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Computer simulation of neutral drift among limbal epithelial stem cells of mosaic mice

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ABSTRACT

The use of mice that are mosaic for reporter gene expression underlies many lineage-tracing studies in stem cell biology. For example, using mosaic *LacZ* reporter mice, it was shown that limbal epithelial stem cells (LESCs) around the periphery of the cornea maintain radial sectors of the corneal epithelium and that radial stripe numbers declined with age. Originally, the corneal results were interpreted as progressive, age-related loss or irreversible inactivation of some LESC clones. In this study we used computer simulations to show that these results could also be explained by stochastic replacement of LESCs by neighbouring LESCs, leading to neutral drift of LESC populations. This was shown to reduce the number of coherent clones of LESCs and hence would coarsen the mosaic pattern in the corneal epithelium without reducing the absolute number of LESCs. Simulations also showed that corrected stripe numbers declined more slowly when LESCs were grouped non-randomly and that mosaicism was rarely lost unless simulated LESC numbers were unrealistically low. Possible reasons why age-related changes differ between mosaic corneal epithelia and other systems, such as adrenal cortices and intestinal crypts, are discussed.

1. Introduction

Two related types of observations with chimaeric or mosaic mice, or from lineage-tracing experiments, suggest that, in some tissues, progressive changes in the pattern of variegation result from stem cells being lost, irreversibly inactivated or replaced. The first observation involved the loss of one of two cell populations, from intestinal crypts of chimaeric mice (Ponder et al., 1985; Schmidt et al., 1988). This loss of mosaicism occurred between birth and adulthood and was termed 'crypt purification' by the authors. The second type of observation is exemplified by the age-related coarsening of variegated patterns in corneal epithelia of adult chimaeric and mosaic mice, comprising two genetically distinct cell populations (Collinson et al., 2002; Mort et al., 2009). This is as shown in Fig. 1A. Equivalent results have been reported recently, using tamoxifen-inducible lineage tracing to label K14-positive progenitor cells with the multi-coloured, R26-confetti marker at 6 weeks (Richardson et al., 2017).

Similar observations have also been made using lineage tracing to label lineages derived from zebrafish skeletal muscle stem cells (Nguyen et al., 2017) and stem cell-derived lineages in other mouse tissues (reviewed by Klein and Simons, 2011), including testis (Nakagawa et al., 2007) and intestinal epithelium (Lopez-Garcia et al., 2010; Snippert et al., 2010). In contrast, however, the stem cell-derived pattern of radial stripes in the adrenal cortex of mosaic transgenic mice did not coarsen with age (Chang et al., 2011).

Lineage tracing in intestinal crypts showed that a progressive coarsening of mosaic patterns in mixed crypts preceded loss of mosaicism, when stem cells were labelled in adults at different ages (Lopez-Garcia et al., 2010; Snippert et al., 2010). This showed that both coarsening and loss of mosaicism were time dependent rather than strictly age dependent. However, we know of no evidence that coarsening of mosaic patterns frequently leads to loss of mosaicism in other tissues and this has not been reported for the corneal epithelium (Collinson et al., 2002; Mort et al., 2009).

There is good evidence that the stem cells that replenish the mouse corneal epithelium, during normal homeostasis, reside in the basal layer of the limbal epithelium (Amitai-Lange et al., 2015; Di Girolamo et al., 2015; Dorà et al., 2015; Kasetti et al., 2016; Lobo et al., 2016; Sun et al., 2010; West et al., 2015). This is a narrow, ring-shaped transition zone between the corneal epithelium and conjunctiva, and the stem

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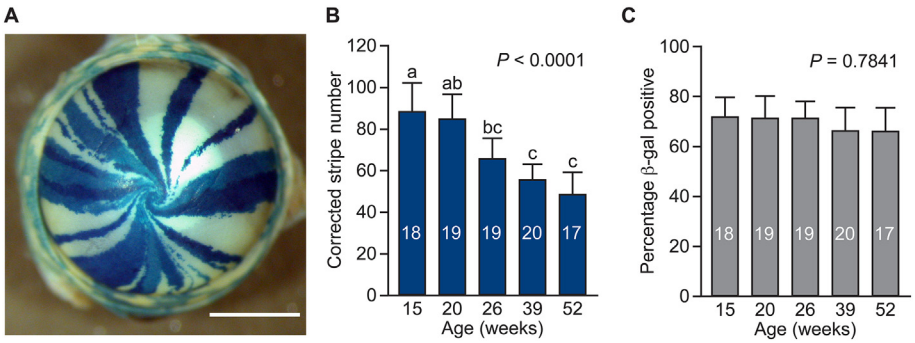

Fig. 1. Stripe patterns in the corneal epithelium of mosaic mice.
Analysis of corneal epithelial stripes in adult *XLacZ*, X-inactivation mosaic mice, modified after Mort et al. (2009) with permission of the authors and using only data from the left eyes. (A) Radial striped pattern of β-galactosidase (β-gal) staining in the corneal epithelium of an intact eye from an adult mosaic mouse. (B) Corrected stripe number in left eyes at 5 ages showing a significant reduction. (C) Percentage of β-gal positive cells in left eyes at 5 ages. Error bars are 95% confidence intervals (CI). 1-way ANOVA *P*-values are shown. Letters above the bars in (B) denote which pairs of ages differ significantly by Bonferroni post-hoc tests. Bars with only different letters (e.g. 15 vs. 26 weeks) differ significantly ($P < 0.05$) but bars with a letter in common (e.g. 15 vs. 20 weeks) do not differ significantly. Sample numbers are shown within each bar. Scale bar: 1 mm.

cells are known as limbal epithelial stem cells (LESCs). The pattern of radial stripes, which occurs in the corneal epithelium of mosaic mice, has been interpreted as clonal lineages of transient (or transit) amplifying cells (TACs), which are produced by LESCs at the periphery and move centripetally to maintain the tissue (Collinson et al., 2002; Mort et al., 2009).

The age-related coarsening of radial stripes in the corneal epithelium of chimaeras and mosaics was quantified by counting the number of stripes. After correction, to factor out the numbers of adjacent stripes of the same population, the corrected stripe number provides an indirect means of comparing LESC function in different groups. This is not a direct estimate of the number of active LESCs but it estimates the number of coherent clones of LESCs and is useful for comparing LESC function at different ages. The corrected stripe number in the corneal epithelium of X-inactivation mosaic mice declined with age (Fig. 1B and Mort et al., 2009). A similar decline was also demonstrated over more limited age ranges for other groups of mosaic and chimaeric mice (Collinson et al., 2002, 2004; Mort et al., 2011).

This age-related decline in corrected stripe numbers in the corneal epithelium was previously interpreted as a decline in the number of active LESC clones caused either by progressive loss or irreversible inactivation of LESCs without replacement, so that each LESC maintained a larger area of the corneal epithelium (Collinson et al., 2002; Mort et al., 2009). Although these mechanisms might also account for progressive coarsening of variegated patterns reported for other tissues, such changes have mostly been attributed to stochastic neutral clonal drift without a reduction in active stem cell numbers (Klein and Simons, 2011; Lopez-Garcia et al., 2010; Nakagawa et al., 2007; Nguyen et al., 2017; Snippert et al., 2010). It has also been suggested that stochastic neutral drift might explain the reported age-related decline in corrected stripe number in the mosaic corneal epithelium (Klein and Simons, 2011; Mort et al., 2012; Richardson et al., 2017). Although this is feasible, there is currently no evidence that favours neutral drift over LESC loss, irreversible inactivation or any combination of these three mechanisms.

Stochastic neutral drift could occur in the corneal limbus if some LESCs were replaced by neighbouring LESC lineages without any net loss in LESC numbers. This might usually require some LESCs to divide symmetrically, to produce two LESCs or two TACs, rather than asymmetrically, to produce one LESC and one TAC. Population asymmetry would be maintained if the two types of symmetric LESC divisions were balanced and this could be regulated either cell-autonomously or by extrinsic factors, as discussed by Klein and Simons (2011). One hypothetical type of extrinsic regulation is illustrated in Fig. 2, to show how LESC replacement might occur.

However, it is unclear whether LESC replacement would affect the quantitative changes in corrected stripe number in the corneal epithelium in the same way as the uncorrected stripe number. This is because LESC replacement is likely to change the proportions of the two LESC populations and this would affect the correction factor and so would alter the relationship between the uncorrected and corrected stripe numbers. Furthermore, as LESC replacement might increase, as well as decrease, the number of stripes in a mosaic corneal epithelium (Fig. 2), it is not intuitively obvious that it would inevitably result in loss of mosaicism, even after a large number of LESC generations.

Our main aim was to test the hypothesis that stochastic LESC replacement, leading to neutral drift, could account for the observed reduction in corrected stripe numbers in the corneal epithelium. For this, we simulated the effects of stochastic LESC loss (without replacement) and stochastic LESC replacement in a simulated mosaic limbal epithelium. This comprised two simulated LESC populations that were distributed around a limbal ring, either randomly or in coherent clonal groups. We then determined the consequences for the proportions of the two LESC populations, the uncorrected stripe number and the corrected stripe number. Our secondary aim was to consider why ageing affected mosaic patterns in the mouse adrenal cortex, corneal epithelium and intestinal crypts differently. For this, we investigated variables that slowed the reduction in corrected stripe numbers or favoured loss of mosaicism in the simulations.

2. Materials and methods

2.1. Assumptions of the computer model

Some β-galactosidase (β-gal) positive stripes in mosaic corneas appear paler than others (Fig. 1A) and this has been attributed to clonal variation in transgene expression (Mort et al., 2009). However, only two populations of stem cells (blue and white) were simulated. This is consistent with biological studies with this mosaic system, which did not distinguish between β-gal-positive cells with different levels of staining (Collinson et al., 2002; Mort et al., 2009).

To mimic our previous studies, the computer model simulates the distribution of two LESC populations ('blue' and 'white') that occupy the basal layer of the narrow ring of limbal epithelium, in a *LacZ* mosaic mouse. In a mouse, the LESCs would produce blue and white TACs, which would move into the corneal epithelium and across the radius to the centre. The mouse corneal epithelium is about 5–6 cells thick but the striped patterns in corneas of mosaic mice (Fig. 1A) are effectively 2-dimensional, because the upper more differentiated layers are derived directly from the underlying basal layer of TACs. Two-dimensional patterns of radial stripes can be represented by 1-dimensional rings so, for example, the ring at the border between the limbus and the corneal epithelium represents the distribution of early generation TACs, where they enter the corneal epithelium. In the mouse, the basal layer of limbal epithelium between the corneal epithelium and conjunctiva forms a narrow 2-dimensional annulus rather than a 1-dimensional ring. However, for the purposes of the simulation, we assume that the LESCs form a 1-dimensional ring and each LESC has only two neighbouring LESCs.

In the simulated 1-dimensional ring of blue and white LESCs, a