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Review

Fibrosis in the lens. Sprouty regulation of TGFβ-signaling prevents lens EMT leading to cataract

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ABSTRACT

Cataract is a common age-related condition that is caused by progressive clouding of the normally clear lens. Cataract can be effectively treated by surgery; however, like any surgery, there can be complications and the development of a secondary cataract, known as posterior capsule opacification (PCO), is the most common. PCO is caused by aberrant growth of lens epithelial cells that are left behind in the capsular bag after surgical removal of the fiber mass. An epithelial-to-mesenchymal transition (EMT) is central to fibrotic PCO and forms of fibrotic cataract, including anterior/posterior polar cataracts. Transforming growth factor β (TGFβ) has been shown to induce lens EMT and consequently research has focused on identifying ways of blocking its action. Intriguingly, recent studies in animal models have shown that EMT and cataract developed when a class of negative-feedback regulators, Sprouty (Spry)1 and Spry2, were conditionally deleted from the lens. Members of the Spry family act as general antagonists of the receptor tyrosine kinase (RTK)-mediated MAPK signaling pathway that is involved in many physiological and developmental processes. As the ERK/MAPK signaling pathway is a well established target of Spry proteins, and overexpression of Spry can block aberrant TGFβ-Smad signaling responsible for EMT and anterior subcapsular cataract, this indicates a role for the ERK/MAPK pathway in TGFβ-induced EMT. Given this and other supporting evidence, a case is made for focusing on RTK antagonists, such as Spry, for cataract prevention. In addition, and looking to the future, this review also looks at possibilities for supplanting EMT with normal fiber differentiation and thereby promoting lens regenerative processes after cataract surgery. Whilst it is now known that the epithelial to fiber differentiation process is driven by FGF, little is known about factors that coordinate the precise assembly of fibers into a functional lens. However, recent research provides key insights into an FGF-activated mechanism intrinsic to the lens that involves interactions between the Wnt-Frizzled and Jagged/Notch signaling pathways. This reciprocal epithelial-fiber cell interaction appears to be critical for the assembly and maintenance of the highly ordered three-dimensional architecture that is central to lens function. This information is fundamental to defining the specific conditions and stimuli needed to recapitulate developmental programs and promote regeneration of lens structure and function after cataract surgery.

Cataract is defined as the loss of transparency of the eye lens, and accounts for much of the world's blindness. Cataract has widely variable phenotypes and hence several classifications, including nuclear, cortical, anterior and posterior polar and total (Francis et al., 1999). Cataracts can appear in association with various systemic diseases (Beiran et al., 1994), presenting diverse phenotypes; however, by far the most common contributing factor for cataract is aging (Mukesh et al., 2006; Vinson, 2006). Over the years much effort has been directed towards understanding the etiology of human cataract. In addition to identifying a strong genetic component (as in congenital cataracts), recent progress has been made in identifying factors that influence the stability of the long-lived proteins in the lens as well as the sites on these proteins that show marked deterioration in age-related cataract. This has led to the view that lens opacification is the result of cumulative age-related modifications to lens proteins (Truscott and Friedrich, 2014). How to prevent or ameliorate these protein modifications provides a major challenge for lens researchers in the future.

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To date, the only way to restore visual loss caused by cataract is surgery (Brian and Taylor, 2001). This is the most common ophthalmic procedure and involves removal of the opaque fiber mass followed by implantation of a synthetic intraocular lens (IOL) for restoration of vision (Awasthi et al., 2009). While modern surgery is largely effective, it is not without complications and the most frequent is posterior capsular opacification (PCO), also referred to as secondary cataract (Awasthi et al., 2009; Spalton, 2013; see Miyamoto et al., 2014). Consequently there is a strong drive towards gaining a greater understanding of the key cellular processes and molecular mechanisms responsible for PCO. This will provide the platform for devising molecular strategies for reducing the incidence of PCO and improving the outcome of cataract surgery. Here, we will consider some of the important molecules and signaling pathways leading to cataract formation, and the importance of tightly regulating them for maintenance of normal lens growth, architecture and function.

1. Epithelial mesenchymal transition (EMT)

A common feature of fibrotic PCO and polar cataracts is the loss of lens epithelial cell integrity, associated with aberrant proliferation, migration and most significantly a change in cell morphology, with cells distancing themselves from their ectodermal epithelial origin and transforming into more mesodermal-derived mesenchymal-like cells (see Fig. 1). This biological process, known as an epithelial to mesenchymal transition (EMT), is normal for the early gastrulating embryo, but also presents itself in tissue repair and pathology, including cancer and cataract (Kalluri and Neilson, 2003). EMT characteristics include the acquisition of a spindle-shaped cellular morphology that is accompanied by accumulation of α-smooth muscle actin (αSMA) and redistribution of actin stress fibers, loss of cell polarity and epithelial markers such as cytokeratin and ZO-1, loss of E-cadherin and expression of transcription factors including Snail (Snai1), Slug (Snai2) and Twist (Fig. 2; Zeisberg and Neilson, 2009). αSMA is a common marker of active fibroblasts, but it is not specific for fibroblasts (Zeisberg and Neilson, 2009). It is one of six actin family members, and its expression has been shown to correlate with the EMT process that occurs during normal development, fibrosis or in cancer progression (Kalluri and Weinberg, 2009). E-cadherin is a calcium-dependent membrane-associated cell–cell adhesion molecule (van Roy and Berx, 2008), predominantly present in epithelial cells (Takeichi, 1991). Localized to the plasma membrane, E-cadherin complexes with β-catenin and αE-catenin, key functional components of adherens junctions (van Roy and Berx, 2008; Wijnhoven et al., 2000); hence, its loss or change in distribution promotes a loss of epithelial phenotype, characteristic of the EMT process. The newly established mesenchymal cell type also possesses elevated migratory and invasive properties, increased resistance to apoptosis, and exaggerated production of extracellular matrix (ECM) components (Kalluri and Weinberg, 2009). It is through this process that we see fibrosis in the lens, characterized primarily by the accumulation of excess connective tissue that obliterates not only normal lens structure but most importantly its function.

2. Growth factors trigger EMT

EMT can be triggered by aberrant signaling of various molecules, such as epidermal growth factor (EGF), fibroblast growth factor (FGF), insulin-like growth factor (IGF-II), hepatocyte growth factor (HGF), and Notch (Savagner et al., 1997; Morali et al., 2001; Strutz et al., 2002; Timmerman et al., 2004; Ahmed et al., 2006; Matsuno et al., 2012); however, it is transforming growth factor beta (TGFβ) that is the most well-known EMT inducer in both normal and pathological conditions (Fig. 2; Zavadil and Bottinger, 2005). The TGFβ superfamily comprises over 30 TGFβ-related members, that include TGFβ isoforms, activins, inhibins, bone morphogenic proteins (BMPs), and many other structurally related factors, in vertebrates, insects and nematodes (Massague et al., 1994; Moustakas et al., 2001). TGFβ is involved in the regulation of cell growth, differentiation, migration, adhesion, organization, senescence and extracellular matrix production. Its signaling normally promotes growth and development during early embryogenesis, whereas in mature tissues, they usually induce either cytostatic or apoptotic responses, depending on the type and state of the cell (Massague and Wotton, 2000). There are 28 genes encoding these members (Venter et al., 2001) and they all show sequence similarity to the prototype TGFβ1 (Massague et al., 1994), which naturally occurs as a secreted homodimeric protein. Three isoforms of TGFβ, namely TGFβ1, TGFβ2 and TGFβ3, have been identified in mammals, and all 3 have been localized in the lens (Jampel et al., 1990; Cousins et al., 1991; Pelton et al., 1991; de Iongh et al., 2001a), with TGFβ2, the predominant isoform in the ocular media (Connor et al., 1989).

TGFβ is secreted as a biologically inactive complex, comprised of a disulfide-bonded homodimer of the mature TGFβ, and another disulfide-bonded homodimer of a prodomain peptide termed the TGFβ latency-associated peptide (LAP; Zhu and Burgess, 2001). The biological activity of TGFβ in the aqueous and vitreous has been reported to be variable in different species, and also in different states of health (Connor et al., 1989; Granstein et al., 1990; Cousins et al., 1991; de Boer et al., 1994; Kurosaka and Nagamot, 1994; Tripathi et al., 1994). Members of the TGFβ superfamily mediate important processes involved in the development of the eye, including the lens, promoting various stages of lens development and differentiation of lens fiber cells (Belecky-Adams et al., 2002; de Iongh et al., 2001a; de Iongh et al., 2001b; Obata et al., 1999;

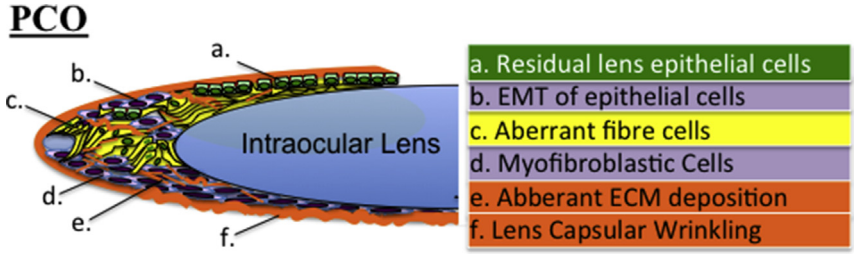

Fig. 1. Complications following cataract surgery primarily lead to posterior capsular opacification (PCO). PCO results from residual lens epithelial cells (a), left behind following fiber cell extraction, that undergo an epithelial to mesenchymal transition (b) and/or aberrant differentiation into fiber cells (c) more commonly referred to as Soemmering's ring and Elschnig's pearls. The resultant myofibroblasts (d), also migrate posteriorly to populate and cover the posterior capsule, invading the visual axis as they further lay aberrant extracellular matrix (e) and modulate the underlying capsule, causing it to fold and wrinkle (f).