DOI: 10.56871/RBR.2024.84.99.009
UDC 617.713-001.3-092-003.93+577.218

MOLECULAR GENETIC ASPECTS OF CORNEAL TISSUE REPAIR

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For citation: Stadnikov AA, Oleynik DV. Molecular genetic aspects of corneal tissue repair. Russian Biomedical Research. 2024;9(2):80-85. DOI: https://doi.org/10.56871/RBR.2024.84.99.009

Received: 15.02.2024 **Revised: 11.04.2024** **Accepted: 20.05.2024**

Abstract. The article is devoted to a scientific literature review on the issues of reparative histogenesis and cell differentiation in the cornea at the genetic level. The most vulnerable membrane in case of eye injuries is the cornea. In this regard, the assessment of its regeneration processes, including at the molecular genetic level, becomes important. Knowledge of the molecular biology of regenerative genes is far from complete, and many aspects remain insufficiently studied. The genes *MKI67, TAB3, PAX6* are involved in the regeneration of corneal tissue, including after injury. This review focuses on these three genes. Ki-67 protein is a universal marker of proliferation and is necessary for maintaining the cell cycle. Pax-6 is an early marker of corneal epithelial cell differentiation. Expression of this gene is suppressed in many tissues of an adult, but it persists in eye cornea, participating in the normal functioning of the cornea. *TAB3* gene, as a correlate of TGF-β activation, helps to increase the intensity of proliferation and migration of epithelial cells and promotes rapid healing of the wound surface. Currently, the study of the patterns of cyto- and histogenesis, differentiation of cells and tissues of the organ of vision, their physiological and reparative regeneration and the regulation of these processes at the molecular genetic level in the aspect of regenerative medicine is being updated.

Keywords: regeneration, cornea, gene expression

МОЛЕКУЛЯРНО-ГЕНЕТИЧЕСКИЕ АСПЕКТЫ РЕПАРАЦИИ ТКАНЕЙ РОГОВИЦЫ

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Для цитирования: Стадников А.А., Олейник Д.В. Молекулярно-генетические аспекты репарации тканей роговицы // Российские биомедицинские исследования. 2024. Т. 9. № 2. С. 80–85. DOI: https://doi.org/10.56871/RBR.2024.84.99.009

Поступила: 15.02.2024 **Одобрена: 11.04.2024** **Принята к печати: 20.05.2024**

Резюме. В статье приводится обзор научной литературы по вопросам репаративного гистогенеза и дифференцировки клеток в роговице на генетическом уровне. Наиболее уязвимой оболочкой при травмах глаза является роговица. Важное значение приобретает оценка процессов ее регенерации, в том числе на молекулярно-генетическом уровне. Знания о молекулярной биологии регенераторных генов далеки от полноты, и многие аспекты остаются недостаточно изученными. В регенерации тканей роговицы, в том числе после травмы, принимают участие гены *MKI67, TAB3, PAX6*. В данном обзоре делается акцент на этих трех генах. Белок Ki-67 — универсальный маркер пролиферации, необходим для поддержания клеточного цикла. Pax-6 — ранний маркер дифференцировки эпителиальных клеток роговицы. Экспрессия данного гена подавляется во многих тканях взрослого человека, но она сохраняется в роговице глаза, участвуя в нормальном ее функционировании. Ген *TAB3* как коррелят активации TGF-β способствует увеличению интенсивности пролиферации

и миграции эпителиоцитов, содействует быстрому заживлению раневой поверхности. В настоящее время актуализируется изучение закономерностей цито- и гистогенезов, дифференцировки клеток и тканей органа зрения, их физиологической и репаративной регенерации и регуляции этих процессов на молекулярно-генетическом уровне в аспекте регенеративной медицины.

Ключевые слова: регенерация, роговица, экспрессия генов

The determining factor in development and functional specialization of tissues is the genetic determination and subsequent differentiation. During the normal course of development, in competent material, under the influence of one or another inducer, initially unstable (labile) determination occurs, and later, irreversible (stable) determination starts [5]. Only after this the rudiment of a certain tissue appears. The tissue determination of cornea is based on the expression of certain tissue-specific genes that determine the synthesis of nucleic acids and proteins [4].

During reparative histogenesis, it is often necessary to observe coordinated gene expression when several specific protein substrates are synthesized in cells or when some humoral factor induces the expression of several genetic loci in cells of different tissue types.

When studying corneal regeneration, it is necessary to dwell on some genes whose action has been described and plays a key role in the development and differentiation of tissues of the eye's anterior segment.

Ki-67 was originally identified as an antigen recognized by a monoclonal antibody generated by immunizing mice with nuclei isolated from the Hodgkin lymphoma cell line L428 [16]. Cloning and sequencing of complementary deoxyribonucleic acid (cDNA) of Ki-67 [15, 37] revealed that the amino acid sequence had little similarity to other known proteins, so the protein was named after the antibody that identified it. Ki originates from Kiel, Germany, where the antibodies were developed, with 67 being the well number on a 96-well plate. In 1996, the entire *Ki-67* gene locus was sequenced and was found to contain approximately 30,000 bases [41].

The *MKI67* gene, encoding a Ki-67 protein, is a universal marker of proliferation and is detected in cells in all phases of the mitotic cycle except G0 [1]. The Ki-67 protein is also necessary for maintaining the cell cycle.

A disadvantage of the MKI67 gene determination method is that mitosis is the fastest phase of cell cycle, which may lead to an underestimation of actual values of its quantity [12]. The protein half-life is approximately 90 minutes [19], so inhibition of protein synthesis for 60 minutes leads to a significant decrease in the Ki-67 protein level [6].

Average messenger ribonucleic acid (mRNA) and Ki-67 protein levels in proliferating cells appear to be independent of cell type. Similar levels of RNA and protein are observed in several human cell lines [30].

Ki-67 expression is a useful marker of early precancerous lesions [7]. Experimental studies have confirmed changes in Ki-67 expression in the eye.

Thus, when exposed to a femtosecond laser (a system capable of generating ultra-short laser pulses lasting 5 femtoseconds or more) on cornea, the Ki-67 expression increases on the first day and reaches a maximum in epithelial cells on the third day [29].

In early stages of corneal damage, cells with high Ki-67 expression are localized predominantly in the region of the growth zone of limbus [3].

In a study of pterygium, it was found that Ki-67 expression increased and depended on the duration of a disease, but did not depend on the extent of spread to cornea and the severity of a disease [22]. It was found that the number of immunopositive cells to Ki-67 in epithelial layer of pterygium is significantly higher than in the normal conjunctiva bordering the cornea [26]. In normal conjunctiva, Ki-67 expression is <5% [27].

In 1991, the PAX6 gene was identified in humans on the short arm of chromosome 11 in the 11p13 region [40]. Pax-6 is an early marker of corneal epithelial cell differentiation [24]. The PAX6 gene is a critical regulatory gene that encodes a specific DNA-binding transcription factor capable of initiating eye development during embryogenesis [20, 42].

PAX6 expression is downregulated in many adult tissues, but it is retained in the adult cornea [23], indicating that PAX6 is required not only for ontogenesis but also for normal corneal function [14], where the cornea is involved in wound maintenance and healing [10, 25].

PAX6 supports the regeneration process by providing differentiation of human corneal epithelial cells [22]. At the same time, the *PAX6* gene plays a key role in maintaining the multipotent state of several types of cells (iris, retinal pigment epithelium, and neuronal retina). This combination of regulatory functions is explained by the presence of different functional domains in its structure [2].

In adults, the PAX6 protein is responsible for maintaining the pool of stem cells in the lens epithelium, corneal limbus, pigment epithelium of the ciliary body and iris [28].

Transcriptome analysis (RNA-seq) of corneal epithelium from mouse embryos confirmed that PAX6 was relatively highly expressed in corneal epithelium, indicating a key role of PAX6 in the development of this cell layer [36].

PAX6 has been identified as a key molecular factor capable of reprogramming rabbit skin epithelial cells to give rise to corneal epithelial cells and repair corneal surface defects [34]. In studies on rabbits, PAX6 has been identified as a cellular molecular factor capable of reprogramming rabbit skin epithelial cells, trans-differentiating them into corneal-like epithelium, and repairing corneal surface defects.

It has been shown that changes in PAX6 expression, both downward and upward, affect cell differentiation, erosion healing response, and corneal transparency [11].

Transforming growth factor beta (TGF-β) is a widely studied cytokine that is synthesized in virtually all cells and tissues of the body.

Expression of the TAB3 gene was considered as a correlate of TGF-β (transforming growth factor beta) activation, which, under conditions of corneal trauma, promotes an increase in the intensity of proliferation and migration of epithelial cells, which contributes to rapid healing of the wound surface [32].

TGF-β cytokines were first discovered in the early 1980s, and three TGF-β isoforms have been identified in mammals (TGF-β1, TGF-β2, and TGF-β3) [17].

TGF-β 1, 2 and 3 have been detected in the aqueous and vitreous humor of a human eye [4, 8]. In addition, these ligands are also expressed in cornea, ciliary epithelium, lens, retina and blood vessels [9].

Depending on the cellular context, TGF-β family members can either inhibit or stimulate proliferation, control extracellular matrix turnover, and participate in epithelial-mesenchymal interactions during embryogenesis. Their activity is also associated with tissue repair and modulation of immune response [18].

An integrated process of cell proliferation, migration, differentiation, desquamation, and apoptosis maintains homeostasis of the adult corneal epithelium, but alterations in these processes result in persistent corneal abnormality and may lead to blindness. TGF-β is usually restricted to healthy intact corneal epithelium [39]. In the injured cornea, TGF-β1 is weakly expressed, while TGF-β2 is expressed. M.I. Huh et al. [21] reported differences in TGF-β3 levels after corneal injury in chickens. TGF-βRI and II are also expressed in the injured stroma, thus participating in the wound healing process in corneal tissue [43]. Following corneal injury, overexpression of TGF-β protein leads to an increase in profibrotic factors and pro-inflammatory cytokines.

Taken together, these observations support a stimulatory role for TGF-β family members in the corneal wound healing process, indicating that they may represent therapeutic targets for the treatment of corneal injury.

TGF-β family proteins that transmit signals through the SMAD pathway (SMAD proteins are signal transducers and transcriptional modulators that mediate several signaling pathways) are likely essential for maintaining corneal epithelial homeostasis [35]. Thus, blocking TGF-β activity at the level of SMAD signaling has been proposed as a treatment option to accelerate corneal wound healing. Indeed, blocking TGF-β protein activity by in vivo gene transfer of soluble TGF-β receptor type RII accelerates corneal injury tissue repair in rats. Blocking TGF-β activity by adenoviral gene transfer of soluble TGF-β receptor type RII results in inhibition of corneal opacification, edema, and angiogenesis [31]. The use of a TGF-β receptor inhibitor (SB431542) also maintains normal endothelial phenotypes in cultured corneal endothelial cells [33].

Monoclonal antibodies are potential treatments for corneal scarring: TGF-β antagonists such as antibodies to TGF-β1 and -β2 have been shown to inhibit cutaneous scar formation in rodent wounds [38]. The use of tranilast, a TGF-β inhibitor, reduced the recurrence of corneal fibrosis or primary pterygium, a degenerative disease of the ocular surface with fibrovascular growth of the bulbar conjunctiva onto the cornea [13].

Thus, there is currently a trend towards optimizing the processes of determining priorities for basic research, including in ophthalmology. This concerns the study of the patterns of cyto- and histogenesis, differentiation of cells and tissues of the visual organ, their physiological and reparative regeneration and regulation of these processes at the molecular-genetic level. In this regard, the given, obviously incomplete, list of genetic markers of elementary histogenetic processes of the structural elements of the cornea will be an objective methodological basis for evidence-based ophthalmology.

ADDITIONAL INFORMATION

Author contribution. Thereby, all authors made a substantial contribution to the conception of the study, acquisition, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study.

Competing interests. The authors declare that they have no competing interests.

Funding source. This study was not supported by any external sources of funding.

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ

Вклад авторов. Все авторы внесли существенный вклад в разработку концепции, проведение исследования и подготовку статьи, прочли и одобрили финальную версию перед публикацией.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

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