

# Effect of a microtubule-targeting drug on cell-cell contacts in bladder epithelial tumour cells

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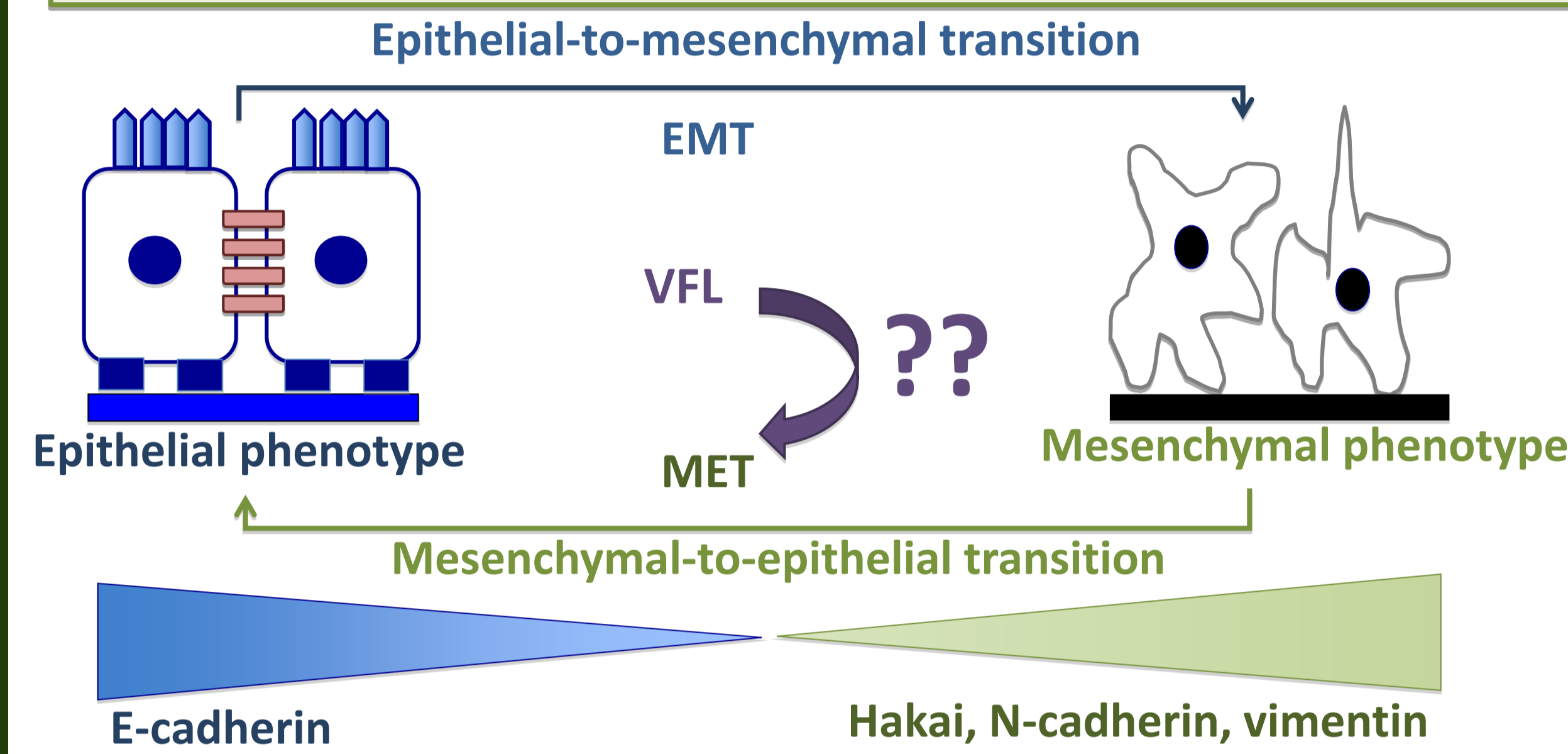
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## Introduction

Bladder cancer is a common malignancy that represents the fifth most common cancer in the world. Transitional cell carcinoma (TCC) represents 95% of these tumours. Transitional epithelium cells establish cell-cell contacts, on which E-cadherin is the prototype and best characterized member of adherens junctions and it is considered a hallmark of epithelial-to-mesenchymal transition (EMT).



*Catharanthus roseus*



Vinflunine (VFL) is a third-generation semi-synthetic vinca alkaloid obtained from *Catharanthus roseus* that suppresses microtubule dynamics both *in vitro* and in living cancer cells. Several lines of evidence underline the influence of the microtubules dynamics on the cadherin-dependent cell-cell adhesions. We hypothesized that VFL can influence on E-cadherin-based cell-cell adhesions of epithelial bladder tumor cells and may impact upon EMT and metastasis.

## Results

Figure 1. Effect of VFL on cytotoxicity of bladder tumour cell lines (HT1376, 5637, SW780, UMUC3 and T24). The dose-dependent inhibition of cell growth (IC50) in human tumour bladder cell lines was determined by MTT assay.

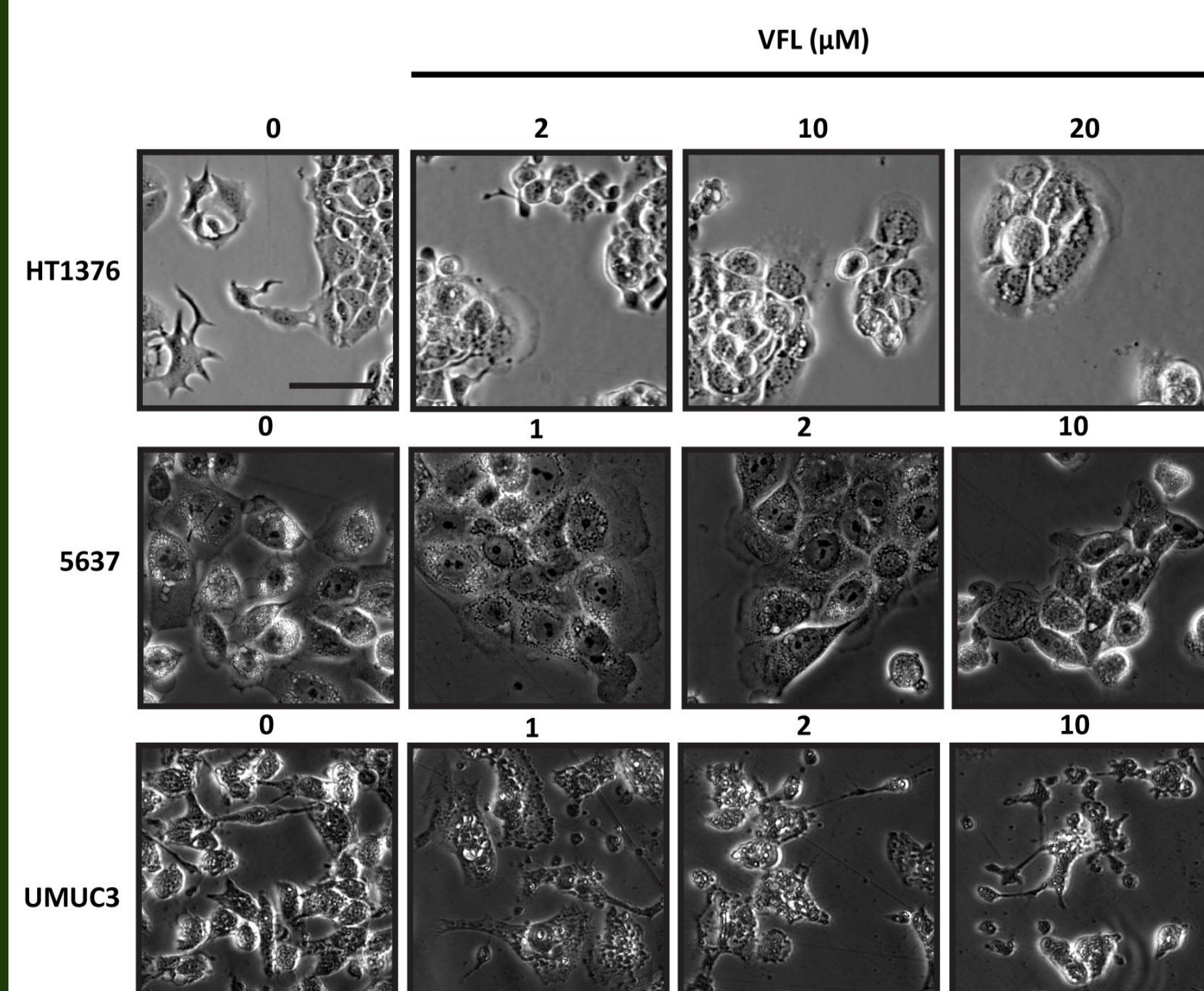
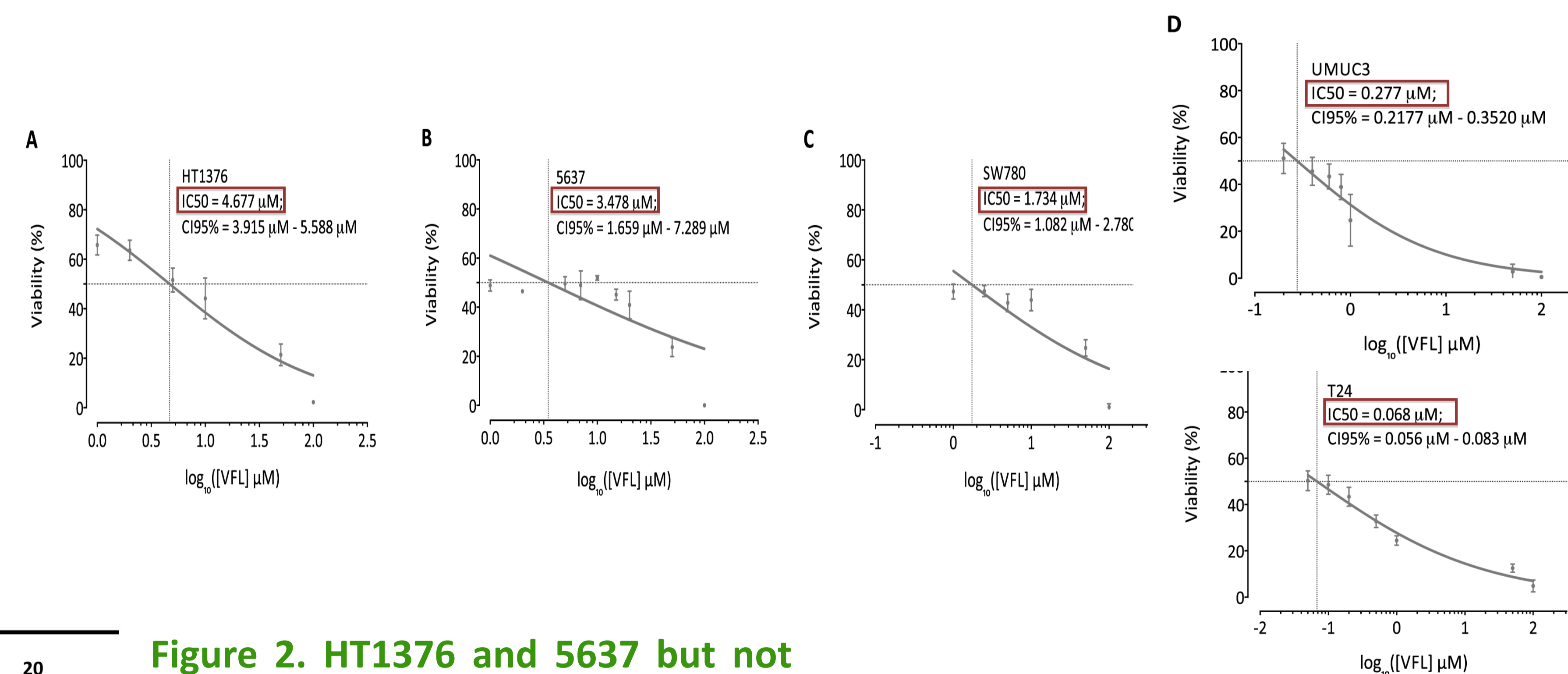


Figure 2. HT1376 and 5637 but not UMUC3, exhibit phenotypic changes suggestive of mesenchymal-to-epithelial under VFL treatment. Phase-contrast microscopy images after treatment with VFL. Scale bar, 100 µm.

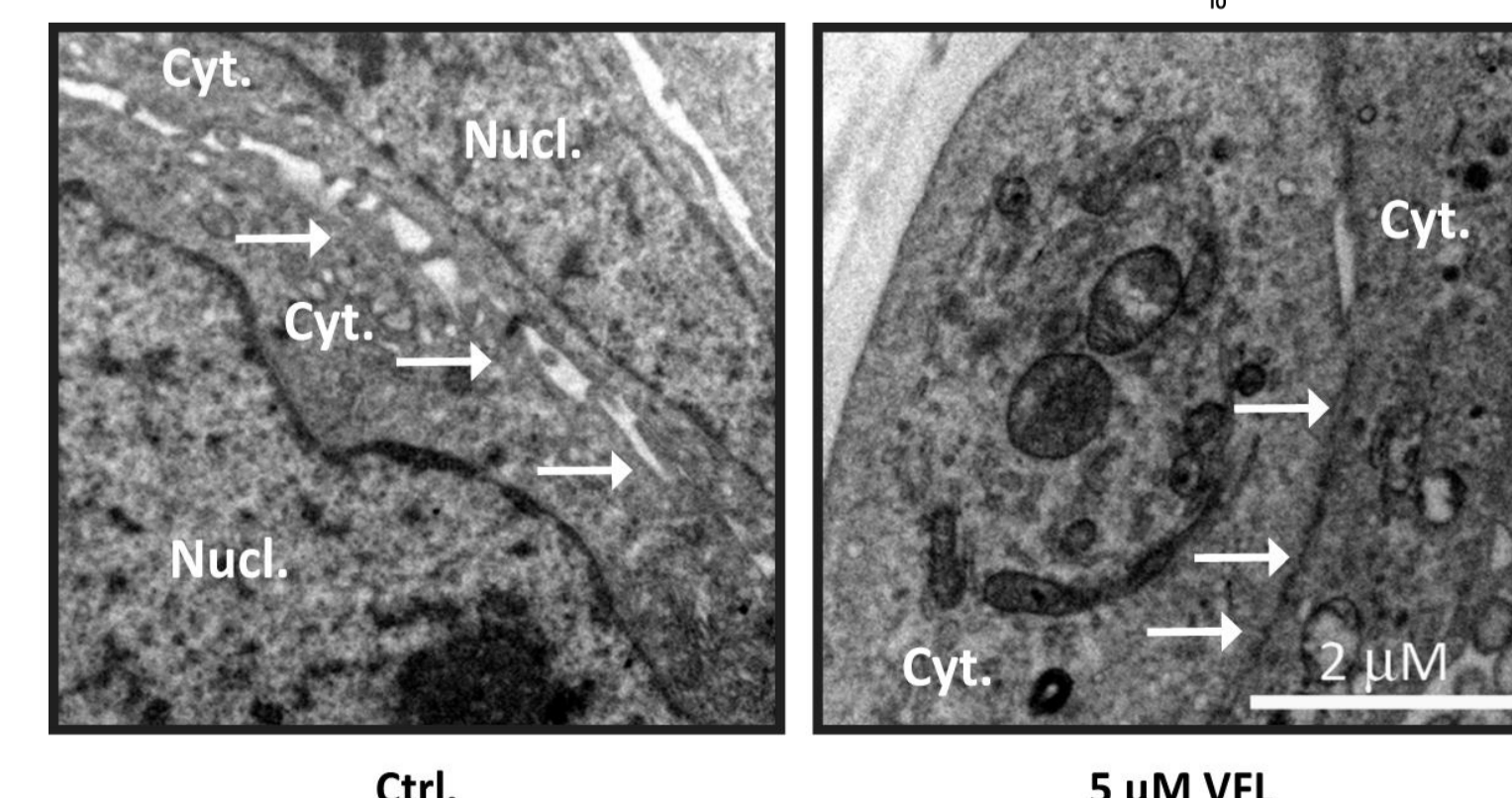


Figure 3. 5637 cells show very closely apposed cell-cell under VFL treatment. Transmission electron microscopy images were taken in 5 µM VFL-treated cell compared to control. Nucl.: nucleus; Cyt.: cytoplasm; Arrowheads: sites of close cell-cell contacts. Scale bar, 2 µm.

Figure 4. E-cadherin is expressed in HT1376, SW780 and 5637, the most resistant cells to VFL. Figure 4A. Expression of epithelial and mesenchymal markers (E-cadherin, Hakai, N-cadherin and Vimentin) were analysed by western blotting. Figure 4B, 4C and 4D. Epithelial E-cadherin marker is increased, while mesenchymal markers are reduced, under VFL treatment. Effect of VFL treatment on 5637 (B), UMUC3 (C) and HT1376 (D) cells analysed by western blotting.

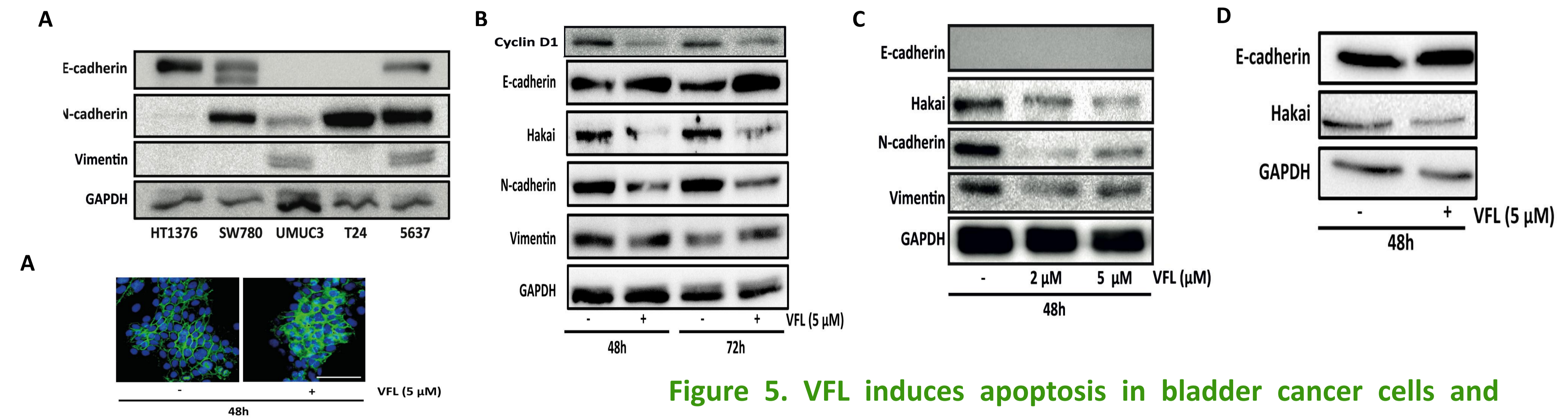
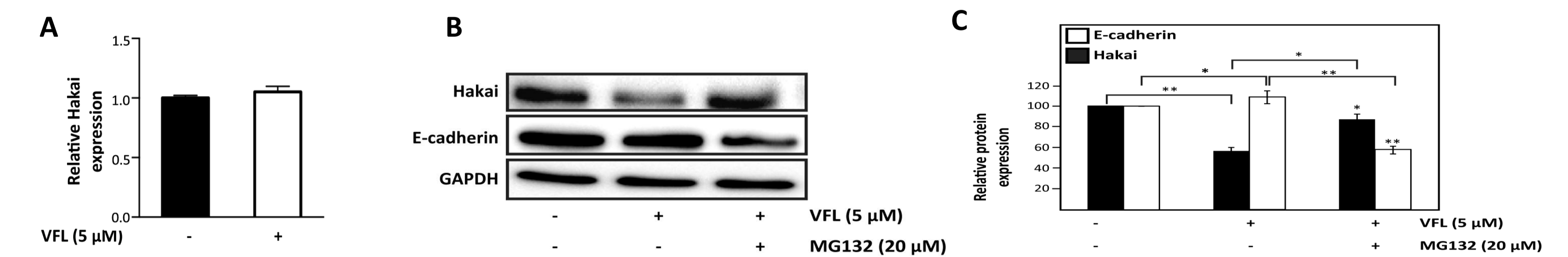


Figure 5. VFL induces apoptosis in bladder cancer cells and activates epithelial differentiation of the remaining living cells. A. Immunofluorescence analysis of E-cadherin expression in VFL-treated HT1376 cells compared to control. B. TUNEL staining for apoptosis was performed following by immunofluorescence of E-cadherin in VFL-treated HT1376 cells compared to control.

Figure 6. VFL promotes proteasome-mediated Hakai degradation. Figure 6A. No differences on RNA expression were detected. Figure 6B and 6C. Effect of proteasome inhibitor MG132 on Hakai expression in 5637 cells after treatment (B) and quantification by densitometry was represented, \*p<0.05, \*\*p<0.01, (C).



## Conclusions

Our findings suggest the existence of a new mechanism of VFL-microtubule targeting drug to cell-cell contacts with potential functional implications in the maintenance of epithelial cell phenotype.

## Acknowledgments

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