Developmental biologists Drs Helen P Makarenkova and Darlene A Dartt describe their collaborative work exploring the morphogenesis of the lacrimal gland, as well as explaining the respective careers that brought them into this field of research.

To begin, can you provide a synopsis of your respective research backgrounds and explain what led you to work together on your present line of investigation?

HM: When I completed my education I became interested in the mechanisms of embryo and organ development. I thought defining the mechanisms regulating these processes would give us an insight into fundamental control of tissue regeneration. The lacrimal gland appeared to be an ideal model system for identifying regulatory factors and signalling pathways that control development and repair. During the last three years, our studies and those of other laboratories have indicated the presence of stem-like cells in the lacrimal gland and the ability of the gland to heal itself following an injury. Recently, our laboratories have identified new putative epithelial cell progenitor (Makarenkova laboratory) and myoepithelial progenitor (Dartt laboratory) populations of cells. We decided to combine our expertise and efforts to dissect stem and progenitor cell function in lineage segregation during development, regeneration and dry eye conditions.

DD: For most of my career I investigated the cellular signalling mechanisms that neurotransmitters use to stimulate lacrimal gland protein secretion. We began to wonder what the role of the contractile myoepithelial cells were in regulating this secretion, so we decided to grow them in culture. During the culture process we noticed there were a lot of small cells that grew out of the tissue explants and eventually differentiated into myoepithelial cells. We isolated the small cells and showed they were progenitor cells that could differentiate into at least three different cell types. Helen’s laboratory showed evidence there was a different type of progenitor, an epithelial one. So we joined forces to determine which type of cell was the progenitor or stem cell in the lacrimal gland.

What are the main aims of your current research?

Our goal is to develop new therapies able to restore lacrimal gland function. We believe that such therapies could be based on determining factors and mechanisms involved in the regulation of gland morphogenesis and regeneration or on isolation and transplanting stem or progenitor cells. Our ongoing work seeks to identify cell progenitors able to restore the function of the ‘diseased’ lacrimal gland as a critical first step in the development of stem cell-based strategies to treat patients with dry eye conditions. If we succeed, it would drastically improve the quality of life of people affected by dry eye.

Do you employ particular methodologies and strategies in your lab?

Both our laboratories use multiple methodologies: developmental, cell, stem cell, molecular biology, mouse genetics and bioinformatics. What is more, we are working with multilevel model systems, exploring cell and organ cultures and animal models. Depending on the question, we choose the model that can give us the most informative answers.

Can you describe your use of the lacrimal gland as a model system?

The control of the lacrimal gland’s branching pattern is mediated by a network of signalling factors, including members of the fibroblast growth factor subfamily 7 (FGF7). In particular, FGF10 – which is expressed in the mesenchyme near the tip of the lacrimal gland’s bud – was found to be important for morphogenesis. FGF7 and FGF10 signal through the same receptor (FGFR2b) but induce very different responses in lacrimal gland epithelium. Using the lacrimal gland as a model system we showed that developmental activities of these growth factor morphogens depend on the gradients they form in the extracellular matrix. A similar mechanism is required for epithelial bud morphogenesis not only in the lacrimal gland, but also in many branched organs including the lungs, thyroid, pituitary, mammary and salivary glands. Our studies also provide a model for how to apply protein engineering to manipulate diffusion and gradient formation by morphogens through the extracellular matrix, with the aim of improving their biological activities and stem cell function.

What do you hope to be the far-reaching implications of your research?

One of the biggest challenges in modern biology is to understand how a single stem or progenitor cell gives rise to all different types of cells during embryonic development and tissue regeneration and how the function of these cells changes in diseased glands. The lacrimal gland is a good system to answer these questions. We hope our joint efforts in this direction can reveal fundamental concepts and create new therapeutic approaches to treat diseases affecting the lacrimal gland.

What will be the direction of your future studies?

A very attractive paradigm of our future research is to define and manipulate the mechanisms controlling stem or progenitor cell specification, proliferation, differentiation and therefore gland regeneration. We would then be able to induce and control regeneration of diseased lacrimal glands and cure dry eye disease.
A helping gland

A laboratory based at Scripps Research Institute in California has joined forces with a second group of researchers in Boston to investigate the possibility of using stem cell therapies to counteract corneal disease; especially dry eye syndrome.

COMPOSED OF A delicate balance of molecular ingredients, the basal tears form a permanent aqueous layer that lubricates and protects the surface of the eye. The lacrimal gland, located above and on the outward side of the eye, is responsible for preparing this complex cocktail and delivers it to the lacrimal sack, which secretes it through the tear duct. When the gland fails to adequately perform its function, the basal tears can become unstable or insufficient, leading to health problems.

Dry eye syndrome affects an estimated 25-30 million Americans every year, with a heavy bias towards the elderly – meaning that incidence is likely to rise as time goes on. The condition causes irritation, pain, inflammation, corneal damage and, if left untreated, even blindness. There is currently no cure and no satisfactory treatment for dry eye, which is often caused by dysfunction or degeneration of the lacrimal gland. Despite the strong link between the lacrimal gland and dry eye syndrome, and the importance of proper gland function to corneal health, historically the lacrimal gland has not often been the subject of medical study, being considered an accessory structure of the eye.

A BINOCULAR PERSPECTIVE

In the arena of stem and progenitor cell research, therefore, medical scientists investigating the cornea have been highly successful in developing novel regenerative measures to repair tissue damage. Far fewer scientists worldwide focus on bringing the same benefits to the repair and restoration of lacrimal gland function, although the impact of such advances, especially for dry eye patients, could be great.

In order to push forward scientific understanding in this area, two US laboratories have come together to study the development and repair processes at work in the formation and maintenance of the lacrimal gland. Dr Helen P Makarenkova’s laboratory at Scripps Research Institute in La Jolla, California, has joined forces with Dr Darlene A Dartt’s team at Schepens Eye Research Institute/Massachusetts Eye and Ear in Boston, bringing together scientists from both coasts of the US in the hunt for crucial stem and progenitor cells at work in the lacrimal gland.

SEARCHING FOR THE SOURCE OF CELLS

Recent studies have indicated that the lacrimal gland, like other exocrine glands such as the pancreas or mammary gland, has a high regenerative potential and is therefore capable of repairing itself after sustaining significant damage. There is also evidence that the gland contains stem/progenitor cells, making it an ideal target for potential regenerative stem cell therapy. The problem is that the source of the regenerative cells the gland uses is unclear, and matters are further complicated by the complexity of the gland itself; within the lacrimal gland’s epithelial component, which is responsible for producing, modifying and secreting the basal tear, there are three different cell types: acinar, ductal and myoepithelial. These cells may have separate progenitors or may share just one.

Both laboratories overcame this problem, each discovering a potential progenitor cell for a different portion of the epithelium. Makarenkova’s team was recently able to identify a species of colony-forming, regenerative cells that may be the progenitors of acinar and ductal cells. Furthermore, they demonstrated that, when transplanted into damaged lacrimal gland tissue, these cells were able to repopulate the gland, proving that progenitor cells could, in principle, be used to treat dry eye conditions. The Dartt team, meanwhile, had also been able to identify myoepithelial progenitor cells – although these were the variety that would make up the myoepithelial cells.

MICE AND METHODS

The California lab has subsequently been successful in developing new methods for the isolation, propagation and transplantation of candidate progenitor cells. Using a simple and efficient technique, the researchers found that progenitor cells could be cultured in a 3D reaggregation, exploiting the cells’ ability to reform into 3D structures in vitro. Within such a culture, the specification, proliferation and survival of the cells is governed by complex cell and matrix interactions.

Using this new approach the scientists are now working towards defining the conditions for restoring lacrimal function in dry eye mouse models. An innovative part of their joint work has been the decision to use a novel model for Sjögren’s syndrome in this process: the thrombospondin-1-null (TSP-1) mouse. Sjögren’s syndrome is an autoimmune condition that sees the body attacking exocrine glands including the lacrimal...
gland, producing dry eye in these mice as they age, although they are normal at birth. In this sense, TSP-1 mice make perfect models of human lacrimal gland deterioration, without the need for injury or stress to the animal; the glands themselves also happen to be much easier to access in mice than humans, providing ideal conditions for study.

DELVING INTO DEVELOPMENT
This has promising implications for possible clinical applications, but the body of study from which these projects have emerged constitutes a much broader and more sustained examination of the lacrimal gland’s developmental and regenerative biology, derived over the course of many years. The California group’s more fundamental work has delved into the genes and signalling pathways responsible for the gland’s branching morphogenesis, which it shares with many other organs within higher organisms. The complex 3D tubular network of the gland is built through repetitive cleft and bud formations and cell proliferation. Dartt’s laboratory used human goblet cell to identify the neurotransmitters that stimulate conjunctival goblet cell mucin production and the components of the signalling pathways. Boston scientists previously found that both nerves – parasympathetic and sympathetic – regulate lacrimal gland secretion. This research focused on studying the role of purinergic receptors, forming channels in the membrane that by themselves or in conjunction with neurotransmitters stimulate secretion from the lacrimal gland. Currently, Dartt’s laboratory is studying purinergic receptors in lacrimal gland myoepithelial cells in a mouse model of dry eye disease.

In a series of papers published as far back as 2000, Makarenkova and her collaborators demonstrated that fibroblast growth factors (FGFs) 7 and 10 signal through the same receptor but induce different cellular responses; the former induces budding in the epithelium while the latter caused these buds to elongate. Subsequently, the researchers found that the diffusion of FGFs through the extracellular matrix was controlled by their ability to bind with heparin sulphate glycoaminoglycans, resulting in various extracellular FGF gradient patterns that can be affected by altering the heparin-binding sites of either FGF7 or FGF10. Through the lacrimal gland, therefore, the California scientists were able to generate a model of morphogenetic gradient operation, supporting the idea that this kind of system is important to the patterning of embryonic tissues in development.

In multiple publications, Dartt and her collaborators investigated potential treatments for dry eye targeted at the mucin layer of the tear film, on conjunctival goblet cell mucin secretion and cell proliferation. Dartt’s laboratory used human goblet cells to identify the neurotransmitters that stimulate conjunctival goblet cell mucin production and the components of the signalling pathways. Boston scientists previously found that both nerves – parasympathetic and sympathetic – regulate lacrimal gland secretion. This research focused on studying the role of purinergic receptors, forming channels in the membrane that by themselves or in conjunction with neurotransmitters stimulate secretion from the lacrimal gland. Currently, Dartt’s laboratory is studying purinergic receptors in lacrimal gland myoepithelial cells in a mouse model of dry eye disease.

With a strong history of fundamental research supporting a new approach to an old clinical problem, the researchers led by Makarenkova and Dartt are primed for success; they have already achieved much in the field, but if their plans for the next few years come to fruition, the results will be clear for all to see.