Commentary: Novel Cell Culture Paradigm Prolongs Mouse Corneal Epithelial Cell Proliferative Activity In Vitro and In Vivo

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A Commentary on

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1 INTRODUCTION

Limbal stem cells (LSCs) are located in the limbal palisades of Vogt between the cornea and conjunctiva. The LSCs exhibit a series of key characteristics of epithelial stem cells, including self-renewal ability, high proliferation potential, and tissue regeneration capacity (Li et al., 2021a). The loss of LSCs and damage to their microenvironment contribute to various corneal diseases such as LSC deficiency (LSCD). LSCD further causes corneal vascularization, opacification, and even blindness (Singh and Sangwan, 2021). Better treatments for LSCD are currently being developed, including LSC culture for autograft. In 2015, Holoclar [ex vivo expanded autologous human corneal epithelial cells (CECs) containing stem cells] successfully gained the marketing authorization from the European Medicines Agency to treat moderate-to-severe LSCD following chemical and thermal eye burns (Yu et al., 2018). The regenerative potential of Holoclar mainly relies upon the highly proliferative and self-renewing properties of holoclones. However, it still needs to be further explored on how to optimize LSC expansion in vitro. In fact, current efforts are focused on the addition of small molecular compounds to improve efficiency of stem cell expansion. These compounds have clear targets, show quick action and reversibility, and are therefore of great value in the research and practical application toward maintaining LSC self-renewal.

2 A NOVEL STRATEGY TO OPTIMIZE SELF-RENEWAL AND EXPANSION OF LIMBAL STEM CELLS IN CULTURE

It is well known that increasing extracellular calcium, serum and air-lifting can lead to terminal differentiation and gradual loss of LSCs (Meyer-Blazejewska et al., 2010). Previously, Xiang et al. has reported the long-term in vitro maintenance of primary human hepatocytes by modulating cell signaling pathways with a combination of five chemicals (5C), including forskolin, SB431542, DAPT,
IWP-2, and LDN-193189 (Xiang et al., 2019). Interestingly, each compound of 5C has been proved to improve corneal epithelium homeostasis. This study by An et al. (2021) compared the effects of 5C and 6C (combined Y-27632 and 5C) on the mouse CECs (mCECs) and found that 6C could increase mCEC proliferation, sustain the expression levels of the progenitor cell function gene, as well as suppress epithelial–mesenchymal transition. The 6C culture method may be applied for improving the availability of CECs to treat LSCD in clinical practice.

The 6C improve the maintenance of mCEC morphology and function in long-term culture, subculture in vitro, and mouse cornea culture ex vivo. Moreover, the 6C culture system conduces to construct tissue-engineered corneal epithelium and promotes healing of corneal epithelial wounds in mice. These small-molecule combinations regulate mCEC proliferation involving in cAMP, TGF-β, BMP, Notch, Wnt/β-catenin, and Rho/ROCK signaling (An et al., 2021). Remarkably, 6C seem to maintain the limbal proliferating stem and progenitor cell phenotype in vivo, which demonstrates that these signaling modulators might regulate LSC functions. Considering that CECs have a finite capacity to replicate and eventually enter irreversible growth arrest, it is more significant to apply this novel strategy to LSC expansion based on the mechanisms underlying LSC self-renewal. Then, specific small-molecule compounds can be optimized to act on the signaling regulatory network for LSC expansion in culture.

Transcription factor (TF) PAX6 is expressed during eye development, which is considered as the master gene for oculogenesis (Ramos et al., 2015). PAX6 plays an essential role in specifying LSCs, in which Wnt7a controls CEC fate determination through PAX6 (Ouyang et al., 2014). GSK3β inhibitors, lithium chloride, and CHIR-99021 can activate the canonical Wnt pathway to improve LSC self-renewal (Bonnet et al., 2021). Specific small molecules such as IIIIC3 (DKK inhibitor) and MFH-ND (Wnt mimic) have been designed to improve LSC expansion in vitro by interacting specifically with the Wnt co-receptors LRPS/6 and FZD (Janda et al., 2017; Gonzalez et al., 2019; Chen et al., 2020; Zhang et al., 2020). TFs RUNX1 and SMAD3 are also required for maintenance of corneal epithelial identity and homeostasis by interactions with PAX6 (Li et al., 2007). Moreover, RUNX1 can shape LSC chromatin architecture via modulating H3K27ac deposition (Li et al., 2021c). RNA sequencing (RNA-seq) and qualitative proteomics identify that miR146a has an opposite regulatory role in the fine-tuning of Notch 1/2 expression to balance LSC self-renewal and differentiation (Poe et al., 2020). The above studies suggest that LSC self-renewal is regulated by a variety of signaling pathways ranging from signaling factor, TF, epigenetic regulator to microRNA, which serve as putative targets of small-molecule compounds during LSC culture.

LSCs certainly need to communicate with their own niche to maintain self-renewal (Li et al., 2007), including specific extracellular matrix (ECM), niche cells, and signaling molecules (Ashworth et al., 2021). The ECM not only anchors the basal epithelium but also mediates intercellular communication and provides distinct mechanical properties that influence LSC phenotype, population, and self-renewal (Gesteira et al., 2017; Gouveia et al., 2019; Zheng et al., 2019; Zhu et al., 2020; Ashworth et al., 2021). Additionally, cell–cell communication analysis reveals the central role of LSCs and their...
The long-term maintenance of cell function requires a sophisticated signaling regulatory network; a chemical strategy using small-molecule combinations confers the advantage of synergistically orchestrating innate signals to achieve spatiotemporal modulations of specific cellular targets (Xu et al., 2015). This is the first report on the 6C culture system prolonging mCEC maintenance in vitro. The chemical approach is simple and easily applied for autologous epithelial sheet transplantation. It also provides a new idea and method for LSC expansion in vitro (Figure 1). More stable LSC populations can be obtained for applications in regenerative medicine research by optimizing specific small-molecule combinations under a niche-mimicking culture condition.

AUTHOR CONTRIBUTIONS

BX conceived the article. XJ and BX wrote the first draft. MZ, TF, and BX reviewed and revised the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

Altshuler, A., Amitai-Lange, A., Tarazi, N., Dey, S., Strinkovsky, L., Hadad-Porat, S., et al. (2021). Discrete Limbal Epithelial Stem Cell Populations Mediate Corneal Homeostasis and Wound Healing. Cell Stem Cell 28, 1248–1261. doi:10.1016/j.stem.2021.04.003

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Ashworth, S., Harrington, J., Hammond, G. M., Bains, K. K., Koudouna, E., Hayes, A. J., et al. (2021). Chondroitin Sulfate as a Potential Modulator of the Stem Cell Niche in Cornea. Front. Cell Dev. Biol. 8, 567358. doi:10.3389/fcell.2020.567358

Bonnet, C., González, S., Roberts, J. S., Robertson, S. Y. T., Ruiz, M., Zheng, J., et al. (2021). Human Limbal Epithelial Stem Cell Regulation, Bioengineering and Function. Prog. Retin. Eye Res. 85, 100956. doi:10.1016/j.preteyeres.2021.100956

Chen, H., Lu, C., Ouyang, B., Zhang, H., Huang, Z., Bhatia, D., et al. (2020). Development of Potent, Selective Surrogate WNT Molecules and Their Application in Defining Frizzled Requirements. Cell Chem. Biol. 27, 598–609. e4. doi:10.1016/j.chembiol.2020.02.009
Janda, C. Y., Dang, L. T., You, C., Chang, J., de Lau, W., Zhong, Z. A., et al. (2017). Surrogate Wnt Agonists that Phenocopy Canonical Wnt and β-catenin Signalling. Nature 545, 234–237. doi:10.1038/nature22306

Kaplan, N., Wang, J., Wray, B., Patel, P., Yang, W., Peng, H., et al. (2019). Single-cell RNA Transcriptome Helps Define the Limbal/corneal Epithelial Stem/early Transplant Amplifying Cells and How Autophagy Affects This Population. Invest. Ophthalmol. Vis. Sci. 60, 3570–3583. doi:10.1167/iovs.19-27656

Langhans, S. A. (2018). Three-dimensional In Vitro Cell Culture Models in Drug Discovery and Drug Repositioning. Front. Pharmacol. 9, 6. doi:10.3389/fphar.2018.00006

Li, W., Hayashida, Y., Chen, Y.-T., and Tseng, S. C. (2007). Niche Regulation of the Epithelium of Human Cornea. Invest. Ophthalmol. Vis. Sci. 48, 3570–3574. doi:10.1167/iovs.06-11371

Li, J.-M., Kim, S., Zhang, Y., Bian, F., Hu, J., Lu, R., et al. (2021b). Single-cell Transcriptomics Identifies Limiting Stem Cell Population and Cell Types Mapping its Differentiation Trajectory in Limbal Basal Epithelium of Human Cornea. Ocul. Surf. 17, 20–32. doi:10.1016/j.jtos.2020.12.004

Li, J.-M., Kim, S., Zhang, Y., Bian, F., Hu, J., Lu, R., et al. (2021b). Single-cell Transcriptomics Identifies a Unique Entity and Signature Markers of Transi Amplifying Cells in Human Corneal Limbus. Invest. Ophthalmol. Vis. Sci. 62, 36. doi:10.1167/iovs.62.9.36

Li, M., Huang, H., Li, L., He, C., Zhu, L., Guo, H., et al. (2021c). Core Transcription Regulatory Circuitry Orchestrates Corneal Epithelial Homeostasis. Nat. Commun. 12, 420. doi:10.1038/s41467-020-20713-2

Meyer-Blazejewska, E. A., Kruse, F. E., Bitterer, K., Meyer, C., Hofmann-Rummelt, C., Wünsch, P. H., et al. (2010). Preservation of the Limbal Stem Cell Phenotype by Appropriate Culture Techniques. Invest. Ophthalmol. Vis. Sci. 51, 765–774. doi:10.1167/iovs.09-4109

Ouyang, H., Yue, Y., Lin, Y., Zhang, X., Xi, L., Patel, S., et al. (2014). WNT7A and PAX6 Define Corneal Epithelium Homeostasis and Pathogenesis. Nature 511, 358–361. doi:10.1038/nature13465

Plass, M., Solana, J., Wolf, F. A., Ayoub, S., Misios, A., Glazier, P., et al. (2018). Cell Type Atlas and Lineage Tree of a Whole Complex Animal by Single-Cell Transcriptomics. Science 360, eaaq1723. doi:10.1126/science.aaq1723

Poe, A. J., Kulkarni, M., Leszcynska, A., Tang, J., Shah, R., Jami-Alahmadi, Y., et al. (2020). Integrated Transcriptome and Proteome Analyses Reveal the Regulatory Role of miR-146a in Human Limbal Epithelium via Notch Signaling. Cells 9, 2175. doi:10.3390/cells9102175

Polisetti, N., Giessler, A., Zankel, M., Heger, L., Dudziak, D., Naschberger, E., et al. (2021). Melanocytes as Emerging Key Players in Niche Regulation of Limbal Epithelial Stem Cells. Ocul. Surf. 22, 172–189. doi:10.1016/j.jtos.2021.08.006

Ramos, T., Scott, D., and Ahmad, S. (2015). An Update on Ocular Surface Epithelial Stem Cells: Cornea and Conjunctiva. Stem Cell Int. 2015, 1–7. doi:10.1155/2015/601731

Singh, A., and Sangwan, V. S. (2021). Mini-review: Regenerating the Corneal Epithelium with Simple Limbal Epithelial Transplantation. Front. Med. 8, 673330. doi:10.3389/fmed.2021.673330

Xiang, C., Du, Y., Meng, G., Soon Yi, L., Sun, S., Song, N., et al. (2019). Long-term Functional Maintenance of Primary Human Hepatocytes In Vitro. Science 364, 399–402. doi:10.1126/science.aau7307

Xu, J., Du, Y., and Deng, H. (2015). Direct Lineage Reprogramming: Strategies, Mechanisms, and Applications. Cell Stem Cell 16, 119–134. doi:10.1016/j.stem.2015.01.013

Yazdapanan, G., Haq, Z., Kang, K., Jabbehadi, S., Rosenblatt, M. I., and Djelilian, A. R. (2019). Strategies for Reconstructing the Limbal Stem Cell Niche. Ocul. Surf. 17, 230–240. doi:10.1016/j.jtos.2019.01.002

Yu, T. T. L., Gupta, P., Ronford, V., Vertès, A. A., and Bayon, Y. (2018). Recent Progress in European Advanced Therapy Medicinal Products and beyond. Front. Bioeng. Biotechnol. 6, 130. doi:10.3389/fbioe.2018.00130

Zhang, C., Mei, H., Robertson, S. Y. T., Lee, H.-J., Deng, S. X., and Zheng, J. J. (2020). A Small-Molecule Wnt Mimic Improves Human Limbal Stem Cell Ex Vivo Expansion. Science 23, 101075. doi:10.1126/science.aau7307

Zheng, M., Tian, C., Fan, T., and Xu, B. (2019). Fibronectin Regulates the Self-Renewal of Rabbit Limbal Epithelial Stem Cells by Stimulating the Wnt11/Fzd7/ROCK1 Canonical Wnt Pathway. Exp. Eye Res. 185, 107681. doi:10.1016/j.exer.2019.05.021

Zhu, J., Wang, L.-y., Li, C.-y., Wu, J.-y., Zhang, Y.-t., Pang, K.-p., et al. (2020). SPARC Promotes Self-Renewal of Limbal Epithelial Stem Cells and Ocular Surface Restoration through JNK and P38-MAPK Signaling Pathways. Stem Cells 38, 134–145. doi:10.1002/stem.3100

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