Letter to the Editor

Lens regeneration in mice: implications in cataracts

Mindy K. Calla, Matthew W. Grogg, Katia Del Rio-Tsonis, Panagiotis A. Tsonis

Laboratory of Molecular Biology, Department of Biology, University of Dayton, Dayton, OH 45469-2320, USA

Department of Zoology, Miami University, Oxford, OH 45056, USA

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Abstract

Lens regeneration in adult mice is possible when the lens capsule is left behind after lentectomy. The lens is regenerated by the remaining adherent lens epithelial cells, which differentiate to form lens fibres within days, showing normal morphology and bow regions. Epithelial to mesenchymal cell transformation is also seen during the early stages. The mouse, therefore, can become an indispensable animal model for cataract research, surgery and therapy.

Keywords: lens; regeneration; mouse; cataract

Traditionally, the newt has been hailed as the most powerful animal model for lens regeneration (Del Rio-Tsonis and Tsonis, 2003). True enough adult newts can always replace their lens upon removal. Lens regeneration in newts is achieved by transdifferentiation of the pigment epithelial cells from the dorsal iris. Other amphibians, such as frogs, are capable of lens regeneration by transdifferentiation of the cornea, but only during a short window of time before metamorphosis (Freeman, 1963). The situation in higher vertebrates, especially in mammals, is very different. Lens regeneration has been shown in rabbits, but only if the lens capsule is left behind (Gwon et al., 1990). Obviously, some lens epithelial cells remain attached to the lens capsule and they differentiate to lens fibres to ‘regenerate’ a lens, which nevertheless is not perfect. Some similar, but limited observations have been seen in cats (Gwon et al., 1993). The studies with rabbits suggest that while lens regeneration does not follow the traditional road of transdifferentiation as in newts, regeneration can nevertheless occur by differentiation of lens epithelial cells remaining on the capsule. Rabbits (or cats), however, are not favorable mammalian animal models for approaching the problem of lens regeneration with the frontline technology of molecular biology. Therefore, we have turned our attention to mice.

We used three different strains in our study, Balb/c, NZW and MRL/MpJ. The mice were sexually mature (8–12 weeks old) of both sexes. Before operation, mice were anesthetized with ketamine (87 mg kg\(^{-1}\)) in combination with xylazine (13 mg kg\(^{-1}\)). Two types of operations were performed. In one set, the lens along with the capsule was removed and in the other set, the capsule was left behind (with only part of the anterior capsule damaged). Integrity and restoration of the normal shape of the capsule is important. To remove the lens, a corneal incision was made with a sharp blade. Due to the small size of the mouse eye, we preferred to remove the lens with fine forceps by applying pressure in the eye. Such an operation results, as in the newts, in removal of the lens but not the capsule. After such extracapsular extraction, we injected saline solution to clean the capsule. Histological preparations of eyes after lentectomy showed a rather clean capsule with adherent lens epithelial cells. After lentectomy, the mice were collected in time intervals starting at 2 days and ending at 30 days post lentectomy. We found that when the whole lens (with the capsule) was removed no lens regeneration resulted in any case (Table 1). In this respect, and as expected, mice do not regenerate their lens by transdifferentiation of the pigment epithelial cells of the dorsal iris.
as seen in adult salamanders. On the contrary, to what was previously assumed, intraperitoneal retinol palmitate injections (50 IU mouse$^{-1}$ in 50 μl solution) every other day, failed to induce lens regeneration from the dorsal iris (Shekhawat et al., 2001). For this experiment we used the same mouse strains and age as indicated above (Table 1).

However, in eyes where the capsule was left behind, regeneration of the lens was achieved in 100% of the cases in all strains (Table 1). The growth of the lens was extremely rapid, the capsule filled with differentiated lens fibres within a few days. When the eyes were examined 30 days post-lentectomy, the regenerated lens was of a considerable size (at least half of the intact lens) with morphology displaying an established equator with well differentiated bow regions (Fig. 1(a)–(c)). Differentiation of lens fibres was evident even at day 2 post-lentectomy, as revealed by histology and staining with a lens fiber-specific antibody to β-crystallin (Sawada et al., 1993) (Fig. 1(a),(d)–(f)). This antibody has been shown to be specific for β-crystallin. The same results were received for all three strains and both sexes with or without vitamin A treatment (Table 1).

Interestingly, we also observed transformation of lens epithelial cells to mesenchymal cells (EMT) at the posterior part of the capsule. EMT, as judged by the marker smooth muscle α-actin, was evident at early stages, but was diminished considerably after 20 days post-lentectomy (Fig. 1(g)–(i)). This argues that in the beginning there is

### Table 1

<table>
<thead>
<tr>
<th>Mouse Strain</th>
<th>With capsule</th>
<th>Without capsule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balb/c</td>
<td>25/25</td>
<td>0/83</td>
</tr>
<tr>
<td>MRL</td>
<td>11/11</td>
<td>0/24</td>
</tr>
<tr>
<td>NZW</td>
<td>9/9</td>
<td>0/24</td>
</tr>
<tr>
<td>Balb/c with vitamin A</td>
<td>30/30</td>
<td>0/24</td>
</tr>
<tr>
<td>MRL with vitamin A</td>
<td>15/15</td>
<td>0/15</td>
</tr>
<tr>
<td>NZW with vitamin A</td>
<td>15/15</td>
<td>0/15</td>
</tr>
</tbody>
</table>

Numbers represent lens/eye.

![Fig. 1](image-url) (a) A histological section through a regenerating lens 2 days post-lentectomy. Note the differentiation of lens fibres (arrows) at the bow region. The posterior capsule is also shown intact (arrowhead) × 200. (b) A regenerated lens 30 days post-lentectomy. Note the normal morphology and size of the regenerated lens × 40. (c) A close-up at the bow region of a regenerated lens 30 days post-lentectomy. Note the normal morphology and differentiation of lens fibres (arrowhead); le, lens epithelium; lc, lens capsule; lf, primary lens fibres × 100. (d–f) Representative sections of regenerating lenses 5, 10 and 20 days post-lentectomy, respectively. Expression of crystallin (green) and type IV collagen (red) (BioDesign) depicting lens fibre differentiation and the lens capsule, respectively. × 200 (d) × 400 (e,f). (g–i) Representative sections of regenerating lenses 5, 10 and 20 days post-lentectomy, respectively. Expression of type IV collagen (red) and smooth muscle α-actin (green) (Sigma). Note that some α-actin positive cells (arrows) can be seen in the posterior part of the regenerating lens during the early stages of lens regeneration, indicating transformation of lens epithelial cells to mesenchymal cells. EMT is largely diminished in the regenerating lens by 20 days post-lentectomy (i) × 400.
a characteristic wound healing response, but later appears to follow a more characteristic differentiation process. Despite the fact that the lens shows normal differentiation, we should stress here that while we call this lens regeneration (as in the case of rabbits) caution should be exercised because of the lack of functional studies.

While several reasons, such as the type of operation (incision of anterior capsule) or age of animals come to mind to explain these positive results, these findings have two major implications. First, they demonstrate that mammals might possess much stronger potential for lens repair than originally thought and, therefore, extending such studies in higher mammals, including humans are now warranted. Indeed, after submission of this manuscript we found out about a similar study using rats (Lois et al., 2003). Second, mouse models might revolutionize cataract research and surgery. The traditional cataract surgery requires that the posterior capsule remains intact to hold the synthetic lens. This, however, could lead to the development of secondary cataracts by opacification of the posterior capsule. This opacification is the result of transformation of the remaining lens epithelial cells to mesenchymal cells (Ibaraki, 1997). Since in our experiments EMT was seen during the early stages but diminished in later ones, mouse lens regeneration could become an indispensable model to study factors that are implicated in the aetiology, inhibition or reversal of EMT. Lens regeneration experiments with knock-out or transgenic mice will open new avenues in this field. Likewise, experimentally induced cataract can be studied in regeneration models and provide insights about possible regeneration therapy.

Acknowledgements

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References