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Strategies for Regenerating the Lacrimal Gland

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Abstract

Purpose of review—Aqueous deficient dry eye disease, a significant cause of morbidity worldwide, is due to dysfunction of the main and accessory lacrimal glands. Recent advances in efforts to regenerate lacrimal gland are reviewed.

Recent findings—Several strategies are being explored: *ex vivo* culture models of human and non-human lacrimal gland epithelial and myoepithelial cells, isolation and characterization of adult precursor cells within lacrimal glands, directed differentiation of stem cells to lacrimal gland cells, and organogenesis and engraftment techniques.

Summary—Conditions for primary cell culture and expansion are being established and will help in the characterization of lacrimal cells. Presumed adult precursor cells have been isolated, laying down foundations for regeneration. Stem cells have been induced to express features of lacrimal gland cells. Engraftment of *ex vivo* cultured lacrimal tissue is proof of concept that lacrimal gland regeneration and repopulation is possible.

Keywords

lacrimal gland; dry eye disease; Sjögren's keratoconjunctivitis; organogenesis; regeneration

Introduction

Dry eye disease (DED) causes significant ocular discomfort and affects 5–35% of people worldwide [1]. Aqueous tear-deficient dry eye is related to inability of the lacrimal gland to produce sufficient water component of tears, leading to poor coating of the cornea. Both the main lacrimal gland and the accessory lacrimal glands, interspersed in the palpebral conjunctiva of the eyelid, contribute to aqueous production. Dry eye can be classified into aqueous-deficient and evaporative causes [1]. Causes of aqueous-deficient dry eye include

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Compliance with Ethical Guidelines

Conflict of Interest

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Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

Sjögren's Syndrome dry eye and non-Sjögren's dry eye, which can be due to loss of lacrimal gland function, for example from chronic dacryoadenitis and lacrimal gland tumors requiring excision. This can lead to deficiency of lacrimal tissue, obstruction of lacrimal gland duct, and block of sensory reflex for tearing. Lacrimal gland function is also important in keeping a stable ocular surface after injury to the conjunctival and corneal surface from Steven's Johnson syndrome, chemical injury, and burns.

Dry eye caused by deficiency of lacrimation places a significant toll on a person's ability to function, sometimes requiring placement of artificial tears every 1–2 hours chronically to provide some relief. In addition, loss of corneal sensation can potentially lead to permanent blindness from corneal infection and ulcers. Patients with severe DED, in whom artificial tear supplementation and other more aggressive measures are unsuccessful may benefit from cell based therapies to reestablish lacrimal aqueous function.

Regeneration of lacrimal tissue would be a potentially longer lasting, more physiologic solution to the current armamentarium of dry eye treatment consisting of topical eye drops and ointments. The aqueous component of tears is a complex fluid containing critical functional proteins, electrolytes and immunoglobulins[1]. Herein we review the advances made in the area of lacrimal gland regeneration.

LACRIMAL GLAND EXPANSION

Human lacrimal gland consists of secretory acinar epithelial cells that secrete water and tear proteins into ducts (figure 1A). These units are organized into lobules by intervening extracellular matrix. The acini and ducts are composed of epithelial cells; acinar epithelial cells are surrounded by myoepithelial cells and ductal epithelial cells help modify the electrolyte content of the secreted fluid.

Development of regenerative strategies requires the recapitulation of all elements of the endogenous lacrimal gland for success. Important initial progress in this process would involve creation of an *ex vivo* source of lacrimal acinar epithelium and stromal elements. Efforts have been made to harvest lacrimal gland tissue for the creation of epithelial cell lines for study, and eventual expansion[2].

Mouse lacrimal gland epithelial cells have been cultured successfully[3, 4]. Since then, Yoshino et al. and others have successfully isolated human lacrimal gland epithelia from cadaveric tissue [5]. Tiwari et al. isolated fresh lacrimal glands from exenteration patients and established *in vitro* conditions for primary cell culture of epithelial cells [2]. These cultured cells expressed phenotypically appropriate markers and sustained epithelial morphology for 30–35 days. They maintained secretory ability. Encouragingly, a subset of these cultured epithelia expressed several stem cell markers, including CD117, ABCG2, and high ALDH levels.

The interaction of epithelia and their surrounding stromal supporting tissues is of critical importance to organogenesis[6]. Isolation of stromal connective tissue elements is also important to recreate a matrix scaffold. Lin et al. decellularized rabbit lacrimal glands, leaving intact the extracellular matrix skeleton[7]. They isolated lacrimal gland cells and

grew lacrimal spheres embedded within a 3D collagen matrix connective tissue scaffold. These cells retained secretory function.

Thus, exciting progress has been made in the isolation and propagation of two important elements of the lacrimal gland- epithelium and stromal tissue. Aside from the importance of these advancements, the isolation and establishment of stable cell lines of lacrimal gland tissue may help in studying the biology of lacrimal function and disease and can be used as a basis for *in vitro* models to test for effects of toxic and therapeutic agents.

ADULT TISSUE RESIDENT PROGENITORS

While modeling of lacrimal gland *ex vivo* is important to the development of cell based strategies, a number of other approaches can facilitate development of cell based therapeutics. One such approach is to manipulate resident adult precursor/slow cycling precursor cell populations. Indeed, lacrimal gland progenitor cells have been isolated from rats [8]. These cells expressed several stem cell markers (Musashi 1, ABCG2, Pax6, Chx10, deltanN p63, and Sox2) and can be induced to differentiate to different cell lineages based on factors in different culture media. Presumptive murine lacrimal gland stem cells have also been grown in culture[9]. In an alternative strategy, whole adult mouse lacrimal tissue was excised, and cells were harvested by sorting[10]. Those with stem cell-like expression was selected and grown in vitro. These cells showed ability to differentiate into acinar and ductile components.

After acute inflammation of the lacrimal gland, patients tend to recover without long term sequelae. The question remains of what cells are involved in lacrimal gland regeneration, and what triggers repair. This is in contrast to chronic inflammatory conditions such as Sjogren's keratoconjunctivitis sicca where destruction of the lacrimal and accessory lacrimal glands (and other cells) leads to severe dry eye.

In mice, an injection of a pro-inflammatory cytokine, IL-1, induced a severe acute inflammatory response in the lacrimal gland leading to programmed cell death of acinar cells that, after 3–7 days, regenerated with return of cell function. The stem cell marker, nestin, showed an acute rise in expression over the saline-injected controls by day 2–3, with decline back to baseline levels by day 7 [11]. Primary lacrimal gland cells with stem-cell markers could be harvested only after injection of IL-1 and not saline, suggesting that stem-cells proliferate after acute injury and help with wound healing[12].

Progress has also been made in the area of resident precursor cell identification in accessory lacrimal gland. Adult human accessory lacrimal glands, located in the palpebral conjunctiva, also express precursor cell markers[13] (figure 1B). Expression of these markers appears to be lower with age, suggesting an association between lacrimal tissue age and precursor abundance.

Although multiple groups have identified cells which express precursor stem cell markers, it is unclear which population of cells contain a precursor cell population. Three potential sources of precursor cells are myoepithelial cells, ductal epithelial cells, other

transdifferentiating cell types and mesenchymal cells from blood, depending on the underlying mechanism of cell loss and disease [14].

On immunohistochemistry, co-localization of cells with anti-smooth muscle actin, a marker of myoepithelial cells, and nestin occurred in IL-1 injected mice [11], implicating that myoepithelial cells may be the source of precursors in an inflammatory model of lacrimal disease. Furthermore, myoepithelial cells harvested from rats can differentiate to different cell lineages based on culture conditions and express stem cell markers[8]. Thus, myoepithelial cells may be a source of stem or progenitor cells that help with repair and regeneration[14].

Mesenchymal tissues interact with epithelial tissue during embryonic development in mice and this interaction can lead to formation of lacrimal gland organ germs *in vitro*[6]. In the setting of acute injury of adult mice, stem-like cells that proliferated during repair expressed vimentin, a marker of mesenchymal cells[12]. Thus, mesenchymal tissue could also be a source of stem or progenitor cells[15].

STEM CELL BASED REGENERATION

Although there have been promising results on establishing *ex vivo* culture systems and isolation of adult precursor cells, new techniques utilizing stem cells as a source of lacrimal epithelia have emerged. The use of human induced pluripotent stem cells (hiPSCs) and human embryonic stem cells (hESCs) have been a source of great inquiry and promise in tissue regeneration [16].

In an effort to create a source of lacrimal epithelial cells, hESCs have been directed successfully to differentiate along a lacrimal epithelial fate pathway. The basis of many of these experiments is an expanding understanding of the developmental signals and critical gene expression pathways involved in lacrimal gland development[17]. Hirayama et al. utilized microarray analysis to identify transcription factors enriched in mouse lacrimal gland epithelial cells[18]. The human analogs of PAX6, SIX1, and FOXC1 mRNA were transfected into human embryonic stem cells. The combined overexpression of all 3 factors lead to morphological changes and expression of several markers found in lacrimal gland and epithelial tissue.

An alternative strategy using small molecular inhibitors of developmental pathways has been successful in directing differentiation of hiPSCs into corneal epithelial cells[19]. A better understanding of the important factors involved in lacrimal gland embryogenesis can help guide potential usage of small molecules in inducing lacrimal gland differentiation. Indeed, analysis of global gene expression from human lacrimal gland compared to other ectodermal derived tissues show factors that are differentially expressed [20](figure 2). These may be potential targets that define lacrimal gland development.

Directed differentiation by the transfection method and the small molecule approaches are similar in that both are targeting key genes that define lacrimal gland epithelial cells. They can have their own pros and cons. Transfection allows for expression of a known factor that may define a lacrimal gland epithelial cell. Differentiation may occur more quickly and

efficiently. It potentially could be more cost efficient as different cell types (iPS and somatostatic cells like fibroblasts) could serve as a source. On the other hand, gene expression levels may be difficult to control. Moreover, inducing expression at a late stage of development may also be more difficult technically. Concerns with oncogenic potential and xeno-elements in some stem cell based methods may also prevent use of genetically engineered tissue in the clinical setting[21]. Meanwhile, small molecules can be added at any time and can theoretically be more titratable in altering the level of expression. However, off target effects can occur and can confound effects from inhibiting or inducing the intended factor or pathway. Current research into the use of “synthetic mRNA” in directing differentiation may be a good compromise of both methods[15]. All of the available methods for directing differentiation of stem cells towards mature fates can be utilized in a complementary fashion and each successive experiment yields advances in the ultimate goal of an efficiently obtained and utilized *ex vivo* source of mature cell types.

LACRIMAL GLAND ORGANOGENESIS AND TRANSPLANTATION

The ability to transform 2-dimensional sheets into 3-dimensional lacrimal gland buds is important for organogenesis and transplantation efforts. In a recent experiment, lacrimal gland tissue was harvested from embryonic mice and separated into mesenchymal and epithelial components. Lacrimal gland germs with branching morphology were generated from *in vitro* co-culturing of the two separated cell populations[6].

These cells generated from embryonic mice were then engrafted successfully into adult mice that lacked extraorbital lacrimal glands (excised) (figure 3). Histology showed the presence of epithelial, myoepithelial, and neuronal cells. Functionally, these mice could be stimulated to produce tears, and reversed the DED phenotype caused by lacrimal gland extirpation[6].

Another strategy for *in vivo* organogenesis was demonstrated by Gromova et al. In this study, progenitor cells were harvested from normal adult mice and transplant to mice with chronic inflammatory damage [10]. After growth *ex vivo* in culture, the cells were injected into TSP1^{-/-} mice, a model organism for chronic lacrimal gland inflammation. A small, but significant percentage of viable cells remained engrafted 40–80 days after injection. Compared to saline-injected mice, a decrease in inflammation was seen with the lacrimal glands injected with epithelial progenitor cells. Thus, engrafted cells were able to rescue glands damaged by inflammation.

CONCLUSIONS

Though the field of lacrimal gland regeneration is young, significant advances have been made from different, but complementary approaches. Work is ongoing to find methods to isolate human lacrimal epithelial and precursor cells, with the intent of later expansion or differentiation into epithelial tissue (respectively). Other groups are studying the complex interaction of epithelial and mesenchymal tissues in the development of lacrimal gland. Still more researchers seek to find methods for directing differentiation of stem cells into mature lacrimal epithelial fates. Finally, organogenesis *in vitro* and transplantation is another

strategy that is currently under investigation. All of these efforts are important for finding alternative, long term solutions to dry eye disease.

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Figure 1.

Human accessory lacrimal gland. A. H&E section of formalin-fixed paraffin-embedded accessory lacrimal gland surrounded by conjunctival tissue, taken from human Muller's Muscle Conjunctival Resection (blepharoptosis surgery) specimens. Epithelial cells are organized into acini with surrounding stromal and myoepithelial tissues. B. Immunofluorescence localization showing expression of potential precursor cell markers, ATP-Binding Cassette Subfamily G Member 2 (ABCG2) and nestin.

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Figure 3.

Schematic of lacrimal gland regeneration *in vitro* and engraftment by the organ germ method[6]. Epithelial (purple arrow “2”) and mesenchymal cells were harvested from embryonic day 16.5 lacrimal germs. They developed branching morphology characteristic of lacrimal gland when co-cultured in vitro. The lacrimal gland germs were engrafted onto adult mice that had lacrimal glands removed. Also depicted are alternative stem cell sources (purple arrow “1”) for epithelial cells for engraftment.