Response to: HCE-T cells express cornea-specific differentiation marker, PAX6 protein

Lorenz Latta1 · Tanja Stachon1 · Berthold Seitz2 · Nóra Szentmáry1

Received: 27 May 2022 / Revised: 27 May 2022 / Accepted: 7 July 2022 / Published online: 25 July 2022
© The Author(s) 2022

Dear Editor,

We thank Araki-Sasaki et al. for their important comments on our study.

The purpose of our experiments was to investigate to which extent the HCE-T cell line [1] is suitable for analysis of corneal epithelial cell differentiation. Therefore, we compared expression of different markers using qPCR and Western blot in the HCE-T cell line, in human primary limbal epithelial cells (LEC), and in differentiated primary human corneal epithelial cells (pCEC). Expression levels of conjunctival- and corneal-specific keratin and adhesion markers (KRT3, KRT12, KRT13, KRT19, DSG1), stem cell and differentiation markers (PAX6, ABCG2, ADH7, TP63, ALDH1A1), and additional (unvalidated) putative differentiation and stem cell markers (CTSV, SPINK7, DKK1) were examined with qPCR. Additionally, KRT3, KRT12, DSG1, and PAX6 protein levels were analyzed with Western blot [2].

The PAX6 measurement results showed a clear difference at mRNA and protein level between the HCE-T cell line, undifferentiated primary LEC, and pCEC. Although PAX6 mRNA and protein expressions were verifiable in the HCE-T cell line (especially using KSFM), these were detected at a much lower level than those in LEC and the pCEC [2]. This was an unexpected result for us.

The actual data presented by Araki-Sasaki is not quantitative. Therefore, it remains unclear whether our HCE-T cell batch has a lower PAX6 level compared to the original cell batch. In our hands, PAX6 expression could also be detected in the oral mucosa (unpublished data), by RT-PCR. Our statement that PAX6 protein is hardly detectable in the HCE-T cell line is a comparison to the expression levels in differentiated LEC and pCEC [2].

In our publication, we also aimed to point out the difference in expression level of several differentiation and stem cell markers between pCEC, LEC, and the HCE-T cell line [2]. Our study demonstrated a lower KRT3, KRT12, KRT13, KRT19 DSG1, ADH7, ALDH1A1, TP63, CTSV, and SPINK7 mRNA expression in the HCE-T cell line than that in differentiated LEC and in pCEC [2].

Due to the low PAX6 expression levels in the HCE-T cell line (compared to LEC and pCEC), we aimed to accentuate that the HCE-T cell line needs to be kept under special consideration, in case of its use for epithelial cell differentiation studies. In fact, the HCE-T cell line is a well-established model to analyze epithelial cell behavior [1, 3–6]. Nevertheless, we aimed to point out carefully where limitations of a model like the HCE-T cell line might lie. The results of our study may of course differ slightly from results in other laboratories, as other types of antibodies may have been used.

It is extremely important for us to emphasize that we do not want to question that the HCE-T cell line is an appropriate model for studying human corneal epithelial cells in vitro, and we expressly apologize for any misunderstandings.

Funding Open Access funding enabled and organized by Projekt DEAL.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated
otherwise in a credit line to the material. If material is not included in
the article’s Creative Commons licence and your intended use is not
permitted by statutory regulation or exceeds the permitted use, you will
need to obtain permission directly from the copyright holder. To view a
copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

1. Araki-Sasaki K, Ohashi Y, Sasabe T, Hayashi K, Watanabe H, Tano Y, Handa H (1995) An SV40-immortalized human corneal
epithelial cell line and its characterization. Invest Ophthalmol Vis
Sci 36(3):614–21
2. Rubelowski AK, Latta L, Katiyar P, Stachon T, Käsmann-
Kellner B, Seitz B, Szentmáry N (2020) HCE-T cell line lacks
cornea-specific differentiation markers compared to primary
limbal epithelial cells and differentiated corneal epithelium.
Graefes Arch Clin Exp Ophthalmol 258(3):565–575
3. Wu MF, Stachon T, Wang J, Song X, Colanesi S, Seitz B, Wagen-
peil S, Langenbucher A, Szentmáry N (2016) Effect of keratocyte
supernatant on epithelial cell migration and proliferation after
corneal crosslinking (CXL). Curr Eye Res 41(4):466–473
4. Wu MF, Stachon T, Langenbucher A, Seitz B, Szentmáry N
(2017) Effect of amniotic membrane suspension (AMS) and
amniotic membrane homogenate (AMH) on human corneal
epithelial cell viability, migration and proliferation in vitro.
Curr Eye Res 42(3):351–357. https://doi.org/10.1080/02713
683.2016.1192193
5. Wu MF, Stachon T, Seitz B, Langenbucher A, Szentmáry N (2017)
Effect of human autologous serum and fetal bovine serum on human
corneal epithelial cell viability, migration and proliferation in vitro.
Int J Ophthalmol 18;10(6):908–913. https://doi.org/10.18240/ijo.
2017.06.12
6. Shi L, Stachon T, Seitz B, Wagenpfeil S, Langenbucher A, Szentmáry N (2018) The effect of antimicrobial agents on
viability, proliferation and migration of human epithelial
cells, keratocytes and endothelial cells, in vitro. Curr Eye Res
43(6):725–733

Publisher’s note Springer Nature remains neutral with regard to jurisdic-
tional claims in published maps and institutional affiliations.