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Focal adhesion kinase knockdown modulates the response of human corneal epithelial cells to topographic cues

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ABSTRACT

A rapidly expanding literature broadly documents the impact of biophysical cues on cellular behaviors. In spite of increasing research efforts in this field, the underlying signaling processes are poorly understood. One of the candidate molecules for being involved in mechanotransduction is focal adhesion kinase (FAK). To examine the role of FAK in the response of immortalized human corneal epithelial (hTCEpi) cells to topographic cues, FAK was depleted by siRNA transfection. Contrary to expectations, FAK knockdown resulted in an enhanced response with a greater number of hTCEpi cells aligned to the long axis of anisotropically ordered surface ridges and grooves. Both underlying topographic features and FAK depletion modulated the migration of corneal epithelial cells. The impact of FAK knockdown on both migration and alignment varied depending on the topographic cues to which the cells were exposed, with the most significant change observed on the biologically relevant size scale (400 nm). Additionally, a change in expression of genes encoding perinuclear Nesprins 1 and 2 (SYNE1, 2) was observed in response to topographic cues. SYNE1/2 expression was also altered by FAK depletion, suggesting that these proteins might represent a link between cytosolic and nuclear signaling processes. The data presented here have relevance to our understanding of the fundamental processes involved in corneal cell behavior to topographic cues. These results highlight the importance of incorporating biophysical cues in the conduction of in vitro studies and into the design and fabrication of implantable prosthetics.

1. Introduction

Corneal diseases affect over 10 million people worldwide and remain a major cause of blindness [1]. At this time, the only well-established treatment for corneal blindness is transplantation with corneal donor tissue or with a biosynthetic corneal prosthesis. However, the number of suitable donor transplants does not meet the demand and there are few widely utilized keratoprosthesis available. Recently, bioengineering approaches for the development of improved corneal prostheses have begun to incorporate biophysical and chemical cues into their design to better mimic the native cellular microenvironment.

Corneal epithelial cells in vivo attach to the underlying stroma through the basement membrane, a specialized extracellular matrix (ECM) which possesses a rich three-dimensional felt-like topographic landscape comprising bumps, fibers, and pores with feature sizes in the nano- and submicron range [2–4]. The impact of topographic cues on fundamental cell behaviors including shape [5], adhesion [6], proliferation [5,7,8], migration [9,10], cell alignment [2,11–13], and survival [8] has been reported by our lab and others, documenting that in addition to chemical signals, cells respond to biophysical cues that are intrinsic attributes of their microenvironment. However, the mechanisms that allow the cells to identify and react to topographic features remain poorly understood. To study the impact of topographic cues on cell behavior, surfaces patterned with anisotropically ordered ridges and grooves spanning the nano- to micron size scale have been developed that have been shown to induce corneal epithelial cells to lose their random orientation and preferentially align parallel or perpendicular to the long axis of the underlying topographic features [9,14].

As cells attach to the extracellular matrix through transmembrane proteins, mechanical stimuli from outside of the cell can be transmitted to the nucleus through the cytoskeleton. Extracellular mechanical stimuli modulate adhesion, migration, and cytoskeletal dynamics, resulting in changes in nuclear shape and hence cellular gene expression patterns. However, the molecular signaling mechanisms involved in these processes are not fully understood. We therefore set out to investigate the effects of micro- to nanoscale topographic features on signaling events both near the extracellular matrix interface and near the nucleus.

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