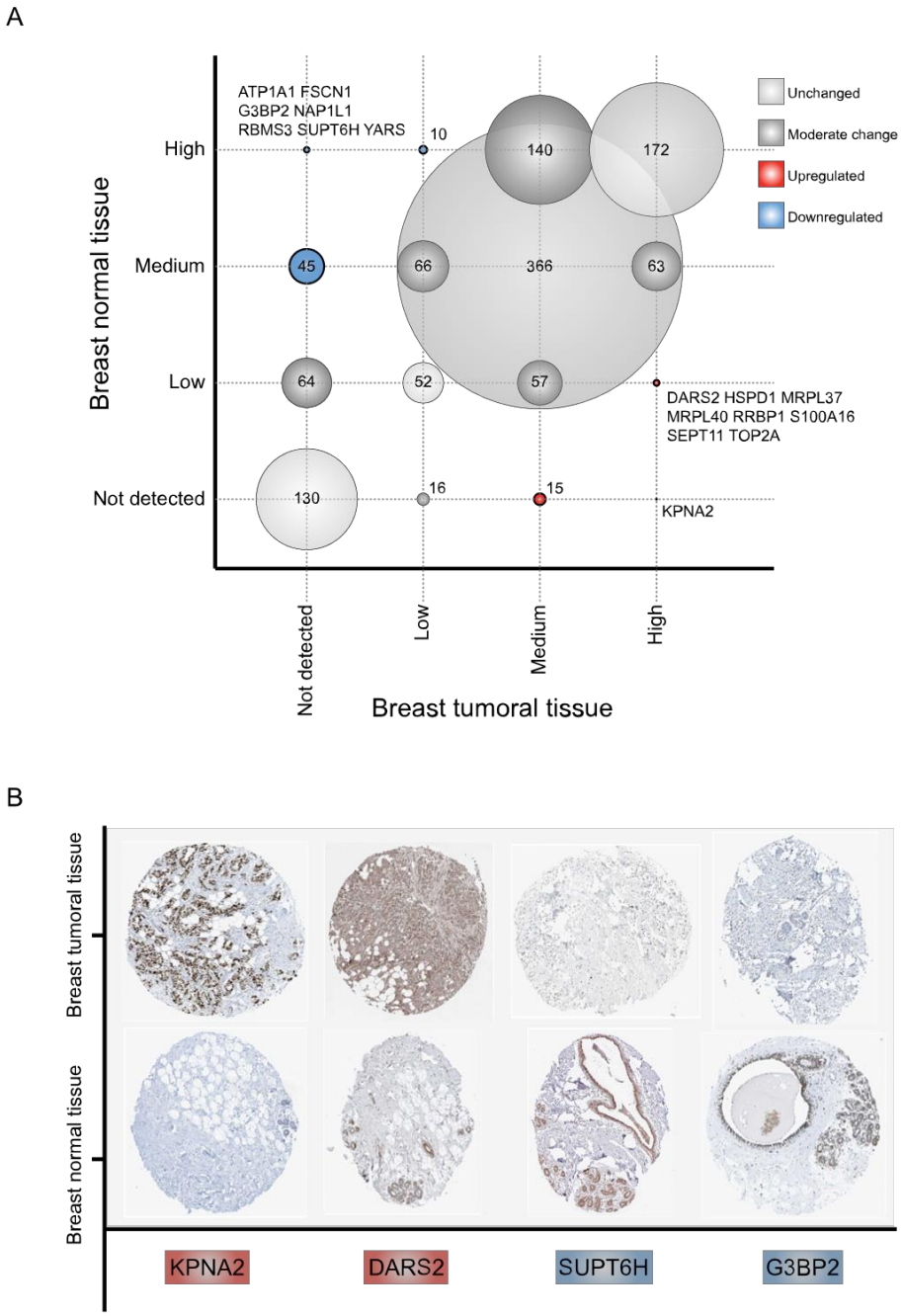

Figure 5. Immunohistochemical protein expression profile of RNA-binding proteins between healthy and tumor breast tissues. (**A**) A correlation plot, comparing RBPs immunohistochemical levels between normal and BC tissues, is presented. Circle sizes correlate with the number of RBPs in each intersection. (**B**) Representative immunohistochemical stains of four RBPS (upregulated: KPNA2 and DARS2; downregulated: G3BP2 and SUPT6H) on normal and tumor breast tissues according to the HPA.

3.5. Exploring RNA-Binding Proteins Breast Cancer Dependencies

Most RBPs present numerous genomic alterations (Figures 2 and 3C,D; Tables S2–S4), making it difficult to detect essential RBPs for cell proliferation and/or survival, i.e., breast cancer RBPs dependencies. Thus, we analyzed 1288 available RBPs on CERES [11] and 1290 available RBPs on DEMETER2 [12,13] through the DepMap portal (https://depmap.org/portal (accessed on 20 June 2021)). Both initiatives report loss-of-function screens performed in several human cancer cell lines [11–13].

Figure 6A shows the distribution of dependency scores of all available RBPs in 82 (DEMETER2 [12,13]) and 28 (CERES [11]) BC cell lines. The dependency score expresses how vital a gene is in a target cell line; if the score is greater than 0.5, the cell line is considered dependent. The genome-scale RNAi loss-of-function screens (DEMETER2 [12,13]) identified 90 essential RBPs (Figure 6A), being SNRPD1, SF3B1, SF3B2, RPL5, ARCN1, EIF3B, RAN, COPB1, RPL14, and VCP (mean dependency scores ranging from −1.3 to −1.5) the top ten essential RBPs for BC survival (Table S8). On the other hand, genome-scale CRISPR-Cas9 loss-of-function screens (CERES [11]) determined 176 essential RBPs (Figure 6A), being RAN, HSPE1, SNRNP200, SNRPD1, SARS, EEF2, RPL37, CCT3, KPNB1, and RPL23 (mean dependency scores ranging from −1.5 to −1.8) the top ten essential RBPs for tumor survival (Table S9). In toto, 207 essential RBPs were identified by both computational methods (Figure 6A; Tables S8 and S9).

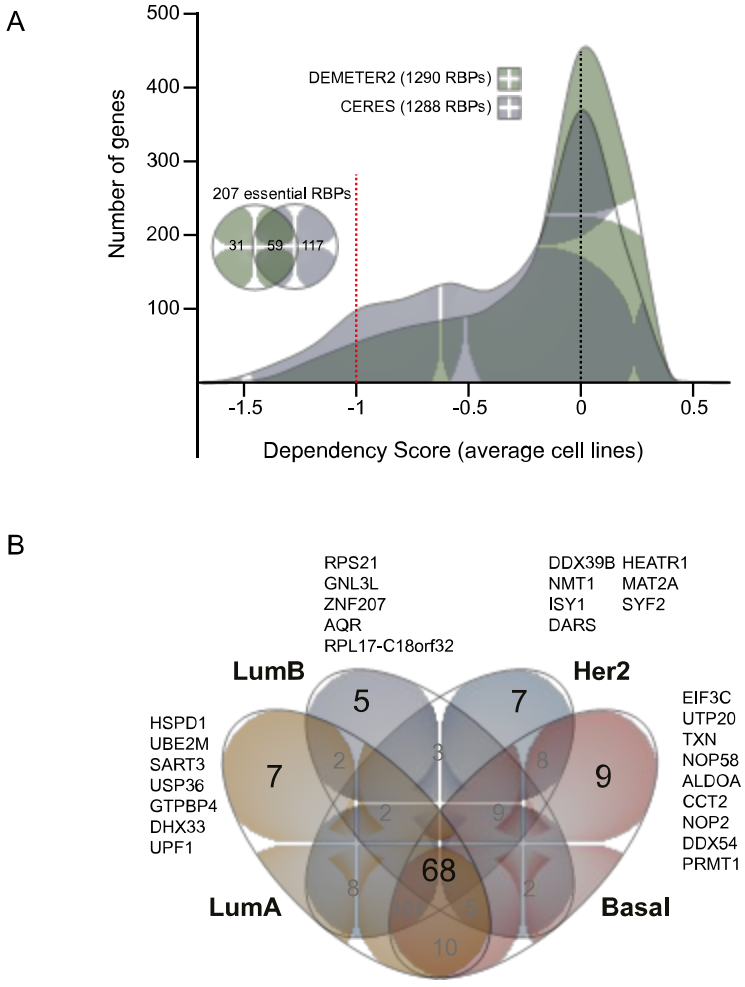

Figure 6. RBPs BC dependencies. (**A**) The distribution of dependency scores of 1290 (DEMETER2) and 1288 RBPs (CERES) is shown. (**B**) A Venn diagram comparing BC essential RBPs per subtype is presented.

To identify essential RBPs per BC molecular subtype, we first updated subtypes by merging data from Smith et al. [38], Dai et al. [39], and Kao et al. [40] (Table S10). We next identified and compared 203 LumA, 96 LumB, 206 Her2, and 212 Basal essential RBPs (Figure 6B; Tables S8 and S9). Thus, we identified essential RBPs for each BC subtype: seven LumA (HSPD1, UBE2M, SART3, USP36, GTPBP4, DHX33, and UPF1), five LumB (RPS21, GNL3L, ZNF207, AQR, and RPL17-C18orf32), seven Her2 (DDX39B, NMT1, ISY1, DARS, HEATR1, MAT2A, and SYF2), and nine Basal (EIF3C, UTP20, TXN, NOP58, ALDOA, CCT2, NOP2, DDX54, and PRMT1) (Figure 6B).

3.6. Unraveling Putative Breast Cancer RNA-Binding Protein

Cancer-related RBPs control hundreds of tumor mRNAs, interact with well-known cancer driver proteins, and appear to be highly altered in cancer genomic databases and tumor tissues [57]. Therefore, we reasoned that the integration of our previous analyses could narrow down the identification of a potential breast cancer RNA-binding protein.

To this end, we focused on RBPs with putative tumor progression profiles. Thus, we overlapped our previous results as follows: (1) 348 RBPs belonging to the first quartile of most genomically altered RBPs concerning tumor-progression-related alterations (mRNA upregulation, genomic amplification, gene mutations, or fusions); (2) all 398 RBPs presenting PPIs with well-known BC proteins (Table S6); (3) 160 RBPs with at least one immunohistochemical variation level towards protein overexpression (e.g., not detected to low); (4) all 207 essential BC RBPs (Figure 6A; Tables S8 and S9).

We found five RBPs presenting the aforementioned tumor-associated characteristics, TFRC, KPNB1, PUF60, NSF, and SF3A3 (Figure 7). TFRC and KPNB1 have been previously implicated in BC [63–66], while PUF60 has been associated with colon and non-small cell lung cancer [67,68]. Interestingly, NSF and SF3A3 have never been studied in cancer. We also found 14 RBPs showing high genomic alterations, PPIs with BC proteins, and altered protein expression profiles in tumoral tissues. Although these proteins are not needed for tumor survival ex vivo (Tables S8 and S9), they could be implicated in other tumoral processes; indeed, 11 of these RBPs have been described as BC tumor progressors [18,69–79]. Interestingly, PLEC has not been related to BC but promotes the migration and invasion of neck squamous cell carcinoma [80]. In addition, PRPF3 and MAGOHB have not been linked to cancer before. In fact, PRPF3 alterations have been related to Retinitis pigmentosa and MAGOHB to Metaphyseal Chondrodysplasia, Schmid Type, and Hermansky–Pudlak Syndrome 3.

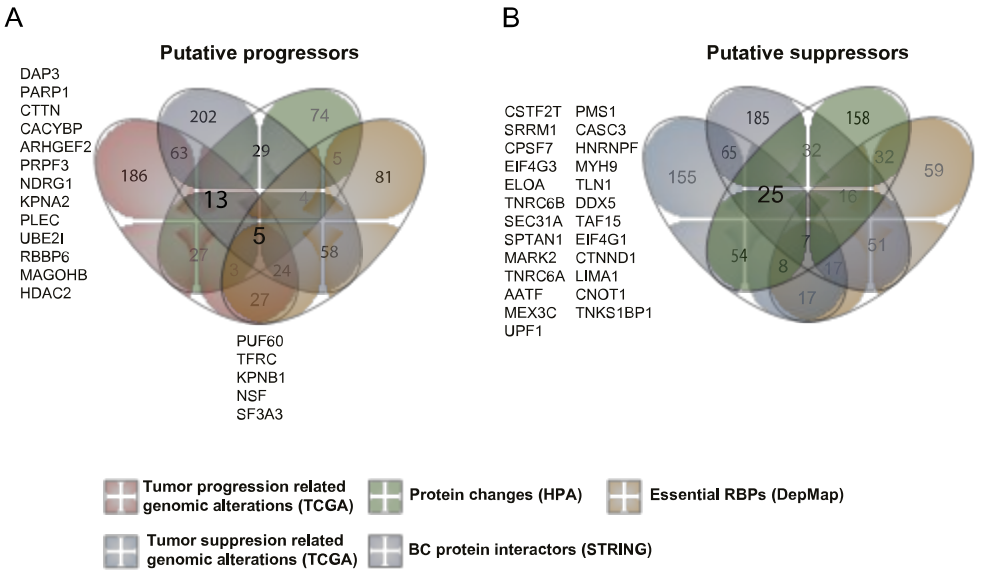

Figure 7. Detecting putative breast cancer RNA-binding proteins. (**A**) A Venn diagram depicting the number of unique and shared RBPs across the four cancer-progression profiles. (**B**) A Venn diagram showing the number of unique and shared RBPs across the four cancer-suppression profiles.

3.7. PUF60 and SF3A3 Are Central Elements of a Spliceosome-Related Network Involving RNA-Binding Proteins and Cancer Driver Genes

To better understand the cellular functions of these prioritized RBPs (TFRC, KPNB1, PUF60, NSF, and SF3A3) in cancer, we next interrogated the HumanNet v2 [81,82]. This tool allowed us to integrate these five RBPs into a disease gene network. We first obtained an initial network of 2231 interactions (Table S11). To narrow down the analysis to cancer-relevant interactions, we then filtered the network by CDGs (n = 202 genes) and RBPs

(n = 125 genes) and used MCODE [41] to find protein complexes within the network; the largest one and more relevant was formed by 36 nodes and 591 edges. The CORUM [42] database identified 34 of these 36 proteins as a part of the spliceosome complex where PUF60 and SF3A3 are central elements interacting with several RBPs and the cancer driver gene (Figure 8).

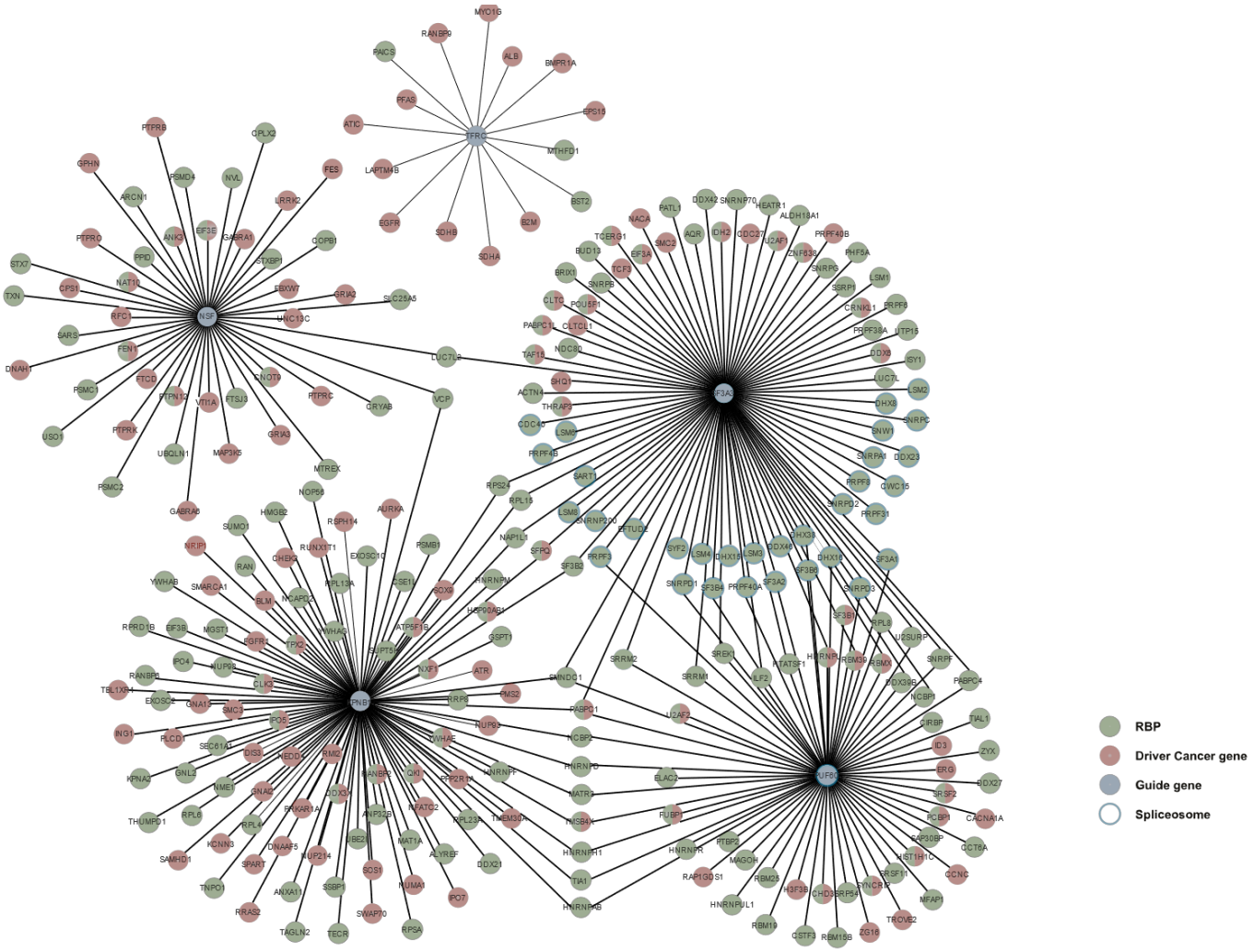

Figure 8. PUF60 and SF3A3 are central elements of a spliceosome-related network involving RNA-binding protein and CDGs. Previously prioritized RBPs (PUF60, TFRC, KPNB1, NSF, and SF3A3) were integrated into a disease gene network (filtered by RBPs and CDGs) using the HumanNet v2 [81,82]. Spliceosome-related proteins and their interactions were determined using MCODE [41] and CORUM [42].

4. Discussion

Current oncological research generates large-scale datasets that contain undiscovered strategic features of molecular mechanisms underlying the growth and metastasis of tumors, and yet these databases are not fully exploited. Integrated in silico analyses of these data could therefore lead to the discovery of new cancer proteins.

We first revealed that RBPs are equally altered as well-known BC proteins (Figure 2A); this was expected since many RBPs are highly altered across cancer types [28] and have been linked in silico to cancer-related cellular processes [83]. We found that most RBPs' genomic alterations in BC are mRNA upregulation (68.7%) and amplification (15.4%) (Figure 2B). This probably will increase RBPs' cellular concentrations, leading to dysfunctional post-transcriptional processes.

To determine how many RBPs have been previously studied in BC, we analyzed the most recent catalog of CDG, NCG6 [31]. Only 14 RBPs were cataloged as BC driver genes (Figure 3A). This indicates that RBPs have been poorly investigated in breast carcinogenesis. Thus, to identify new putative breast cancer RNA-binding proteins, we first explored their genomic alteration profiles associated with tumor progression or suppression (Tables 1 and S2). As expected, we identified well-known BC-progressor and -suppressor proteins, such as DAP3 [18], MTDH [19], or CCAR2 [20], which validate our strategy (Table 1). On the contrary, our strategy revealed RBPs that have not been associated with tumorigenesis, and yet they are highly altered in BC (e.g., TFB2M, C1ORF131, or DDX19A) (Table 1). Interestingly, the most altered RBP in our analysis, MRPL13, has never been studied in cancer. MRPL13, along with other highly altered RBPs (Table 1), has only been shown to interact with ESR2, a tumor suppressor in breast and other cancer types [43]. This observation led us to investigate how many RBPs interact with ESR2; strikingly, we found that 30% of all RBPs interact with this receptor (Figure 3B) [43]. ESR2 could probably exert its suppressive activity through post-transcriptional mechanisms involving several RBPs; nevertheless, more research is needed to understand this observation.

Second, to further characterize RBPs associated with BC subtypes and staging, we analyzed RBPs' genomic alterations (Figure 3C,D). Interestingly, RBPs' genomic alterations gradually increased from the Normal to Basal subtype (Figure 3C), i.e., from a low to high proliferation stage [2]. Concordantly, metastasized tumors (Stage IV) showed high frequencies of RBPs' genomic alterations compared to non-metastasized samples (Stage I to III) (Figure 3D). It seems, therefore, that RBPs are acting as BC progressors rather than suppressors, which agrees with their genomic-alteration profiles (Figure 2B). This analysis also revealed highly altered RBP per subtype or staging (Figure 3C,D; Tables S4 and S5), which could lead to the discovery of new clinical biomarkers or therapeutic targets. Indeed, SCAMP3 and HEATR6, which have not been studied in BC, presented similar degrees of genomic alterations (Figure 3D) compared to well-known metastasis drivers, ARF1 [58] and PARP1 [59]. In hepatocellular carcinoma cells, SCAMP3 knockdown has been shown to suppress cell proliferation [84], while HEATR6 has never been associated with tumorigenesis. Thus, more research is needed to understand their role in BC.

Interaction networks are useful for identifying crucial tumoral proteins [57]. In this regard, by analyzing PPIs between RBP and well-known BC proteins, we identified SF3B1 and CDC5L at the core of two main networks (Figure 4). While SF3B1 has been previously implicated in BC [85], CDC5L, which interacts with 14 BC proteins, has not been studied in this malignancy. However, CDC5L has been related to other cancer types, such as osteosarcoma [86] and prostate cancer [87].

We next exploited the HPA database [14–16] to identify differentially expressed RBPs in tumor breast tissues. We found 24 upregulated and 62 downregulated RBPs compared with normal tissues. Unsurprisingly, our analyses revealed RBPs that were already related to breast cancer. For instance, KPNA2, which has been known to enhance BC metastasis ex vivo [21], is highly expressed in BC tissues (7 out of 12 tumor samples are classified as high) (Figure 5B, Table S6). On the contrary, G3BP2 expression is reduced in tumoral breast tissues (Figure 5B, Table S6); accordingly, the loss of G3BP2 enhances tumor invasion and metastasis in vivo [22]. Interestingly, DARS2, which has never been related to BC, is upregulated in our analysis (10 out 12 tumor samples are classified as high) (Figure 5B, Table S6) and has been associated with hepatocarcinogenesis [88], demonstrating its putative implication in BC. In addition, SUPT6H protein expression is diminished in breast tumoral tissues (Figure 5B, Table S6) and has not been linked to this malignancy. Furthermore, SUPT6H knockdown is associated with DNA damage via the formation of RNA: DNA hybrids (R-loops) in HeLa cells [89], showing its possible role in breast tumorigenesis.

To identify essential RBPs for tumor survival, we next analyzed ex vivo loss-of-function screens, CERES [11] and DEMETER2 [12,13]. In toto, we identified 207 essential RBPs for tumor survival. This was expected since RBPs control every trait of RNA metabolism. However, only 59 were characterized as essential by both computational methods (Figure 6A;

Tables S8 and S9). Although CERES [11] and DEMETER2 [12,13] did not test all human RBPs, future therapeutic post-transcriptional BC research could be focused on these 59 RBPs. However, more investigation is needed to deeply understand their carcinogenic roles. We also revealed essential RBPs per BC molecular subtype (Figure 6B) that could be analyzed to better understand subtype-related post-transcriptional processes.

In extending the scope of our previous analyses, we finally reasoned that the integration of all the databases examined could narrow down the identification of potential breast cancer RNA-binding proteins. As discussed before and depicted in Figures 2B and 3C,D, RBPs seem to act as cancer progressors rather than suppressors. Thus, we focused on RBPs with putative tumor progression profiles and distinguished 19 RBPs with tumorigenic characteristics according to our analyses (Figure 7). As expected, most of them (13 out 19) have been described as BC tumor progressors, controlling different cellular processes such as migration, invasion, and metastasis. Interestingly, NSF, SF3A3, PRPF3, and MAGOHB have never been studied in cancer. While on the other hand, PUF60 has been associated with colon and non-small cell lung cancer [67,68], and PLEC has been shown to promote the migration and invasion of neck squamous cell carcinoma [80].

As depicted in Figure 7, we prioritized 5 RBPs according to our previous analyses. These putative BC progressor RBPs (PUF60, TFRC, KPNB1, NSF, and SF3A3) were integrated into a disease gene network to shed light on their molecular and cellular functions in cancer (Figure 8). Thus, we obtained a very intricate network of 2231 interactions (Table S11), which emphasized the robust and complex network formed between RBP–RBP, RBP–CDG, and CDG–CDG. In addition to this complexity, some of these RBPs are also CDGs. Quattrone and Dassi already established that the RBP network is a hierarchical structure that is formed by clusters and chains that cooperate and compete on common target mRNAs controlling different cellular processes (e.g., splicing) [90]. This is also observed in our densely interconnected network, where PUF60 and SF3A3 are central elements of a spliceosome-related cluster involving RBPs and CDGs.

5. Conclusions

In sum, individual and integrated analysis of the aforementioned databases led us to identify RBPs that have never been studied in BC but displayed defined tumorigenic functions in other cancer types. Thus, based on their tumorigenic characteristics presented in this study and their roles in other cancer types, we identified five new putative breast cancer RBPs: PUF60, TFRC, KPNB1, NSF, and SF3A3. However, further research should focus on the mechanisms by which these proteins promote breast tumorigenesis, which holds the potential to discover new therapeutic pathways along with novel drug-development strategies.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/biology11040481/s1. Table S1: Gene sets analyzed in this study. Table S2: RNA-binding protein genomic alterations in invasive breast carcinoma (TCGA, PanCancer Atlas) and breast cancer (CPTAC). Table S3: RNA-binding proteins and their status in the Network of Cancer Genes 6.0 (NCG6). Table S4: RNA-binding protein genomic alterations in invasive breast carcinoma subtypes (TCGA, PanCancer Atlas) and breast cancer (CPTAC). Table S5: RNA-binding protein genomic alterations in invasive breast carcinoma stages (TCGA, PanCancer Atlas) and breast cancer (CPTAC). Table S6: STRING interactions between RNA-binding proteins and breast cancer proteins (experimental data and databases, interaction score: 0.9). Table S7: RBP protein level in normal and tumoral samples. Table S8: DEMETER2 score of 1290 RNA-binding proteins in 82 breast cancer cell lines. Table S9: CERES score of 1288 RNA-binding proteins in 28 breast cancer cell lines. Table S10: Subtypes of breast cancer cell lines analyzed by DEMETER2 and CERES. Table S11: Total interactions between candidate genes and RNA-binding proteins and cancer driver genes.

Author Contributions: Conceptualization, S.G., A.L.-C., A.I. and J.M.G.-C.; methodology, S.G., A.I., A.L.-C. and J.M.G.-C.; investigation, S.G., A.J.-A. and J.M.G.-C.; data curation, S.G. and I.A.-C., writing—original draft preparation, S.G. and J.M.G.-C.; writing—review and editing, A.P.-V., I.A.-C., A.L.-C., A.J.-A. and A.I.; visualization, S.G., A.P.-V., I.A.-C. and J.M.G.-C.; supervision, S.G. and A.L.-C. All authors have read and agreed to the published version of the manuscript.

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References

1. Bray, F.; Ferlay, J.; Soerjomataram, I.; Siegel, R.L.; Torre, L.A.; Jemal, A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* **2018**, *68*, 394–424. [CrossRef] [PubMed]
2. Harbeck, N.; Penault-Llorca, F.; Cortes, J.; Gnant, M.; Houssami, N.; Poortmans, P.; Ruddy, K.; Tsang, J.; Cardoso, F. Breast cancer. *Nat. Rev. Dis. Prim.* **2019**, *5*, 66. [CrossRef] [PubMed]
3. Guerrero, S.; López-Cortés, A.; Indacochea, A.; García-Cárdenas, J.M.; Zambrano, A.K.; Cabrera-Andrade, A.; Guevara-Ramírez, P.; González, D.A.; Leone, P.E.; Paz-y-Miño, C. Analysis of Racial/Ethnic Representation in Select Basic and Applied Cancer Research Studies. *Sci. Rep.* **2018**, *8*, 13978. [CrossRef] [PubMed]
4. Hiatt, R.A.; Brody, J.G. Environmental Determinants of Breast Cancer. *Annu. Rev. Public Health* **2018**, *39*, 113–133. [CrossRef]
5. Hoadley, K.A.; Yau, C.; Hinoue, T.; Wolf, D.M.; Lazar, A.J.; Drill, E.; Shen, R.; Taylor, A.M.; Cherniack, A.D.; Thorsson, V.; et al. Cell-of-Origin Patterns Dominate the Molecular Classification of 10,000 Tumors from 33 Types of Cancer. *Cell* **2018**, *173*, 291–304.e6. [CrossRef]
6. Ellrott, K.; Bailey, M.H.; Saksena, G.; Covington, K.R.; Kandoth, C.; Stewart, C.; Hess, J.; Ma, S.; Chiotti, K.E.; McLellan, M.; et al. Scalable Open Science Approach for Mutation Calling of Tumor Exomes Using Multiple Genomic Pipelines. *Cell Syst.* **2018**, *6*, 271–281.e7. [CrossRef]
7. Taylor, A.M.; Shih, J.; Ha, G.; Gao, G.F.; Zhang, X.; Berger, A.C.; Schumacher, S.E.; Wang, C.; Hu, H.; Liu, J.; et al. Genomic and Functional Approaches to Understanding Cancer Aneuploidy. *Cancer Cell* **2018**, *33*, 676–689.e3. [CrossRef]
8. Gao, Q.; Liang, W.W.; Foltz, S.M.; Mutharasu, G.; Jayasinghe, R.G.; Cao, S.; Liao, W.W.; Reynolds, S.M.; Wyczalkowski, M.A.; Yao, L.; et al. Driver Fusions and Their Implications in the Development and Treatment of Human Cancers. *Cell Rep.* **2018**, *23*, 227–238.e3. [CrossRef]
9. Liu, J.; Lichtenberg, T.; Hoadley, K.A.; Poisson, L.M.; Lazar, A.J.; Cherniack, A.D.; Kovatich, A.J.; Benz, C.C.; Levine, D.A.; Lee, A.V.; et al. An Integrated TCGA Pan-Cancer Clinical Data Resource to Drive High-Quality Survival Outcome Analytics. *Cell* **2018**, *173*, 400–416.e11. [CrossRef]
10. Sanchez-Vega, F.; Mina, M.; Armenia, J.; Chatila, W.K.; Luna, A.; La, K.C.; Dimitriadoy, S.; Liu, D.L.; Kantheti, H.S.; Saghafinia, S.; et al. Oncogenic Signaling Pathways in The Cancer Genome Atlas. *Cell* **2018**, *173*, 321–337.e10. [CrossRef]
11. Meyers, R.M.; Bryan, J.G.; McFarland, J.M.; Weir, B.A.; Sizemore, A.E.; Xu, H.; Dharia, N.V.; Montgomery, P.G.; Cowley, G.S.; Pantel, S.; et al. Computational correction of copy number effect improves specificity of CRISPR–Cas9 essentiality screens in cancer cells. *Nat. Genet.* **2017**, *49*, 1779–1784. [CrossRef] [PubMed]
12. Tsherniak, A.; Vazquez, F.; Montgomery, P.G.; Weir, B.A.; Kryukov, G.; Cowley, G.S.; Gill, S.; Harrington, W.F.; Pantel, S.; Krill-Burger, J.M.; et al. Defining a Cancer Dependency Map. *Cell* **2017**, *170*, 564–576.e16. [CrossRef]
13. McFarland, J.M.; Ho, Z.V.; Kugener, G.; Dempster, J.M.; Montgomery, P.G.; Bryan, J.G.; Krill-Burger, J.M.; Green, T.M.; Vazquez, F.; Boehm, J.S.; et al. Improved estimation of cancer dependencies from large-scale RNAi screens using model-based normalization and data integration. *Nat. Commun.* **2018**, *9*, 4610. [CrossRef] [PubMed]
14. Uhlén, M.; Fagerberg, L.; Hallström, B.M.; Lindskog, C.; Oksvold, P.; Mardinoglu, A.; Sivertsson, Å.; Kampf, C.; Sjöstedt, E.; Asplund, A.; et al. Proteomics. Tissue-based map of the human proteome. *Science* **2015**, *347*, 1260419. [CrossRef] [PubMed]
15. Thul, P.J.; Åkesson, L.; Wiking, M.; Mahdessian, D.; Geladaki, A.; Ait Blal, H.; Alm, T.; Asplund, A.; Björk, L.; Breckels, L.M.; et al. A subcellular map of the human proteome. *Science* **2017**, *356*, eaal3321. [CrossRef] [PubMed]
16. Uhlen, M.; Zhang, C.; Lee, S.; Sjöstedt, E.; Fagerberg, L.; Bidkhori, G.; Benfeitas, R.; Arif, M.; Liu, Z.; Edfors, F.; et al. A pathology atlas of the human cancer transcriptome. *Science* **2017**, *357*, eaan2507. [CrossRef] [PubMed]
17. Abdel-Wahab, O.; Gebauer, F. Editorial overview: Cancer genomics: RNA metabolism and translation in cancer pathogenesis and therapy. *Curr. Opin. Genet. Dev.* **2018**, *48*, iv–vi. [CrossRef]
18. Wazir, U.; Sanders, A.J.; Wazir, A.M.; Ye, L.; Jiang, W.G.; Ster, I.C.; Sharma, A.K.; Mokbel, K. Effects of the knockdown of death-associated protein 3 expression on cell adhesion, growth and migration in breast cancer cells. *Oncol. Rep.* **2015**, *33*, 2575–2582. [CrossRef]

19. Yu, J.; Wang, J.-G.; Zhang, L.; Yang, H.-P.; Wang, L.; Ding, D.; Chen, Q.; Yang, W.-L.; Ren, K.-H.; Zhou, D.-M.; et al. MicroRNA-320a inhibits breast cancer metastasis by targeting metadherin. *Oncotarget* **2016**, *7*, 38612–38625. [CrossRef]
20. Qin, B.; Minter-Dykhouse, K.; Yu, J.; Zhang, J.; Liu, T.; Zhang, H.; Lee, S.; Kim, J.; Wang, L.; Lou, Z. DBC1 Functions as a Tumor Suppressor by Regulating p53 Stability. *Cell Rep.* **2015**, *10*, 1324–1334. [CrossRef]
21. Noetzel, E.; Rose, M.; Bornemann, J.; Gajewski, M.; Knüchel, R.; Dahl, E. Nuclear transport receptor karyopherin-α2 promotes malignant breast cancer phenotypes in vitro. *Oncogene* **2012**, *31*, 2101–2114. [CrossRef]
22. Wei, S.C.; Fattet, L.; Tsai, J.H.; Guo, Y.; Pai, V.H.; Majeski, H.E.; Chen, A.C.; Sah, R.L.; Taylor, S.S.; Engler, A.J.; et al. Matrix stiffness drives epithelial-mesenchymal transition and tumour metastasis through a TWIST1-G3BP2 mechanotransduction pathway. *Nat. Cell Biol.* **2015**, *17*, 678–688. [CrossRef]
23. Schackmann, R.C.J.; Klarenbeek, S.; Vlug, E.J.; Stelloo, S.; van Amersfoort, M.; Tenhagen, M.; Braumuller, T.M.; Vermeulen, J.F.; van der Groep, P.; Peeters, T.; et al. Loss of p120-catenin induces metastatic progression of breast cancer by inducing anoikis resistance and augmenting growth factor receptor signaling. *Cancer Res.* **2013**, *73*, 4937–4949. [CrossRef] [PubMed]
24. Jiang, W.G.; Martin, T.A.; Lewis-Russell, J.M.; Douglas-Jones, A.; Ye, L.; Mansel, R.E. Eplin-alpha expression in human breast cancer, the impact on cellular migration and clinical outcome. *Mol. Cancer* **2008**, *7*, 71. [CrossRef] [PubMed]
25. Sharma, M. Apoptosis-antagonizing transcription factor (AATF) gene silencing: Role in induction of apoptosis and down-regulation of estrogen receptor in breast cancer cells. *Biotechnol. Lett.* **2013**, *35*, 1561–1570. [CrossRef] [PubMed]
26. Hentze, M.W.; Castello, A.; Schwarzl, T.; Preiss, T. A brave new world of RNA-binding proteins. *Nat. Rev. Mol. Cell Biol.* **2018**, *19*, 327–341. [CrossRef] [PubMed]
27. Wang, K.; Li, L.; Fu, L.; Yuan, Y.; Dai, H.; Zhu, T.; Zhou, Y.; Yuan, F. Integrated Bioinformatics Analysis the Function of RNA Binding Proteins (RBPs) and Their Prognostic Value in Breast Cancer. *Front. Pharmacol.* **2019**, *10*, 140. [CrossRef]
28. Wang, Z.L.; Li, B.; Luo, Y.X.; Lin, Q.; Liu, S.R.; Zhang, X.Q.; Zhou, H.; Yang, J.H.; Qu, L.H. Comprehensive Genomic Characterization of RNA-Binding Proteins across Human Cancers. *Cell Rep.* **2018**, *22*, 286–298. [CrossRef]
29. Hunt, S.E.; McLaren, W.; Gil, L.; Thormann, A.; Schuilenburg, H.; Sheppard, D.; Parton, A.; Armean, I.M.; Trevanion, S.J.; Flicek, P.; et al. Ensembl variation resources. *Database* **2018**, *2018*, bay119. [CrossRef]
30. Zerbino, D.R.; Achuthan, P.; Akanni, W.; Amode, M.R.; Barrell, D.; Bhai, J.; Billis, K.; Cummins, C.; Gall, A.; Girón, C.G.; et al. Ensembl 2018. *Nucleic Acids Res.* **2018**, *46*, D754–D761. [CrossRef]
31. Repana, D.; Nulsen, J.; Dressler, L.; Bortolomeazzi, M.; Kuppili Venkata, S.; Tourna, A.; Yakovleva, A.; Palmieri, T.; Ciccarelli, F.D. The Network of Cancer Genes (NCG): A comprehensive catalogue of known and candidate cancer genes from cancer sequencing screens. *Genome Biol.* **2019**, *20*, 1. [CrossRef] [PubMed]
32. Piazza, R.; Ramazzotti, D.; Spinelli, R.; Pirola, A.; De Sano, L.; Ferrari, P.; Magistroni, V.; Cordani, N.; Sharma, N.; Gambacorti-Passerini, C. OncoScore: A novel, Internet-based tool to assess the oncogenic potential of genes. *Sci. Rep.* **2017**, *7*, 46290. [CrossRef] [PubMed]
33. Cerami, E.; Gao, J.; Dogrusoz, U.; Gross, B.E.; Sumer, S.O.; Aksoy, B.A.; Jacobsen, A.; Byrne, C.J.; Heuer, M.L.; Larsson, E.; et al. The cBio Cancer Genomics Portal: An open platform for exploring multidimensional cancer genomics data. *Cancer Discov.* **2012**, *2*, 401–404. [CrossRef] [PubMed]
34. Gao, J.; Aksoy, B.A.; Dogrusoz, U.; Dresdner, G.; Gross, B.; Sumer, S.O.; Sun, Y.; Jacobsen, A.; Sinha, R.; Larsson, E.; et al. Integrative Analysis of Complex Cancer Genomics and Clinical Profiles Using the cBioPortal. *Sci. Signal.* **2013**, *6*, pl1. [CrossRef] [PubMed]
35. Krug, K.; Jaehnig, E.J.; Satpathy, S.; Blumenberg, L.; Karpova, A.; Anurag, M.; Miles, G.; Mertins, P.; Geffen, Y.; Tang, L.C.; et al. Proteogenomic Landscape of Breast Cancer Tumorigenesis and Targeted Therapy. *Cell* **2020**, *183*, 1436. [CrossRef]
36. Szklarczyk, D.; Franceschini, A.; Wyder, S.; Forslund, K.; Heller, D.; Huerta-Cepas, J.; Simonovic, M.; Roth, A.; Santos, A.; Tsafou, K.P.; et al. STRING v10: Protein-protein interaction networks, integrated over the tree of life. *Nucleic Acids Res.* **2015**, *43*, D447–D452. [CrossRef]
37. Shannon, P.; Markiel, A.; Ozier, O.; Baliga, N.S.; Wang, J.T.; Ramage, D.; Amin, N.; Schwikowski, B.; Ideker, T. Cytoscape: A software environment for integrated models of biomolecular interaction networks. *Genome Res.* **2003**, *13*, 2498–2504. [CrossRef]
38. Smith, S.E.; Mellor, P.; Ward, A.K.; Kendall, S.; McDonald, M.; Vizeacoumar, F.S.; Vizeacoumar, F.J.; Napper, S.; Anderson, D.H. Molecular characterization of breast cancer cell lines through multiple omic approaches. *Breast Cancer Res.* **2017**, *19*, 65. [CrossRef]
39. Dai, X.; Cheng, H.; Bai, Z.; Li, J. Breast Cancer Cell Line Classification and Its Relevance with Breast Tumor Subtyping. *J. Cancer* **2017**, *8*, 3131–3141. [CrossRef]
40. Kao, J.; Salari, K.; Bocanegra, M.; Choi, Y.-L.; Girard, L.; Gandhi, J.; Kwei, K.A.; Hernandez-Boussard, T.; Wang, P.; Gazdar, A.F.; et al. Molecular Profiling of Breast Cancer Cell Lines Defines Relevant Tumor Models and Provides a Resource for Cancer Gene Discovery. *PLoS ONE* **2009**, *4*, e6146. [CrossRef]
41. Bader, G.D.; Hogue, C.W.V. An automated method for finding molecular complexes in large protein interaction networks. *BMC Bioinform.* **2003**, *4*, 2. [CrossRef]
42. Giurgiu, M.; Reinhard, J.; Brauner, B.; Dunger-Kaltenbach, I.; Fobo, G.; Frishman, G.; Montrone, C.; Ruepp, A. CORUM: The comprehensive resource of mammalian protein complexes—2019. *Nucleic Acids Res.* **2019**, *47*, D559–D563. [CrossRef] [PubMed]
43. Giurato, G.; Nassa, G.; Salvati, A.; Alexandrova, E.; Rizzo, F.; Nyman, T.A.; Weisz, A.; Tarallo, R. Quantitative mapping of RNA-mediated nuclear estrogen receptor β interactome in human breast cancer cells. *Sci. Data* **2018**, *5*, 180031. [CrossRef] [PubMed]

44. Chin, S.F.; Teschendorff, A.E.; Marioni, J.C.; Wang, Y.; Barbosa-Morais, N.L.; Thorne, N.P.; Costa, J.L.; Pinder, S.E.; van de Wiel, M.A.; Green, A.R.; et al. High-resolution aCGH and expression profiling identifies a novel genomic subtype of ER negative breast cancer. *Genome Biol.* **2007**, *8*, R215. [CrossRef] [PubMed]
45. Cao, J.; Hou, P.; Chen, J.; Wang, P.; Wang, W.; Liu, W.; Liu, C.; He, X. The overexpression and prognostic role of DCAF13 in hepatocellular carcinoma. *Tumor Biol.* **2017**, *39*, 101042831770575. [CrossRef]
46. Li, Y.; Zou, L.; Li, Q.; Haibe-Kains, B.; Tian, R.; Li, Y.; Desmedt, C.; Sotiriou, C.; Szallasi, Z.; Iglehart, J.D.; et al. Amplification of LAPTM4B and YWHAZ contributes to chemotherapy resistance and recurrence of breast cancer. *Nat. Med.* **2010**, *16*, 214–218. [CrossRef]
47. Chang, C.-C.; Zhang, C.; Zhang, Q.; Sahin, O.; Wang, H.; Xu, J.; Xiao, Y.; Zhang, J.; Rehman, S.K.; Li, P.; et al. Upregulation of lactate dehydrogenase a by 14-3-3ζ leads to increased glycolysis critical for breast cancer initiation and progression. *Oncotarget* **2016**, *7*, 35270–35283. [CrossRef]
48. Jacques, C.; Fontaine, J.-F.; Franc, B.; Mirebeau-Prunier, D.; Triau, S.; Savagner, F.; Malthiery, Y. Death-associated protein 3 is overexpressed in human thyroid oncocytic tumours. *Br. J. Cancer* **2009**, *101*, 132–138. [CrossRef]
49. Drosos, Y.; Kouloukoussa, M.; Østvold, A.; Grundt, K.; Goutas, N.; Vlachodimitropoulos, D.; Havaki, S.; Kollia, P.; Kittas, C.; Marinos, E.; et al. NUCKS overexpression in breast cancer. *Cancer Cell Int.* **2009**, *9*, 19. [CrossRef]
50. Shi, C.; Qin, L.; Gao, H.; Gu, L.; Yang, C.; Liu, H.; Liu, T. NUCKS nuclear elevated expression indicates progression and prognosis of ovarian cancer. *Tumor Biol.* **2017**, *39*, 101042831771463. [CrossRef]
51. Li, J.; Huang, C.; Li, J.; Yuan, J.; Chen, Q.; Zhang, W.; Xu, Z.; Liu, Y.; Li, Y.; Zhan, M.; et al. Knockdown of metadherin inhibits cell proliferation and migration in colorectal cancer. *Oncol. Rep.* **2018**, *40*, 2215–2223. [CrossRef] [PubMed]
52. Best, S.A.; Nwaobasi, A.N.; Schmults, C.D.; Ramsey, M.R. CCAR2 Is Required for Proliferation and Tumor Maintenance in Human Squamous Cell Carcinoma. *J. Investig. Dermatol.* **2017**, *137*, 506–512. [CrossRef] [PubMed]
53. Jen, J.; Lin, L.-L.; Chen, H.-T.; Liao, S.-Y.; Lo, F.-Y.; Tang, Y.-A.; Su, W.-C.; Salgia, R.; Hsu, C.-L.; Huang, H.-C.; et al. Oncoprotein ZNF322A transcriptionally deregulates alpha-adducin, cyclin D1 and p53 to promote tumor growth and metastasis in lung cancer. *Oncogene* **2016**, *35*, 2357–2369. [CrossRef] [PubMed]
54. Gala, K.; Li, Q.; Sinha, A.; Razavi, P.; Dorso, M.; Sanchez-Vega, F.; Chung, Y.R.; Hendrickson, R.; Hsieh, J.J.; Berger, M.; et al. KMT2C mediates the estrogen dependence of breast cancer through regulation of ERα enhancer function. *Oncogene* **2018**, *37*, 4692–4710. [CrossRef] [PubMed]
55. Xia, M.; Xu, L.; Leng, Y.; Gao, F.; Xia, H.; Zhang, D.; Ding, X. Downregulation of MLL3 in esophageal squamous cell carcinoma is required for the growth and metastasis of cancer cells. *Tumor Biol.* **2015**, *36*, 605–613. [CrossRef]
56. Kim, S.; Alsaidan, O.A.; Goodwin, O.; Li, Q.; Sulejmani, E.; Han, Z.; Bai, A.; Albers, T.; Beharry, Z.; Zheng, Y.G.; et al. Blocking Myristoylation of Src Inhibits Its Kinase Activity and Suppresses Prostate Cancer Progression. *Cancer Res.* **2017**, *77*, 6950–6962. [CrossRef]
57. Wurth, L.; Papasaikas, P.; Olmeda, D.; Bley, N.; Calvo, G.T.; Guerrero, S.; Cerezo-Wallis, D.; Martinez-Useros, J.; García-Fernández, M.; Hüttelmaier, S.; et al. UNR/CSDE1 Drives a Post-transcriptional Program to Promote Melanoma Invasion and Metastasis. *Cancer Cell* **2016**, *30*, 694–707. [CrossRef]
58. Xie, X.; Tang, S.-C.; Cai, Y.; Pi, W.; Deng, L.; Wu, G.; Chavanieu, A.; Teng, Y. Suppression of breast cancer metastasis through the inactivation of ADP-ribosylation factor 1. *Oncotarget* **2016**, *7*, 58111–58120. [CrossRef]
59. Zimmer, A.S.; Gillard, M.; Lipkowitz, S.; Lee, J.-M. Update on PARP Inhibitors in Breast Cancer. *Curr. Treat. Options Oncol.* **2018**, *19*, 21. [CrossRef]
60. Rodríguez, M.I.; Peralta-Leal, A.; O'Valle, F.; Rodriguez-Vargas, J.M.; Gonzalez-Flores, A.; Majuelos-Melguizo, J.; López, L.; Serrano, S.; de Herreros, A.G.; Rodríguez-Manzaneque, J.C.; et al. PARP-1 Regulates Metastatic Melanoma through Modulation of Vimentin-induced Malignant Transformation. *PLoS Genet.* **2013**, *9*, e1003531. [CrossRef]
61. Szklarczyk, D.; Gable, A.L.; Lyon, D.; Junge, A.; Wyder, S.; Huerta-Cepas, J.; Simonovic, M.; Doncheva, N.T.; Morris, J.H.; Bork, P.; et al. STRING v11: Protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res.* **2019**, *47*, D607–D613. [CrossRef] [PubMed]
62. Ri Reimand, J.; Kull, M.; Peterson, H.; Hansen, J.; Vilo, J. g:Profiler-a web-based toolset for functional profiling of gene lists from large-scale experiments. *Nucleic Acids Res.* **2007**, *35*, W193–W200. [CrossRef] [PubMed]
63. Jian, J.; Yang, Q.; Huang, X. Src Regulates Tyr^{20} Phosphorylation of Transferrin Receptor-1 and Potentiates Breast Cancer Cell Survival. *J. Biol. Chem.* **2011**, *286*, 35708–35715. [CrossRef]
64. Singh, M.; Mugler, K.; Hailoo, D.W.; Burke, S.; Nemesure, B.; Torkko, K.; Shroyer, K.R. Differential Expression of Transferrin Receptor (TfR) in a Spectrum of Normal to Malignant Breast Tissues. *Appl. Immunohistochem. Mol. Morphol.* **2011**, *19*, 417–423. [CrossRef] [PubMed]
65. Habashy, H.O.; Powe, D.G.; Staka, C.M.; Rakha, E.A.; Ball, G.; Green, A.R.; Aleskandarany, M.; Paish, E.C.; Douglas Macmillan, R.; Nicholson, R.I.; et al. Transferrin receptor (CD71) is a marker of poor prognosis in breast cancer and can predict response to tamoxifen. *Breast Cancer Res. Treat.* **2010**, *119*, 283–293. [CrossRef]
66. Sheng, C.; Qiu, J.; He, Z.; Wang, H.; Wang, Q.; Guo, Z.; Zhu, L.; Ni, Q. Suppression of Kpnβ1 expression inhibits human breast cancer cell proliferation by abrogating nuclear transport of Her2. *Oncol. Rep.* **2017**, *39*, 554–564. [CrossRef]

67. Kobayashi, S.; Hoshino, T.; Hiwasa, T.; Satoh, M.; Rahmutulla, B.; Tsuchida, S.; Komukai, Y.; Tanaka, T.; Matsubara, H.; Shimada, H.; et al. Anti-FIRs (PUF60) auto-antibodies are detected in the sera of early-stage colon cancer patients. *Oncotarget* **2016**, *7*, 82493–82503. [CrossRef]
68. Müller, B.; Bovet, M.; Yin, Y.; Stichel, D.; Malz, M.; González-Vallinas, M.; Middleton, A.; Ehemann, V.; Schmitt, J.; Muley, T.; et al. Concomitant expression of far upstream element (*FUSE*) binding protein (*FBP*) interacting repressor (FIR) and its splice variants induce migration and invasion of non-small cell lung cancer (NSCLC) cells. *J. Pathol.* **2015**, *237*, 390–401. [CrossRef]
69. Ray Chaudhuri, A.; Nussenzweig, A. The multifaceted roles of PARP1 in DNA repair and chromatin remodelling. *Nat. Rev. Mol. Cell Biol.* **2017**, *18*, 610–621. [CrossRef]
70. Wang, Y.; Zhang, J.; Wu, L.; Liu, W.; Wei, G.; Gong, X.; Liu, Y.; Ma, Z.; Ma, F.; Thiery, J.P.; et al. Tricho-rhino-phalangeal syndrome 1 protein functions as a scaffold required for ubiquitin-specific protease 4-directed histone deacetylase 2 de-ubiquitination and tumor growth. *Breast Cancer Res.* **2018**, *20*, 83. [CrossRef]
71. Montanaro, L.; Calienni, M.; Ceccarelli, C.; Santini, D.; Taffurelli, M.; Pileri, S.; Treré, D.; Derenzini, M. Relationship between dyskerin expression and telomerase activity in human breast cancer. *Cell. Oncol.* **2008**, *30*, 483–490. [CrossRef] [PubMed]
72. Jiang, B.-H.; Tseng, W.-L.; Li, H.-Y.; Wang, M.-L.; Chang, Y.-L.; Sung, Y.-J.; Chiou, S.-H. Poly(ADP-Ribose) Polymerase 1: Cellular Pluripotency, Reprogramming, and Tumorogenesis. *Int. J. Mol. Sci.* **2015**, *16*, 15531–15545. [CrossRef] [PubMed]
73. Dedes, K.J.; Lopez-Garcia, M.-A.; Geyer, F.C.; Lambros, M.B.K.; Savage, K.; Vatcheva, R.; Wilkerson, P.; Wetterskog, D.; Lacroix-Triki, M.; Natrajan, R.; et al. Cortactin gene amplification and expression in breast cancer: A chromogenic in situ hybridisation and immunohistochemical study. *Breast Cancer Res. Treat.* **2010**, *124*, 653–666. [CrossRef] [PubMed]
74. Wang, N.; Ma, Q.; Wang, Y.; Ma, G.; Zhai, H. CacyBP/SIP Expression is Involved in the Clinical Progression of Breast Cancer. *World J. Surg.* **2010**, *34*, 2545–2552. [CrossRef] [PubMed]
75. Ridgway, L.D.; Wetzel, M.D.; Ngo, J.A.; Erdreich-Epstein, A.; Marchetti, D. Heparanase-Induced GEF-H1 Signaling Regulates the Cytoskeletal Dynamics of Brain Metastatic Breast Cancer Cells. *Mol. Cancer Res.* **2012**, *10*, 689–702. [CrossRef]
76. Sevinsky, C.J.; Khan, F.; Kokabee, L.; Darehshouri, A.; Maddipati, K.R.; Conklin, D.S. NDRG1 regulates neutral lipid metabolism in breast cancer cells. *Breast Cancer Res.* **2018**, *20*, 55. [CrossRef]
77. Ma, A.; Tang, M.; Zhang, L.; Wang, B.; Yang, Z.; Liu, Y.; Xu, G.; Wu, L.; Jing, T.; Xu, X.; et al. USP1 inhibition destabilizes KPNA2 and suppresses breast cancer metastasis. *Oncogene* **2019**, *38*, 2405–2419. [CrossRef]
78. Zhu, S.; Sachdeva, M.; Wu, F.; Lu, Z.; Mo, Y.-Y. Ubc9 promotes breast cell invasion and metastasis in a sumoylation-independent manner. *Oncogene* **2010**, *29*, 1763–1772. [CrossRef]
79. Moela, P.; Choene, M.M.S.; Motadi, L.R. Silencing RBBP6 (Retinoblastoma Binding Protein 6) sensitises breast cancer cells MCF7 to staurosporine and camptothecin-induced cell death. *Immunobiology* **2014**, *219*, 593–601. [CrossRef]
80. Katada, K.; Tomonaga, T.; Satoh, M.; Matsushita, K.; Tonoike, Y.; Kodera, Y.; Hanazawa, T.; Nomura, F.; Okamoto, Y. Plectin promotes migration and invasion of cancer cells and is a novel prognostic marker for head and neck squamous cell carcinoma. *J. Proteom.* **2012**, *75*, 1803–1815. [CrossRef]
81. Lee, I.; Blom, U.M.; Wang, P.I.; Shim, J.E.; Marcotte, E.M. Prioritizing candidate disease genes by network-based boosting of genome-wide association data. *Genome Res.* **2011**, *21*, 1109–1121. [CrossRef] [PubMed]
82. Hwang, S.; Kim, C.Y.; Yang, S.; Kim, E.; Hart, T.; Marcotte, E.M.; Lee, I. HumanNet v2: Human gene networks for disease research. *Nucleic Acids Res.* **2019**, *47*, D573–D580. [CrossRef] [PubMed]
83. Moore, S.; Järvelin, A.I.; Davis, I.; Bond, G.L.; Castello, A. Expanding horizons: New roles for non-canonical RNA-binding proteins in cancer. *Curr. Opin. Genet. Dev.* **2018**, *48*, 112–120. [CrossRef] [PubMed]
84. Zhang, X.; Sheng, J.; Zhang, Y.; Tian, Y.; Zhu, J.; Luo, N.; Xiao, C.; Li, R. Overexpression of SCAMP3 is an indicator of poor prognosis in hepatocellular carcinoma. *Oncotarget* **2017**, *8*, 109247–109257. [CrossRef]
85. Maguire, S.L.; Leonidou, A.; Wai, P.; Marchiò, C.; Ng, C.K.Y.; Sapino, A.; Salomon, A.V.; Reis-Filho, J.S.; Weigelt, B.; Natrajan, R.C. SF3B1 mutations constitute a novel therapeutic target in breast cancer. *J. Pathol.* **2015**, *235*, 571–580. [CrossRef]
86. Lu, X.-Y.; Lu, Y.; Zhao, Y.-J.; Jaeweon, K.; Kang, J.; Xiao-Nan, L.; Ge, G.; Meyer, R.; Perlaky, L.; Hicks, J.; et al. Cell Cycle Regulator Gene CDC5L, a Potential Target for 6p12-p21 Amplicon in Osteosarcoma. *Mol. Cancer Res.* **2008**, *6*, 937–946. [CrossRef]
87. Li, X.; Wang, X.; Song, W.; Xu, H.; Huang, R.; Wang, Y.; Zhao, W.; Xiao, Z.; Yang, X. Oncogenic Properties of NEAT1 in Prostate Cancer Cells Depend on the CDC5L–AGRN Transcriptional Regulation Circuit. *Cancer Res.* **2018**, *78*, 4138–4149. [CrossRef]
88. Qin, X.; Li, C.; Guo, T.; Chen, J.; Wang, H.-T.; Wang, Y.-T.; Xiao, Y.-S.; Li, J.; Liu, P.; Liu, Z.-S.; et al. Upregulation of DARS2 by HBV promotes hepatocarcinogenesis through the miR-30e-5p/MAPK/NFAT5 pathway. *J. Exp. Clin. Cancer Res.* **2017**, *36*, 148. [CrossRef]
89. Nojima, T.; Tellier, M.; Foxwell, J.; Ribeiro de Almeida, C.; Tan-Wong, S.M.; Dhir, S.; Dujardin, G.; Dhir, A.; Murphy, S.; Proudfoot, N.J. Deregulated Expression of Mammalian lncRNA through Loss of SPT6 Induces R-Loop Formation, Replication Stress, and Cellular Senescence. *Mol. Cell* **2018**, *72*, 970–984.e7. [CrossRef]
90. Quattrone, A.; Dassi, E. The Architecture of the Human RNA-Binding Protein Regulatory Network. *iScience* **2019**, *21*, 706–719. [CrossRef]

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Data mining identifies novel RNA-binding proteins involved in colon and rectal carcinomas

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Colorectal adenocarcinoma (COREAD) is the second most deadly cancer and third most frequently encountered malignancy worldwide. Despite efforts in molecular subtyping and subsequent personalized COREAD treatments, multidisciplinary evidence suggests separating COREAD into colon cancer (COAD) and rectal cancer (READ). This new perspective could improve diagnosis and treatment of both carcinomas. RNA-binding proteins (RBPs), as critical regulators of every hallmark of cancer, could fulfill the need to identify sensitive biomarkers for COAD and READ separately. To detect new RBPs involved in COAD and READ progression, here we used a multidata integration strategy to prioritize tumorigenic RBPs. We analyzed and integrated 1) RBPs genomic and transcriptomic alterations from 488 COAD and 155 READ patients, 2) ~ 10,000 raw associations between RBPs and cancer genes, 3) ~ 15,000 immunostainings, and 4) loss-of-function screens performed in 102 COREAD cell lines. Thus, we unraveled new putative roles of NOP56, RBM12, NAT10, FKBP1A, EMG1, and CSE1L in COAD and READ progression. Interestingly, FKBP1A and EMG1 have never been related with any of these carcinomas but presented tumorigenic features in other cancer types. Subsequent survival analyses highlighted the clinical relevance of FKBP1A, NOP56, and NAT10 mRNA expression to predict poor prognosis in COREAD and COAD patients. Further research should be performed to validate their clinical potential and to elucidate their molecular mechanisms underlying these malignancies.

KEYWORDS

RNA-binding proteins (RBPs), colorectal adenocarcinoma (COREAD), rectum, colon, biomarkers, cancer, data mining, multi-omics

Introduction

Colorectal adenocarcinoma (COREAD) has been ranked as the second most deadly cancer and the third most common malignancy worldwide with an estimated 1.9 million cases and 0.9 million deaths in 2020 (Xi and Xu, 2021). Over the past 10 years, significant advances were achieved in personalized treatments for COREAD patients based on the molecular subtyping (Cohen et al., 2020; López-Cortés et al., 2020; Assis et al., 2022). For example, metastatic COREAD patients, harboring *BRAF*^{V600E} mutation, have now better treatment options (Mauri et al., 2021). Despite these efforts, molecular subtyping has been insufficient to address the heterogeneity of colon and rectal tumors (Cohen et al., 2020; Liu Z. et al., 2021; Assis et al., 2022). In that context, Paschke *et al.*, after analyzing ~2000 publications and the results of two large clinical trials, suggested stopping using the term COREAD and started separating into two different tumor identities: colon cancer (COAD) and rectal cancer (READ). Paschke *et al.*, reached this conclusion by describing obvious differences between COAD and READ concerning molecular carcinogenesis, pathology, surgical topography and procedures, and multimodal treatment. This new perspective could improve the identification of new biomarkers and therapeutic targets for both types of cancer (Paschke et al., 2018).

A new emerging understanding of RNA-binding proteins (RBPs) have addressed them as critical modulators of every hallmark of cancer (Abdel-Wahab and Gebauer, 2018; García-Cárdenas et al., 2019; Hanahan, 2022). RBPs can modulate the expression levels of oncogenes and tumor suppressors (Hentze et al., 2018; García-Cárdenas et al., 2019; Kang et al., 2020) by controlling all aspects of their mRNA processing and metabolism, such as capping, polyadenylation, alternative splicing, subcellular localization, nucleocytoplasmic transport, stability, and degradation (Hentze et al., 2018; García-Cárdenas et al., 2019; Kang et al., 2020; Mestre-Farràs et al., 2022). Thus, identification of tumorigenic RBPs could fulfill the need to discover more accurate and sensitive therapeutic targets for COAD and READ (Cohen et al., 2020; Liu Z. et al., 2021; Assis et al., 2022).

In that respect, we previously performed a literature review to identify RBPs implicated in COREAD (García-Cárdenas et al., 2019). As a result, we found 35 RBPs (out of 1,392 described by Hentze et al., 2018) involved in different aspects of COREAD progression, such as angiogenesis, metastasis, or chemotherapy resistance (Hentze et al., 2018). We also showed that these RBPs are implicated in a complex interconnected network where a single RBP can bind to thousands of RNAs. For instance, ELAVL1 targets 21,578 RNAs, whereas KHDRBS1 interacts with 962. These results pointed out the potential of RBPs to regulate cancerous cellular processes and thereby to be used as COAD or READ biomarkers.

In extending the scope of our previous work and discovering new RBPs involved in COAD and READ separately, here we used our previously published multidata integration strategy to prioritized tumorigenic RBPs (García-Cárdenas et al., 2022). Thus, we assembled data from several resources: The Cancer Genome Atlas (Tomczak et al., 2015), The Human Protein Atlas (Pontén et al., 2008), STRING (Szklarczyk et al., 2019), Depmap (Yu et al., 2016) and HumanNet (Kim et al., 2022), and revealed new RBPs associated with both types of cancer. Our results provide a better understanding of COAD and READ biology and potentially unveil new targets for cancer therapy and prognostic biomarkers.

Methods

Gene sets

Hentze et al., compiled all published RNA interactomes and stringently curated a list of 1,393 RBPs (Hentze et al., 2018). After checking for new annotations using Ensembl (http://www.ensembl.org), we found one duplicate (ENSG00000100101 and ENSG00000273899, both corresponded to NOL12), leaving a final list of 1,392 RBPs. The cancer driver genes (n = 2,372) were retrieved from the Network of Cancer Genes 7.0 (NCG7, http://ncg.kcl.ac.uk/) (Dressler et al., 2022) and filtered by COREAD genes (n = 156) (Supplementary Table S1).

Genomic and transcriptomic data exploration

The cBioPortal for Cancer Genomics (https://www.cbioportal.org; accessed on 04 March 2022) was used to analyze and retrieve genomic and transcriptomic alterations of RBPs from datasets that clinically differentiate COAD and READ. Specifically, we used the Colorectal Adenocarcinoma dataset (TCGA, PanCancer Atlas; Hoadley et al., 2018) which has 378 COAD patients and 155 READ patients. We also analyzed the Colon Cancer study (CPTAC-2 Prospective; Vasaikar et al., 2019) which has 110 complete COAD samples. To compare the aforementioned gene sets, genomic and transcriptomic alterations were corrected by the number of patients. As COAD and READ sets have different number of patients, we divided the number of alterations per RBP by number of patients, i.e., the mean of genomic and transcriptomic alterations per RBP. A Mann–Whitney U test was applied when comparing clinical characteristics or genomic and transcriptomic alterations between gene sets (colon vs. rectum) and within each group (COAD and READ stages and subtypes) (Supplementary Tables S2–S7). Additionally, mRNA Z-scores of aberrantly expressed RBPs in COAD and READ were collected and compared using a Mann–Whitney U test (Supplementary Tables S8, S9). A z-score of < -2 or >2 (p-value = <0.05; confidence level 95%) was used as the criteria for RBPs being determined as down/upregulated, respectively.

Gene network construction

Experimental and database interactions between RBPs (n = 1,392) and COREAD proteins (n = 156) (Supplementary Table S10), having an interaction score of 0.9 (highest confidence), were predicted with the STRING database (Szklarczyk et al., 2015; Repana et al., 2019). Then, the network was visualized using the Cytoscape 3.9.1 (Seattle, USA) platform (Shannon et al., 2003).

Protein expression analysis

Protein immunohistochemical levels were extracted from The Human Protein Atlas version 21.1 (https://www.proteinatlas.org; accessed on 15 March 2022) (Uhlén et al., 2015; Thul et al., 2017; Uhlen et al., 2017). We obtained protein

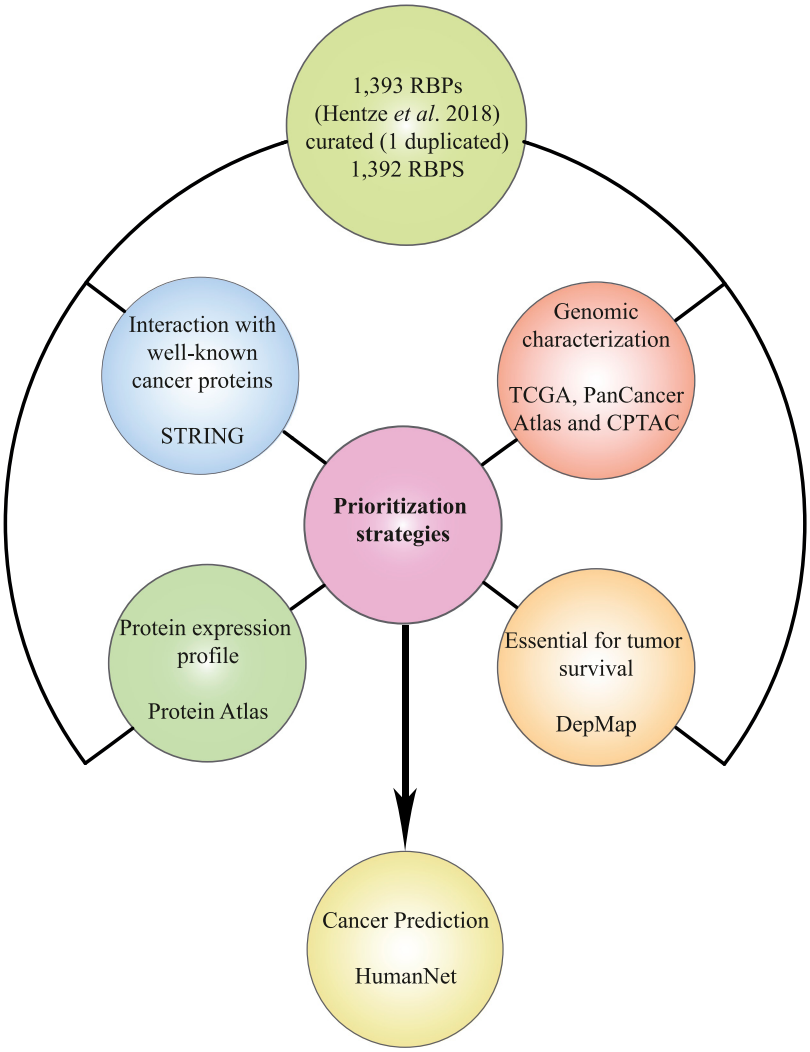

FIGURE 1
Schematic representation of the data mining strategy. All databases interrogated for prioritization: The Cancer Genome Atlas (Tomczak et al., 2015), The Human Protein Atlas (Pontén et al., 2008), STRING (Szklarczyk et al., 2019), and DepMap (Yu et al., 2016), and further cancer association analysis (HumanNet; Kim et al., 2022) are depicted in the multidata integration workflow.

expression levels (not detected, low, medium, and high) for 608 RBPs in COAD and 609 RBPs in READ tissues. Protein expression levels of normal tissues were taken from glandular cells, while a consensus level was manually generated for COAD and READ tissues (Supplementary Tables S11, S12) based on the expression levels. Both normal and tumor tissues had antibody validation parameters. Only enhanced and supported parameters were considered for this analysis.

Cancer genetic dependency analysis

RBPs cancer dependency scores from CERES (Meyers et al., 2017) (1,341 available RBPs) and DEMETER2 (Tsherniak et al., 2017; McFarland et al., 2018) (1,255 available RBPs) were obtained from the Dependency Map (DepMap) portal (https://depmap.org/portal) (Yu et al., 2016). A score of 0 = not essential gene for cell survival, whereas a score of −1 corresponds to the median of all common essential genes, i.e., genes whose principal cellular processes are involved in fundamental cell survival pathways. These scores were calculated from gene knock-out (CERES) and knock-down (Demeter) experiments performed in cancer cell lines. CERES reported data from 42 COAD cell lines and three READ cell lines, while DEMETER2 obtained data from 47 COAD cell lines and 10 READ cell lines (Supplementary Tables S13–S16).

Integrative gene network

The prioritized RBPs for COAD and READ were integrated into a disease gene network by using the HumanNet XC (functional gene network extended network by co-citation) latest version (v3 software) (https://www.inetbio.org/humannet) (Kim et al., 2022) and visualized through Cytoscape 3.9.1 (Shannon et al., 2003) (Supplementary Tables S17, S18).

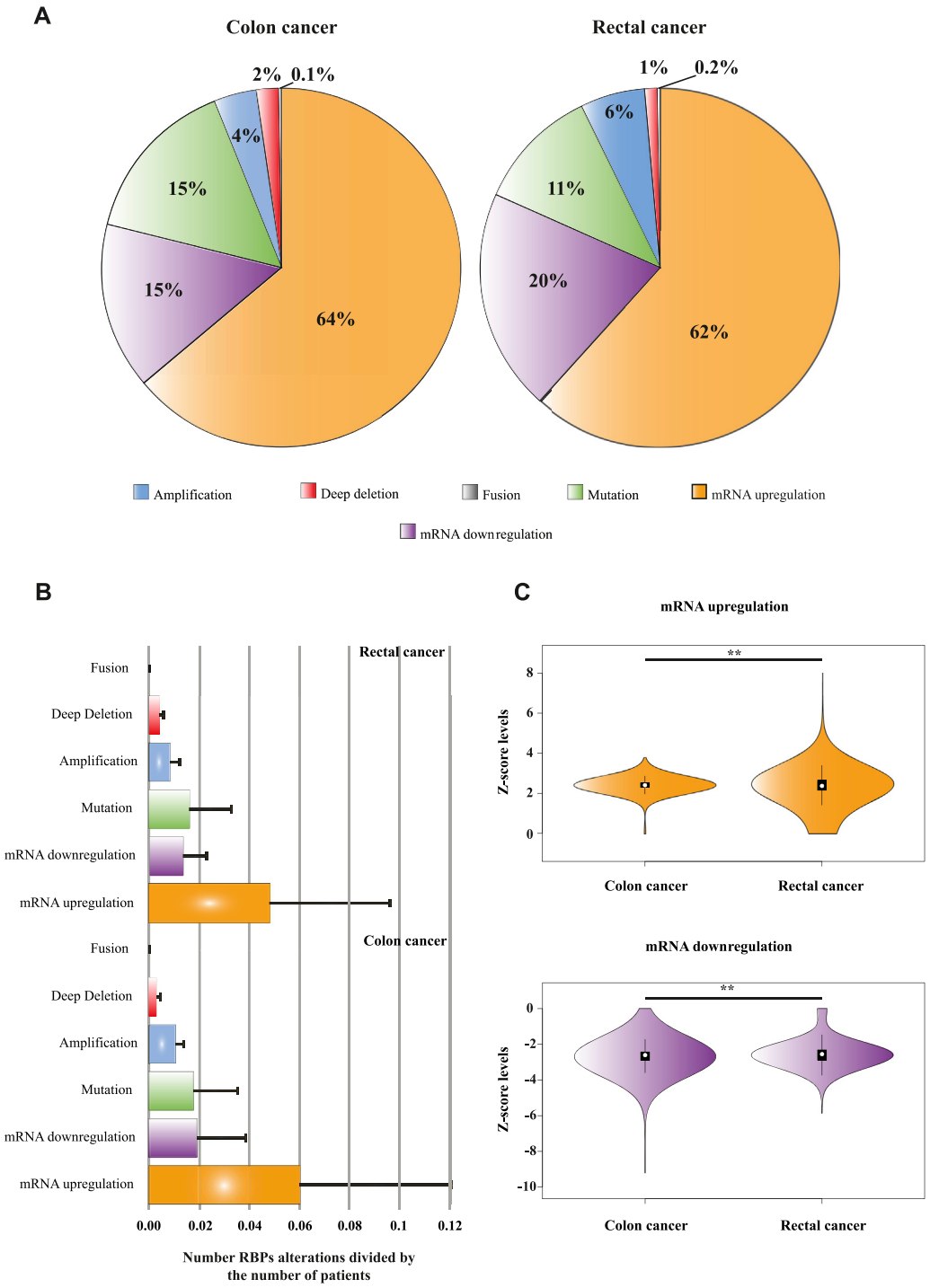

FIGURE 2
Genome and transcriptome alterations of RNA Binding Proteins. **(A)** A pie chart illustrating different types of RBPs genomic and transcriptomic alterations and their percentages in colon and rectal tumors. Data was obtained from Colorectal Adenocarcinoma study (TCGA, PanCancer Atlas; Hoadley et al., 2018) and Colon Cancer study (CPTAC-2 Prospective; Vasaikar et al., 2019). **(B)** Number of RBPs alterations (corrected by number of patients, arbitrary units) separated by type. A Mann–Whitney *U* test was performed to compare alterations between datasets (COAD vs. READ alterations). All possible comparisons between sets presented significant statistically differences ($p < 0.001$) except mutations and fusions; ns = not significant. **(C)** Violin plots portraying the differences of mRNA levels (Z-scores) between colon and rectal tumors, a Mann–Whitney *U* test was performed to compare these data sets. ** = high statistically significant difference.

TABLE 1 Most frequently altered RNA-binding proteins in colon and rectal cancer. Data was obtained from Colorectal Adenocarcinoma study (TCGA, PanCancer Atlas; Hoadley et al., 2018) and Colon Cancer study (CPTAC-2 Prospective; Vasaikar et al., 2019).

Genomic and transcriptomic alterations	Protein name	COAD (C) or READ (R)/Number of alterations	Known COAD or READ molecular and cellular functions	Related to other cancer types/
upregulation + fusion + mutations	STAU1	C/295	No	Yes Marcellus et al. (2021)
		R/291		
	YTHDF1	C/291	Yes. YTHDF1 Regulates Tumorigenicity in Human Colorectal Carcinoma Bai et al. (2019); Chen P et al. (2021); Yan et al. (2021)	Yes Cao et al. (2017)
		R/146		
	CHMP4B	C/272	No	Yes Hu et al. (2015)
		R/127		
	DDX27	C/266	Yes. DDX27 promotes Colorectal cancer growth and metastasis Tang et al. (2018)	Yes Li et al. (2021)
		R/138		
	CSTF1	R/128	No	No
	DIDO1	C/250	Yes. DIDO1 promotes carcinoma progression Sillars-Hardebol et al. (2012)	Yes Forghanifard et al. (2020)
		R/117		
	RBM39	C/248	Yes. RBM39 promotes carcinoma progression Sillars-Hardebol et al. (2012)	Yes Xu et al. (2022)
	EIF2S2	C/247	Yes. EIF2S2 may promote glycolysis in CRC Yang et al. (2021)	Yes Ji et al. (2021)
		R/121		
	RPRD1B	C/245	Yes. Overexpression of RPRD1B confers colorectal cancer sensitivity to fluorouracil (Kuang et al., 2018)	Yes Wen et al. (2020)
	LSM14B	C/244	No	Yes Ta et al. (2021)
		R/125		
	NCOA5	C/243	Yes. NCOA5 promotes proliferation, migration and invasion of colorectal cancer cells Sun et al. (2017)	Yes Tan et al. (2021)
		R/127		
	PRPF6	R/114	Yes. PRPF6 is essential for tumor growth Adler et al. (2014)	Yes Liu W et al. (2021)
Suppressors Deep deletion + mRNA downregulation + fusion + mutations	CCAR2	C/140	Yes. CCAR2 mediates colon cancer progression Kim et al. (2018)	Yes Chen L et al. (2021)
		R/76		
	NARS	C/116	Yes. Novel genes associated with colorectal cancer Eldai et al. (2013)	No
	FXR2	C/100	Yes. Differentially Expressed Profiles of mRNA N6-Methyladenosine in Colorectal Cancer Li N et al. (2022)	No
		R/46		
	ATP5F1A	C/99	No	Yes Feichtinger et al. (2018)

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TABLE 1 (*Continued*) Most frequently altered RNA-binding proteins in colon and rectal cancer. Data was obtained from Colorectal Adenocarcinoma study (TCGA, PanCancer Atlas; Hoadley et al., 2018) and Colon Cancer study (CPTAC-2 Prospective; Vasaikar et al., 2019).

Genomic and transcriptomic alterations	Protein name	COAD (C) or READ (R)/Number of alterations	Known COAD or READ molecular and cellular functions	Related to other cancer types/
		R/60		
	SYNE1	C/94	Yes. SYNE1 mutations are related with worse survival outcomes Zhou et al. (2020)	Yes Qu et al. (2021)
		R/31		
	GTF2E2	C/84	No	Yes Bi et al. (2021)
		R/42		
	ALKBH5	C/76	Yes. Related to tumor immunity in colon adenocarcinoma Yan et al. (2021)	Yes Guo et al. (2020)
	NCBP3	C/71	No	No
	RTF1	C/71	No	No
	ELAC2	C/63	No	Yes Noda et al. (2006)
		R/60		
	LRRC47	R/37	No	Yes Mu et al. (2022)
	YWHAE	R/31	Yes. YWHAE mediated the function of miR-6778–5p in the proliferation of colorectal cancer cells Li et al. (2019)	Yes Yang et al. (2019)
	MRM3	R/30	No	Yes Schmidlin et al. (2016)
	DHX33	R/29	Yes. It promotes colon cancer development downstream of Wnt signaling Zhu et al. (2020)	Yes Tian et al. (2016)

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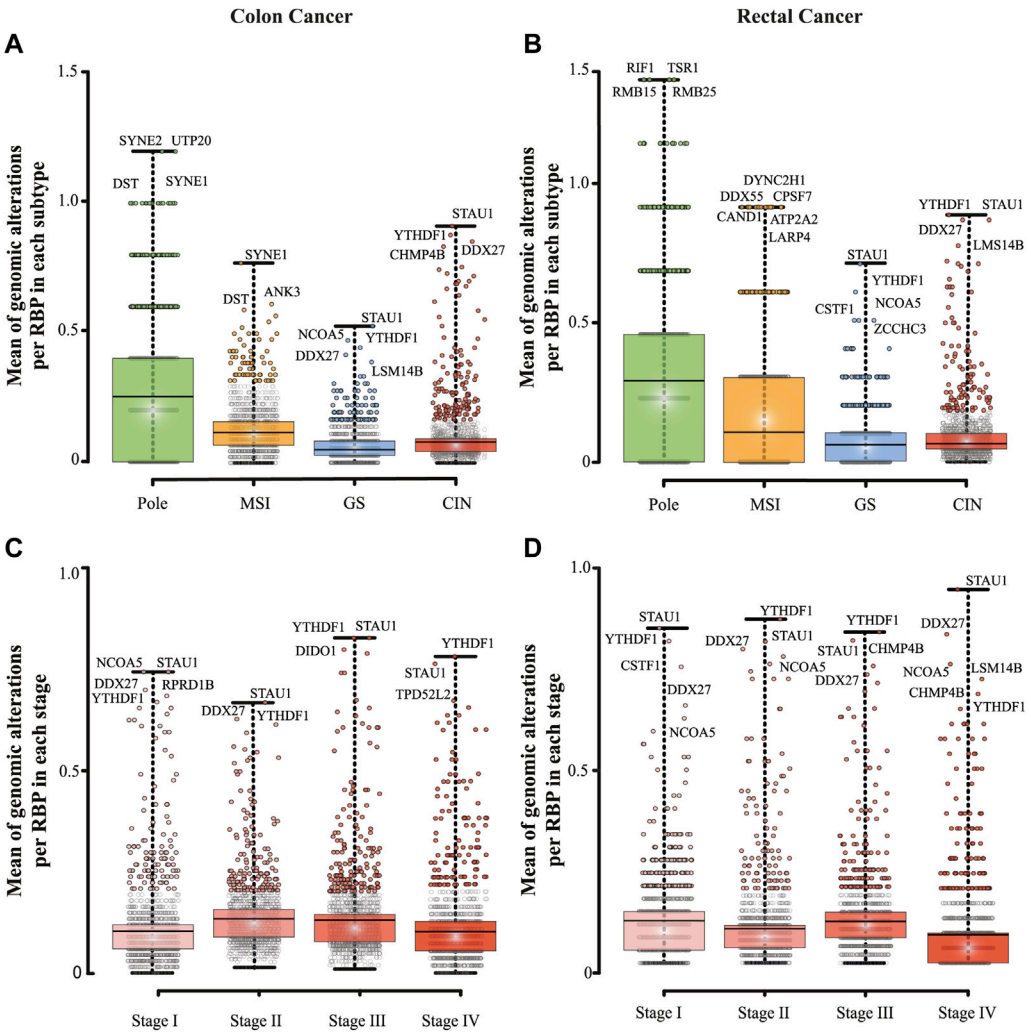

FIGURE 3
Detection of highly altered RNA-binding proteins in colon and rectal carcinomas. RBPs genomic and transcriptomic alterations per subtype **(A, B)** and stage **(C, D)** are depicted in boxplots. Data was obtained from Colorectal Adenocarcinoma study (TCGA, PanCancer Atlas; Hoadley et al., 2018) and Colon Cancer study (CPTAC-2 Prospective; Vasaikar et al., 2019). The number of RBPs alterations was divided by the number of patients in each set of data. A Mann–Whitney *U* test was performed to compare genomic and transcriptomic alterations between sets. All possible comparisons between colon and rectum and within each data set in both carcinomas presented high statistically significant difference ($p < 0.001$), except for GS subtype when comparing colon vs. rectum, stage 1 vs. stage 4 (in colon cancer), and stage 1 vs. 3 (in rectal cancer); ns = not significant.

Clinical analysis

The TCGA, PanCancer Atlas (Hoadley et al., 2018) colorectal cancer database was inspected for mRNA expression of prioritized RBPs in colon and rectum patients. Probability of overall survival (OS) and disease-free survival (DFS) were calculated. Curves were obtained by dividing samples in two groups using median z-score as a cutoff in COREAD, COAD, and READ patients. These two groups represent 1) patients with RBP mRNA upregulation (blue lines) and 2) patients with RBP mRNA downregulation (red lines). Differences between groups were calculated using log rank test. Graphical representations and statistical analysis were performed with IBM SPSS, version 22. Only significant comparisons with an N > 20 per group is shown.

Results

Multidata integration strategy to prioritized tumorigenic RNA-binding proteins

We previously published a multidata integration strategy that allowed us to identify PUF60 and SF3A3 as new spliceosome-related breast cancer RBPs (García-Cárdenas et al., 2022). In this work, we used the same strategy to prioritize tumorigenic RBPs that could be used as COAD or READ biomarkers. First, we performed individual analysis of several databases such as The Cancer Genome Atlas (Tomczak et al., 2015), The Human Protein Atlas (Pontén et al., 2008), STRING (Szklarczyk et al., 2019), and Depmap (Yu et al., 2016) to identify RBPs with different cancer-related characteristics: 1) high genomic and transcriptomic alterations, 2) interactions with well-known cancer

TABLE 2 Mean of genomic and transcriptomic alterations per stage and subtypes and sample number in COAD and READ patients.

Subtypes	Colon (number of patients/Mean)	Rectum (number of patients/Mean)
Pole	55/0.252	4/0.319
MSI	44/0.128	3/0.122
CIN	36/0.092	9/0.097
GS	202/0.061	99/0.076
Stages		
I	78/0.104	29/0.113
II	186/0.135	50/0.091
III	150/0.131	44/0.112
IV	62/0.103	25/0.074

proteins, 3) aberrant protein expression levels compared with normal tissues, and 4) essential for tumor survival. Next, we performed a rigorous analysis to detect RBPs with all the aforementioned attributes, and thereby prioritizing potential COAD and READ RBPs. Finally, we predicted how these prioritized RBPs are correlated with cancer phenotypes using the HumanNet (Kim et al., 2022) database (Figure 1).

Detection of highly altered RNA-binding proteins in colon and rectal tumors

To globally determine the genomic and transcriptomic alterations of RBPs in COAD and READ patients, we interrogated two independent datasets, TCGA, PanCancer Atlas (Hoadley et al., 2018) and Colon Cancer study CPTAC-2 Prospective (Vasaikar et al., 2019) including *in toto* 488 COAD and 155 READ patients. First, we compared RBPs (n = 1,392) genomic and transcriptomic alterations between COAD and READ. Once we corrected by the number of patients (i.e., mean of genomic and transcriptomic alterations per RBP), we found significant differences ($p < 0.001$) between COAD and READ. In Figure 2A is depicted the percentages of each alteration, where mRNA upregulation accounted for most of the genomic and transcriptomic modifications in COAD and READ. In colon tumors we found that mRNA downregulation and mutations presented equal percentages (15%), whereas mRNA downregulation (20%) occupied the second place followed by mutations (11%) in READ.

Although the pattern of RBPs genomic and transcriptomic alterations in COAD and READ are similar (Figure 2A), when they are analyzed separately, significant differences were found ($p < 0.001$) except for mutations and fusions in COAD vs. READ (Figure 2B; Supplementary Tables S2, S3). Even though RBPs mRNA upregulations account for 64% in COAD vs. 62% in READ, mRNA Z-scores were higher in READ than in COAD ($p < 0.001$) (Figure 2C; Supplementary Tables S8, S9). The contrary is appreciated when analyzing mRNA downregulation, COAD RBPs z-scores distribution shows a wider mRNA downregulation compared to READ RBPs ($p < 0.001$) (Figure 2C).

To identify RBPs involved in tumor progression or suppression, RBPs genomic and transcriptomic alterations categories were classified accordingly. In malignant cells, mRNA upregulation and genomic amplifications are related to tumor progressors, while mRNA downregulation and genomic deletions are connected with suppressors (Wurth et al., 2016; Mestre-Farràs et al., 2022). Gene fusion and mutations have been detected in both tumor progressors and suppressors. Based on these principles, we listed the most frequently altered RBPs in COAD and READ (Table 1, Supplementary Tables S2, S3). As expected, most of them have already been related to COAD or READ, and thereby validating our strategy (Table 1). Interestingly, STAU1 was the most altered RBP in both carcinomas, and yet it has never been correlated with COAD or READ; however, it has been associated with prostate cancer (Marcellus et al., 2021). Similarly, some progressors (CHMP4B, CSTF1, and LSM14B) and suppressors RBPs (ATP5F1A, GTF2E2, RTF1, ELAC2, LRRC47, and MRM3) have never been associated with COAD or READ, but present oncogenic properties in other cancer types (Table 1, Supplementary Tables S2, S3). Worthy of note, we also identified RBPs that are unique for each cancer type and others (e.g., CSTF1) that have been never related to cancer (Table 1).

We, next, determined genomic and transcriptomic alterations of RBPs by subtypes, pole (polymerase ε), microsatellite instability (MSI), genomically stable (GS), and chromosomal instability (CIN) (Figures 3A,B; Table 2; Supplementary Tables S4, S5) and stages (Stage I to IV, Table 2; Figures 3C,D; Supplementary Tables S6, S7). We found statistically significant differences between all subtypes in both carcinomas (Mann–Whitney U, $p < 0.001$; Figures 3A,B). Pole was the most altered subtype, followed by MSI, CIN, and GS in both malignancies (Figures 3A,B). When comparing subtypes between COAD and READ, we also found statistically significant differences except for COAD GS vs. READ GS subtype (Mann–Whitney U, $p < 0.001$; Figures 3A,B). In COAD, we also observed equally altered RBPs among subtypes. For example, DST and SYNE1 were highly altered in Pole and MSI, while DDX27, STAU1, and YTHDF1 in GS and CIN. It is important to mention that some of these RBPs

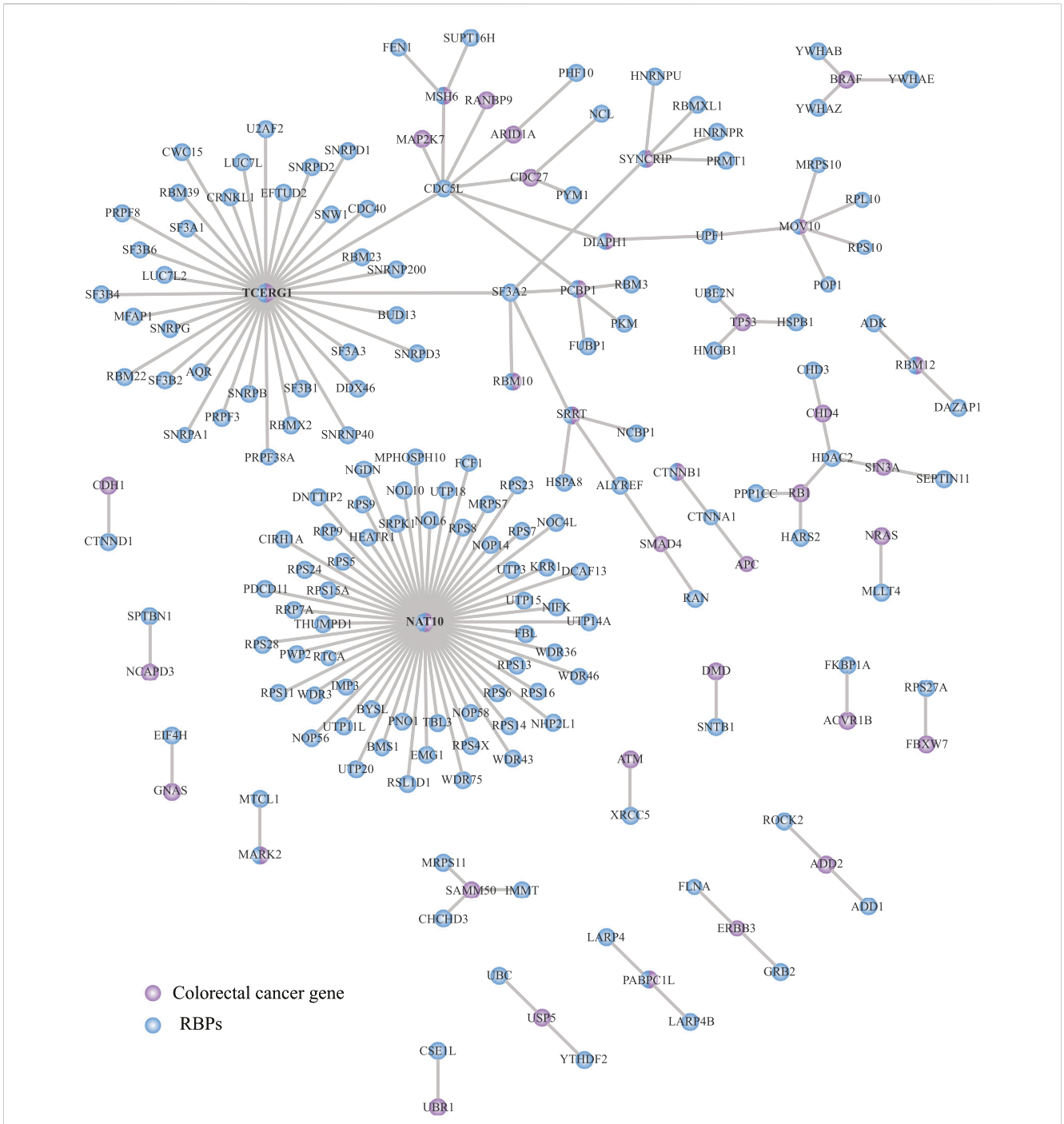

FIGURE 4
Interaction network between RBPs and colorectal cancer driver proteins. STRING portal (Szklarczyk et al., 2019) was used to analyze protein-protein interactions obtained from experiments and databases. A total of 153 RBPs were identified to be associated with 37 COREAD proteins. Blue = RBPs, purple = COREAD proteins.

were subtype specific such as SYNE2 and UTP20 for Pole, and ANK3 for MSI (Figure 3A). Similarly, we found subtype specific RBPs within READ subtypes that have never related to COREAD. For instance, DYNC2H1, DDX55, CPSF7, CAND1 and LARP4 were found in MSI subtype, TSR1 in Pole, ZCCHC3 in GS, and LSM14B in CIN (Figure 3B).

We also detected statistically significant differences between all stages in both cancer types (Mann–Whitney U, $p < 0.001$; Figures 3C,D). In COAD the highest mean of RBPs genomic and transcriptomic alterations were detected in stage II, while in READ they were found in stage I (Figures 3C,D; Table 2). Interestingly, stage IV showed the lowest number of RBPs

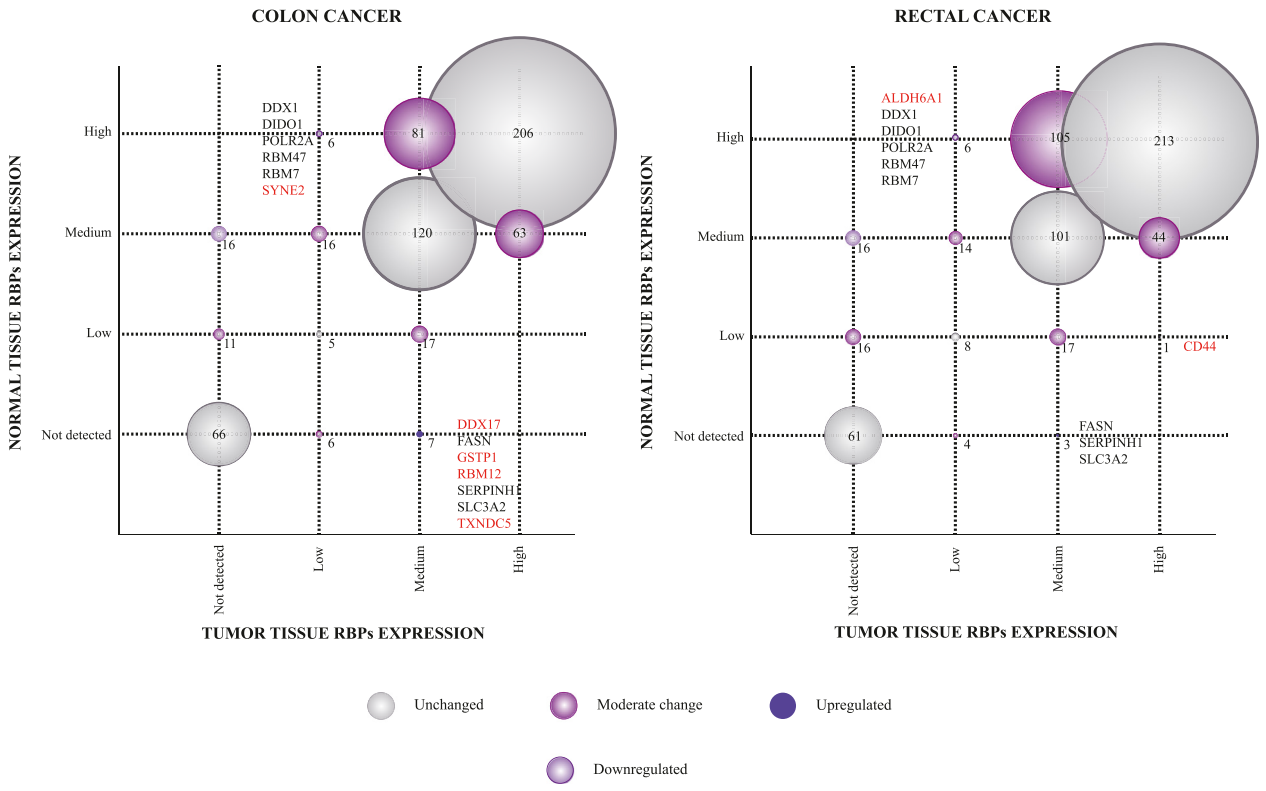

FIGURE 5
Immunohistochemical protein expression profiles of RNA-binding proteins between healthy and tumoral tissues. Scatterplots comparing RBPs immunohistochemical levels between normal and tumoral tissues are presented. The size of the circle is associated with the number of RBPs found in each correlation. RBPs in red are those which are unique for each type of cancer.

alterations in both cancers. Despite these differences, STAU1 and YTHDF1 were constantly altered in all stages. This was also observed in GS and CIN subtypes (Figures 3A,B; Table 2). Contrary to STAU1, YTHDF1 has previously been associated with COREAD development (Bai et al., 2019; Chen P. et al., 2021; Yan et al., 2021), showing the potential of STAU1 to be involved in COREAD progression too.

Networking analysis of RNA-Binding proteins vs. colorectal drivers

Protein-protein interaction (PPIs) networks have been proved to be effective in detecting tumorigenic RNA regulons (Wurth et al., 2016; Indacochea et al., 2021). Thus, we next interrogated the STRING database (Szklarczyk et al., 2019) to understand the relationship between RBPs (n = 1,392) and COREAD drivers (n = 139) (Repana et al., 2019) and outline key interactions among them. We identified 37 COREAD proteins interacting with 153 RBPs (Figure 4; Supplementary Table S10). The interactions were obtained from experiments and databases using a highest confidence threshold (interaction score = 0.9). As shown in Figure 4, we detected two main interaction networks around TCERG1 and NAT10. Interestingly, these proteins not only have the ability to bind RNA but also, they were catalogued as COREAD drivers. Interestingly, TCERG1 binds to CDCL5 which in turn binds to five COREAD drivers.

RNA-binding proteins expression levels in colon and rectal tissues

The Human Protein Atlas (HPA) constitutes a large-scale resource to study antibody-based protein expression patterns in human tissues (Uhlén et al., 2015; Thul et al., 2017; Uhlen et al., 2017). We, therefore, used this tool to identify differentially expressed RBPs between tumoral and normal colon and rectal tissues. Thus, we compared protein immunohistochemical levels (not detected, low, medium, and high) of 608 available RBPs in COAD and 609 RBPs in READ tissues. We detected 211 in colon and 226 in rectum RBPs having at least one variation level (e.g., not detected to low or medium to high) between malignant and healthy colon and rectal tissues, respectively (Figure 5; Supplementary Tables S11, S12).

To detect highly altered RBPs, we next categorized these proteins as up or downregulated based on a two-variation level difference. As a result, we found seven upregulated RBPs (DDX17, FASN, GSTP1, RBM12, SERPINH1, SLC3A2, and

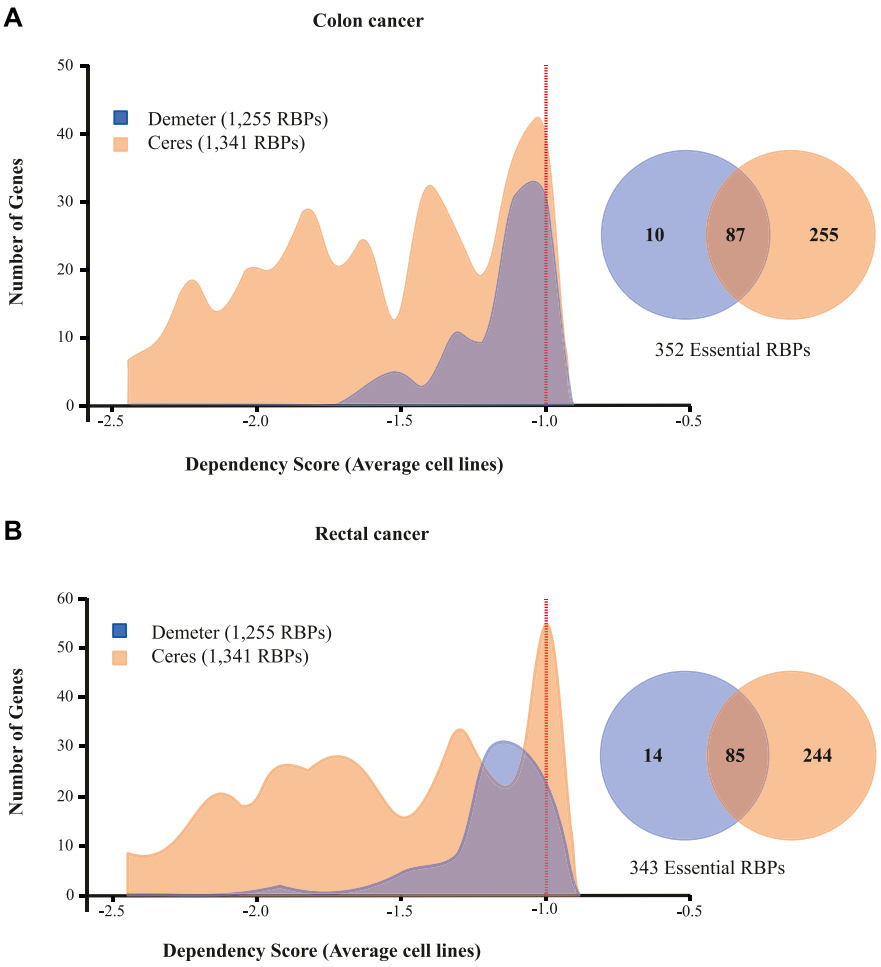

FIGURE 6
RBPs **(A)** colon and **(B)** rectal cancer dependencies. Dependency scores of 1,255 (DEMETER2; Tsherniak et al., 2017; McFarland et al., 2018) and 1,341 RBPs (CERES; Meyers et al., 2017) from 89 colon and 13 rectal cancer cell lines are presented.

TABLE 3 CRISPR-Cas9 (CERES; Meyers et al., 2017) and RNAi (DEMETER2; Tsherniak et al., 2017; McFarland et al., 2018) cell lines and top five essential RBPs.

	Ceres			Demeter		
	Cell lines	Mean DepScore	Top 5	Cell lines	Mean DepScore	Top 5
Colon	47	−1.6	RAN, RPL15, SNRPB, HSPE1, and RPL4	42	−1.18	SF3B2, RPL7, SNRPD1, RPL14, and EIF3B
Rectum	10	−1.6	RAN, RPL15, HSPE1, RPL23, and RPS6	3	−1.23	SNRPD1, SF3B2, RPL5, COPB1, and SRSF3

TXNDC5) and six downregulated (DDX1, DIDO1, POLR2A, RBM47, RBM7, and SYNE2) in colon tissues. As anticipated, our strategy identified well-known COREAD proteins, such as DDX17 (Li X. N. et al., 2018), FASN (Yu et al., 2020) or GSTP1 (Sameer et al., 2012), validating our analysis. It is noteworthy to mention that RBM12 and RBM7 have never been implicated in COAD or READ before. Regarding rectal tissues, we identified four upregulated (CD44, FASN, SERPINH1, and SLC3A2) and six downregulated RBPs (ALDH6A1, DDX1, DIDO1, POLR2A, RBM47, and RBM7). Also, several of these proteins have been previously studied in COREAD as ALDH6A1 (Li X. et al., 2022) or DDX1 (Tanaka et al., 2018). Interestingly, CD44, a well-established COREAD protein (Herrlich et al., 1995; Wielenga et al., 2000), was upregulated only in rectal tumors showing its potential to distinguish rectal from colon carcinomas.

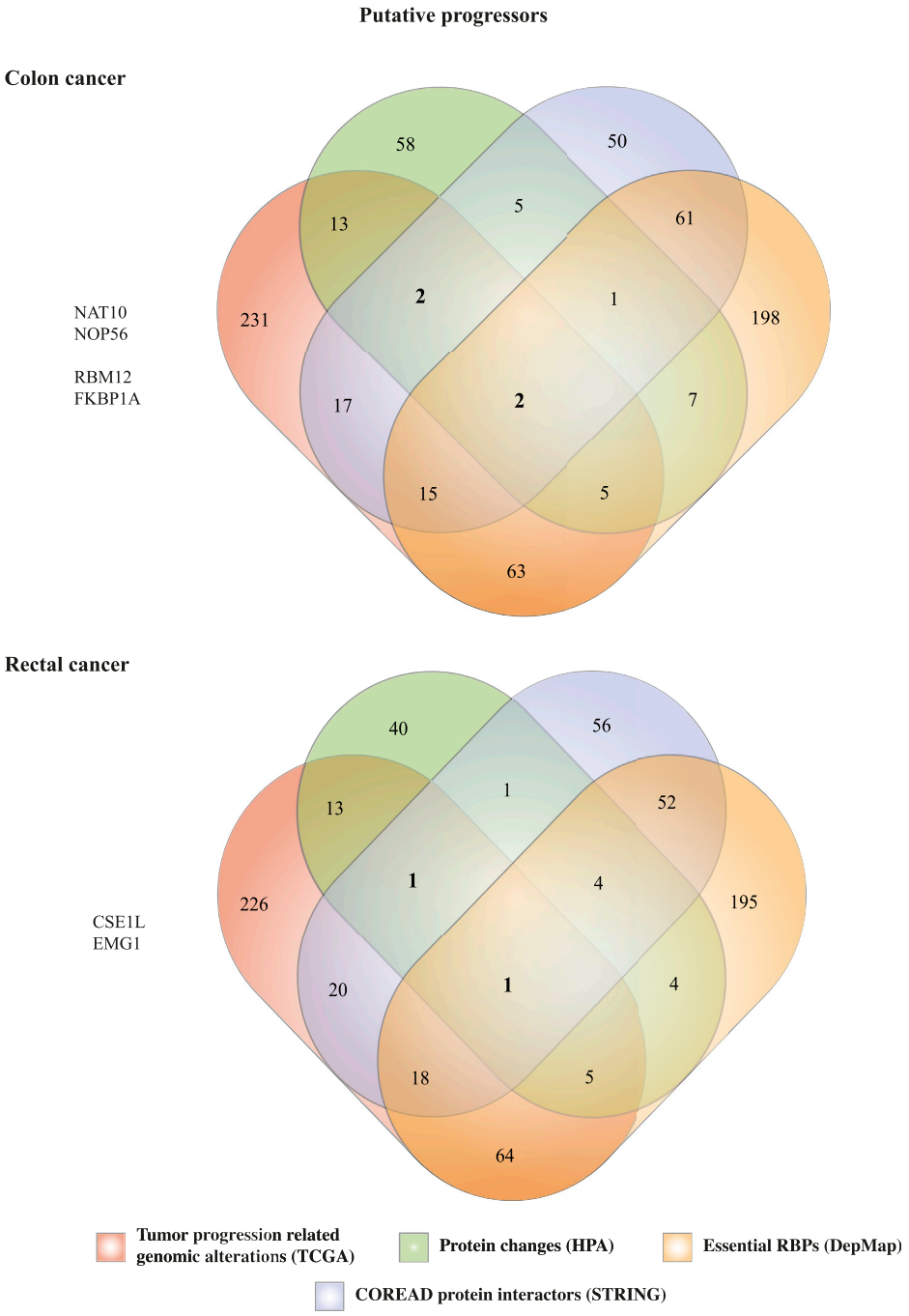

FIGURE 7
Identification of novel RNA-binding proteins involved in colon and rectal carcinomas progression. Prioritization of RBPs in COAD and READ was based on four cancer-related characteristics 1) high genomic and transcriptomic alterations, 2) interactions with well-known cancer proteins, 3) aberrant protein expression levels compared with normal tissues, and/or 4) essential for tumor survival.

Identification of RNA binding proteins involved in COAD and READ cell survival

To identify essential RBPs for COAD and READ cell survival, we interrogated two large-scale CRISPR-Cas9 (CERES; Meyers et al., 2017) and RNAi (DEMETER2; Tsherniak et al., 2017; McFarland et al., 2018) loss-of-function screens using the DepMap portal (https://depmap.org/portal/). (Yu et al., 2016) CERES contains data of 1,341 RBPs in 47 colon and 10 rectal cancer cell lines, while DEMETER2 presents data of 1,255 RBPs in 42 colon and three rectal cancer cell lines. These initiatives calculate a dependency score that represents how vital a gene is to cell survival. A score of 0 indicates non-essentiality, whereas a score

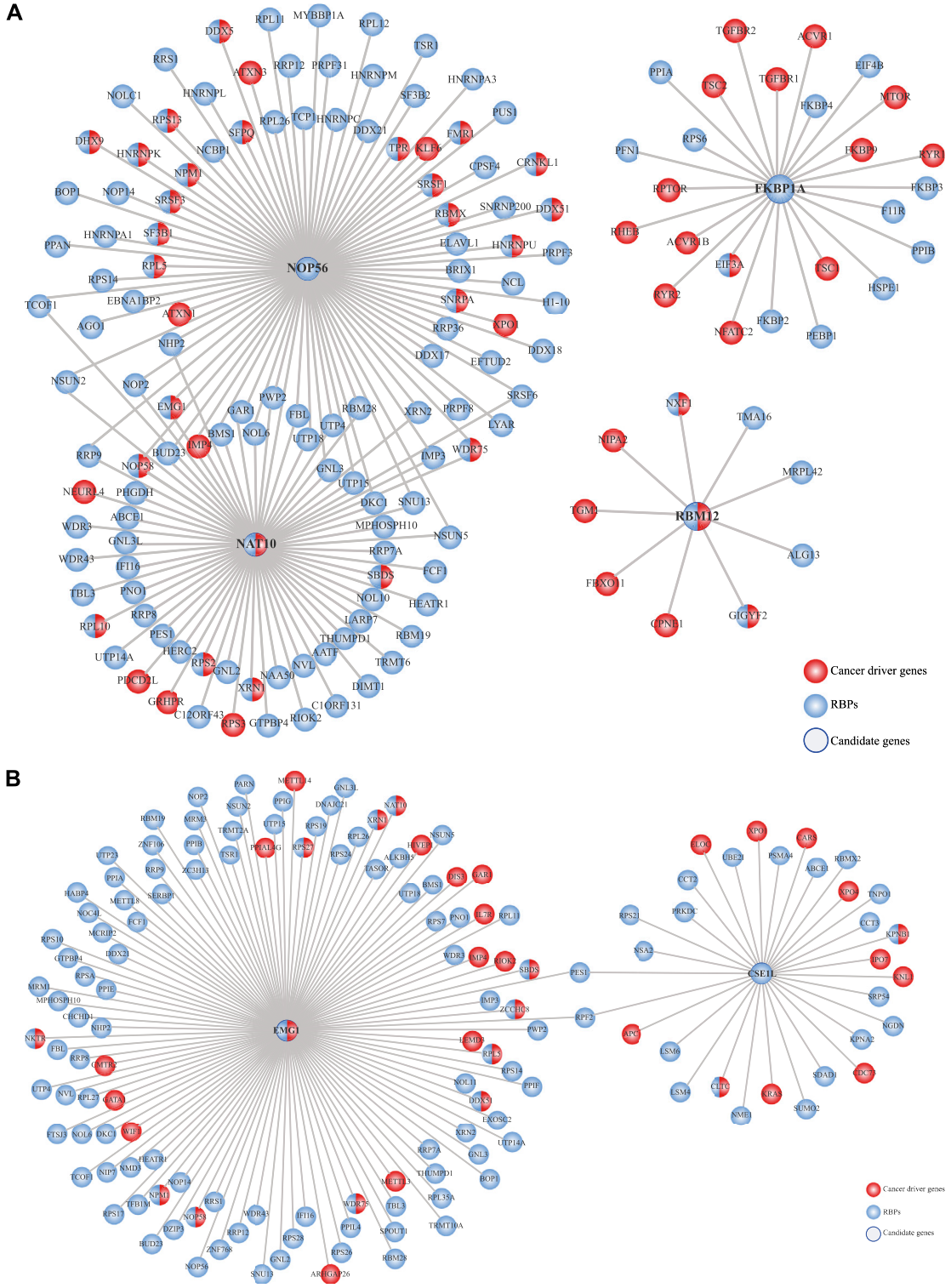

FIGURE 8
Prioritized RBPs correlate with cancer genes. Previously prioritized RBPs in **(A)** colon cancer (NAT10, NOP56, RBM12, and FKBP1A) and **(B)** rectal cancer (CSE1L and EMG1) were correlated with cancer genes by networking analysis using the HumanNet v3 database (Kim et al., 2022).

of ≤ −1 corresponds to the median of all pan-essential genes. Thus, we identified 352 (87 detected by both screens) and 343 (85 detected by both screens) essential RBPs in colon and rectal tumors, respectively (Figures 6A,B; Supplementary Tables S13–S16). Additionally, Table 3 shows the top five essential RBPs for colon and rectal carcinomas.

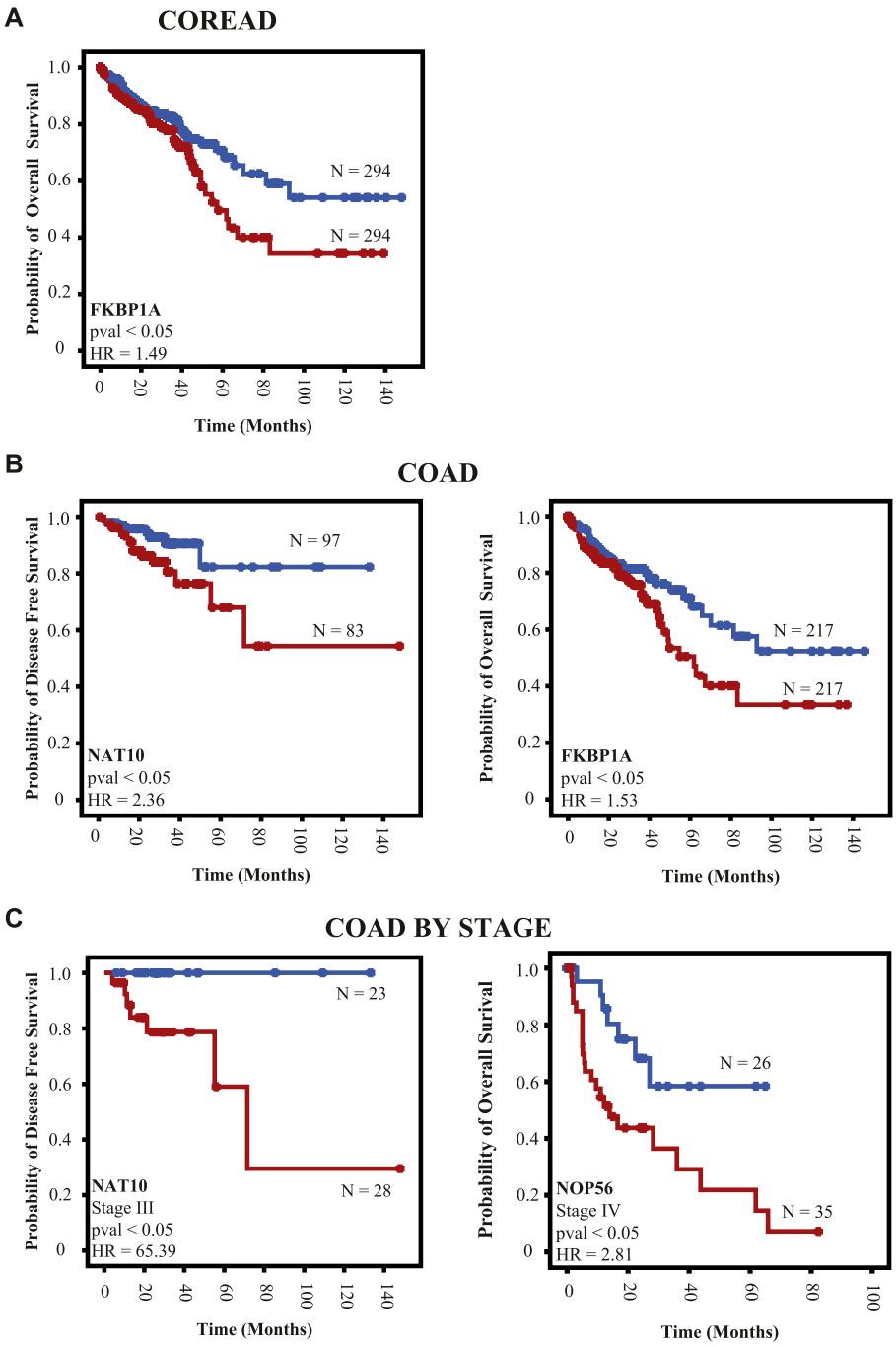

FIGURE 9
RBPs expression as a determinant of clinical outcome. We interrogated TCGA data using as a cutoff point the median of mRNA expression. **(A)** Prognosis in COREAD population **(B)** prognosis in colon cancer patients regardless the stage, and **(C)** colon cancer patients by stage. Red and blue lines indicate downregulation and upregulation mRNA, respectively.

Prioritization of RNA-binding proteins in colon and rectal carcinomas

As more and more studies reveal the complex roles of RBPs in cancer progression, new data mining strategies come forward to narrowed down the identification of tumorigenic RBPs (Xing et al., 2021; Chen et al., 2022). To identify potential colon and rectal cancer tumor progressors RBPs, we next used our previously published multidata integration strategy (Figure 1) (García-Cárdenas et al., 2022). Thus, we merged our previous results as follows: 1) first quartile of most genomic and transcriptomic altered RBPs ($n = 348$) concerning tumor progression-related alterations (mRNA upregulation + genomic amplification + gene mutations + fusions), 2) 153 RBPs presenting PPIs with colorectal proteins, 3)

93 (colon) and 69 (rectum) RBPs having moderate or upregulated immunohistochemical variation, 4) 352 (colon) and 343 (rectum) essential RBPs (Figure 7).

In COAD, we identified two RBPs (NAT10 and NOP56) having all four tumor-associated characteristics presented in this study. NAT10 has already been implicated in colorectal cancer in several studies (Zhang et al., 2014; Liu et al., 2016; 2019; Cao et al., 2020), while NOP56 has been found differentially expressed in COREAD by a different data mining approach (Liang et al., 2021). Also, we were able to retrieve two other RBPs (RBM12 and FKBP1A) that were not essential for tumor cell survival, but they could be implicated in any other hallmark of cancer. For instance, FKBP1A overexpression has been correlated with apoptosis inhibition in prostate cancer (Leng et al., 2020); we did not find any reports of its involvement in COREAD. On the other hand, RBM12 has been found to be hypermutated in 619 COREAD tumors (Giannakis et al., 2016), but no further experimentation has been performed.

Similarly, we prioritized two RBPs in rectal cancer, CSE1L which presents all the above-mentioned characteristics, and EMG1 that was not essential for tumor cell survival. CSE1L has been related to colorectal cancer before (Sillars-Hardebol et al., 2012; Tai et al., 2013; Pimiento et al., 2016; Xu et al., 2020), while EMG1 has been poorly studied. Interestingly, NOP56, NAT10, and CSEL1 have been classified as common essential by DepMap survival algorithm. Thus, therapeutic targeting of these RBPs could have a greater impact in cell survival due to their implications in RNA-dependent basic cellular processes.

Finally, we intended to understand how these prioritized RBPs could correlate with cancer in general terms. To that end, we used the HumanNet v3 (Lee et al., 2011; Hwang et al., 2019) to generate networks where only cancer genes and RBPs were considered. We observed intricated connections between these prioritized RBPs with several cancer genes and other RBPs, showing their potential to promote cancer (Figures 8A,B; Supplementary Tables S17, S18).

RBPs mRNA expression levels as determinants of clinical outcomes

To explore the clinical relevance of RBPs mRNA expression (upregulation vs. downregulation) in COREAD, COAD, and READ patients, we have interrogated TCGA, PanCancer Atlas (Hoadley et al., 2018) database for mRNA expression of prioritized RBPs (NAT10, NOP56, RBM12, FKBP1A, CSE1L, and EMG1) in those populations and calculated several clinical aspects (OS and DFS). In COREAD population, we only found FKBP1A expression is related with an adverse outcome in overall survival (OS; $p < 0.05$) (Figure 9A).

Due to the fact that colon and rectum cancer are different clinical entities (Paschke et al., 2018), we have explored whether these relevant RBPs could predict clinical outcomes. In COAD patients, NAT10 and FKBP1A are related with adverse outcome in DFS and OS, respectively ($p < 0.05$; Figure 9B). We also found that NAT10 is related with poor outcome in DFS of stage III COAD patients ($p < 0.05$; Figure 9C). Moreover, high expression of NOP56 in primary tumors are related with bad outcome in OS of stage IV COAD patients ($p < 0.05$; Figure 9C). No other clinical outcomes were predicted by RBPs mRNA expression in COREAD, COAD, and READ patients.

Discussion

The development and implementation of multi-omics approaches along with modern bioinformatic technologies have provided new insights in COREAD biology (Chierici et al., 2020; Yin et al., 2020; Heo et al., 2021). In that respect, several studies have attempted to molecularly characterize COREAD tumors (Schlicker et al., 2012; Budinska et al., 2013; De Sousa E Melo et al., 2013; Marisa et al., 2013; Sadanandam et al., 2013; Roepman et al., 2014). Guinney et al., by integrating several subtyping algorithms, proposed the Consensus Molecular Subtypes of colorectal cancer to establish a baseline for clinical decision making (Guinney et al., 2015). Nevertheless, according to Paschke et al., COREAD has been treated as one entity when several clinical and molecular aspects (e.g., epidemiology, carcinogenic risk, molecular carcinogenesis, etc.) indicate the contrary (Paschke et al., 2018). Consequently, Paschke et al., suggested separating COREAD into COAD and READ, implementing a new perspective to discover novel biomarkers for both cancer types, which holds the potential to improve subtype-based clinical interventions (Paschke et al., 2018). In this regard, RBPs as emerging regulators of cancerous processes (Wurth and Gebauer, 2015; Corrado et al., 2016; Hentze et al., 2018; Kang et al., 2020; Zhang et al., 2020; Chen et al., 2022), could fulfill this need.

In this work, we used our previously published multidata integration strategy to prioritized tumorigenic RBPs in COAD and READ separately (García-Cárdenas et al., 2022). First, we determined genomic and transcriptomic alteration profiles of RBPs in COAD and READ patients. As is shown in Figure 2A, most of the alterations were found in mRNA levels: mRNA upregulation (64% in colon and 62% in rectum) followed by mRNA downregulation (15% in colon and 20% in rectum). Even though genomic and transcriptomic alteration profiles of COAD and READ were similar, we found significant differences among alteration types (Figure 2B) and the level of up and downregulation (Figure 2C), supporting Paschke *et al.*, findings at least regarding RNA metabolism (Paschke et al., 2018). These results also agree with a comprehensive transcriptomic analysis performed by Zhang *et al.*, in which RBPs are predominantly upregulated across cancer types (Zhang et al., 2020). These alterations will probably influence key post-transcriptional processes involved in COAD and READ development.

Despite the genomic and transcriptomic alteration similarities among the subtypes of both carcinomas, most altered RBPs in each subtype differed (Figures 3A,B). At least in READ, most of the highly altered RBPs are unique for this type of cancer, e.g., TSR1, TSR1, DYNC2H1, DDX55, CPSF7, CAND1, LARP4, ZCCHC3, and LSM14B and they have never been associated with COREAD. These findings support Paschke *et al.*, suggestion (Paschke et al., 2018), several RBPs and maybe not only RBPs could have been

ignored when we studied as COREAD but, when they are separated in COAD and READ, new putative biomarkers are discovered.

Unlike subtypes, we observed dissimilar patterns of RBPs genomic and transcriptomic alterations among stages in colon and rectal tumors. Stage II was the most altered in COAD, whereas in READ the most altered one was stage I (Figures 3C,D; Table 2). Despite these differences, we found proteins that were constantly altered in all stages. For example, STAU1 and YTHDF1 were highly altered across stages in both carcinomas. Contrary to STAU1, YTHDF1 has previously been associated with COREAD development (Bai et al., 2019; Chen P. et al., 2021; Yan et al., 2021). Interestingly, STAU1 has been related with pancreatic cancer (Marcellus et al., 2021) and its misregulation impacts cell cycle regulation (Bonnet-Magnaval and DesGroseillers, 2021), showing the potential of this protein to also be involved in COREAD progression.

Networking analysis has been shown to be a powerful tool to identify tumorigenic proteins in cancer (Wurth et al., 2016; Indacochea et al., 2021; García-Cárdenas et al., 2022). Similarly, PPIs between RBPs and COREAD drivers allowed us to determine two functional modules, such as NAT10 and TCERG1, which were central elements (Figure 4). These RBPs have also been catalogued as COREAD drivers. In fact, NAT10 suppresses tumor proliferation by activating p53; in COREAD, NAT10 activity is decreased resulting in p53 malfunction and, therefore, uncontrollable cell division (Liu et al., 2016). Concerning TCERG1 subnetwork, we observed intricated connections between RBPs and COREAD drivers. For example, TCERG1 interacts with CDC5L which in turn connects with six COREAD drivers (MAP2K7, RANBP9, ARID1A, CDC27, MSH6, and DIAPH1). Interestingly, CDC5L has been related to other cancer types, such as prostate cancer (Li X. et al., 2018) and osteosarcoma (Lu et al., 2008).

We next examined protein immunohistochemical levels (the Human Protein Atlas database) to identify differentially expressed RBPs in colon and rectal tumor tissues (Pontén et al., 2008). In fact, immunohistochemistry (IHC) is a widely used approach in histopathology for cancer diagnosis. Thus, we found DDX17, GSTP1, RBM12, and TXNDC5 to be overexpressed only in COAD, while CD44 is exclusively upregulated in READ. Similarly, we found SYNE2 and ALDH6A1 to be exclusively downregulated in COAD and READ, respectively. Further IHC studies should be performed to address their potential as diagnostic biomarkers of colon and rectal tumors, separately. As anticipated, our strategy also identified well-known COREAD proteins (Figure 5), such as DDX17 (Li X. N. et al., 2018), ALDH6A1 (Li X. et al., 2022), DDX1 (Tanaka et al., 2018), FASN (Yu et al., 2020), and GSTP1 (Sameer et al., 2012), validating our analysis. It is noticeable to mention that RBM12 and RBM7 have never been implicated in COAD or READ before.

Then, we explored RBPs-based COAD and READ cell dependencies by interrogating two large-scale loss-of-function screens CERES (Meyers et al., 2017) and DEMETER2 (Tsherniak et al., 2017; McFarland et al., 2018). In colon we found 352 essentials RBPs, while in READ we identified 343 RBPs (both CRISPR-Cas9 and RNAi methods included) (Figures 6A,B). In other words, 25% of all known RBPs are essentials for oncogenic cells survival, unsurprisingly given the crucial role of RBPs in RNA metabolism. In Table 3, we listed the top five essentials RBPs in both types of cancer based on the DepScore. The same scenario of the previous analyses is repeated, we obtained RBPs that have been related to COREAD (e.g., SF3B2, RPL7, and SNRPD1), and others that have not (e.g., COPB1) (Supplementary Tables S13–S16) (Boleij et al., 2010; Fijneman et al., 2012; Xu et al., 2021).

Compelling studies have shown the potential of RBPs to promote cancer development. RBPs are widely altered in cancer cells, control hundreds to thousands RNAs, and interact with cancer driver proteins (Wurth and Gebauer, 2015; Hentze et al., 2018; García-Cárdenas et al., 2019). With that in mind, we reasoned that the integration of our previous analyses could narrow down the identification of potential COAD and READ RBPs. Our data mining strategy (Figure 1) allowed us to identify four proteins in COAD (NAT10, NOP56, RBM12, and FKBP1A) and two in READ (CSE1L and EMG1) (Figure 7). NAT10 and CSE1L have already been involved in COREAD (Sillars-Hardebol et al., 2012; Tai et al., 2013; Zhang et al., 2014; Liu et al., 2016; 2019; Pimiento et al., 2016; Cao et al., 2020; Xu et al., 2020). NOP56 and RBM12 were already identified in COREAD by different data mining approaches (Liang et al., 2021), validating our results. Additionally, to the best of our knowledge, no prior studies have associated FKBP1A and EMG1 with cancer before. To highlight their relevance in cancer, we interrogated the HumanNet v3 and found that NOP56, RBM12, FKBP1A and EMG1 are highly interconnected with cancer genes and other RBPs, showing their potential to form tumorigenic RNA-regulons (Figures 8A,B).

Finally, we explored the clinical implications of these prioritized RBPs. Upregulation of FKBP1A, NAT10, and NOP56 mRNA expression could predict clinical outcomes in COREAD and COAD patients. Similarly, mRNA upregulation of NAT10 and NOP56 are related with poor outcomes depending on COAD staging. This is clinically relevant since COAD therapy is defined by stage (Argilés et al., 2020; Cervantes et al., 2022). Stage II COAD patients are not always candidate of adjuvant therapy. However, adjuvant chemotherapy is a relevant treatment for stage III COAD patients because it decreases the risk of relapse (Argilés et al., 2020). In daily clinical practice there are no specific biomarker to predict a relapse. According to our results, NAT10 mRNA upregulation is related with adverse outcome in DFS of stage III COAD patients. Similarly, NOP56 overexpression in primary tumors is associated with poor prognosis in DFS and OS of stage IV COAD patients. These results highlight the clinical relevance of FKBP1A, NAT10, and NOP56. Despite these promising findings, a high number of patients are needed to validate these results in specifical clinical scenarios.

In summary, we analyzed and integrated data from 488 COAD and 155 READ patients, 102 cancer cell lines, more than 15,000 immunostainings, and ~10,000 raw associations between RBPs and cancer genes to unravel new RBPs involved in COAD

(NOP56, NAT10, RBM12, and FKBP1A) and READ (EMG1 and CSE1L). Further analyses allowed us to identify potential clinical applications of FKBP1A, NAT10, and NOP56 as biomarkers of specific outcomes.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

Conceptualization, SG, AI, and JG-C; methodology, SG, AI, and JG-C; investigation, SG, AI, and JG-C; data curation, SG and AL-C. writing original draft preparation, SG and JG-C; writing editing, NG-C, IA-C, AL-C, AI, and DP-C; visualization, SG, NG-C, IA-C, and DP-C; supervision, SG and AI All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcell.2023.1088057/full#supplementary-material

References

Abdel-Wahab, O., and Gebauer, F. (2018). Editorial overview: Cancer genomics: RNA metabolism and translation in cancer pathogenesis and therapy. *Curr. Opin. Genet. Dev.* 48, iv. vi. doi:10.1016/j.gde.2018.01.007

Adler, A. S., McCleland, M. L., Yee, S., Yaylaoglu, M., Hussain, S., Cosino, E., et al. (2014). An integrative analysis of colon cancer identifies an essential function for PRPF6 in tumor growth. *Genes Dev.* 28, 1068–1084. doi:10.1101/gad.237206.113

Argilés, G., Tabernero, J., Labianca, R., Hochhauser, D., Salazar, R., Iveson, T., et al. (2020). Localised colon cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann. Oncol. Off. J. Eur. Soc. Med. Oncol.* 31, 1291–1305. doi:10.1016/J.ANNONC.2020.06.022

Assis, J. V. de, Coutinho, L. A., Oyeyemi, I. T., Oyeyemi, O. T., and Grenfell, R. F. e. Q. (2022). Diagnostic and therapeutic biomarkers in colorectal cancer: A review. *Am. J. Cancer Res.* 12, 661.

Bai, Y., Yang, C., Wu, R., Huang, L., Song, S., Li, W., et al. (2019). YTHDF1 regulates tumorigenicity and cancer stem cell-like activity in human colorectal carcinoma. *Front. Oncol.* 9, 332. doi:10.3389/FONC.2019.00332

Bi, G., Zhu, D., Bian, Y., Huang, Y., Zhan, C., Yang, Y., et al. (2021). Knockdown of GTF2E2 inhibits the growth and progression of lung adenocarcinoma via RPS4X *in vitro* and *in vivo*. *Cancer Cell Int.* 21, 181. doi:10.1186/S12935-021-01878-Z

Boleij, A., Roelofs, R., Schaeps, R. M. J., Schülin, T., Glaser, P., Swinkels, D. W., et al. (2010). Increased exposure to bacterial antigen RpL7/L12 in early stage colorectal cancer patients. *Cancer* 116, 4014–4022. doi:10.1002/CNCR.25212

Bonnet-Magnaval, F., and DesGroseillers, L. (2021). The Staufen1-dependent cell cycle regulon or how a misregulated RNA-binding protein leads to cancer. *Biol. Rev. Camb. Philos. Soc.* 96, 2192–2208. doi:10.1111/BRV.12749

Budinska, E., Popovici, V., Tejpar, S., D'Ario, G., Lapique, N., Sikora, K. O., et al. (2013). Gene expression patterns unveil a new level of molecular heterogeneity in colorectal cancer. *J. Pathol.* 231, 63–76. doi:10.1002/PATH.4212

Cao, J., Hou, P., Chen, J., Wang, P., Wang, W., Liu, W., et al. (2017). The overexpression and prognostic role of DCAF13 in hepatocellular carcinoma. *Tumor Biol.* 39, 1010428317705753. doi:10.1177/1010428317705753

Cao, Y., Yao, M., Wu, Y., Ma, N., Liu, H., and Zhang, B. (2020). N-acetyltransferase 10 promotes micronuclei formation to activate the senescence-associated secretory phenotype machinery in colorectal cancer cells. *Transl. Oncol.* 13, 100783. doi:10.1016/J.TRANON.2020.100783

Cervantes, A., Adam, R., Roselló, S., Arnold, D., Normanno, N., Taïeb, J., et al. (2022). Metastatic colorectal cancer: ESMO clinical practice guideline for diagnosis, treatment and follow-up. *Ann. Oncol.* 34, 10–32. doi:10.1016/J.ANNONC.2022.10.003/ATTACHMENT/5A57CC70-CCE4-401B-A657-C12E03275943/MMC1.PDF

Chen, L., Zhou, S. J., Xu, Y., Liao, Q. M., Zou, Y. S., and Pei, H. (2021). CCAR2 promotes a malignant phenotype of osteosarcoma through Wnt/β-catenin-dependent transcriptional activation of SPARC. *Biochem. Biophys. Res. Commun.* 580, 67–73. doi:10.1016/J.BBRC.2021.09.070

Chen, P., Liu, X. Q., Lin, X., Gao, L. Y., Zhang, S., and Huang, X. (2021). Targeting YTHDF1 effectively re-sensitizes cisplatin-resistant colon cancer cells by modulating GLS-mediated glutamine metabolism. *Mol. Ther. Oncolytics* 20, 228–239. doi:10.1016/J.OMTO.2021.01.001

Chen, Y., Qin, H., and Zheng, L. (2022). Research progress on RNA–binding proteins in breast cancer. *Front. Oncol.* 12, 4303. doi:10.3389/fonc.2022.974523

Chierici, M., Bussola, N., Marcolini, A., Francescatto, M., Zandonà, A., Trastulla, L., et al. (2020). Integrative network fusion: A multi-omics approach in molecular profiling. *Front. Oncol.* 10, 1065. doi:10.3389/fonc.2020.01065

Cohen, R., Pudlarz, T., Delattre, J. F., Colle, R., and André, T. (2020). Molecular targets for the treatment of metastatic colorectal cancer. *Cancers (Basel)* 12, 2350–2418. doi:10.3390/CANCERS12092350

Corrado, G., Tebaldi, T., Costa, F., Frasconi, P., and Passerini, A. (2016). RNAcommender: Genome-wide recommendation of RNA-protein interactions. *Bioinformatics* 32, 3627–3634. doi:10.1093/bioinformatics/btw517

De Sousa E Melo, F., Wang, X., Jansen, M., Fessler, E., Trinh, A., De Rooij, L. P. M. H., et al. (2013). Poor-prognosis colon cancer is defined by a molecularly distinct subtype and develops from serrated precursor lesions. *Nat. Med.* 195 19, 614–618. doi:10.1038/nm.3174

Dressler, L., Bortolomeazzi, M., Keddar, M. R., Misetic, H., Sartini, G., Acha-Sagredo, A., et al. (2022). Comparative assessment of genes driving cancer and somatic evolution in non-cancer tissues: An update of the network of cancer genes (NCG) resource. *Genome Biol.* 231 23, 35–22. doi:10.1186/S13059-022-02607-Z

Eldai, H., Periyasamy, S., Al Qarni, S., Al Rodayyan, M., Muhammed Mustafa, S., Deeb, A., et al. (2013). Novel genes associated with colorectal cancer are revealed by high resolution cytogenetic analysis in a patient specific manner. *PLoS One* 8, e76251. doi:10.1371/JOURNAL.PONE.0076251

Feichtinger, R. G., Schäfer, G., Seifarth, C., Mayr, J. A., Kofler, B., and Klocker, H. (2018). Reduced levels of ATP synthase subunit ATP5F1A correlate with earlier-onset prostate cancer. *Oxid. Med. Cell. Longev.* 2018, 1347174. doi:10.1155/2018/1347174

Fijneman, R. J. A., De Wit, M., Pourghiasian, M., Piersma, S. R., Pham, T. V., Warmoes, M. O., et al. (2012). Proximal fluid proteome profiling of mouse colon tumors reveals biomarkers for early diagnosis of human colorectal cancer. *Clin. Cancer Res.PROTEOME-PROFILING-OF-MOUSE-COLON* 18, 2613–2624. doi:10.1158/1078-0432.CCR-11-1937

Forghanifard, M. M., Naeimi Khorasanizadeh, P., Abbaszadegan, M. R., Javdani Mallak, A., and Moghbeli, M. (2020). Role of DIDO1 in progression of esophageal squamous cell carcinoma. *J. Gastrointest. Cancer* 51, 83–87. doi:10.1007/S12029-019-00212-1

García-Cárdenas, J. M., Armendáriz-Castillo, I., Pérez-Villa, A., Indacochea, A., Jácome-Alvarado, A., López-Cortés, A., et al. (2022). Integrated in silico analyses identify PUF60 and SF3A3 as new spliceosome-related breast cancer RNA-binding proteins. *Biol* 11, 481. *Page 481* 11. doi:10.3390/BIOLOGY11040481

García-Cárdenas, J. M., Guerrero, S., López-Cortés, A., Armendáriz-Castillo, I., Guevara-Ramírez, P., Pérez-Villa, A., et al. (2019). Post-transcriptional regulation of colorectal cancer: A focus on RNA-binding proteins. *Front. Mol. Biosci.* 6, 65. doi:10.3389/fmolb.2019.00065

Giannakis, M., Mu, X. J., Shukla, S. A., Qian, Z. R., Cohen, O., Nishihara, R., et al. (2016). Genomic correlates of immune-cell infiltrates in colorectal carcinoma. *Cell Rep.* 15, 857–865. doi:10.1016/J.CELREP.2016.03.075

Guinney, J., Dienstmann, R., Wang, X., de Reyniès, A., Schlicker, A., Soneson, C., et al. (2015). The consensus molecular subtypes of colorectal cancer. *Nat. Med.* 21, 1350–1356. doi:10.1038/nm.3967

Guo, X., Li, K., Jiang, W., Hu, Y., Xiao, W., Huang, Y., et al. (2020). RNA demethylase ALKBH5 prevents pancreatic cancer progression by posttranscriptional activation of PER1 in an m6A-YTHDF2-dependent manner. *Mol. Cancer* 19, 91. doi:10.1186/S12943-020-01158-W

Hanahan, D. (2022). Hallmarks of cancer: New dimensions. *Cancer Discov.* 12, 31–46. doi:10.1158/2159-8290.CD-21-1059

Hentze, M. W., Castello, A., Schwarzl, T., and Preiss, T. (2018). A brave new world of RNA-binding proteins. *Nat. Rev. Mol. Cell Biol.* 19, 327–341. doi:10.1038/nrm.2017.130

Heo, Y. J., Hwa, C., Lee, G.-H., Park, J.-M., and An, J.-Y. (2021). Integrative multi-omics approaches in cancer research: From biological networks to clinical subtypes. *Mol. Cells* 44, 433–443. doi:10.14348/molcells.2021.0042

Herrlich, P., Pals, S., and Ponta, H. (1995). CD44 in colon cancer. *Eur. J. Cancer* 31A, 1110–1112. doi:10.1016/0959-8049(95)00252-E

Hoadley, K. A., Yau, C., Hinoue, T., Wolf, D. M., Lazar, A. J., Drill, E., et al. (2018). Cell-of-Origin patterns dominate the molecular classification of 10,000 tumors from 33 types of cancer. *Cell* 173, 291–304.e6. doi:10.1016/j.cell.2018.03.022

Hu, B., Jiang, D., Chen, Y., Wei, L., Zhang, S., Zhao, F., et al. (2015). High CHMP4B expression is associated with accelerated cell proliferation and resistance to doxorubicin in hepatocellular carcinoma. *Tumour Biol.* 36, 2569–2581. doi:10.1007/S13277-014-2873-1

Hwang, S., Kim, C. Y., Yang, S., Kim, E., Hart, T., Marcotte, E. M., et al. (2019). HumanNet v2: Human gene networks for disease research. *Nucleic Acids Res.* 47, D573–D580. doi:10.1093/nar/gky1126

Indacochea, A., Santiago, G., Ureña, M., Araujo, F., Coll, O., Lleonart, M. E., et al. (2021). Cold-inducible RNA binding protein promotes breast cancer cell malignancy by regulating Cystatin C levels. *RNA* 27, 190–201. doi:10.1261/RNA.076422.120

Ji, P., Wang, H., Cheng, Y., and Liang, S. (2021). Prognostic prediction and gene regulation network of EIF2S2 in hepatocellular carcinoma based on data mining. *J. Gastrointest. Oncol.* 12, 3061–3078. doi:10.21037/JGO-21-748

Kang, D., Lee, Y., and Lee, J. S. (2020). RNA-binding proteins in cancer: Functional and therapeutic perspectives. *Cancers (Basel)* 12, 2699–2733. doi:10.3390/CANCERS12092699

Kim, C. Y., Baek, S., Cha, J., Yang, S., Kim, E., Marcotte, E. M., et al. (2022). HumanNet v3: An improved database of human gene networks for disease research. *Nucleic Acids Res.* 50, D632–D639. doi:10.1093/NAR/GKAB1048

Kim, H. J., Moon, S. J., Kim, S. H., Heo, K., and Kim, J. H. (2018). DBC1 regulates Wnt/β-catenin-mediated expression of MACC1, a key regulator of cancer progression, in colon cancer. *Cell Death Dis.* 9, 831. doi:10.1038/S41419-018-0899-9

Kuang, Y. S., Wang, Y., Ding, L. D., Yang, L., Wang, Y., Liu, S. H., et al. (2018). Overexpression of CREPT confers colorectal cancer sensitivity to fluorouracil. *World J. Gastroenterol.* 24, 475–483. doi:10.3748/WJG.V24.I4.475

Lee, I., Blom, U. M., Wang, P. I., Shim, J. E., and Marcotte, E. M. (2011). Prioritizing candidate disease genes by network-based boosting of genome-wide association data. *Genome Res.* 21, 1109–1121. doi:10.1101/gr.118992.110

Leng, W., Liu, Q., Zhang, S., Sun, D., and Guo, Y. (2020). LncRNA AFAP1-AS1 modulates the sensitivity of paclitaxel-resistant prostate cancer cells to paclitaxel via miR-195-5p/FKBP1A axis. *Cancer Biol. Ther.* 21, 1072–1080. doi:10.1080/15384047.2020.1829266

Li, H., Jin, X., Liu, B., Zhang, P., Chen, W., and Li, Q. (2019). CircRNA CBL.11 suppresses cell proliferation by sponging miR-6778-5p in colorectal cancer. *BMC Cancer* 19, 826. doi:10.1186/S12885-019-6017-2

Li N, N., Guo, Q., Zhang, Q., Chen, B. J., Li, X. A., and Zhou, Y. (2022). Comprehensive analysis of differentially expressed profiles of mRNA N6-methyladenosine in colorectal cancer. *Front. Cell Dev. Biol.* 9, 760912. doi:10.3389/FCELL.2021.760912

Li, S., Ma, J., Zheng, A., Song, X., Chen, S., and Jin, F. (2021). DEAD-box helicase 27 enhances stem cell-like properties with poor prognosis in breast cancer. *J. Transl. Med.* 19, 334. doi:10.1186/S12967-021-03011-0

Li, X. N., Wang, Z. J., Ye, C. X., Zhao, B. C., Li, Z. L., and Yang, Y. (2018). RNA sequencing reveals the expression profiles of circRNA and indicates that circDDX17 acts as a tumor suppressor in colorectal cancer. *J. Exp. Clin. Cancer Res.* 37, 325. doi:10.1186/S13046-018-1006-X

Li, X., Wang, N., Wu, Y., Liu, Y., and Wang, R. (2022). ALDH6A1 weakens the progression of colon cancer via modulating the RAS/RAF/MEK/ERK pathway in cancer cell lines. *Gene* 842, 146757. doi:10.1016/J.GENE.2022.146757

Li, X., Wang, X., Song, W., Xu, H., Huang, R., Wang, Y., et al. (2018). Oncogenic properties of NEAT1 in prostate cancer cells depend on the cdc5l–AGRN transcriptional regulation circuit. *Cancer Res.* 78, 4138–4149. doi:10.1158/0008-5472.CAN-18-0688

Liang, Q., Du, X., Mao, L., and Wang, G. (2021). Molecular characterization of colorectal cancer: A five-gene prognostic signature based on RNA-binding proteins. *Saudi J. Gastroenterol.* 27, 223–233. doi:10.4103/SJG.SJG_530_20

Liu, W., Wang, C., Wang, S., Zeng, K., Wei, S., Sun, N., et al. (2021). PRPF6 promotes androgen receptor/androgen receptor-variant 7 actions in castration-resistant prostate cancer cells. *Int. J. Biol. Sci.* 17, 188–203. doi:10.7150/IJBS.50810

Liu, X., Tan, Y., Zhang, C., Zhang, Y., Zhang, L., Ren, P., et al. (2016). NAT10 regulates p53 activation through acetylating p53 at K120 and ubiquitinating Mdm2. *EMBO Rep.* 17, 349–366. doi:10.15252/EMBR.201540505

Liu, Z., Liu, X., Li, Y., Ren, P., Zhang, C., Wang, L., et al. (2019). miR-6716-5p promotes metastasis of colorectal cancer through downregulating NAT10 expression. *Cancer Manag. Res.* 11, 5317–5332. doi:10.2147/CMAR.S197733

Liu, Z., Zhang, Y., Dang, Q., Wu, K., Jiao, D., Li, Z., et al. (2021). Genomic alteration characterization in colorectal cancer identifies a prognostic and metastasis biomarker: FAM83A|Ido1. *Front. Oncol.* 11, 607. doi:10.3389/fonc.2021.632430

López-Cortés, A., Paz-y-Miño, C., Guerrero, S., Jaramillo-Koupermann, G., León Cáceres, Á., Intriago-Baldeón, D. P., et al. (2020). Pharmacogenomics, biomarker network, and allele frequencies in colorectal cancer. *Pharmacogenomics J.* 20, 136–158. doi:10.1038/s41397-019-0102-4

Lu, X.-Y., Lu, Y., Zhao, Y.-J., Jaeweon, K., Kang, J., Xiao-Nan, L., et al. (2008). Cell cycle regulator gene CDC5L, a potential target for 6p12-p21 amplicon in osteosarcoma. *Mol. Cancer Res.* 6, 937–946. doi:10.1158/1541-7786.MCR-07-2115

Marcellus, K. A., Crawford Parks, T. E., Almasi, S., and Jasmin, B. J. (2021). Distinct roles for the RNA-binding protein Staufen1 in prostate cancer. *BMC Cancer* 21, 120. doi:10.1186/S12885-021-07844-2

Marisa, L., de Reyniès, A., Duval, A., Selves, J., Gaub, M. P., Vescovo, L., et al. (2013). Gene expression classification of colon cancer into molecular subtypes: Characterization, validation, and prognostic value. *PLOS Med.* 10, e1001453. doi:10.1371/JOURNAL.PMED.1001453

Mauri, G., Bonazzina, E., Amatu, A., Tosi, F., Bencardino, K., Gori, V., et al. (2021). The evolutionary landscape of treatment for $BRAF^{V600E}$ mutant metastatic colorectal cancer. *Cancers (Basel)* 13, 137–215. doi:10.3390/CANCERS13010137

McFarland, J. M., Ho, Z. V., Kugener, G., Dempster, J. M., Montgomery, P. G., Bryan, J. G., et al. (2018). Improved estimation of cancer dependencies from large-scale RNAi screens using model-based normalization and data integration. *Nat. Commun.* 9, 4610. doi:10.1038/s41467-018-06916-5

Mestre-Farràs, N., Guerrero, S., Bley, N., Rivero, E., Coll, O., Borràs, E., et al. (2022). Melanoma RBPome identification reveals PDIA6 as an unconventional RNA-binding protein involved in metastasis. *Nucleic Acids Res.* 50, 8207–8225. doi:10.1093/NAR/GKAC605

Meyers, R. M., Bryan, J. G., McFarland, J. M., Weir, B. A., Sizemore, A. E., Xu, H., et al. (2017). Computational correction of copy number effect improves specificity of CRISPR–Cas9 essentiality screens in cancer cells. *Nat. Genet.* 49, 1779–1784. doi:10.1038/ng.3984

Mu, W., Xie, Y., Li, J., Yan, R., Zhang, J., Liu, Y., et al. (2022). High expression of PDZ-binding kinase is correlated with poor prognosis and immune infiltrates in hepatocellular carcinoma. *World J. Surg. Oncol.* 20, 22. doi:10.1186/S12957-021-02479-W

Noda, D., Itoh, S., Watanabe, Y., Inamitsu, M., Dennler, S., Itoh, F., et al. (2006). ELAC2, a putative prostate cancer susceptibility gene product, potentiates TGF-beta/Smad-induced growth arrest of prostate cells. *Oncogene* 25, 5591–5600. doi:10.1038/SJ.ONC.1209571

Paschke, S., Jafarov, S., Staib, L., Kreuser, E. D., Maulbecker-Armstrong, C., Roitman, M., et al. (2018). Are colon and rectal cancer two different tumor entities? A proposal to abandon the term colorectal cancer. *Int. J. Mol. Sci.* 19, 2577. doi:10.3390/IJMS19092577

Pimiento, J. M., Neill, K. G., Henderson-Jackson, E., Eschrich, S. A., Chen, D. T., Husain, K., et al. (2016). Knockdown of CSE1L gene in colorectal cancer reduces tumorigenesis *in vitro*. *Am. J. Pathol.* 186, 2761–2768. doi:10.1016/J.AJPATH.2016.06.016

Pontén, F., Jirström, K., and Uhlen, M. (2008). The human protein atlas—A tool for pathology. *J. Pathol.* 216, 387–393. doi:10.1002/path.2440

Qu, Y., Gao, N., and Wu, T. (2021). Expression and clinical significance of SYNE1 and MAGI2 gene promoter methylation in gastric cancer. *Med. Baltim.* 100, e23788. doi:10.1097/MD.0000000000023788

Repana, D., Nulsen, J., Dressler, L., Bortolomeazzi, M., Kuppili Venkata, S., Tourna, A., et al. (2019). The network of cancer genes (NCG): A comprehensive catalogue of known and candidate cancer genes from cancer sequencing screens. *Genome Biol.* 20, 1–12. doi:10.1186/s13059-018-1612-0

Roepman, P., Schlicker, A., Tabernero, J., Majewski, I., Tian, S., Moreno, V., et al. (2014). Colorectal cancer intrinsic subtypes predict chemotherapy benefit, deficient mismatch repair and epithelial-to-mesenchymal transition. *Int. J. Cancer* 134, 552–562. doi:10.1002/IJC.28387

Sadanandam, A., Lyssiotis, C. A., Homicsko, K., Collisson, E. A., Gibb, W. J., Wullschleger, S., et al. (2013). A colorectal cancer classification system that associates cellular phenotype and responses to therapy. *Nat. Med.* 195 19, 619–625. doi:10.1038/nm.3175

Sameer, A. S., Qadri, Q., and Siddiqi, M. A. (2012). GSTP1 I105V polymorphism and susceptibility to colorectal cancer in Kashmiri population. *DNA Cell Biol.* 31, 74–79. doi:10.1089/DNA.2011.1297

Schlicker, A., Beran, G., Chresta, C. M., McWalter, G., Pritchard, A., Weston, S., et al. (2012). Subtypes of primary colorectal tumors correlate with response to targeted treatment in colorectal cell lines. *BMC Med. Genomics* 5, 66. doi:10.1186/1755-8794-5-66

Schmidlin, T., Garrigues, L., Lane, C. S., Mulder, T. C., van Doorn, S., Post, H., et al. (2016). Assessment of SRM, MRM(3), and DIA for the targeted analysis of phosphorylation dynamics in non-small cell lung cancer. *Proteomics* 16, 2193–2205. doi:10.1002/PMIC.201500453

Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., Ramage, D., et al. (2003). Cytoscape: A software environment for integrated models of biomolecular interaction networks. *Genome Res.* 13, 2498–2504. doi:10.1101/gr.1239303

Sillars-Hardebol, A. H., Carvalho, B., Belién, J. A. M., De Wit, M., Delis-Van Diemen, P. M., Tijssen, M., et al. (2012). CSE1L, DIDO1 and RBM39 in colorectal adenoma to carcinoma progression. *Cell. Oncol. (Dordr).* 35, 293–300. doi:10.1007/S13402-012-0088-2

Sun, K., Wang, S., He, J., Xie, Y., He, Y., Wang, Z., et al. (2017). NCOA5 promotes proliferation, migration and invasion of colorectal cancer cells via activation of PI3K/AKT pathway. *Oncotarget* 8, 107932–107946. doi:10.18632/ONCOTARGET.22429

Szklarczyk, D., Franceschini, A., Wyder, S., Forslund, K., Heller, D., Huerta-Cepas, J., et al. (2015). STRING v10: Protein-protein interaction networks, integrated over the tree of life. *Nucleic Acids Res.* 43, D447–D452. doi:10.1093/nar/gku1003

Szklarczyk, D., Gable, A. L., Lyon, D., Junge, A., Wyder, S., Huerta-Cepas, J., et al. (2019). STRING v11: Protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res.* 47, D607–D613. doi:10.1093/nar/gky1131

Ta, H. D. K., Wang, W. J., Phan, N. N., Ton, N. T. A., Anuraga, G., Ku, S. C., et al. (2021). Potential therapeutic and prognostic values of LSM family genes in breast cancer. *Cancers (Basel)* 13, 4902. doi:10.3390/CANCERS13194902

Tai, C. J., Su, T. C., Jiang, M. C., Chen, H. C., Shen, S. C., Lee, W. R., et al. (2013). Correlations between cytoplasmic CSE1L in neoplastic colorectal glands and depth of tumor penetration and cancer stage. *J. Transl. Med.* 11, 29. doi:10.1186/1479-5876-11-29

Tan, Y., Liu, F., and Xu, P. (2021). Knockdown of NCOA5 suppresses viability, migration and epithelial-mesenchymal transition, and induces adhesion of breast cancer cells. *Oncol. Lett.* 22, 694. doi:10.3892/OL.2021.12955

Tanaka, K., Ikeda, N., Miyashita, K., Nuriya, H., and Hara, T. (2018). DEAD box protein DDX1 promotes colorectal tumorigenesis through transcriptional activation of the LGR5 gene. *Cancer Sci.* 109, 2479–2489. doi:10.1111/CAS.13661

Tang, J., Chen, H., Wong, C. C., Liu, D., Li, T., Wang, X., et al. (2018). DEAD-box helicase 27 promotes colorectal cancer growth and metastasis and predicts poor survival in CRC patients. *Oncogene* 37, 3006–3021. doi:10.1038/S41388-018-0196-1

Thul, P. J., Åkesson, L., Wiking, M., Mahdessian, D., Geladaki, A., Ait Blal, H., et al. (2017). A subcellular map of the human proteome. *Science* 356, eaal3321. doi:10.1126/science.aal3321

Tian, Q. H., Zhang, M. F., Luo, R. G., Fu, J., He, C., Hu, G., et al. (2016). DHX33 expression is increased in hepatocellular carcinoma and indicates poor prognosis. *Biochem. Biophys. Res. Commun.* 473, 1163–1169. doi:10.1016/J.BBRC.2016.04.033

Tomczak, K., Czerwińska, P., and Wiznerowicz, M. (2015). The cancer genome atlas (TCGA): An immeasurable source of knowledge. *Contemp. Oncol. Pozn.* 19, 68–77. doi:10.5114/wo.2014.47136

Tsherniak, A., Vazquez, F., Montgomery, P. G., Weir, B. A., Kryukov, G., Cowley, G. S., et al. (2017). Defining a cancer dependency map. *Cell* 170, 564–576.e16. doi:10.1016/j.cell.2017.06.010

Uhlén, M., Fagerberg, L., Hallström, B. M., Lindskog, C., Oksvold, P., Mardinoglu, A., et al. (2015). Proteomics. Tissue-based map of the human proteome. *Science* 347, 1260419. doi:10.1126/science.1260419

Uhlen, M., Zhang, C., Lee, S., Sjöstedt, E., Fagerberg, L., Bidkhori, G., et al. (2017). A pathology atlas of the human cancer transcriptome. *Science* 357, eaan2507. doi:10.1126/science.aan2507

Vasaikar, S., Huang, C., Wang, X., Petyuk, V. A., Savage, S. R., Wen, B., et al. (2019). Proteogenomic analysis of human colon cancer reveals new therapeutic opportunities. *Cell* 177, 1035–1049.e19. doi:10.1016/J.CELL.2019.03.030

Wen, N., Bian, L., Gong, J., and Meng, Y. (2020). Overexpression of cell-cycle related and expression-elevated protein in tumor (CREPT) in malignant cervical cancer. *J. Int. Med. Res.* 48, 300060519895089. doi:10.1177/0300060519895089

Wielenga, V. J. M., Van der Neut, R., Offerhaus, G. J. A., and Pals, S. T. (2000). CD44 glycoproteins in colorectal cancer: Expression, function, and prognostic value. *Adv. Cancer Res.* 77, 169–187. doi:10.1016/S0065-230X(08)60787-3

Wurth, L., and Gebauer, F. (2015). RNA-binding proteins, multifaceted translational regulators in cancer. *Biochim. Biophys. Acta - Gene Regul. Mech.* 1849, 881–886. doi:10.1016/j.bbagrm.2014.10.001

Wurth, L., Papasaikas, P., Olmeda, D., Bley, N., Calvo, G. T., Guerrero, S., et al. (2016). UNR/CSDE1 drives a post-transcriptional program to promote melanoma invasion and metastasis. *Cancer Cell* 30, 694–707. doi:10.1016/j.ccell.2016.10.004

Xi, Y., and Xu, P. (2021). Global colorectal cancer burden in 2020 and projections to 2040. *Transl. Oncol.* 14, 101174. doi:10.1016/J.TRANON.2021.101174

Xing, Q., Liu, S., Luan, J., Wang, Y., and Ma, L. (2021). A novel 13 RNA binding proteins (RBPs) signature could predict prostate cancer biochemical recurrence. *Pathol. - Res. Pract.* 225, 153587. doi:10.1016/J.PRP.2021.153587

Xu, B., Yang, N., Liu, Y., Kong, P., Han, M., and Li, B. (2020). Circ_cse1l inhibits colorectal cancer proliferation by binding to eIF4A3. *Med. Sci. Monit.* 26, e923876. doi:10.12659/MSM.923876

Xu, H., Wong, C. C., Li, W., Zhou, Y., Li, Y., Wang, L., et al. (2021). RING-finger protein 6 promotes colorectal tumorigenesis by transcriptionally activating SF3B2. *Oncogene* 40, 6513–6526. doi:10.1038/S41388-021-01872-9

Xu, Y., Nijhuis, A., and Keun, H. C. (2022). RNA-binding motif protein 39 (RBM39): An emerging cancer target. *Br. J. Pharmacol.* 179, 2795–2812. doi:10.1111/BPH.15331

Yan, G., An, Y., Xu, B., Wang, N., Sun, X., and Sun, M. (2021). Potential impact of ALKBH5 and YTHDF1 on tumor immunity in colon adenocarcinoma. *Front. Oncol.* 11, 1. doi:10.3389/fonc.2021.670490

Yang, J. W., Yuan, L. L., Gao, Y., Liu, X. S., Wang, Y. J., Zhou, L. M., et al. (2021). 18 F-FDG PET/CT metabolic parameters correlate with EIF2S2 expression status in colorectal cancer. *J. Cancer* 12, 5838–5847. doi:10.7150/JCA.57926

Yang, Y. F., Lee, Y. C., Wang, Y. Y., Wang, C. H., Hou, M. F., and Yuan, S. S. F. (2019). YWHAE promotes proliferation, metastasis, and chemoresistance in breast cancer cells. *Kaohsiung J. Med. Sci.* 35, 408–416. doi:10.1002/KJM2.12075

Yin, Z., Yan, X., Wang, Q., Deng, Z., Tang, K., Cao, Z., et al. (2020). Detecting prognosis risk biomarkers for colon cancer through multi-omics-based prognostic analysis and target regulation simulation modeling. *Front. Genet.* 11, 524. doi:10.3389/fgene.2020.00524

Yu, C., Mannan, A. M., Yvone, G. M., Ross, K. N., Zhang, Y.-L., Marton, M. A., et al. (2016). High-throughput identification of genotype-specific cancer vulnerabilities in mixtures of barcoded tumor cell lines. *Nat. Biotechnol.* 34, 419–423. doi:10.1038/nbt.3460

Yu, W., Ling, J., Yu, H., Du, J., and Liu, T. (2020). AZGP1 suppresses the process of colorectal cancer after upregulating FASN expression via mTOR signal pathway. *Gen. Physiol. Biophys.* 39, 239–248. doi:10.4149/GPB_2019061

Zhang, B., Babu, K. R., Lim, C. Y., Kwok, Z. H., Li, J., Zhou, S., et al. (2020). A comprehensive expression landscape of RNA-binding proteins (RBPs) across 16 human cancer types. *RNA Biol.* 17, 211–226. doi:10.1080/15476286.2019.1673657

Zhang, H., Hou, W., Wang, H. L., Liu, H. J., Jia, X. Y., Zheng, X. Z., et al. (2014). GSK-3β-regulated N-acetyltransferase 10 is involved in colorectal cancer invasion. *Clin. Cancer Res.* 20, 4717–4729. doi:10.1158/1078-0432.CCR-13-3477

Zhou, Y., Cheng, X., Zhang, F., Chen, Q., Chen, X., Shen, Y., et al. (2020). Integrated multi-omics data analyses for exploring the co-occurring and mutually exclusive gene alteration events in colorectal cancer. *Hum. Mutat.* 41, 1588–1599. doi:10.1002/HUMU.24059

Zhu, Y., Du, Y., and Zhang, Y. (2020). DHX33 promotes colon cancer development downstream of Wnt signaling. *Gene* 735, 144402. doi:10.1016/J.GENE.2020.144402

4 Global Discussion

Cancer is a collection of diseases characterized by the uncontrolled proliferation and division of abnormal cells, which can be life-threatening due to their ability to invade and spread to other parts of the body [1,2]. BC and COREAD are notable examples of cancer types [3,4]. BC is the leading cause of cancer-related deaths and the most frequently diagnosed cancer among women worldwide [5], while COREAD ranks as the third most prevalent malignant tumor and the second deadliest cancer [6,7]. Extensive efforts have been made to unravel the underlying molecular mechanisms of carcinogenesis [2,4,8]. However, these malignancies lack distinct biomarkers that can reliably indicate treatment response and disease progression, thereby presenting challenges in monitoring the status of the disease and assessing the effectiveness of treatments [9–11]. Additionally, Paschke *et al.* based on several molecular, pathological, and surgical differences suggested to treat COAD and READ separately [12]. This differentiation has the potential to enhance the discovery of novel biomarkers and therapeutic targets for both types of cancer.

The role of RNA biology in cancer has been relatively understudied[13,14]. However, RBPs are emerging as crucial regulators of various cancer hallmarks, exerting control over multiple aspects of RNA metabolism [15–17]. RBPs can influence the expression of oncogenes and tumor suppressors, impacting key aspects of cancer progression [17,18]. Despite their significance, only a small fraction of the identified human RBPs (1,393 in total) have been linked to BC, COAD, and READ [19]. Thus, there is a need to further explore the involvement of RBPs in these specific cancer types to understand their contributions to carcinogenesis and develop possible clinical or biotechnological applications.

There has been a significant increase in the amount of cancer omics data in recent years due to advancements in high-throughput technologies. This rapid growth of data has introduced the big data in cancer research, which requires extensive computational resources for analysis and has the potential to provide new perspectives on important inquiries. The integration of big data, bioinformatics, and artificial intelligence has already resulted in remarkable progress in our fundamental comprehension of cancer biology and practical applications. A notable illustration of such extensive data is the dataset amassed by The Cancer Genome Atlas (TCGA) [20]. TCGA encompasses a staggering 2.5 petabytes of unprocessed data. Moreover, from its initial launch in 2008 until March 2022, a PubMed search revealed that TCGA was referenced in no fewer than 10,242 articles [20]. This serves as a testament to its significant impact as a communal asset, effectively propelling advancements in cancer research. Therefore, we have used different omics data types from large-scale cancer projects and processed them using several bioinformatic tools (e.g., cBioportal [21,22] and DepMap [23]) to unravel putative biomarkers in BC, COAD and READ.

First, to determine how many RBPs have been previously studied in BC and COREAD, we analyzed the most recent catalog of CDG, NCG6 [24] and performed a comprehensive literature review [25], respectively. Only 14 RBPs were cataloged as BC driver genes and only 35 from 1,392 RBPs have undergone prior investigation in COREAD. The existing body of research suggests that RBPs have received limited attention in the context of breast and colorectal cancer development. This demonstrates a gap in our understanding of the role of these proteins in cancer progression and their potential clinical utility. It becomes apparent that there is a need to deeper

enquire into identifying novel RBPs that could serve as valuable biomarkers for diagnosis, prognosis, and treatment strategies in these types of cancer.

Regarding BC, we prioritized five potential RBPs (PUF60, TFRC, KPNB1, NSF, and SF3A3) using a complex integration strategy of several omics' resources (Figure 1). As expected, some of these proteins have been previously implicated in BC (TFRC and KPNB1) [26–29], while PUF60 has been associated with colon and non-small cell lung cancer, but not with BC [30,31]. Interestingly, NSF and SF3A3 have never been studied in cancer and yet they possess cancer-related characteristics according to our analyses. Then, these proteins were integrated into a disease gene network to gain insights into their molecular and cellular functions within cancer. As a result, we obtained a highly intricate network between RBPS and cancer related genes. Previous studies by Quattrone and Dassi have already established that the RBP network exhibits a hierarchical structure, characterized by clusters and chains that collaborate and compete on shared target mRNAs to regulate various cellular processes, such as splicing [32]. This hierarchical structure is also evident in our densely interconnected network, where PUF60 and SF3A3 serve as central elements within a cluster related to the spliceosome, involving both RNA-binding proteins and cancer proteins.

In the context of COAD and READ, our data mining strategy successfully identified four proteins in COAD (NAT10, NOP56, RBM12, and FKBP1A) and two in READ (CSE1L and EMG1). Notably, NAT10 and CSE1L have been previously implicated in COREAD, validating our findings [33–40]. On the other hand, NOP56 and RBM12 were identified in COREAD through different our data mining approaches [41], further supporting the credibility of our results. Importantly, FKBP1A and EMG1 have not been associated with cancer in prior studies, underscoring their novelty in this context. To emphasize their significance in cancer, we observed that NOP56, RBM12, FKBP1A, and EMG1 exhibit strong connections with cancer genes and other RBPs, indicating their potential to form tumorigenic RNA-regulons. Additionally, we explored the clinical implications of these prioritized RBPs. Elevated mRNA expression of FKBP1A, NAT10, and NOP56 was found to be predictive of clinical outcomes in COREAD and COAD patients. Similarly, increased expression of NAT10 and NOP56 mRNA was associated with poor outcomes depending on the staging of COAD. This is particularly relevant in clinical practice, as COAD therapy is determined by the stage of the disease [42,43]. While stage II COAD patients may not always require adjuvant therapy, stage III patients can benefit from adjuvant chemotherapy as it reduces the risk of relapse [42]. One critical aspect in daily clinical practice is the absence of specific biomarkers to predict relapse. Our results highlight the potential of NAT10 mRNA upregulation to indicate an adverse outcome in disease-free survival (DFS) among stage III COAD patients. Additionally, overexpression of NOP56 in primary tumors is linked to poor prognosis in both DFS and overall survival (OS) among stage IV COAD patients. These findings highlight the clinical relevance of FKBP1A, NAT10, and NOP56 and their possible biotechnological applications in personalized medicine.

Big data has become increasingly important in cancer research, revolutionizing the way scientists and clinicians understand and treat the disease. Big data analytics has enabled us to evaluate massive amounts of genomic and molecular data. *In toto* in these studies, we have analyzed and integrated data from 1,116 BC, 488 COAD, and 155 READ patients, 212 cancer cell lines, more than 44,501 immunostainings, and ~ 24,000 raw associations between RBPs and cancer genes to unravel new RBPs involved in BC (PUF60, TFRC, KPNB1, NSF, and SF3A3), COAD (NOP56,

NAT10, RBM12, and FKBP1A), and READ (EMG1 and CSE1L). Further analyses allowed us to identify potential clinical applications of FKBP1A, NAT10, and NOP56 as biomarkers of specific outcomes.

Discussion References

1. Hanahan, D. Hallmarks of Cancer: New Dimensions. *Cancer Discov.* **12**, 31–46 (2022).
2. Guerrero, S. *et al.* Analysis of Racial/Ethnic Representation in Select Basic and Applied Cancer Research Studies. *Sci. Rep.* **8**, 13978 (2018).
3. Sung, H. *et al.* Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA. Cancer J. Clin.* **71**, 209–249 (2021).
4. Harbeck, N. *et al.* Breast cancer. *Nat. Rev. Dis. Prim.* **5**, 66 (2019).
5. Globocan. All cancers. *World Health Organization. International Agency for Research on Cancer* https://gco.iarc.fr/today (2020).
6. Gao, C. *et al.* Downregulation of Msi1 suppresses the growth of human colon cancer by targeting p21cip1. *Int. J. Oncol.* **46**, 732–740 (2015).
7. Xi, Y. & Xu, P. Global colorectal cancer burden in 2020 and projections to 2040. *Transl. Oncol.* **14**, 101174 (2021).
8. Hiatt, R. A. & Brody, J. G. Environmental Determinants of Breast Cancer. *Annu. Rev. Public Health* **39**, 113–133 (2018).
9. Assis, J. V. de, Coutinho, L. A., Oyeyemi, I. T., Oyeyemi, O. T. & Grenfell, R. F. e Q. Diagnostic and therapeutic biomarkers in colorectal cancer: a review. *Am. J. Cancer Res.* **12**, 661 (2022).
10. Cohen, R., Pudlarz, T., Delattre, J. F., Colle, R. & André, T. Molecular Targets for the Treatment of Metastatic Colorectal Cancer. *Cancers (Basel).* **12**, 1–18 (2020).
11. Liu, Z. *et al.* Genomic Alteration Characterization in Colorectal Cancer Identifies a Prognostic and Metastasis Biomarker: FAM83A|IDO1. *Front. Oncol.* **11**, 607 (2021).
12. Paschke, S. *et al.* Are Colon and Rectal Cancer Two Different Tumor Entities? A Proposal to Abandon the Term Colorectal Cancer. *Int. J. Mol. Sci.* **19**, (2018).
13. Lukong, K. E., Chang, K.-W., Khandjian, E. W. & Phane Richard, S. RNA-binding proteins in human genetic disease. *Cell* **24**, 416–425 (2008).
14. Morris, A. R. *et al.* Alternative Cleavage and Polyadenylation during Colorectal Cancer Development. *Clin. Cancer Res.* **18**, 5256–5266 (2012).
15. Wurth, L. *et al.* UNR/CSDE1 Drives a Post-transcriptional Program to Promote Melanoma Invasion and Metastasis. *Cancer Cell* **30**, 694–707 (2016).
16. Martinez-Useros, J. *et al.* UNR/CSDE1 Expression Is Critical to Maintain Invasive Phenotype of Colorectal Cancer through Regulation of c-MYC and Epithelial-to-Mesenchymal Transition. *J. Clin. Med.* **8**, 560 (2019).
17. Abdel-Wahab, O. & Gebauer, F. Editorial overview: Cancer genomics: RNA metabolism and translation in cancer pathogenesis and therapy. *Curr. Opin. Genet. Dev.* **48**, iv–vi (2018).
18. Wurth, L. & Gebauer, F. RNA-binding proteins, multifaceted translational regulators in cancer. *Biochim. Biophys. Acta - Gene Regul. Mech.* **1849**, 881–886 (2015).
19. Hentze, M. W., Castello, A., Schwarzl, T. & Preiss, T. A brave new world of RNA-binding proteins. *Nat. Rev. Mol. Cell Biol.* **19**, 327–341 (2018).

20. Weinstein, J. N. *et al.* The cancer genome atlas pan-cancer analysis project. *Nat. Genet.* **45**, 1113–1120 (2013).
21. Cerami, E. *et al.* The cBio Cancer Genomics Portal: An open platform for exploring multidimensional cancer genomics data. *Cancer Discov.* **2**, 401–404 (2012).
22. Gao, J. *et al.* Integrative Analysis of Complex Cancer Genomics and Clinical Profiles Using the cBioPortal. *Sci. Signal.* **6**, pl1 (2013).
23. McFarland, J. M. *et al.* Improved estimation of cancer dependencies from large-scale RNAi screens using model-based normalization and data integration. *Nat. Commun.* **9**, 4610 (2018).
24. Repana, D. *et al.* The Network of Cancer Genes (NCG): a comprehensive catalogue of known and candidate cancer genes from cancer sequencing screens. *Genome Biol.* **20**, 1–12 (2019).
25. García-Cárdenas, J. M. *et al.* Post-transcriptional Regulation of Colorectal Cancer: A Focus on RNA-Binding Proteins. *Front. Mol. Biosci.* **6**, (2019).
26. Jian, J., Yang, Q. & Huang, X. Src Regulates Tyr ^{20} Phosphorylation of Transferrin Receptor-1 and Potentiates Breast Cancer Cell Survival. *J. Biol. Chem.* **286**, 35708–35715 (2011).
27. Singh, M. *et al.* Differential Expression of Transferrin Receptor (TfR) in a Spectrum of Normal to Malignant Breast Tissues. *Appl. Immunohistochem. Mol. Morphol.* **19**, 417–423 (2011).
28. Habashy, H. O. *et al.* Transferrin receptor (CD71) is a marker of poor prognosis in breast cancer and can predict response to tamoxifen. *Breast Cancer Res. Treat.* **119**, 283–293 (2010).
29. Sheng, C. *et al.* Suppression of Kpnβ1 expression inhibits human breast cancer cell proliferation by abrogating nuclear transport of Her2. *Oncol. Rep.* **39**, 554–564 (2017).
30. Kobayashi, S. *et al.* Anti-FIRs (PUF60) auto-antibodies are detected in the sera of early-stage colon cancer patients. *Oncotarget* **7**, 82493–82503 (2016).
31. Müller, B. *et al.* Concomitant expression of far upstream element (*FUSE*) binding protein (*FBP*) interacting repressor (FIR) and its splice variants induce migration and invasion of non-small cell lung cancer (NSCLC) cells. *J. Pathol.* **237**, 390–401 (2015).
32. Quattrone, A. & Dassi, E. The Architecture of the Human RNA-Binding Protein Regulatory Network. *iScience* **21**, 706–719 (2019).
33. Cao, Y. *et al.* N-Acetyltransferase 10 Promotes Micronuclei Formation to Activate the Senescence-Associated Secretory Phenotype Machinery in Colorectal Cancer Cells. *Transl. Oncol.* **13**, (2020).
34. Zhang, H. *et al.* GSK-3β-regulated N-acetyltransferase 10 is involved in colorectal cancer invasion. *Clin. Cancer Res.* **20**, 4717–4729 (2014).
35. Liu, Z. *et al.* miR-6716-5p promotes metastasis of colorectal cancer through downregulating NAT10 expression. *Cancer Manag. Res.* **11**, 5317–5332 (2019).
36. Liu, X. *et al.* NAT10 regulates p53 activation through acetylating p53 at K120 and ubiquitinating Mdm2. *EMBO Rep.* **17**, 349–366 (2016).
37. Sillars-Hardebol, A. H. *et al.* CSE1L, DIDO1 and RBM39 in colorectal adenoma to carcinoma progression. *Cell. Oncol. (Dordr).* **35**, 293–300 (2012).
38. Tai, C. J. *et al.* Correlations between cytoplasmic CSE1L in neoplastic colorectal glands and depth of tumor penetration and cancer stage. *J. Transl. Med.* **11**, (2013).
39. Pimiento, J. M. *et al.* Knockdown of CSE1L Gene in Colorectal Cancer Reduces

Tumorigenesis in Vitro. *Am. J. Pathol.* **186**, 2761–2768 (2016).
40. Xu, B. *et al.* Circ_cse1l Inhibits Colorectal Cancer Proliferation by Binding to eIF4A3. *Med. Sci. Monit.* **26**, (2020).
41. Liang, Q., Du, X., Mao, L. & Wang, G. Molecular characterization of colorectal cancer: A five-gene prognostic signature based on RNA-binding proteins. *Saudi J. Gastroenterol.* **27**, 223 (2021).
42. Argilés, G. *et al.* Localised colon cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann. Oncol. Off. J. Eur. Soc. Med. Oncol.* **31**, 1291–1305 (2020).
43. Cervantes, A. *et al.* Metastatic colorectal cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up†. *Ann. Oncol.* **34**, 10–32 (2022).

5 Conclusions

i. The detailed review of the available public data on RNA binding proteins (RBPs), their regulation by microRNAs, data form xenograft studies and the clinical implications of their alterations has allowed us to conclude that RPBs are crucial in carcinogenesis although only 35, out of 1,392, had been associated to colorectal cancer (COREAD) previously.

ii. This research has also reinforced the notion, supported by numerous studies, of the pivotal part RNA Binding Proteins (RBPs) play in regulating gene expression. In conclusion, we have enquired into the complex interconnections of RNA regulons and confirmed that RBPs establish interactions with other RBPs, cancer-driving genes, and with hundreds or thousands of RNAs (from 900 to 21,000). It is fascinating to note that these RNAs either encode proteins or engage in RNA-associated processes such as RNA binding or mRNA transport, further expanding the RNA regulon model. Consequently, any irregularities in RBPs could lead to significant disruptions in cellular balance and contribute to cancer progression.

iii. By integrating multi-omics approaches, advanced bioinformatics, and data mining techniques carried out in this thesis, significant insights have emerged concerning previously unassociated RBPs like PUF60, TFRC, KPNB1, NSF, and SF3A3 in BC, NOP56, NAT10, RBM12, and FKBP1A in COAD, and EMG1 and CSE1L in READ, revealing their involvement in tumorigenesis across diverse cancer types. Subsequent survival analyses highlighted the clinical relevance of FKBP1A, NOP56, and NAT10 mRNA expression to predict poor prognosis in COREAD and COAD patients. Despite these promising findings, a high number of patients are needed to validate these results in specifical clinical scenarios.

6 References

References are listed at the end of the introduction and discussion pages 3 and 64

7 Resumen extenso en español

El cáncer es un grupo de enfermedades complejas y devastadoras que se caracterizan por el crecimiento descontrolado de células, conduciendo a la formación de tumores y, potencialmente, a la metástasis [1,2]. El cáncer de mama (BC, por sus siglas en inglés) y el adenocarcinoma colorrectal (COREAD, por sus siglas en inglés) son dos ejemplos destacados, siendo el BC la principal causa de muerte asociada al cáncer y el carcinoma más comúnmente diagnosticado entre las mujeres en todo el mundo [3–5]. COREAD, por otro lado, se ubica como el tercer tumor maligno más común y el segundo cáncer más letal, afectando a millones de personas en todo el mundo [6,7]. La complejidad de esta enfermedad surge de la interacción de factores ambientales, rasgos biológicos y desregulación genética [2,4,8]. Se han logrado avances en tratamientos personalizados y subtipificación molecular, pero la heterogeneidad molecular de estos tumores impide abordar totalmente la complejidad del cáncer de mama, colon y recto [9–11].

La biología del ARN representa un aspecto poco investigado del cáncer [12,13]. Las proteínas de unión al ARN (RBPs, por sus siglas en inglés) están emergiendo como moduladores críticos de todas las características del cáncer, desempeñando un papel importante en los regulones del ARN postranscripcionales y controlando todos los aspectos del metabolismo del ARN [14–16]. Las RBPs pueden modular los niveles de expresión de oncogenes y genes supresores de tumores, influyendo en varios aspectos de la progresión del cáncer, como la angiogénesis, la metástasis o la resistencia a la quimioterapia [16,17]. A pesar de la importancia de las RBPs en el cáncer, solo una pequeña fracción de las 1.393 RBPs humanas identificadas se han implicado en el proceso carcinogénico, y mucho menos en BC y COREAD [18]. Además, dadas las diferencias entre el cáncer de colon (COAD, por sus siglas en inglés) y el cáncer de recto (READ, por sus siglas en inglés) con respecto a su carcinogénesis molecular, la patología, la topografía, los procedimientos quirúrgicos y el tratamiento multimodal, Paschke *et al*. han sugerido separar estas dos identidades tumorales para mejorar la identificación de nuevos biomarcadores y dianas terapéuticas para ambos tipos de cáncer. Una mejor comprensión de las funciones de las RBPs en COAD y READ puede ayudar a encontrar dianas terapéuticas más precisas y sensibles para estos cánceres [19].

Los esfuerzos por comprender mejor el papel de las RBPs en el cáncer han llevado al análisis e integración de conjuntos de datos a gran escala, como el Atlas del Genoma del Cáncer (TCGA) [20–25], el Mapa de Dependencia del Cáncer (DepMap) [26–28] y el Atlas de Proteínas Humanas (HPA) [29–31], los cuales han proporcionado información valiosa sobre los procesos moleculares del cáncer y han redefinido el desarrollo, diagnóstico y tratamiento de fármacos contra el cáncer. Sin embargo, aún quedan por descubrir características fundamentales adicionales de la oncogénesis, el crecimiento tumoral y la diseminación, especialmente en el campo de la regulación post-transcripcional de la tumorigénesis [16]. El estudio de las RBPs y su papel en cánceres como el de mama, colon y recto ayudarán a mejorar la comprensión de la biología tumoral e identificar nuevas dianas terapéuticas y biomarcadores pronósticos [3,4,6,7]. Mediante la integración de conjuntos de datos a gran escala los investigadores pueden explorar las complejas interacciones entre las RBPs y varios aspectos del desarrollo y progresión del cáncer [18,32,33]. Además, la separación del cáncer de colon y el cáncer de recto en identidades tumorales distintas podría mejorar aún más la especificidad y eficacia del tratamiento individualizado [19].

A medida que el campo de la biología del ARN y el estudio de las RBPs continúan evolucionando [18,34–38], es crucial mantener un enfoque interdisciplinario que combine métodos computacionales de vanguardia, técnicas experimentales y datos clínicos para poder descubrir nuevas RBPs implicadas en la progresión del cáncer de mama, colon y recto. Específicamente, se examinó exhaustivamente la participación de las RBPs en el cáncer colorrectal, incluida su regulación por microARNs, estudios de xenoinjertos y sus posibles implicaciones clínicas. Así como también, se investigó las características comunes de las RBPs en el cáncer colorrectal, centrándonos concretamente en sus dominios proteicos, interacciones proteína-proteína (PPI, por sus siglas en inglés), dianas de ARN y capacidades oncogénicas. Finalmente, se pretendió descubrir RBPs *in-silico* que pueden utilizarse como biomarcadores de pronóstico y diagnóstico de cáncer de mama, colon y recto y, posiblemente, como nuevas dianas terapéuticas.

De esta manera, para poder cumplir con estos objetivos, esta tesis se enfocó en conocer el estado del arte de las RBPs que habían sido ya relacionadas con el cáncer colorrectal. Para este fin se hizo una revisión bibliográfica en la que encontramos que solo 35 RBPs de las 1.392 han sido estudiadas y relacionadas con COREAD [32]. También nos dirigimos a recabar información sobre ciertas características comunes como son su regulación por microARNs, estudios de xenotrasplantes e implicaciones clínicas potenciales. En García-Cárdenas *et al.* se puede apreciar que de las 35 RBPs que habían sido estudiadas, muy pocas estaban totalmente caracterizadas. Además, para vislumbrar mejor las posibles relaciones funcionales entre las 35 RBPs que se han asociado con COREAD, se indagó en las PPI[32]. Nuestro análisis reveló una red de interacción compleja que involucraba a 17 de las RBPs. Estos hallazgos sugieren que las RBPs asociadas a COREAD poseen diversos dominios funcionales y participan en una red compleja de interacciones, lo que puede influir en el desarrollo y la progresión de COREAD. Finalmente, para comprender mejor el potencial oncogénico de las RBPs antes mencionadas, el enfoque se dirigió hacia la determinación de sus características oncogénicas. De esta manera se pudo determinar que la mayoría de las RBPs presentan propiedades que promueven la progresión tumoral, mientras que solo un pequeño subconjunto de las 35 RBPs identificadas poseen capacidades supresoras de tumores. Es así como se concluyó que a través de complejas redes conocidas como regulones de ARN, las RBPs desempeñan un papel crucial en la regulación de la expresión génica. Cualquier alteración en las RBPs tiene el potencial de interrumpir gravemente la homeostasis celular [1,16,32].

La investigación oncológica actual genera una gran cantidad de datos [20,21,30,31,22–29] que contienen características estratégicas no descubiertas de los mecanismos moleculares que subyacen al crecimiento tumoral y la metástasis, sin embargo, estas bases de datos no se explotan por completo. Es así como se propuso estudiar en esta tesis todas las 1.393 RBPs tanto en BC como en COAD y READ a través de la minería de datos para poder priorizar e identificar posibles RBPs relacionadas con la progresión tumoral de estas enfermedades.

Para determinar cuántas RBPs se han estudiado previamente en BC, se analizó el catálogo más reciente de los genes conductores del cáncer (CDG, por sus siglas en inglés) en el Network Cancer Gene 6 [39]. Solo 14 RBPs se han catalogado como CDG de BC. Esto indica que las RBPs se han investigado poco en la carcinogénesis de mama al igual que en COREAD. Por lo tanto, para identificar nuevas RBPs de BC putativas, primero exploramos su perfil de alteraciones genómica asociadas con la progresión o supresión de la tumorigénesis. En este sentido se identificó que las RBPs están igualmente alteradas como los CDG de BC; esto era de esperar, dado que muchas RBPs están bastante alteradas en todos los tipos de cáncer y se han relacionado *in-silico* con

procesos celulares relacionados con el cáncer. Se encontró que la mayoría de las alteraciones genómicas de las RBPs en BC son la sobreexpresión del ARNm (68,7%) y las amplificaciones. Esto probablemente aumentará las concentraciones celulares de las RBPs, lo que conducirá a procesos postranscripcionales disfuncionales. En el mismo sentido, cuando analizamos los perfiles de alteración transcriptómica y genómica de las RBPs en pacientes con COAD y READ, encontramos que la mayor parte de la alteración estaba en los niveles de ARNm: sobreexpresión del ARNm (64 % en colon y 62 % en el recto), seguida de la baja expresión del ARNm (15 % en colon y 20% en recto).

En segundo lugar, para caracterizar aún más las RBPs asociadas con los subtipos y la estadificación de BC, se analizaron las alteraciones genómicas de las RBPs. Curiosamente, las alteraciones genómicas de las RBPs aumentaron gradualmente del subtipo Normal al Basal, es decir, de una etapa proliferativa baja hacia una etapa proliferativa alta. En concordancia, en estadio IV mostraron altas frecuencias de alteraciones genómicas en comparación con los estadios I a III. Por lo tanto, parece que las RBPs actúan promoviendo la progresión de BC en lugar de suprimirlo, lo que concuerda con sus perfiles de alteración genómica. En cuanto al mismo análisis realizado con pacientes COAD y READ, a pesar de las similitudes en las alteraciones genómicas y transcriptómicas entre los subtipos de ambos carcinomas, la mayoría de las RBPs alteradas en cada subtipo diferían. Al menos en READ, la mayoría de las RBPs altamente alteradas son exclusivas para este tipo de cáncer. Estos hallazgos respaldan la sugerencia de Paschke *et al.*, y además se evidencia que varias RBPs y tal vez no solo las RBPs podrían haber sido ignoradas cuando las estudiamos en conjunto en cáncer de colon y recto, pero cuando se separan en COAD y READ, se pueden descubrir nuevos biomarcadores putativos [19].

Las redes de interacción son útiles para identificar proteínas tumorales cruciales [14,40,41]. En este sentido, mediante el análisis de PPI entre RBPs y proteínas conductoras del BC, identificamos a SF3B1 y CDC5L en el núcleo de dos redes principales. Si bien SF3B1 se ha implicado previamente en BC [42], CDC5L, no se ha estudiado en esta malignidad. Sin embargo, CDC5L se ha relacionado con otros tipos de cáncer, como el osteosarcoma [43]. y el cáncer de próstata [44]. Del mismo modo, las PPIs entre RBPs y COREAD nos permitieron determinar dos módulos funcionales, como NAT10 y TCERG1, que eran elementos centrales. Estas RBPs también han sido catalogadas como conductoras de COREAD [45].

A continuación, exploramos la base de datos del Human Protein Atlas [29–31] para identificar RBPs expresadas diferencialmente en tejidos mamarios tumorales así como también en COAD y en READ. Encontramos varias RBPs sobreexpresadas y otras bajamente expresadas en comparación con los tejidos normales. Como era de esperar, nuestros análisis revelaron RBPs que ya estaban relacionadas con BC, COAD y READ y otras que no. También descubrimos RBPs que se sobreexpresan solo en COAD, mientras que otras están sobreexpresadas exclusivamente en READ.

Para identificar las RBPs esenciales para la supervivencia del tumor en BC, se analizaron las pruebas de pérdida de función *ex vivo*, CERES y DEMETER2 [26–28]. En total, se identificaron 207 RBPs esenciales para la supervivencia del tumor. Esto era de esperar, dado que las RBPs controlan todos los rasgos del metabolismo del ARN. Sin embargo, solo 59 se caracterizaron como esenciales por ambos métodos computacionales. Aunque CERES y DEMETER2 [26–28] aún no han validado todas las RBPs, la futura investigación terapéutica postranscripcional de BC podría

centrarse en estas 59 RBPs. También se encontró RBPs esenciales por subtipo molecular en BC que podrían analizarse para comprender mejor los procesos postranscripcionales relacionados con cada subtipo. Aplicando el mismo análisis a COAD y READ mediante dos pruebas de pérdida de función a gran escala (CERES y DEMETER [26–28]), se identificaron en COAD 352 RBPs esenciales, mientras que en READ identificamos 343 RBPs. En otras palabras, el 25% de las RBPs son esenciales para la supervivencia de las células oncogénicas. Hicimos una lista de las 5 principales RBPs esenciales en ambos tipos de cáncer según el DepScore. Se repitió el mismo escenario de los análisis anteriores, obtuvimos RBPs que se han relacionado con COREAD y otras que no.

Al ampliar el alcance de nuestros análisis anteriores, finalmente consideramos la integración de todas las bases de datos examinadas para definir mejor las posibles RBPs implicadas en BC. Como se discutió antes, las RBPs parecen actuar como promotores del cáncer en lugar de supresores. Por lo tanto, nos enfocamos en las RBPs que tienen perfiles putativos de progresión tumoral y distinguimos 19 RBPs con características tumorigénicas. Como era de esperar, la mayoría de ellas (13 de 19) se han descrito como promotores de tumores en BC, dado que controlan diferentes procesos celulares como la migración, la invasión y la metástasis [46]. Curiosamente, NSF, SF3A3, PRPF3 y MAGOHB nunca se han estudiado en el cáncer. Mientras que, por otro lado, PUF60 se ha asociado con el cáncer de pulmón de células no pequeñas y de colon [47,48] y se ha demostrado que PLEC promueve la migración y la invasión del carcinoma de células escamosas del cuello [49].

Como resultados de nuestros análisis priorizamos 5 RPBs putativamente asociadas a BC (PUF60, TFRC, KPNB1, NSF y SF3A3) que se integraron en una red de genes asociados a enfermedades para entender sus funciones moleculares y celulares en el cáncer. Así, obtuvimos una red muy intrincada de 2.231 interacciones, que enfatizaron la red robusta y compleja formada entre RBP - RBP, RBP - CDG y CDG - CDG. Además de esta complejidad, algunas de estas RBPs también son CDG. Quattrone y Dassi ya establecieron que la red de RBPs es una estructura jerárquica que está formada por grupos y cadenas, que cooperan y compiten por ARNm comunes que controlan diferentes procesos celulares (por ejemplo, el proceso de empalme durante la maduración de RNAm) [50]. Esto también se observó en nuestra red densamente interconectada donde PUF60 y SF3A3 son elementos centrales de un grupo relacionado con el espliceosoma que involucra RBPs y CDGs.

Aplicamos el mismo criterio de integración a los datos obtenidos para COAD y READ. Nuestra estrategia de minería de datos nos permitió identificar cuatro proteínas en COAD (NAT10, NOP56, RBM12 y FKBP1A) y dos en READ (CSE1L y EMG1). NAT10, CSE1L, NOP56 y RBM12 ya han sido involucradas en COREAD COREAD [45,51–58], validando nuestros resultados. Además, hasta donde sabemos, ningún estudio previo ha asociado FKBP1A y EMG1 con cáncer. Para resaltar su relevancia en el cáncer, interrogamos a HumanNet [38] y descubrimos que NOP56, RBM12, FKBP1A y EMG1 están altamente interconectados con los genes del cáncer y otras RBPs, lo que demuestra su potencial para formar regulones de ARN tumorigénicos.

Finalmente, se exploró las implicaciones clínicas de estas RBPs priorizadas. La sobreexpresión del ARNm de FKBP1A, NAT10 y NOP56 puede predecir los resultados clínicos en pacientes con COREAD y COAD. De manera similar, la sobreexpresión del ARNm de NAT10 y NOP56 está relacionada con un mal pronóstico según el estadio de COAD. Esto es clínicamente

relevante ya que la terapia de COAD se define por etapa. Los pacientes con COAD en estadio II no siempre son candidatos para la terapia adyuvante. Sin embargo, la quimioterapia adyuvante es un tratamiento relevante para los pacientes con COAD en estadio III porque disminuye el riesgo de recaída. En la práctica clínica diaria no existen biomarcadores específicos para predecir una recaída. Según nuestros resultados, la sobreexpresión del ARNm de NAT10 está relacionada con un resultado adverso en la supervivencia libre de la enfermedad (DFS, siglas en inglés) de los pacientes con COAD en estadio III. De manera similar, la sobreexpresión de NOP56 en tumores primarios se asocia con un mal pronóstico en DFS y la supervivencia promedio de pacientes con COAD en estadio IV. Estos resultados destacan la relevancia clínica de FKBP1A, NAT10 y NOP56.

En resumen, el BC y COREAD representan grandes retos de salud a nivel mundial. A pesar de los avances significativos en la comprensión de sus variedades moleculares y aspectos genéticos, encontrar una cura continúa siendo un desafío. Se ha incrementado el interés en el papel que desempeñan las RBPs en el desarrollo y avance de estos tipos de cáncer. Las RBPs, que son reguladoras esenciales de todas las características del cáncer, podrían actuar como biomarcadores sensibles para el diagnóstico, pronóstico y utilizarse como posibles dianas terapéuticas. En el caso del COREAD, la integración de diferentes tipos de datos ha permitido identificar 6 RBPs en la progresión del COAD y READ. En el BC, se han identificado de igual manera cinco RBPs que participan en la progresión tumoral. Dos de estas RBPs se identificaron como elementos clave en un grupo vinculado con espliceosoma que incluye RBPs y CDG. Las RBPs presentan un alto potencial como dianas para el diagnóstico, pronóstico y terapias clínicas en BC, COAD y READ. Es esencial continuar la investigación experimental sobre estas RBPs para descubrir sus mecanismos moleculares, confirmar su utilidad clínica y desarrollar nuevas estrategias de tratamiento.

Conclusiones

i. La revisión detallada de los datos públicos disponibles sobre las proteínas de unión al ARN (RBPs), su regulación por microARNs, datos de estudios con xenoinjertos y las implicaciones clínicas de sus alteraciones nos ha permitido concluir que las RBPs son cruciales en la carcinogénesis, aunque solo 35, de 1.392, se habían asociado previamente al cáncer colorrectal (COREAD) a pesar de que la mayoría de las RBPs poseen propiedades oncogénicas

ii. Esta investigación también ha reforzado la noción, respaldada por numerosos estudios, de la parte fundamental que juegan las RBPs en la regulación de la expresión génica. En conclusión, hemos indagado en las complejas interconexiones de los regulones de ARN y confirmado que las RBPs establecen interacciones con otras RBPs, con genes conductores de cáncer y con cientos o miles de ARNs (desde 900 hasta 21,000). Es fascinante notar que estos ARNs codifican proteínas o participan en procesos asociados al ARN, como la unión al ARN o el transporte de ARNm, ampliando aún más el modelo de regulón de ARN. Por consiguiente, cualquier irregularidad en las RBPs podría llevar a importantes alteraciones en el equilibrio celular y contribuir a la progresión del cáncer.

iii. Al integrar enfoques multiómicos, bioinformática avanzada y técnicas de minería de datos llevadas a cabo en esta tesis, han surgido perspectivas significativas respecto a las RBPs previamente no asociadas, como PUF60, TFRC, KPNB1, NSF y SF3A3 en BC, NOP56, NAT10, RBM12 y FKBP1A en COAD, y EMG1 y CSE1L en READ, revelando su participación en la tumorigénesis en diversos tipos de cáncer. Los análisis de supervivencia subsiguientes destacaron la relevancia clínica de la expresión del ARNm de FKBP1A, NOP56 y NAT10 para predecir un pronóstico desfavorable en pacientes con COREAD y COAD. A pesar de estos hallazgos prometedores, se necesita un gran número de pacientes para validar estos resultados en escenarios clínicos específicos.

Resumen extenso References

1. Hanahan, D. Hallmarks of Cancer: New Dimensions. *Cancer Discov.* **12**, 31–46 (2022).
2. Guerrero, S. *et al.* Analysis of Racial/Ethnic Representation in Select Basic and Applied Cancer Research Studies. *Sci. Rep.* **8**, 13978 (2018).
3. Sung, H. *et al.* Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA. Cancer J. Clin.* **71**, 209–249 (2021).
4. Harbeck, N. *et al.* Breast cancer. *Nat. Rev. Dis. Prim.* **5**, 66 (2019).
5. Globocan. All cancers. *World Health Organization. International Agency for Research on Cancer* https://gco.iarc.fr/today (2020).
6. Gao, C. *et al.* Downregulation of Msi1 suppresses the growth of human colon cancer by targeting p21cip1. *Int. J. Oncol.* **46**, 732–740 (2015).
7. Xi, Y. & Xu, P. Global colorectal cancer burden in 2020 and projections to 2040. *Transl. Oncol.* **14**, 101174 (2021).
8. Hiatt, R. A. & Brody, J. G. Environmental Determinants of Breast Cancer. *Annu. Rev. Public Health* **39**, 113–133 (2018).
9. Assis, J. V. de, Coutinho, L. A., Oyeyemi, I. T., Oyeyemi, O. T. & Grenfell, R. F. e Q. Diagnostic and therapeutic biomarkers in colorectal cancer: a review. *Am. J. Cancer Res.* **12**, 661 (2022).
10. Cohen, R., Pudlarz, T., Delattre, J. F., Colle, R. & André, T. Molecular Targets for the Treatment of Metastatic Colorectal Cancer. *Cancers (Basel).* **12**, 1–18 (2020).
11. Liu, Z. *et al.* Genomic Alteration Characterization in Colorectal Cancer Identifies a Prognostic and Metastasis Biomarker: FAM83A|IDO1. *Front. Oncol.* **11**, 607 (2021).
12. Lukong, K. E., Chang, K.-W., Khandjian, E. W. & Phane Richard, S. RNA-binding proteins in human genetic disease. *Cell* **24**, 416–425 (2008).
13. Morris, A. R. *et al.* Alternative Cleavage and Polyadenylation during Colorectal Cancer Development. *Clin. Cancer Res.* **18**, 5256–5266 (2012).
14. Wurth, L. *et al.* UNR/CSDE1 Drives a Post-transcriptional Program to Promote Melanoma Invasion and Metastasis. *Cancer Cell* **30**, 694–707 (2016).
15. Martinez-Useros, J. *et al.* UNR/CSDE1 Expression Is Critical to Maintain Invasive Phenotype of Colorectal Cancer through Regulation of c-MYC and Epithelial-to-Mesenchymal Transition. *J. Clin. Med.* **8**, 560 (2019).
16. Abdel-Wahab, O. & Gebauer, F. Editorial overview: Cancer genomics: RNA metabolism and translation in cancer pathogenesis and therapy. *Curr. Opin. Genet. Dev.* **48**, iv–vi (2018).

17. Wurth, L. & Gebauer, F. RNA-binding proteins, multifaceted translational regulators in cancer. *Biochim. Biophys. Acta - Gene Regul. Mech.* **1849**, 881–886 (2015).
18. Hentze, M. W., Castello, A., Schwarzl, T. & Preiss, T. A brave new world of RNA-binding proteins. *Nat. Rev. Mol. Cell Biol.* **19**, 327–341 (2018).
19. Paschke, S. *et al.* Are Colon and Rectal Cancer Two Different Tumor Entities? A Proposal to Abandon the Term Colorectal Cancer. *Int. J. Mol. Sci.* **19**, (2018).
20. Ellrott, K. *et al.* Scalable Open Science Approach for Mutation Calling of Tumor Exomes Using Multiple Genomic Pipelines. *Cell Syst.* (2018) doi:10.1016/j.cels.2018.03.002.
21. Taylor, A. M. *et al.* Genomic and Functional Approaches to Understanding Cancer Aneuploidy. *Cancer Cell* **33**, 676-689.e3 (2018).
22. Liu, J. *et al.* An Integrated TCGA Pan-Cancer Clinical Data Resource to Drive High-Quality Survival Outcome Analytics. *Cell* (2018) doi:10.1016/j.cell.2018.02.052.
23. Sanchez-Vega, F. *et al.* Oncogenic Signaling Pathways in The Cancer Genome Atlas. *Cell* **173**, 321-337.e10 (2018).
24. Gao, Q. *et al.* Driver Fusions and Their Implications in the Development and Treatment of Human Cancers. *Cell Rep.* **23**, 227-238.e3 (2018).
25. Hoadley, K. A. *et al.* Cell-of-Origin Patterns Dominate the Molecular Classification of 10,000 Tumors from 33 Types of Cancer. *Cell* **173**, 291-304.e6 (2018).
26. Meyers, R. M. *et al.* Computational correction of copy number effect improves specificity of CRISPR–Cas9 essentiality screens in cancer cells. *Nat. Genet.* **49**, 1779–1784 (2017).
27. Tsherniak, A. *et al.* Defining a Cancer Dependency Map. *Cell* **170**, 564-576.e16 (2017).
28. McFarland, J. M. *et al.* Improved estimation of cancer dependencies from large-scale RNAi screens using model-based normalization and data integration. *Nat. Commun.* **9**, 4610 (2018).
29. Uhlén, M. *et al.* Proteomics. Tissue-based map of the human proteome. *Science* **347**, 1260419 (2015).
30. Thul, P. J. *et al.* A subcellular map of the human proteome. *Science* **356**, eaal3321 (2017).
31. Uhlen, M. *et al.* A pathology atlas of the human cancer transcriptome. *Science* **357**, eaan2507 (2017).
32. García-Cárdenas, J. M. *et al.* Post-transcriptional Regulation of Colorectal Cancer: A Focus on RNA-Binding Proteins. *Front. Mol. Biosci.* **6**, (2019).
33. Kang, D., Lee, Y. & Lee, J. S. RNA-Binding Proteins in Cancer: Functional and Therapeutic Perspectives. *Cancers (Basel).* **12**, 1–33 (2020).
34. Tomczak, K., Czerwińska, P. & Wiznerowicz, M. The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge. *Contemp Oncol (Pozn)* **19**, 68–77 (2015).
35. Pontén, F., Jirström, K. & Uhlen, M. The Human Protein Atlas—a tool for pathology. *J. Pathol.* **216**, 387–393 (2008).
36. Szklarczyk, D. *et al.* STRING v11: Protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res.* **47**, D607–D613 (2019).
37. Yu, C. *et al.* High-throughput identification of genotype-specific cancer vulnerabilities in mixtures of barcoded tumor cell lines. *Nat. Biotechnol.* **34**, 419–423 (2016).
38. Kim, C. Y. *et al.* HumanNet v3: an improved database of human gene networks for disease research. *Nucleic Acids Res.* **50**, D632–D639 (2022).
39. Repana, D. *et al.* The Network of Cancer Genes (NCG): a comprehensive catalogue of known and candidate cancer genes from cancer sequencing screens. *Genome Biol.* **20**, 1–12

(2019).
40. Indacochea, A. *et al.* Cold-inducible RNA binding protein promotes breast cancer cell malignancy by regulating Cystatin C levels. *RNA* **27**, 190–201 (2021).
41. García-Cárdenas, J. M. *et al.* Integrated In Silico Analyses Identify PUF60 and SF3A3 as New Spliceosome-Related Breast Cancer RNA-Binding Proteins. *Biol. 2022, Vol. 11, Page 481* **11**, 481 (2022).
42. Maguire, S. L. *et al.* SF3B1 mutations constitute a novel therapeutic target in breast cancer. *J. Pathol.* **235**, 571–580 (2015).
43. Lu, X.-Y. *et al.* Cell Cycle Regulator Gene CDC5L, a Potential Target for 6p12-p21 Amplicon in Osteosarcoma. *Mol. Cancer Res.* **6**, 937–946 (2008).
44. Li, X. *et al.* Oncogenic Properties of NEAT1 in Prostate Cancer Cells Depend on the CDC5L–AGRN Transcriptional Regulation Circuit. *Cancer Res.* **78**, 4138–4149 (2018).
45. Liu, X. *et al.* NAT10 regulates p53 activation through acetylating p53 at K120 and ubiquitinating Mdm2. *EMBO Rep.* **17**, 349–366 (2016).
46. Ray Chaudhuri, A. & Nussenzweig, A. The multifaceted roles of PARP1 in DNA repair and chromatin remodelling. *Nat. Rev. Mol. Cell Biol.* **18**, 610–621 (2017).
47. Kobayashi, S. *et al.* Anti-FIRs (PUF60) auto-antibodies are detected in the sera of early-stage colon cancer patients. *Oncotarget* **7**, 82493–82503 (2016).
48. Müller, B. *et al.* Concomitant expression of far upstream element (*FUSE*) binding protein (*FBP*) interacting repressor (FIR) and its splice variants induce migration and invasion of non-small cell lung cancer (NSCLC) cells. *J. Pathol.* **237**, 390–401 (2015).
49. Wang, Y. *et al.* Tricho-rhino-phalangeal syndrome 1 protein functions as a scaffold required for ubiquitin-specific protease 4-directed histone deacetylase 2 de-ubiquitination and tumor growth. *Breast Cancer Res.* **20**, 83 (2018).
50. Quattrone, A. & Dassi, E. The Architecture of the Human RNA-Binding Protein Regulatory Network. *iScience* **21**, 706–719 (2019).
51. Cao, Y. *et al.* N-Acetyltransferase 10 Promotes Micronuclei Formation to Activate the Senescence-Associated Secretory Phenotype Machinery in Colorectal Cancer Cells. *Transl. Oncol.* **13**, (2020).
52. Zhang, H. *et al.* GSK-3β-regulated N-acetyltransferase 10 is involved in colorectal cancer invasion. *Clin. Cancer Res.* **20**, 4717–4729 (2014).
53. Liu, Z. *et al.* miR-6716-5p promotes metastasis of colorectal cancer through downregulating NAT10 expression. *Cancer Manag. Res.* **11**, 5317–5332 (2019).
54. Sillars-Hardebol, A. H. *et al.* CSE1L, DIDO1 and RBM39 in colorectal adenoma to carcinoma progression. *Cell. Oncol. (Dordr).* **35**, 293–300 (2012).
55. Tai, C. J. *et al.* Correlations between cytoplasmic CSE1L in neoplastic colorectal glands and depth of tumor penetration and cancer stage. *J. Transl. Med.* **11**, (2013).
56. Pimiento, J. M. *et al.* Knockdown of CSE1L Gene in Colorectal Cancer Reduces Tumorigenesis in Vitro. *Am. J. Pathol.* **186**, 2761–2768 (2016).
57. Xu, B. *et al.* Circ_cse1l Inhibits Colorectal Cancer Proliferation by Binding to eIF4A3. *Med. Sci. Monit.* **26**, (2020).
58. Liang, Q., Du, X., Mao, L. & Wang, G. Molecular characterization of colorectal cancer: A five-gene prognostic signature based on RNA-binding proteins. *Saudi J. Gastroenterol.* **27**, 223 (2021).