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Conditioned Medium from Human Uterine Cervical Stem Cells Regulates Oxidative Stress and Angiogenesis of Retinal Pigment Epithelial Cells

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Keywords
Mesenchymal stem cells · Human uterine cervical stem cells · Conditioned medium · Secretome · Retinal pigment epithelium · Oxidative stress · Angiogenesis

Abstract
Introduction: Retinal homeostasis is essential to avoid retinal pigment epithelium (RPE) damage resulting in photoreceptor death and blindness. Mesenchymal stem cells-based cell therapy could contribute to the maintenance of the retinal homeostasis. We have explored the effect of human uterine cervical stem cells (hUCESCs)-conditioned medium (hUCESC-CM) on RPE cells under oxidative stress condition. ***Methods:*** ARPE-19 cells were treated with hydrogen peroxide (H_2O_2) in the presence or absence of hUCESC-CM. qRT-PCR and Western blot were used to evaluate the expression of oxidative stress-related (HO-1, GCLC, and HSPB1) and vasculogenesis-related (VEGFA, PDGFA, and PDGFB) factors. Also, we assessed in vitro effects of hUCESC-CM on endothelial-cell (HUVEC) tube formation. ***Results:*** mRNA expression of HO-1, GCLC, HSPB1, VEGFA, PDGFA, and PDGFB were significantly increased in ARPE-19 cells treated with H_2O_2 + hUCESC-CM compared to cells treated with H_2O_2 only. Regarding the tube formation assay, HUVEC treated with supernatant from ARPE-19 cells treated with H_2O_2 + hUCESC-CM showed a significant increase in average vessel length, number of capillary-like junctions, and average of vessels area compared with HUVEC treated with supernatant from ARPE-19 cells treated with H_2O_2 only. ***Conclusion:*** Our results show potential therapeutic effects of hUCESC-CM on RPE, such as protection from damage by oxidative stress, stimulation of detoxifying genes, and a better vascularization.

Introduction

The retinal pigment epithelium (RPE) is a single layer of epithelial cells that interacts with photoreceptors and choriocapillaris. RPE displays a key function in retinal homeostasis [1]. Maintenance of retinal homeostasis is essential to avoid RPE damage, resulting in photorecep-

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tor death and blindness. Due to its location, RPE is exposed to phototoxic blue light [2] and to high oxygen tension, leading to reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) [3].

In retinal diseases, oxidative stress is responsible for the occurrence of several simultaneous biological processes such as abnormal vascularization, which can cause blindness due to the destruction of the retinal structure. This occurs especially not only in age-related macular degeneration and in diabetic retinopathy but also in retinal vein occlusion and uveitis [4]. Retinal cells have defense mechanisms to counteract both the formation of ROS and their impact when antioxidant capacity is insufficient. Under extreme circumstances, oxidative stress leads to apoptosis through the inhibition of mitochondrial respiratory chain transition and increasing mitochondrial membrane permeability [5]. In addition, oxidative stress also affects retinal vasculature through two main mechanisms, directly on the HIF-VEGF/VEGFR2 signaling pathway or via VEGF-independent mechanism. Considered a key regulator for angiogenesis, VEGF and its pathway are used as primary targets for antiangiogenic therapy [6]. The currently more widely used therapy, based on anti-VEGF antibodies or VEGFR inhibitors, also seems to have its limitations [6]. Inhibition of the VEGF pathway may alter the vasculature homeostasis since the pathological VEGF/VEGFR pathway share similarities with physiological angiogenesis. In addition, diverse VEGF-independent pathways have been implicated in pathological angiogenesis in the last years. Therefore, a more balanced and complete approach is required, avoiding the inhibition of angiogenesis and regulating a nonaberrant angiogenesis.

Cell therapies developed for the treatment of retinal degeneration, using RPE as a target, were focused on replacement (cell replacement by iPS-derived RPE cells, mesenchymal stem cells [MSCs]-derived RPE cells, or retinal stem cell-derived RPE cells) or protection of RPE [7]. Regarding the latter aspect, it is known that MSCs can act at different levels such as the promotion of angiogenesis and extracellular matrix remodeling, the regulation of immune response, the activation of adjacent host stem cells, and the secretion of neurotrophins [8, 9].

Recently, we have isolated and characterized a new population of MSCs obtained from the transition zone of the uterine cervix of healthy women by means of Pap cervical smears called human uterine cervical stem cells (hUCESCs) [10–12], which exhibits a high proliferation rate. The biological niche can define, in part, the therapeutic potential of MSCs [13, 14]; the site of origin of hUCESCs, the human cervical transition zone, which has unique features [11], can biologically explain the different actions of its conditioned medium (CM) (hUCESC-CM). Previously, we have described that hUCESC-CM shows antitumor effects by reducing aggressive breast cancer cells' proliferation and invasiveness and inducing apoptosis [10]. An important immunoregulatory effect of hUCESC-CM was also found, such as acting on monocyte-macrophage differentiation and on macrophage-monocyte dedifferentiation [10] and also on leucocytes infiltration on a rat model of uveitis [15]. Also, hUCESC-CM has shown a regenerative capacity on epithelial healing in a rat model [15] and in a rabbit model of dry eye. The epithelial regeneration promoted by hUCESC-CM seems to be mediated by TIMP-1 and TIMP-2 and the reduction of corneal pro-inflammatory factors at mRNA levels such as MIP-1α and TNF-α [16]. Importantly, treatment of eyes with hUCESC-CM did not produce corneal vascularization in these two models, which is a required condition for optical transparency. hUCESC-CM contains high levels of cytokines with known regenerative, anti-inflammatory [15, 16], antimicrobial [17], and antitumoral effect [10], which explain its therapeutic potential. On the other hand, the use of cell-free therapies such as hUCESC-CM has advantages over stem cell-based applications like (i) the resolution of safety issues associated with the transplantation of proliferating cells; (ii) hUCESC-CM may be tested for safety, dosage, and potency like conventional drugs; and (iii) unlike cells, the storage of hUCESC-CM can be done without toxic cryopreservative agents and without loss of potency for a long period of time [18]. In the present study, we aimed to determine the effect of hUCESC-CM on RPE cells under oxidative stress condition, regarding to the expression of oxidative stress related factors and regarding to the regulation of angiogenesis.

Materials and Methods

Patients, Samples, and Ethics Statement

hUCESCs were obtained by cervical smears from women during a routine gynecological checkup at Fundación Hospital de Jove of Gijón, Spain. All women provided informed written consent. This study was approved by "Comité Ético de Investigación Clínica Regional del Principado de Asturias" – reference: 100/13.

Cell Culture and CM Production

hUCESCs were obtained and cultured as previously described [10]. In brief, cells were grown at 1,500–2,000 cells/cm^2 in a 182-cm^2 tissue culture flask in DMEM-F12 supplemented with 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin in a hu-

Table 1. RealTime ready custom single assays

Gene symbol	Gene name	References (assay ID)
HO-1	Heme oxygenase (decycling) 1	110977
GCLC	Glutamate-cysteine ligase, catalytic subunit	115192
VEGFA	Vascular endothelial growth factor A	140396
PDGFA	Platelet-derived growth factor alpha polypeptide	147202
PDGFB	Platelet-derived growth factor beta polypeptide	110713
β-actina	Actin, beta	143636
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	102052

midified air-CO_2 (95:5) atmosphere at 37°C. CM from hUCESCs was obtained by culturing the cells to 80% confluence. Then, cells were washed three times with phosphate-buffered saline (PBS) and maintained in DMEM-F12 without FBS and penicillin/streptomycin. After 48 h, the medium was centrifuged for 5 min at 300 g, the supernatant was collected, aliquoted and lyophilized, and then stored at −80°C until used. For the use of CM from hUCESCs, aliquots were resuspended in the same volume before the lyophilization with sterile water for cell culture. hUCESC-CM is produced under standardized conditions, establishing a quality control to validate it.

The human cell line arising retinal pigment epithelium (ARPE-19) (American Type Culture Collection; ATCC, Manassas, VA, USA) was seeded in 6 wells at 1.6×10^5 cells per well for gene expression analysis and 2.5×10^5 cells per well for Western blot analysis in DMEM-F12 culture medium with 10% fetal bovine serum (FBS), 0.1 mg/mL streptomycin, and 100 U/mL penicillin in a humidified air-CO_2 (95:5) atmosphere at 37°C for 24 h. Then, cells were washed with PBS and treated for 4 h with 400 μM of H_2O_2 (conclusion of a preconceptual study with different times [2 h, 4 h, and 24 h] and concentrations [200 μM, 400 μM, and 1,000 μM] of H_2O_2) (see online suppl. Fig. S1; for all online suppl. material, see www.karger.com/doi/10.1159/000524484) in the presence of DMEM-F12 without FBS and penicillin/streptomycin or in the presence of 100% hUCESC-CM. Cells in complete medium and treated with DMEM-F12 without FBS and penicillin/streptomycin were used as the control. Subsequently, cells and supernatant were collected and preserved for future experiments. The primary human umbilical vein endothelial cells (HUVEC) (ATCC, Rockville, MD, USA) were cultured in VascuLife Basal Medium supplemented with LifeFactors VascuLife EnGS (Lifeline Cell Technology, Frederick, MD, USA) in a humidified air-CO_2 (95:5) atmosphere at 37°C.

Quantitative Real-Time PCR

Following stress treatment, an RNeasy Mini Kit (QIAGEN, Hilden, Germany) was used for total RNA extraction following the manufacturer's instructions. The Transcriptor First Strand cDNA Synthesis Kit (Roche, Mannheim, Germany) was used for cDNA synthesis. Reverse transcription was carried out as previously reported [19]. qRT-PCR was performed using RealTime ready custom single assays (Roche) containing the specific primers and probes for the factors studied and for two reference genes (Table 1). The mRNA levels were measured in a LightCycler 480 II (Roche) with the following cycling conditions: 95°C for 10 min, 45 cycles of 95°C for 10 s, 60°C for 30 s, and 72°C for 1 s.

Western Blot

Following the treatment of ARPE-19 cells with H_2O_2, cells were washed with PBS and lysed in 30 μL of RIPA buffer (Thermo Fisher Scientific, Waltham, MA, USA) at 4°C in constant agitation. Cell lysate was then centrifuged at 14,000 g for 15 min at 4°C, and the resulting supernatant was collected. Protein concentration was determined by the BCA method. Ten or 20 μg of total protein was subjected to SDS-PAGE electrophoresis and transferred to a PVDF membrane (Amersham™ Hybond™; GE Healthcare Life Science, Chicago, IL, USA). The blots were then blocked in TBS +0.1% Tween-20 containing 5% skimmed milk at room temperature for 1 h and incubated at 4°C overnight with the following mouse antihuman antibodies: anti-HO-1 (dilution 1:500; sc-136960), anti-GCLC (dilution, 1:1,000, sc-166382), anti-HSPB1 (dilution 1:5,000, sc-13132), and anti-GAPDH (dilution 1:5,000, sc-32233) (Santa Cruz Biotechnology, Inc., Dallas, TX, USA), all diluted in TBS +0.1% Tween-20 containing 5% BSA (Sigma, St. Louis, MO, USA). Membranes were then incubated with HRP-conjugated goat anti-mouse immunoglobulin (dilution 1:1,500; Dako, Agilent Technologies, Inc., Santa Clara, CA, USA) at room temperature for 1 h. The signal was detected with the Pierce™ ECL Western blotting substrate (Thermo Fisher Scientific) and visualized by placing the blot in contact with high-performance chemiluminescence films (Amersham Hyperfilm™ ECL; GE Healthcare Life Science). Protein expression was quantified using the ImageJ software (National Institutes of Health, Bethesda, MD, USA); values were normalized to GAPDH, and fold change was calculated in relation to the first condition analyzed.

Tube Formation Assay/Vasculogenesis Assay

Tube formation assay was performed in Matrigel (BD Biosciences) as previously described [20]. Briefly, first growth factor-reduced Matrigel matrix was loaded into a 96-well plate. Once polymerized, HUVECs (4×10^5 cells) were seeded and incubated at 37°C for 5 h together with ARPE-19 cell culture supernatant as described above (ARPE-19 treated or not with H_2O_2 and in the presence or not of hUCESC-CM) and using their own medium as control (VascuLife medium). DMEM-F12 (vehicle of hUCESC-CM) was used as additional control. The formation of capillary-like structures was assessed under a fluorescence microscope with CellTracker Red CMTPX Dye (0.5 μM; Thermo Fisher Scientific). The quantification of vessel lengths and junction numbers was done using AngioTool software.

Human Cytokine Antibody Array

RayBio® Human Cytokine Antibody Array G-Series 2000 was used to profile hUCESC-CM following the manufacturer's instruc-

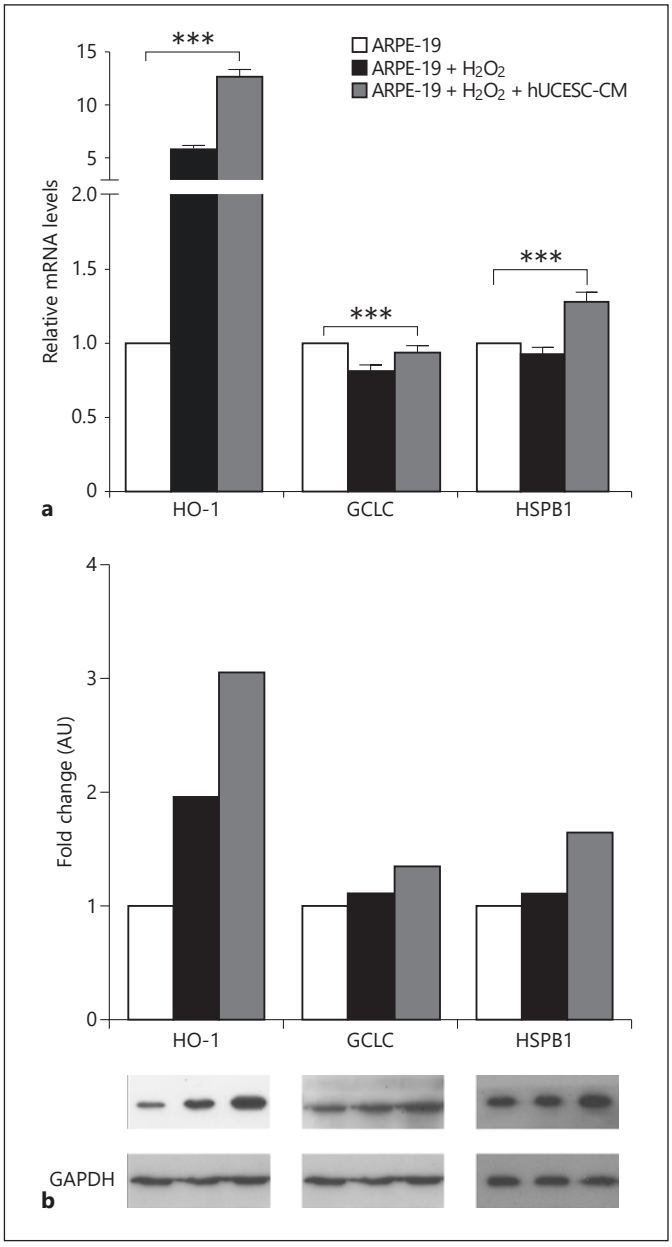

Fig. 1. a qRT-PCR analysis of HO-1, GCLC, and HSPB1 in ARPE-19 cells, ARPE-19 cells treated with H_2O_2, and ARPE-19 cell treated with H_2O_2 in the presence of hUCESC-CM. Data represent mean ± SD (*$p \leq 0.05$). **b** Western blot analysis of HO-1, GCLC, and HSPB1 in ARPE-19 cells, ARPE-19 cells treated with H_2O_2, and ARPE-19 cell treated with H_2O_2 in the presence of hUCESC-CM. SD, standard deviation.

tions, as described previously. DMEM-F12 without FBS was used as negative control. Data are shown as relative fluorescent unit.

Statistics

All values are expressed as mean ± standard deviation. The distribution of variables was analyzed by the Kolmogorov-Smirnov test. On the basis of this analysis, parametric methods, such as Student's *t* test and one-way ANOVA (Bonferroni post hoc test), were used for comparison between groups. The SPSS 18.0 (PASW Statistics 18) program was used for all calculations. Significant differences were established at $p \leq 0.05$.

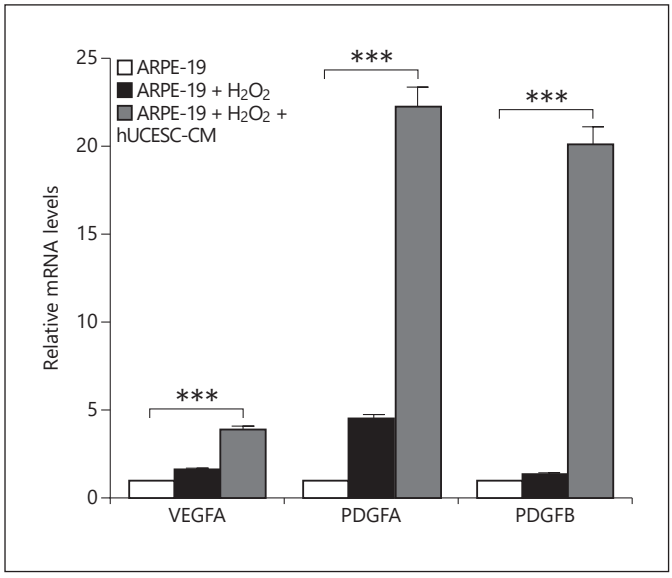

Fig. 2. qRT-PCR analysis of VEGFA, PDGFA, and PDGFB in ARPE-19 cells, ARPE-19 cells treated with H_2O_2, and ARPE-19 cell treated with H_2O_2 in the presence of hUCESC-CM. Data represent mean ± SD (*$p \leq 0.05$). SD, standard deviation.

Results

Effect of hUCESC-CM on ARPE-19 Oxidative Stress Injury

In order to study the effect of hUCESC-CM on the expression of antioxidant genes under oxidative stress conditions, we compared the expression levels of heme oxygenase-1 (HO-1), glutamate-cysteine ligase (GCL) catalytic subunit (GCLC), and heat-shock protein family B (small) member 1 (HSPB1) after H_2O_2 treatment in the presence or not of hUCESC-CM, by qRT-PCR under various concentration and treated time of H_2O_2 (online suppl. Fig. S1). The exposure of ARPE-19 cells to H_2O_2 during 24 h or to high concentration (1,000 μM) of H_2O_2 shows a decrease of cell viability, which seems to affect the secreted factors by ARPE cells in response to hUCESC-CM, and then the intermediate dose and time exposure (400 μM H_2O_2 and 4 h) has been selected. As shown in Figure 1a, mRNA expressions of HO-1, GCLC, and HSPB1 were significantly increased in cells treated with H_2O_2 + hUCESC-CM compared with cells treated with

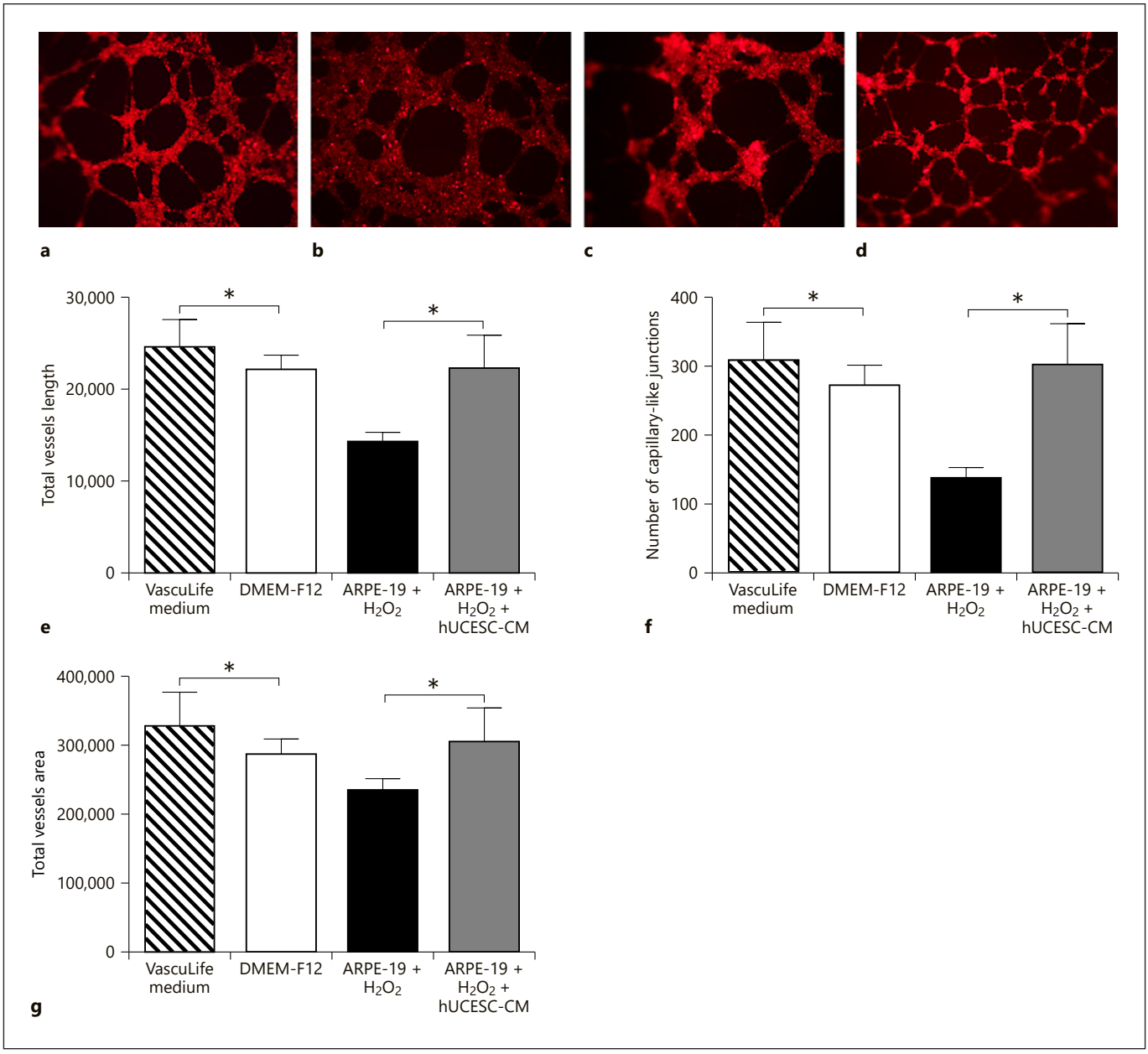

Fig. 3. HUVEC tube formation assay. Representative images of HUVEC cultured for 5 h with VascuLife medium (control) (**a**), DMEM-F12 medium (**b**), ARPE-19 cell culture supernatant treated with H_2O_2 (**c**), and ARPE-19 cell culture supernatant treated with H_2O_2 in the presence of hUCESC-CM (**d**), after which total vessels length (mm) (**e**), total numbers of junctions (/mm^2) (**f**), and vessels areas (mm^2) (**g**) were measured using Angiotool software. Data represent mean ± SD from three independent experiments (*$p \leq 0.05$). SD, standard deviation.

H_2O_2 only ($p < 0.0001$). Regarding the protein expression analyzed by Western blot (Fig. 1b) and confirming gene expression data, we observed that HO-1, GCLC, and HSPB1 were increased in cells treated with H_2O_2 + hUCESC-CM compared with cells treated with H_2O_2 only.

Effect of hUCESC-CM on Vasculogenesis

Due to the importance of vasculogenesis on RPE homeostasis, we studied the effect of hUCESC-CM on the expression of vascular-related factors such as vascular endothelial growth factor A (VEGFA), platelet-derived growth factor subunit A (PDGFA), and platelet-derived

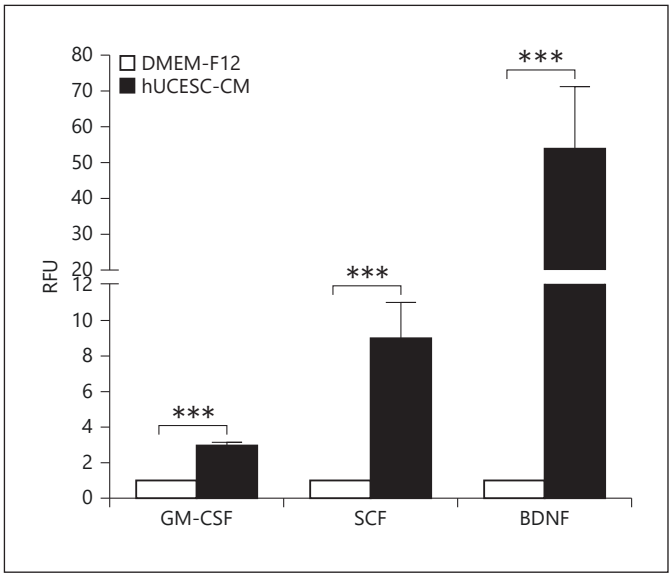

Fig. 4. Relative cytokine expression in hUCESC-CM compared with control media. Data represent mean ± SD of RFU (*$p \leq 0.001$). RFU, relative fluorescent unit; SD, standard deviation.

growth factor subunit B (PDGFB). As shown in Figure 2, the expression of VEGFA, PDGFA, and PDGFB were significantly increased in ARPE-19 cell treated with H_2O_2 + hUCESC-CM compared to cells treated with H_2O_2 only ($p < 0.0001$).

Due to the increased expression of angiogenic factors in ARPE-19 cells treated with hUCESC-CM, we studied the effect on HUVEC. Components of supernatants can accelerate or inhibit the formation of tubes by HUVEC. HUVEC treated with VascuLife medium are able to form tubes that materialize as new blood vessels at the end of the process (Fig. 3a). The treatment of HUVEC with DMEM-F12 (Fig. 3b) showed visually worse tube formation than treatment with VascuLife medium. After 5 h in culture, HUVEC treated with supernatant from ARPE-19 cells treated with H_2O_2 + hUCESC-CM seemed to performed tubes with thin wall more similar to healthy vessels (Fig. 3d) than HUVEC treated with supernatant from ARPE-19 cells treated with H_2O_2 (Fig. 3c).

In addition, HUVEC showed a significant increased average vessels length ($p = 0.003$) (Fig. 3e), number of capillary-like junctions ($p = 0.04$) (Fig. 3f) as well as increased average of vessels area ($p = 0.007$) (Fig. 3g) when they were incubated in supernatant from ARPE-19 treated with H_2O_2 + hUCESC-CM compared with HUVEC incubated in supernatant from ARPE-19 treated with H_2O_2 only. In addition, no significant differences were observed between HUVEC incubated in VascuLife medium or DMEM-F12 and HUVEC incubated in supernatant from ARPE-19 treated with H_2O_2 + hUCESC-CM and HUVEC incubated in the control medium (VascuLife medium) (Fig. 3e–g).

hUCESC-CM Contains Cytokines Potentially Contributing to Damage Protection and Angiogenic Homeostasis of RPE

The presence and semiquantification of factors potentially involved in the RPE homeostasis and protection was evaluated using a human cytokine antibody array. Three molecular factors seem to be relevant, due to their high expression in hUCESC-CM and their known neurotrophic role. Indeed, we observed at least 3–55-fold increase in the level of granulocyte-macrophage colony-stimulating factor, stem cell factor, and brain-derived neurotrophic factor (BDNF) in hUCESC-CM compared with control (DMEM-F12) (Fig. 4).

Discussion

The present study shows that treatment of ARPE-19 cells under oxidative stress condition with hUCESC-CM enhances the gene expression of detoxifying genes as HO-1, GCLC, and HSPB1. In addition, hUCESC-CM showed physiological angiogenic properties on HUVEC. All our data suggest that hUCESC-CM could be of great interest for the treatment of ocular diseases associated with oxidative stress and/or vascular disorders.

The retina is a complex multilayered neural tissue, and its degeneration is a leading cause of blindness. The RPE is a single cell layer located between photoreceptors and the choroidal vasculature [1]. The anatomical location of the RPE exposed it to potential damage related to oxidative stress, which compromise the balance between pro- and antioxidative signaling and induces dysregulation of functional networks, resulting in visual impairment [21].

In the present study, we show that hUCESC-CM upregulates the expression of various defense enzymes against oxidative stress, such as HO-1, GCLC, and HSPB1. HO-1 catalyzes heme breakdown to release, among others, biliverdin which is reduced to bilirubin, a potent radical scavenger. HO-1 expression has been established as a defense mechanism against oxidative stress damage and due to its antioxidative, anti-inflammatory, and antiapoptotic effects, HO-1 provides an extensive tissue protection. In the retina, the enhancement of HO-1 activity exerts beneficial effects by protecting cells from

oxidative stress, therefore promoting cell survival [22]. In this sense, the role of HO-1 in cardiovascular and cerebrovascular disease and organ transplantation, among others, is being increasingly investigated [23]. In addition, GCL, which has a catalytic subunit (GCLC) and a modifier subunit (GCLM), can regulate the biosynthesis of glutathione, a protective factor from oxidative stress [24], and as it was shown in the present study, hUCESC-CM upregulates GCLC expression. Interestingly, GCLC and HO-1 are both target genes of Nrf2 [25], which is known as a major regulator of oxidative responses. The regulation of the Nrf2/HO-1/GCLC pathway is implicated in many processes including neurodegenerative diseases such as Parkinson's disease or ischemic stroke [26] and therefore proposed as a therapeutic option to slow the progression and ameliorate symptoms of neurodegenerative disorders or neuroinflammation. May be other mechanisms, not studied in the present work, are involved in the protection, which is a limitation of this research. Among the heat shock proteins, HSPB1, also called Hsp27, is one of the most strongly induced after stress due to its cytoprotective effects [27]. HSPB1 modulates the intracellular redox potential, probably increasing intracellular levels of glutathione [28] and inhibits apoptosis through interactions with apoptotic proteins among others [29].

In this sense, hUCESC-CM shows high amounts of neurotrophic factors known to protect the retina and photoreceptors from injuries, enhancing cell survival, such as granulocyte-macrophage colony-stimulating factor, stem cell factor, and BDNF [30–37]. BDNF may potentially act as a neuroprotective factor, and considering the retina as an extension of the central nervous system, BDNF may have protective effects on retinal cell death. In fact, various practical approaches have been studied to deliver purified BDNF to the posterior segment of eye as the intravitreal injection of viral vectors or a cell-based therapy [38]. Also, it has been described that a preexisting level of oxidative stress in vitro can impair the stem cell functions and alter their secretome therapeutic potential [39, 40].

The accumulation of toxic agents, such as ROS, breaks down the retinal homeostasis which can derive in blindness. In this regard, the two vascular beds (retinal and choroidal capillary networks) are responsible of waste removal contributing and then the elimination of toxic agents and the maintenance of RPE homeostasis. The correct vasculature maintenance is essential for RPE homeostasis; however, aging changes occur in the retinal and choroidal vasculature. Age-related alterations in perfusion of capillaries, or in exchange of factors between capillaries and tissues, could contribute to the loss of retinal functions, including the loss of neurons from the inner layers of the retina. These changes include a decreased number of vessels around the fovea, the thickening and hyalinization of the vessel walls probably due to the loss of cellularity in the vessel wall [41]. The increase in thickness during senescence may impair the exchange of factors between the capillary lumens and the tissues they serve. These changes are related to retinal vascular disease, such as branch retinal vein occlusion and hypertensive retinopathy, as well as outer retinal ischemia secondary to choroidal vascular insufficiency [41]. In this sense, HUVEC treated with supernatant from ARPE-19 cells treated with H_2O_2 + hUCESC-CM performed tubes with a thin wall compared with the treatment without hUCESC-CM. This suggests that hUCESC-CM could contribute to a better exchange of factors, favoring the detoxification of tissues under oxidative stress condition, and this should be investigated in normal condition. No significant difference was found between HUVEC incubated in control medium (VascuLife medium) or incubated in supernatant from ARPE-19 treated with H_2O_2 + hUCESC-CM, which indicates that hUCESC-CM, even after oxidative damage on RPE, established condition to protect endothelial cells and therefore to maintain a normal vascularization similar to physiological conditions. In addition, the increase in average vessel length, in number of capillary-like junctions, and in average of vessels area when HUVEC were incubated in supernatant from ARPE-19 treated with H_2O_2 + hUCESC-CM could be counteracting vessels changes associated with aging and with pathological conditions related to oxidative stress. In this respect, hUCESC-CM shows high levels of factors related to the angiogenesis process such as IFG-BP6 [42], IP-10 [43], TIMP-1, and TIMP-2 [44, 45], as described previously, and induces an upregulation of VEGFA, PDGFA, and PDGFB in ARPE-19 cells under oxidative stress conditions, thus allowing for the modulation of angiogenesis. Data not shown derived from other our previous investigations indicate that no abnormal vascularization was reported in vivo in eyes of rats or rabbits after the administration of hUCESC-CM [15, 16]. In addition, it could be considered that high levels of VEGF and PDGF secreted by RPE after hUCESC-CM treatment might strongly promote the abnormal vascular proliferation in retina, but this was not observed in the present study. Further investigations are necessary to investigate the possible influence or other factors present in the hUCESC-CM as well as their mechanisms, especially in

in vivo models. The CM is composed by a set of molecules secreted by MSCs that allows maintaining or promoting the reestablishment of physiological conditions, so a single factor or a small set of them is not solely responsible for its effects. For all of that, MSC-based cell therapy is considered a new therapeutic approach, as has been observed in several clinical trials, including in ocular pathologies (www.clinicaltrials.gov). In conclusion, our results lead us to consider that hUCESC-CM could be a good candidate for further studies in ophthalmology based on cell-free therapy. Nevertheless, more knowledge is needed on several aspects related to the administration mode. Recently, it was reported that a single intravitreal delivery of CM from human adipose-derived stem/stromal cells significantly reduces signs of oxidative and neuroinflammatory injury processes and reduces the retinal injury and disfunction associated with repetitive ocular blast injury in mice [46]. Another alternative to avoid the possible injury of repetitive injections in the retina could be the intravitreal administration of a depot solution containing lyophilized CM. It is also noteworthy that our previous results indicate that topical administration of hUCESC-CM (eye drops) significantly reduces retinal tissue inflammation in an animal model of uveitis [15].

Conclusion

It is well known that oxidative stress play essential roles during the progression of diseases. This is relevant in the aging population, where neurodegenerative diseases, glaucoma, uveitis, and diabetic retinopathy, among others, or in youth population where Stargardt disease or retinopathy of prematurity are pathologies that require pharmacological interventions, targeting multiple pathways involved in these diseases. hUCESC-CM has shown to have diverse therapeutic effects as inmunoregulatory [15], regenerative [44], and now, protective effect against oxidative stress injury. Therefore, we consider that the present work opens the possibility of further studies in animal models to explore the potential interest of hUCESC-CM to protect RPE cells viability, barrier integrity, or phagocytic function for shedding the outer segment of photoreceptors. In addition, further studies could be carried out on neurodegenerative diseases, due to the capacity of hUCESC-CM to protect from damage by oxidative stress, to stimulate detoxifying genes and to allow to the possibility of a better vascularization.

Statement of Ethics

hUCESCs were obtained by cervical smears from women during a routine gynecological checkup at Fundación Hospital de Jove of Gijón, Spain. All women provided informed written consent. This study was approved by "Comité Ético de Investigación Clínica Regional del Principado de Asturias" – reference: 100/13.

Conflict of Interest Statement

The authors declare the following competing interests: F.J.V., R.P.F., and N.E. are coinventors of a patent ("Human uterine cervical stem cell population and uses thereof") owned by GiStem Research, of which N.E., J.S.L., S.C., J.S., M.A.B., R.P.F., and F.J.V are shareholders. The founding sponsors had no role in the design of this article, in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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Author Contributions

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Data Availability Statement

All data generated or analyzed during this study are included in this article and its online supplementary material. Further inquiries can be directed to the corresponding author.

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