Reviewer's report

Title: Attempting to distinguish between endogenous and contaminating cytokeratins in a corneal proteomic study

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Reviewer: Katerina Jirsova

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The paper by Mikkel Lyngholm et al., examines the possibility of discriminating between endogenous cytokeratins (CKs) and contaminating CKs in specimens of central corneal and peripheral limbal epithelium in a proteomics study using two-dimensional gel electrophoresis (2D-PAGE) in combination with mass spectrometry (MS).

The aim of the study was to identify the truly endogenous CKs in the described specimens in order to better characterize potential limbal stem cell markers while excluding contaminating CKs.

Although this manuscript presents some interesting findings on CK detection using MS in the corneal and limbal epithelium, the following revisions are suggested.

The following points still remain to be elucidated:

Comments:

1. The authors hypothesized that the higher the frequency that a certain CK is identified by MS from a group of 2D-PAGE spots, the more likely it is to be a contaminating protein. They conclude “that the CKs that were identified with a frequency of <5%, i.e. less than one in every 20th sample, are likely to be endogenous CK and thereby represent a „biologically significant identification”. It should be clarified how this cut-off of 5% was selected and why the contaminating CKs are present in the assessed specimens at a higher frequency than CKs whose expression in the corneal and limbal epithelium has been repeatedly confirmed. Is it really the frequency of CKs or of the detected spots? This should be explained clearly in the Abstract or Introduction section.

2. Due to the varying size of human corneas (10-12 mm usually), the uniform discarding of an area 8-10 mm does not guarantee that the authors examined only the limbal epithelium. It may be that contaminating tissue from the peripheral corneal epithelium is present in some limbal specimens. This fact should be mentioned.

Moreover, the limbus is considered as the border between the cornea and the conjunctiva, but not as the peripheral part of the cornea. I suggest using the term “corneal peripheral epithelium and limbal epithelium” instead of “central and peripheral (limbal) corneal epithelium”. For example:
Background, pg.2: "In the search for stem cell markers, we previously investigated the differences in protein expression between central and peripheral (limbal) corneal epithelium by a proteomic approach" change to: In the search for stem cell markers, we previously investigated the differences in protein expression between the central corneal epithelium and the limbal epithelium by a proteomic approach.

The limbal cornea (located between conjunctiva and the central cornea) is believed to be a niche for the corneal epithelial stem cells. change to: The limbus, which is located between the conjunctiva and the cornea, is a niche for corneal epithelial stem cells.

Fig. 2. Immunohistochemical staining against various antigens. (A) CK 15 and (B) CK 19 appear in limbal corneal epithelium. change to the limbal epithelium

3. The results concerning CK 7, 8, 11, 17 and 18 (compared to those for CK 3, 4, 12, 13 and 15) are not presented or discussed in the text. It is necessary to provide information as to where these CKs were found (central corneal epithelium versus limbal fraction or both); moreover, an appropriate discussion must be added.

4. The differences in the presence of particular CKs in the central corneal and limbal epithelium should be described in the Results and Discussion sections.

5. CKs 5, 6, 14 and 16, which the authors of this study suggest to be contaminants, have already been described in the literature as being present in the corneal epithelial layers, having been repeatedly detected using immunohistochemistry:


Conversely, CK 7 is mostly considered as a conjunctival but not as a corneal epithelium protein.

These discrepancies should be explained/discussed more thoroughly than was already done in the Results and Discussion sections.

6. Immunohistochemistry
The data obtained using immunohistochemistry (CK 19, 3/12 and CK 15) have been published repeatedly and do not provide any new insights. Thus, these data could be deleted in order to allow space for the more interesting results concerning CKs 7, 8, 11, 17 and 18 to be added.

7. Although the manuscript is written in clear English, throughout the text, there are a few small mistakes, for example:
pg.5, line 47: immunohistochemistry... change to: immunohistochemistry
Level of interest: An article of middle importance in its field
Statistical review: I did not assess the statistics in my review.

**Declaration of competing interests:**
I declare that I have no competing interests.