REVIEW ARTICLE

TOWARDS AN INTEGRATIVE VIEW OF CORNEAL PROTEOMICS IN EPITHELIAL WOUND HEALING

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ABSTRACT

Corneal epithelial wound healing is a continuous and multistep process involves cell migration proliferation and differentiation after an injury. Any defect in these processes will result in loss of corneal transparency and function. Around 135 million people are visually impaired, which illustrate the need for better understanding of corneal healing mechanisms and development of efficient ways to accelerate and improve wound healing. Delayed corneal wound healing contains the risk of bacterial infection causes corneal opacity and neovascularization that could lead to corneal blindness. It is therefore important that epithelium should rapidly regenerate after an injury. Reepithelialization involves cell migration proliferation and differentiation to restore the cornea to its highly organized architecture. The molecular mechanisms underlying these processes are yet to be established. Hence the identification of various proteins that could associate with healing process is of great significance.

KEY WORDS: Corneal Epithelium, Proliferation, Wound Healing, Migration

INTRODUCTION

The human cornea is a transparent multilayered tissue composed mainly of five layers among which epithelium is the outermost layer[1].The optical properties of cornea depend upon the tight functional integrity of corneal epithelial cells which enables cornea to act as a functional barrier against the noxious agents[2]. The most distinguished property of corneal epithelium is to undergo continuous renewal process along with rapid and developmental response to injury which plays an important role in corneal homeostasis[3].The process of cell migration involves number of phases. Initially prior to cell migration there is a lag phase which is characterized by the synthesis of several cytoskeletal proteins such as, vinculin, actin, talin, and integrin[2]. Cell surface glycoproteins like integrins and fibronectin has an important role in regenerating corneal epithelium[4-6]. After the completion of lag phase epithelial cells adjacent to the wound started to migrate as a sheet towards the margin followed by the proliferation of basal cells which moved upward to restore the normal thickness of epithelium[7] (Fig.1).

The wound healing process of corneal epithelium is essential to uphold corneal functions and prevent severe consequences. Delayed corneal wound healing and ulcers results in corneal opacity and neovascularization, thus leading to visual loss[8,9]. It is therefore, important that epithelium should rapidly regenerate following an injury. An immense amount of data regarding the identification, localization and functions of proteins/genes has been accumulated during the process of migration[10-12]. This dataset provides a useful background to comprehend the molecular mechanisms behind corneal epithelial disorders. In addition protein expression profiles also provide a definite role with differences in expression among the normal and wounded corneal epithelium which may open avenues for therapeutic interventions. We provide here a brief review of proteins mediating cellular connections crucial for the migration of corneal epithelial cells during wound healing process.

DISCUSSION

Reepithelialization involves cell migration proliferation and differentiation to restore the cornea to its highly organized architecture. The molecular mechanisms underlying these processes are yet to be established. Hence the identification of various proteins that could associate with healing process was of great significance like vinculin, keratin, lumican, fibronectin, tenascin and thrombospon-

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din-I, which are upregulated during migration and are believed to modulate cell adhesion[10].

Multiple types of cells, extracellular matrix and cytokine mediators are involved in wound healing process with a complex and unique series of interaction[11,12].To get knowledge of these mechanisms different wound healing models have been employed which revealed that the renewal process has various distinctive phases involving the movement of a superficial cells to cover the wound area, cell proliferation, stratification and reestablishment of epithelial layers[13-20].Cell migration, a prerequisite to the wound healing process is regulated by the synthesis of an intricate cytoplasmic array of actin - rich stress fibers, the inhibition of which block the migration process[2,21].

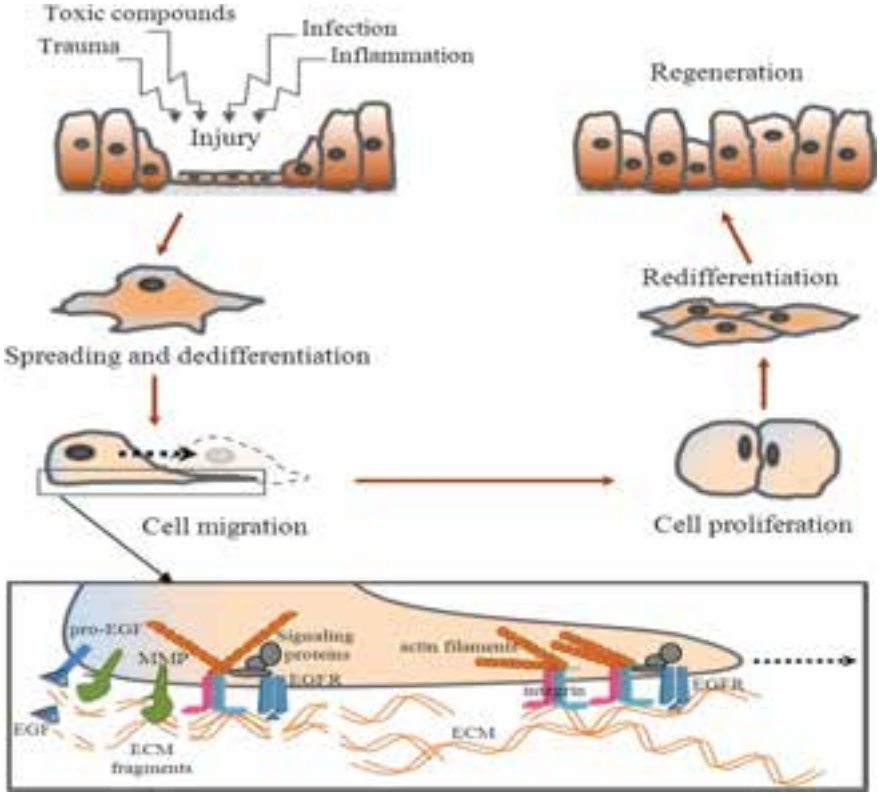

Fig.1 Mediation of Cellular Migration Proliferation and Differentiation of Corneal Epithelial Cells upon Injury. (Adapted from American Journal of physiology-Cell Physiology 2014; 3064: 307-19)

Role of Proteins Involved in Wound Healing Process

The advancement of proteomic and genomic techniques made it possible to develop better understanding towards the coordinated expression and functional association of differentially expressed proteins modulating cellular processes, initiating signal transduction and regulating gene transcription patterns. Using combination of 2D gel electrophoresis, mass spectrometry together with microarray analysis, several classes of proteins have been identified and characterized in corneal epithelium and extracellular matrix. These include growth factors, chemokines, proteoglycans, glycoproteins, basement membrane proteins, interfibrillar matrix proteins, matrix metalloproteinases and growth factors[22-25].

Multiple growth factors are expressed and interact with one another to synchronize the healing process[3]. The epidermal growth factor which is a low molecular weight polypeptide has been extensively studied[26-31]. Upon binding to its receptor it dimerizes and facilitates the activation of mitogen activated proteins which in turn activates the MAPK pathway to regulate the process of proliferation and differentiation in maintaining corneal transparency[2,29,32]. Other growth factors including hepatocyte growth factor (HGF), platelets derived growth factor (PDGF) keratinocyte growth factor, nerve growth factor also stimulate the epithelial growth and trigger epithelial cell apoptosis[2,33-35]. These growth factors are released from corneal stroma and lacrimal glands have shown a paracrine effects[2]. Recently it has been found that recombinant human Granulocyte-macrophage colony-stimulating factor (GM-CSF) accelerated corneal epithelial wound healing both in vitro and in vivo[36].These cytokines and growth factors togeth-

er with adhesion and extracellular matrix proteins regulate the complex but organized mechanism of wound healing in corneal epithelium.

The adhesion molecules including the components of desmosomes, hemidemosomes and gap junction molecules play an important role in intercellular communications. They provide pathways for signal transduction and tissue homeostasis [37, 38]. Previous studies reported the involvement of several proteins like laminin, desmoglein, cadhherins, integrins, vinculin, actin, talin, fibronectin to mediates cell to cell and cell to matrix interactions[39,40]. It has been established that wound healing is progressed under the rigid control of adhesion molecules. As the wound healing proceeds, the previous adhesion junctions are lost with the formation of provisional focal contacts to cover the wounded surface[41] and the metabolic activity of the individual corneal epithelial cells was increased. The hemidesmosome attachment was lost with the morphological changes in the basal cells which turned into more elongated shape with extended lamellipodia at the wound margin. The strength of cell-cell adhesion interactions in the migrating epithelial sheets must be sufficient to withstand the forces generated during the process. As the basal cells change their shape and begin to move out over the wound bed, the neighboring cells appear to be pulled along behind them. As a result the entire sheet of cells move and the epithelium becomes progressively thinner with cells flatten out in an attempt to cover the exposed wound bed. The metabolic activity of cell decreases followed by proliferation to restratify the tissue after the wound edges meet and epithelial integrity is again established[40].

Integrins plays an important role in maintaining cell shape and integrity by mediating the interaction of alpha-actinin and talin to the actin filaments of the cytoskeleton[43-45].These proteins present in the cell membrane at sites of cell-cell interaction, serve as components of the hemidesmosomes in unwounded epithelia and may be available for rapid conscription as epithelial cell migration proceeds[17], whereas many recent studies have shown an important role of beta integrins in mediating epithelial cell migration and differentiation in vitro[45-47].
In addition fibronectin, an important component of extracellular matrix also plays a key role in the regulation of the adhesion and migration of corneal epithelial cells (CEC) [38, 48-51]. Upon injury fibronectin provides a temporary matrix to which corneal cells adhere to and migrate to cover the area of defect[37,50,51].Fibronectin coupled with vinculin whose synthesis has increased dramatically as the migration proceeds, interacts to integrin receptors in focal contacts and enhances the activation of variety of signal transduction pathway[17].PARP-1 gene expression was strongly activated by fibronectin through MAPK and P13K signaling pathway suggesting that PARP-1 may play an important role during the highly prolifertativephase[52]. Recently it has been observed that the migratory effects of fibronectin on adhesion and cellular proliferation has mediated by Beta Pix which contribute to the regulation of cellular migration and proliferation of CEC[53]. Fibronectin also enhances the movement of corneal epithelial cells through the peptide derived from the second cell binding domain (PHSRN)[51,54].

On the basis of these observation we can wrap up that fibronectin-integrin system plays an important part in corneal epithelial wound healing by providing a connective matrix for the attachment and migration of corneal epithelial cells and secondly by the activation of these cells via natural agents[47]. Knowledge of these processes may lead to the development of therapeutic agents used for the treatment of various corneal disorders. Recently PHSRN eye drops were found to be helpful for the management of corneal perforation due to the persistent epithelial defects (PED) with quick reepithelialization being followed by full restoration of stromal thickness [55].The eye drops containing the blend of substance P and IGF-1 are also reported to be successful for the management of corneal PEDs[47].

CONCLUSION

The corneal epithelial wound healing is a complex and multistep process required the plethora of proteins working together to mediate the renewal process. The coordinated expression analysis and the functional association of differentially expressed proteins will lead to the discovery of specific diagnostic biomarkers and new therapeutic targets as well. In addition the specific knowledge of corneal proteome during regular and pathological conditions may also guide to enhanced molecular classifications of corneal diseases and assist the expansion of latest treatments together with gene therapy and the proposal of synthetic corneas for transplantation.

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