

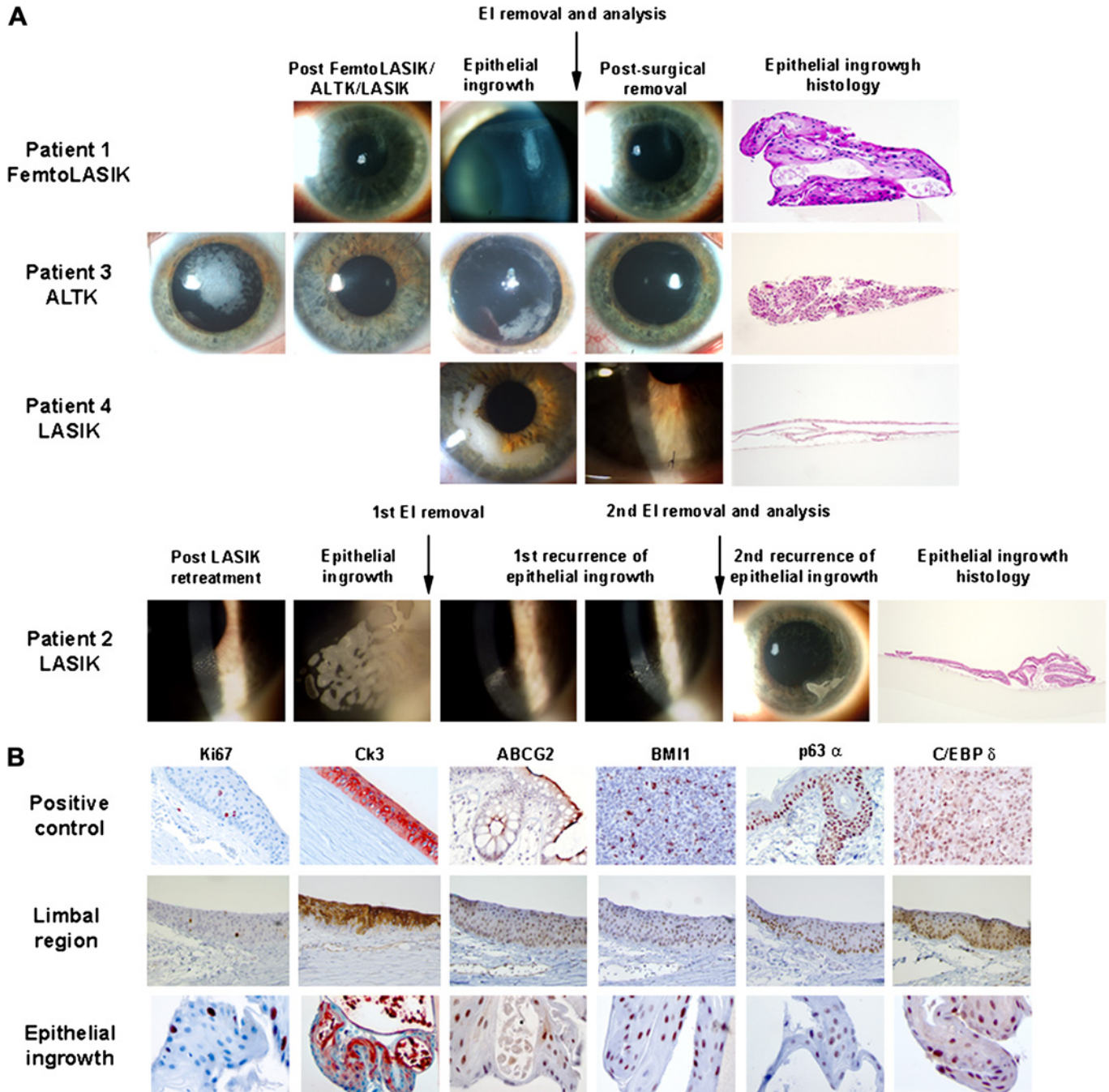
**LETTER**

Epithelial ingrowth cells after LASIK/ALTK (automated lamellar therapeutic keratoplasty): are they corneal epithelial stem cells?

Epithelial ingrowth (EI) is a severe and incompletely understood complication after laser-assisted in situ keratomileusis (LASIK). Its incidence is variable and cases requiring surgical removal occur with a frequency of 0.92%–2.2%.<sup>1,2</sup> Here, we report the clinical, morphological and immunohistological features of EI cells of four patients, two LASIKs, one FemtoLASIK and one ALTK,

with EI that needed surgical removal (figure 1A). Immunohistochemistry was performed using antibodies against CK3, Muc5AC, CK15 and CK19 (differentiation markers) and against p63, BMI1, C/EBP  $\delta$  and BCRP/ABCG2 (stem cell markers) and Ki67 (proliferation marker).<sup>3</sup>

Cytoplasm of superficial squamous cells strongly expressed CK3 with no expression

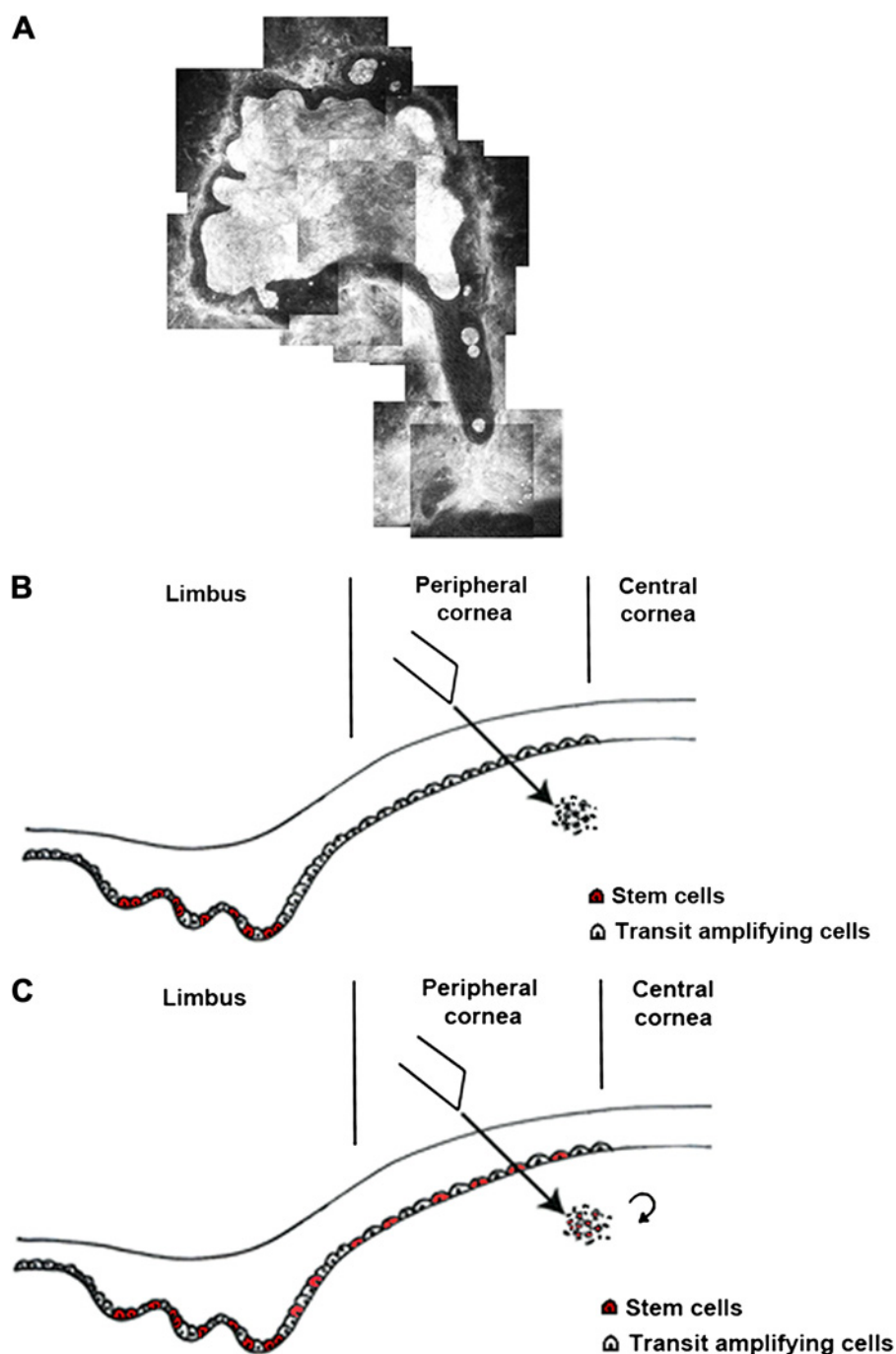


**Figure 1** Slit lamp images of the patients, histopathology and immunostaining of epithelial ingrowth (EI) specimens. (A) Case 1 underwent a Femtolaser-assisted in situ keratomileusis (FemtoLASIK) procedure for hyperopia, cases 2 and 4 a LASIK for myopia and hyperopia, respectively, and case 3 had an automated lamellar therapeutic keratoplasty (ALTK) for a choristoma of the cornea in the context of trisomy 8 in mosaic at 6 years old. All four cases had persistent visually significant EI that needed surgical removal. EI specimens in patients 1, 2 and 3 consist of a 3–4-layered epithelium with basal polyhedral cells and flattened squamous superficial cells whereas in patient 4 it only includes flattened squamous epithelial cells (EI OM 20 $\times$ ). (B) Ki-67 labelling shows an estimated proliferative index of 4%. Immunostaining with antibody anti-CK3 indicates corneal epithelial differentiation. Immunostaining with antibodies anti-ABCG2, anti-Bmi-1, anti-p63  $\alpha$  and anti-C/EBP  $\delta$  suggest stem cell characteristics of the EI cells (positive control and limbal region, OM 20 $\times$  and EI OM 40 $\times$ ).

of Muc5AC, CK19 or CK15. Nuclear expression of BMI1, p63  $\alpha$  and  $\kappa$  and C/EBP  $\delta$  was seen in a majority of cells on EI specimens tested. BCRP/ABCG2 was expressed on the cell membrane and in the cytoplasm and in the nucleus (figure 1B).

There are two hypotheses in the literature to explain the mechanism of EI. The first hypothesis suggests that epithelial cells are introduced in the interface of the corneal flap from the periphery of the cornea. This could happen during cutting, lifting or manipulating the flap. The second hypothesis suggests that EI results from cells migrating continuously from the border of the flap to maintain the epithelial cells in the area of the ingrowth.<sup>2-4</sup> In our cases, EI were separated by a clear area from the flap edge with no visible connection to the surface epithelium. This was confirmed by *in vivo* confocal microscopy in patient N°2 (figure 2A). In patient N°1, the EI was located at the midpoint of a superior hinge more than 2 mm away from the nearest flap edge. These results are in favour of the first hypothesis: EI is an iatrogenic introduction of peripheral corneal epithelial cells in the interface of the flap during surgery (figure 2B). Theoretically, in the periphery of the cornea, epithelial cells are transient amplifying cells (TAC) with a limited potential of self-renewal and no expression of limbal stem cells markers. Surprisingly, our results showed that EI can survive for a long time, up to 36 months, which could not be explained by the limited proliferation potential of TAC, but could only be explained by the presence of cells with stem cell properties in the EI. Furthermore, we showed that these cells are positive for putative stem cells markers (figure 1B). Taken together, these results suggest that epithelial cells in the EI have stem cells or 'stem-like' cells characteristics. These results address another comment: epithelial stem cells in the EI should come from the periphery of the cornea that was reported to be pluripotent epithelial-progenitor cell-free. This postulate is not always true according to recent data in the literature that have shown in mammals the presence of epithelial stem cells throughout the ocular surface. We have published experimental data in animal models that indicate the existence of oligopotent stem cells in the whole corneal epithelium of mammals, suggesting that the renewal of central corneal epithelium is not different from other squamous epithelia.<sup>5</sup> Moreover, clinical reports of the persistence of a clear central corneal epithelium despite a 360° rim of limbal stem cell deficiency provide additional support to the presence of stem or stem-like cells both in the limbus and cornea.<sup>6</sup>

In this study, we present new results that support the first hypothesis. Clinical features of EI (longevity and cells phenotype) are explained by sliding under the flap during surgery corneal epithelial stem cells from the periphery of the cornea (figure 2C).



**Figure 2** Confocal microscopy of epithelial ingrowth (EI) and model of EI formation. (A) Reconstruction of confocal microscopy pictures of patient N°2 showing an isolate of cells composed of epithelial cells, suggesting no connection with the border of the flap. (B) Model of EI formation. Epithelial cells coming from the peripheral cornea, a stem cell-free region, are dragged into the interface of the flap during surgery. (C) New model of EI formation. Epithelial cells that are stem cells or with stem cells characteristics are dragged into the interface of the flap during surgery. These cells have the characteristics of stem cells: they are able to self-renew for a long period of time and they express stem cells markers.

These observations need to be confirmed by additional clinical and experimental data.

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## REFERENCES

1. **Lin RT**, Maloney RK. Flap complications associated with lamellar refractive surgery. *Am J Ophthalmol* 1999;**127**:129–36.
2. **Wang MY**, Maloney RK. Epithelial ingrowth after laser in situ keratomileusis. *Am J Ophthalmol* 2000;**129**:746–51.
3. **Takacs L**, Toth E, Berta A, *et al*. Stem cells of the adult cornea: from cytometric markers to therapeutic applications. *Cytometry A* 2009;**75**:54–66.
4. **Helena MC**, Meisler D, Wilson SE. Epithelial growth within the lamellar interface after laser in situ keratomileusis (LASIK). *Cornea* 1997;**16**:300–5.
5. **Majo F**, Rochat A, Nicolas M, *et al*. Oligopotent stem cells are distributed throughout the mammalian ocular surface. *Nature* 2008;**456**:250–4.
6. **Dua HS**, Miri A, Alomar T, *et al*. The role of limbal stem cells in corneal epithelial maintenance: testing the dogma. *Ophthalmology* 2009;**116**:856–63.