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Review

Signaling during lens regeneration

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Abstract

The newt is one of the few organisms that is able to undergo lens regeneration as an adult. This review will examine the signaling pathways that are involved in this amazing phenomenon. In addition to outlining the current research involved in elucidating the key signaling molecules in lens regeneration, we will also highlight some of the similarities and differences between lens regeneration and development.

Keywords: Regeneration; Newt; Transdifferentiation

Contents

1. Introduction

Regeneration of lost body parts is a remarkable phenomenon that few organisms can undergo. Some salamanders, among them the adult newt, *Notophthalmus viridescens*, have remarkable regenerative abilities. The newt is capable of regenerating most tissues, one of which is the lens. Lens regeneration occurs through a process of transdifferentiation. In this process, pigmented epithelial cells (PECs) of the dorsal iris dedifferentiate and ultimately differentiate into a completely different cell type to form a lens. While much attention has been paid to the process of lens regeneration in the newt, the exact mechanism by which this feat is accomplished is not yet fully understood. One thing that is apparent, however, is the importance of signal transduction mechanisms in inducing lens regeneration. Most speculation on the signal transduction pathways involved in lens regeneration comes from developmental studies. It is a widely held belief that the signaling and induction events that occur during eye and lens development play similar roles in lens regeneration as both processes accomplish the same feat. In recent years, research has started to delineate some of the signaling involved in lens regeneration. Many of the common signal transduction pathways have not been thoroughly examined dur-

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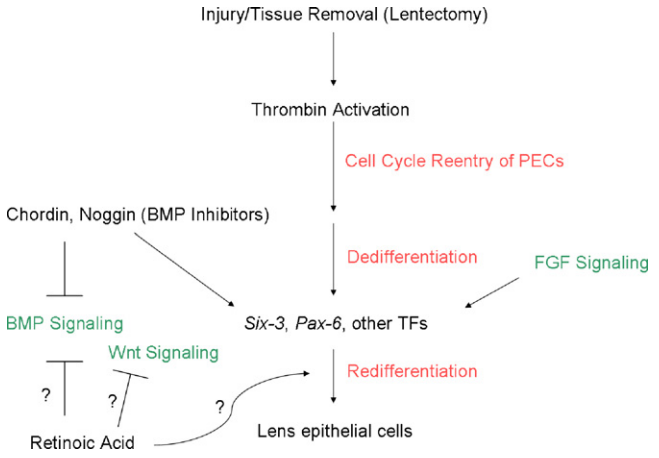

Fig. 1. Outline of events and pathways likely to be involved in lens regeneration. Upon lens removal or injury, PECs must re-enter the cell cycle and thrombin is suspected to play a role in this. Following proliferation, the PECs dedifferentiate and then redifferentiate. The BMP, Wnt and FGF pathways play pivotal roles in lens development. BMP antagonists have been shown to upregulate TFs such as *Six*-3 in the newt during regeneration as well as induce lens regeneration. RA has been shown to inhibit BMP and Wnt signaling in other systems, but the mechanism through which it works in inducing (along with *Six*-3) newt lens regeneration is unclear. Question marks depict possible roles of RA for induction of lens regeneration.

ing lens regeneration and much more remains to be discovered (Fig. 1). The fact that the newt genome is not sequenced has hindered this to some extent, but despite this progress is being made. In this review, we will highlight what is known regarding cell signaling in lens regeneration.

2. Process of lens regeneration

Following removal of the lens, the regenerative process begins with dedifferentiation of the PECs of the dorsal iris. Dedifferentiation marks the beginning of cell cycle re-entry and proliferation. At approximately 10 days post-lentectomy depigmented PECs form a vesicle at the tip of the dorsal iris [1,2]. Cells in the inner layer of the vesicle begin to elongate and differentiate into primary lens fiber cells at 12–16 days post-lentectomy. This time period is also marked by proliferation and crystallin synthesis. At day 15–20 post-lentectomy, primary lens fiber cells continue to grow from the inner layer while cells from the outer layer of the vesicle begin to form secondary fibers. A complete lens is formed 25 days following removal [3,4].

3. Thrombin signaling pathway

One area of signaling often overlooked in regeneration is the injury response cascade, particularly the coagulation pathway involving thrombin. Injury leads to the activation of thrombin by the release of prothrombin (inactive form) from the vasculature. While injury results in the release of prothrombin, it is the presence of a membrane protein, Tissue Factor (clotting factor III), which is actually responsible for the activation of thrombin [5]. Thrombin is known to play a role in cell cycle re-entry on newt cells. Treatment of newt skeletal myotubes with thrombin induces proliferation, whereas mouse myotubes do not exhibit the same response [6]. This species-specific response led to speculation that thrombin might have an important role in the cell cycle re-entry of postmitotic cells known to occur in regenerating tissues. This speculation was further supported by Simon and Brockes when they showed that pigmented epithelial cells (PECs), both dorsal and ventral, of adult newt iris could be stimulated to re-enter S-phase of mitosis with thrombin treatment in vitro [7]. Perhaps the strongest support for a role of thrombin in lens regeneration came from Imokawa and Brockes when they were able to show thrombin activation at the pupillary margin of the dorsal iris during lens regeneration in the newt from 20 min post-lentectomy until about 7 days post-lentectomy [8]. No thrombin activation was seen in the regeneration incompetent ventral iris at any stage [8]. Importantly, inhibition of thrombin led to a loss of cell cycle re-entry at the dorsal margin and inhibition of or incomplete lens regeneration [8,9]. The final support for a role of thrombin signaling in lens regeneration comes from the axolotl, another urodele capable of limb, but not lens, regeneration. Activated thrombin was detected in the mesenchymal tissue of the blastema during limb regeneration, but not seen at any stage in the iris following lentectomy. Future studies on the expression pattern of Tissue Factor, the activator of prothrombin, will be important to clarify this issue. Based on their findings, Imokawa and Brockes suggest that Tissue Factor should be expressed in the dorsal margin of the newt during regeneration and absent in the axolotl iris [8,9]. These studies provide strong evidence for a role of thrombin in postmitotic cell cycle re-entry of newt iris PECs both in vitro and in vivo.

4. FGF signaling pathway

Fibroblast growth factors and their receptors are critical for various stages of lens development, including induction, proliferation and differentiation. FGFs play a dominant role in initiating fiber differentiation and regulating the spatial and temporal pattern of crystallin gene expression [10,11]. In chicks, ectopic FGF8 expression in the distal optic vesicle leads to the expansion of the lens field [12]. Targeted overexpression of FGFs in lenses of transgenic mice leads to inappropriate proliferation and differentiation of the lens epithelium [13–15].

Several of the FGF ligands have also been found to be involved in lens regeneration. Del Rio-Tsonis et al. were the first to show the production of a second lens after FGF4 treatment in lentectomized eyes. Treatment of lentectomized newts with both FGF1 and FGF4 induced lenses with abnormal polarity due to differentiation of lens epithelial cells to lens fibers and double lens formation from the dorsal iris [16]. These abnormalities in the regenerating lenses appeared to be similar to the lens polarity abnormalities induced during lens development in transgenic FGF mice [17]. FGF2 and FGF4 have been shown to be essential for in vitro lens regeneration from the pigmented cells of the dorsal iris [18]. Dorsal iris cellular reaggregates cultured on collagen coated dishes developed lenses in vitro only when treated with FGF2/4 [18]. Hayashi et al. have shown that intraocular injection of recombinant FGF2 can trigger lens regeneration from the dorsal iris without previously removing the host lens.