Location of corneal epithelial stem cells

Arising from: Majo, F., Rochat, A., Nicolas, M., Jaoude, G. A. & Barrandon, Y. *Nature* **456,** 250–254 (2008).

The longstanding concept that corneal epithelial stem cells reside mainly in the limbus is supported by the absence of major corneal epithelial differentiation markers, that is, K3 and K12 keratins, in limbal basal cells (these markers are expressed, however, in corneal basal cells, thus distinguishing the mode of keratin expression in corneal epithelium from that of all other stratified epithelia), the centripetal migration of corneal epithelial cells, the exclusive location of slow-cycling cells in the limbal basal layer, the superior *in vitro* proliferative potential of limbal epithelial cells, and the transplanted limbal cells' ability to reconstitute corneal epithelium *in vivo* (reviewed in refs 1–4). Moreover, previous data indicate that corneal and conjunctival epithelia represent two separate cell lineages (reviewed in refs 1–4). Majo *et al.*[5] suggested, however, that corneal and conjunctival epithelia are equipotent, and that identical oligopotent stem cells are present throughout the corneal, limbal and conjunctival epithelia. We point out here that these suggestions are inconsistent with many known growth, differentiation and cell migration properties of the anterior ocular epithelia.

Majo *et al.* suggested that corneal and conjunctival stem cells are equipotent because corneal epithelial cells could form goblet cells, and because cultured (thus somewhat 'de-differentiated') pig corneal and conjunctival cells shared a similar phenotype[5]. They may have overlooked, however, reports showing that cultured rabbit corneal/limbal epithelial cells, but not conjunctival cells, expressed K3/K12 keratins[6–8]; conversely, conjunctival epithelial cells, but not corneal cells, formed goblet cells when transplanted into athymic mice[8,9]. Similar phenotypic specificity was preserved in cultured human limbal/corneal and conjunctival cells[10]. Moreover, human and rabbit studies showed that limbal epithelial cells, but not conjunctival cells, could restore a true corneal epithelium (reviewed in ref. 4). These data have established that limbal/corneal and conjunctival epithelia are not equipotent and that they represent two distinct cell lineages governed by their own stem cells (reviewed in refs 4, 9 and 10).

Majo *et al.* suggested that corneal epithelium contained stem cells because corneal epithelium gave rise to large colonies, serially transplanted mouse central corneal epithelium could regenerate, and transplanted mouse limbal cells did not migrate centripetally[5]. Although their data showed that some pig corneal cells have significant proliferative potential, this property is not unique to stem cells: some transit amplifying cells such as hair matrix are known to be able to divide numerous times. Hence, a more meaningful test is to compare the growth potential of corneal and limbal cells by serially passaging them under identical culture conditions. Such studies have established that rabbit and human limbal cells have a much greater proliferative capacity than corneal cells[7,10]. Moreover, Majo *et al.*'s data (see figure 3b in ref. 5) showed that although corneal cells of rabbit, pig and sheep grew well in primary culture, those of human[10] and calf did not. Such a major species variation argues against the idea that corneal epithelium contains stem cells (which, if they exist, cannot be slow-cycling given that they are undetectable as label-retaining cells[2]). Regarding the ability of corneal epithelium to self-sustain, Huang and Tseng showed that, after limbal removal, rabbit central corneal epithelium can remain apparently intact for a long time until it is wounded, indicating that central corneal cells have a significant maintenance potential until it is perturbed[11]. Finally, Majo *et al.*'s negative finding that limbal cells do not migrate centripetally contradicts many reports establishing that, in intact human[12] and mouse eyes[13,14] (that have not been surgically manipulated) corneal epithelial cells undergo centripetal migration. Collectively, the existing data strongly suggest that corneal epithelial stem cells reside mainly, if not exclusively, in the limbus.

Finally, Majo *et al.*'s model hypothesized that both corneal and conjunctival epithelial cells migrated towards the limbus (the 'tectonic plate confrontation model'). They may have overlooked, however, several reports showing that conjunctival cells do not migrate[15], while corneal cells undergo centripetal, rather than centrifugal, migration[12–14]. We conclude that this model, which suggests (1) that corneal and conjunctival epithelia are equipotent, (2) that identical oligopotent stem cells are distributed throughout the anterior ocular surface epithelium including the central corneal epithelium, and (3) that corneal and conjunctival epithelial cells migrate towards the limbus, is incompatible with existing data.

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Majo et al. *reply*

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Our claim is not that there are no stem cells in the limbus, but that there is more to corneal renewal than the limbus and that the double-dome-shaped structure of the cornea and physical constraints have a crucial impact on cell dynamics[1].

Sun and colleagues[2] imply that in our paper[3] we misused the term 'holoclones' that we defined as stem cells[4]; the central cornea of the pig contains numerous true holoclones, meaning that the cornea of the pig has extensive growth potential and the ability to be serial passaged *in vitro*. We agree that there are species differences among mammals; nonetheless, all corneas that we have investigated, including calf and human, contain colony-forming cells. Fifty cell doublings in pig cornea is not trivial and contradicts the model proposed by Sun and colleagues[5]; we quote their abstract "we demonstrate the existence of a hierarchy of TA cells; those of peripheral cornea undergo at least two rounds of DNA synthesis before they become post-mitotic, whereas those of central cornea are capable of only one round of division". It also does not agree with Huang and Tseng's experiment[6] showing "that, after limbal removal, rabbit central corneal epithelium can remain apparently intact for a long time until it is wounded, indicating that central cornea cells have a significant maintenance potential".

Our results show that corneal cells can form goblet cells when they migrate onto a conjunctival environment (in mouse) or generate true goblet cell colonies when cloned (in pig). Corneal differentiation is found in human conjunctiva[7], conjunctival cells may be successfully transplanted in the human to replace cornea[8], and there are reports of cornea remaining transparent for years in limbal deficiency[9]. Furthermore, corneal cells[10], like conjunctival cells (our unpublished results), can form hairy skin when exposed to an inductive skin microenvironment, indicating a greater plasticity than anticipated and that stem cell fate strongly depends on stromal signals.

We are not aware of any paper that clearly demonstrates stem cell migration from the limbus. Buck[11] in his landmark paper has not demonstrated basal cell migration; we quote his abstract: "the median distance migrated was about 17 μm per day. This figure represents the distance through which superficial and wing cells had migrated; the distance migrated by basal cells was not determined". Nagazaki and Zhao[12] have presented evidence of movement in the cornea but not that the migrating cells actually originated from the limbus ('from' is not the same as 'near'). An overcrowding of the corneal epithelium, a source of tension and sliding as previously emphasized by Sun and colleagues[13], or sequential activation of the β-actin promoter can easily explain these observations. Similarly, the spiral stripe organization mixing clockwise and counterclockwise clones[14] is highly reminiscent of centrifugal growth originating from a small number of stem cells originally located in central cornea. This biological model occurs widely in nature, for instance in the growth of a daisy, as the easiest and most efficient way to fill space, a notion supported by mathematical models[15] and a clothoid growth model (Euler spiral).

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