Diabetic keratopathy and nuclear proteins (Review)

HAIYING XU[1*], ZHI-LIANG JIANG[2*], YUEHONG WANG[3], XIAOLI HOU[1], WEIXIA DONG[1], YANFANG CHEN[1], QIUYING ZHANG[1], XINYING JI[4,5], SHAOPING JI[1,6] and YALONG DANG[7-9]

[1]Department of Histology and Pathology, Henan Provincial Research Center of Engineering Technology for Nuclear Protein Medical Detection, School of Medicine and Health Sciences, Zhengzhou Health College, Zhengzhou, Henan 450000, P.R. China; [2]School of Clinical Medicine, Henan University, Kaifeng, Henan 475004, P.R. China; [3]Department of Ophthalmology, The First People's Hospital of Hangzhou, Hangzhou, Zhejiang 310003, P.R. China; [4]Department of Microbiology and Immunology, Henan Provincial Research Center of Engineering Technology for Nuclear Protein Medical Detection, School of Medicine and Health Sciences, Zhengzhou Health College, Zhengzhou, Henan 450000, P.R. China; [5]Department of Nuclear Medicine, Henan International Joint Laboratory for Nuclear Protein Regulation, The First Affiliated Hospital, Henan University College of Medicine, Kaifeng, Henan 475004, P.R. China; [6]Laboratory of Cell Signal Transduction, Department of Biochemistry and Molecular Biology, School of Basic Medical Sciences, Henan University, Kaifeng, Henan 475004, P.R. China; [7]Department of Ophthalmology, Sanmenxia Central Hospital, Sanmenxia, Henan 472001, P.R. China; [8]Department of Ophthalmology, Sanmenxia Eye Hospital, Sanmenxia, Henan 472099, P.R. China; [9]Department of Ophthalmology, Henan University of Science and Technology School of Medicine, Luoyang, Henan 471023, P.R. China

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Abstract. Diabetic keratopathy (DK) is an ocular complication of diabetes mellitus (DM). Driven by DM-induced chronic hyperglycemia and its associated metabolic changes, DK is characterized by progressive damage to the corneal epithelium, nerves, stroma and endothelium, manifesting as corneal epitheliopathy, neuropathy, stromal lesions and endotheliopathy. Nuclear proteins (NPs) play essential roles in the regulation of gene expression and physiological activities in the nucleus, and have been implicated in the occurrence and development DM and its complications. The present review provides an overview of DK and highlights the role of core NPs in its pathogenesis, including peroxisome proliferator-activated receptors, high-mobility group box 1, enhancer of zeste homolog, phosphatase and tensin homolog and sirtuins. The review underscores that the roles of these NPs in DK remain incompletely understood and highlights the need for further mechanistic studies and clinical trials to advance DK management. Therefore, it is suggested that future research should focus on elucidating the molecular mechanisms of NPs in DK, and developing novel detection techniques and treatment strategies to provide more effective outcomes for patients with DK.

Correspondence to: Professor Xinying Ji, Department of Microbiology and Immunology, Henan Provincial Research Center of Engineering Technology for Nuclear Protein Medical Detection, School of Medicine and Health Sciences, Zhengzhou Health College, 6 Gongming Road, Erqi, Zhengzhou, Henan 450000, P.R. China
E-mail: 10190096@vip.henu.edu.cn

Professor Yalong Dang, Department of Ophthalmology, Sanmenxia Eye Hospital, 227 Xiaoshan Road, Hubin, Sanmenxia, Henan 472099, P.R. China
E-mail: dangyalong@126.com

*Contributed equally

Abbreviations: DK, diabetic keratopathy; DM, diabetes mellitus; NPs, nuclear proteins; PPARs, peroxisome proliferator-activated receptors; HMGB1, high-mobility group box 1; EZH2, enhancer of zeste homolog 2; PTEN, phosphatase and tensin homolog; SIRT, sirtuin; DR, diabetic retinopathy; NLSs, nuclear localization signals; NPC, nuclear pore complex; CCT, central corneal thickness; ECD, endothelial cell density; AGEs, advanced glycosylation end products; PCNP, PEST-containing nuclear protein; DCN, diabetic corneal neuropathy; PRC2, polycomb repressive complex 2

Key words: diabetic keratopathy, nuclear proteins, diabetes mellitus, diabetic retinopathy, PEST-containing nuclear protein

Contents

1. Introduction

Diabetes mellitus (DM) is a prevalent metabolic and endocrine disorder characterized by the persistent elevation of blood sugar levels, termed hyperglycemia, occurring as a result of impaired insulin secretion and/or function (1). It is

estimated that >10.5% of the global adult population now have this condition (2). DM is a major public health burden associated with high health care and societal costs, early death and serious morbidity. Sustained hyperglycemia gradually induces and aggravates damage to the nervous system, cardiovascular, kidneys, eyes, and other systemic tissues and organs, resulting in complications such as cardiovascular and cerebrovascular disease, diabetic kidney disease (DKD), diabetic retinopathy (DR) and diabetic neuropathy (DN) (1,3).

Diabetic eye complications mainly include DR, diabetic cataract and diabetic keratopathy (DK), the latter of which has historically been neglected. DK is classified as either primary and secondary, with primary DK developing due to chronic hyperglycemia, and secondary DK occurring due to diabetic trauma and following surgery (4). Chronic hyperglycemia leads to progressive damage to multiple organ systems, including corneal tissues (5). DK has been reported to occur in 47-64% of diabetic patients (6,7). The clinical manifestations of DK include dry eye, persistent corneal epithelial erosion, superficial punctate keratopathy, delayed epithelial regeneration and decreased corneal sensitivity (8,9). In more advanced stages, corneal ulceration and scarring may occur, leading to corneal opacity, and ultimately impaired vision or permanent vision loss (10).

Nuclear proteins (NPs) are synthesized in the cytoplasm and specifically targeted to the nucleus of a cell (11). They include histones and non-histone proteins, such as structural proteins of the nuclear matrix and lamina, RNA and DNA polymerases, and gene regulatory proteins (12). The transport of NPs from the cytoplasm into the nucleus is mediated by nuclear localization signals (NLSs), which induce the NPs to pass through the nuclear pore complex (NPC) (13). NPs play important roles in the transmission of genetic information, such as in DNA replication, RNA transcription and processing, and DNA damage repair (14). Certain NPs have been implicated as mediators of pathological conditions affecting the eye, including DR, DK and optic neuropathy; examples include peroxisome proliferator-activated receptors (PPARs) (15,16), high-mobility group box 1 (HMGB1) (17) and enhancer of zeste homolog 2 (EZH2) (18). Therefore, the roles of NPs in DK are of considerable research interest. In the present review, the pathogenesis of DK is described and the roles of various NPs in the pathophysiological processes of DK are discussed.

2. Structure and function of the cornea

The eye is a sensory organ responsible for visual perception, transmitting light-derived information to the brain to generate visual images. The eyeball consists of three distinct anatomical layers: The outer layer including the cornea and sclera; the middle layer consisting of the iris, ciliary body and choroid; and the inner neural layer known as the retina. The intraocular cavity contains the anterior chamber, posterior chamber and vitreous cavity, and the major intraocular contents include the aqueous humor, lens and vitreous body (15,19).

The cornea, forming the anterior portion of the eye, is an avascular, transparent tissue that allows light to enter the visual system. It not only protects the inner eye but also provides approximately two-thirds of the total refractive power of the eye (20-22). The cornea is a convex structure composed of five distinct layers, which are, in order from the anterior to the posterior surface: Epithelium, Bowman's layer, stroma, Descemet's membrane and endothelium (23). Its anterior surface is covered by the tear film, which provides lubrication, protection through soluble immune factors, and a smooth optical surface (24,25).

The epithelium is ~53 μm thick and consists of 5-7 cellular layers composed of three distinct cell types, namely squamous cells, wing cells and basal epithelial cells that adhere to the underlying basement membrane (26). The basal epithelial cells have proliferative capacity, which is important for cell renewal and repair (27). Bowman's layer, located posterior to the epithelial basement membrane, is 8-12 μm thick, acellular and lacks the ability to regenerate (28). It is composed of randomly arranged type I, III, V and XII collagen fibrils together with proteoglycans (29).

Beneath Bowman's layer lies the corneal stroma, constituting ~90% of the corneal thickness (30). The stroma primarily consists of extracellular matrix (ECM) and a small number of keratocytes. The keratocytes produce type I and type V collagen, along with proteoglycans, to assemble collagen fibrils and maintain the ECM (31). The stroma is highly organized, to ensure both mechanical strength and light transparency. The subepithelial nerve plexus, located between Bowman's layer and the anterior stroma, comprises stromal nerve branches that perforate Bowman's layer and form the sub-basal epithelial nerve plexus that supplies the corneal epithelium (32).

Underlying the stroma is Descemet's membrane, a 3-μm, acellular fibrous layer secreted by the underlying endothelial cells (33). Descemet's membrane not only provides an 'adhesive scaffold' and barrier protection for corneal endothelial cells, but also maintains the shape and hydration of the cornea (33). The corneal endothelium, located on the posterior surface of the cornea, consists of a single layer of flat, hexagonal cells that are connected in a mosaic honeycomb pattern (15). These cells preserve corneal clarity and visual acuity by maintaining the relatively dehydrated status of the stroma through high-density ionic pumps in their basolateral membranes (27). Since the corneal endothelium has very limited regenerative capacity, damage to the endothelial cells results in permanent loss, leading to corneal edema and blindness (Fig. 1).

3. DK

Diabetes induces morphological and functional alterations in the cornea. Continuous hyperglycemia can affect all corneal layers, including the epithelium, nerves, stroma and endothelium, with the nerves and epithelial cells being particularly vulnerable (10). DK exhibits several clinical manifestations, including persistent corneal epithelial erosion, superficial punctate keratopathy, delayed epithelial regeneration, corneal edema and decreased corneal sensitivity (8,34). Persistent corneal epithelial defects can progress to corneal scarring and ulceration, ultimately leading to corneal opacity and the risk of decreased visual acuity or permanent vision loss (35,36).

Diabetic corneal epitheliopathy. Corneal epitheliopathy is one of the most common and long-term complications of

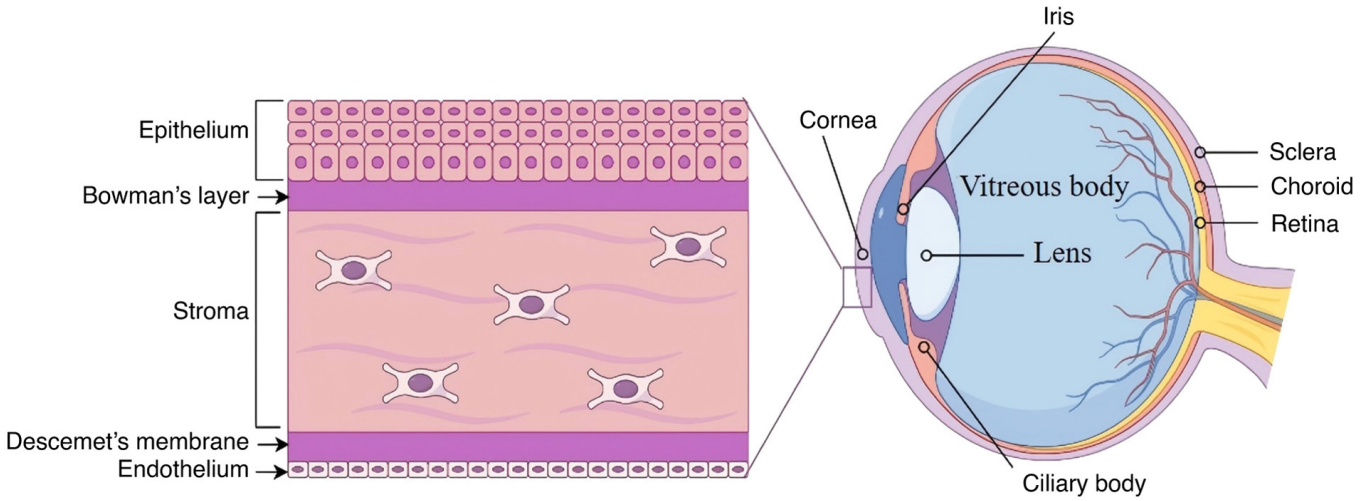

Figure 1. Structure of the eyeball and cornea. The image was created using Figdraw (version 2.0; Beijing Figdraw Technology Co., Ltd.).

DM (37). The rapid repair ability of the epithelium is essential for the maintenance of corneal transparency and homeostasis, particularly in the event of injury or infection (8). However, DM can increase susceptibility to spontaneous corneal trauma, and epithelial lesions take longer to heal in patients with DM. Disrupted epithelial cell junctions, cellular edema and reduced microvilli have been observed in the corneas of diabetic rats by scanning electron microscopy (38).

Hyperglycemia can disrupt the tight-junction complexes of corneal epithelial cells, leading to epithelial dysfunction and stromal edema (39,40). The density of corneal basal epithelial cells has been found to be significantly reduced in patients with both type 1 DM (T1DM) and type 2 DM (T2DM), which impairs the ability of the endothelium to regulate the corneal fluid balance, leading to corneal edema (41).

Hyperglycemia has been demonstrated to impair epidermal growth factor receptor signaling in corneal epithelial cells, ultimately contributing to delayed wound healing (42). The activity of proteolytic enzymes such as matrix metalloproteinases (MMPs) is increased during the wound-healing process in corneal epithelial cells exposed to high glucose (HG) and in the corneal epithelium of diabetic rats, thereby interfering with wound healing and tissue remodeling (43). Moreover, Di *et al* (44) reported that an excessive inflammatory response resulted in increased levels of proinflammatory cytokines and delayed corneal epithelial wound healing in diabetic model mice.

Diabetic corneal neuropathy (DCN). The cornea is the most richly innervated structure in the human body. Corneal nerves provide protective and trophic functions, including the maintenance of corneal sensitivity and the regulation of corneal wound healing through the release of neuropeptides, neurotrophins and growth factors (8). DCN results from trigeminal nerve impairment caused by chronic hyperglycemia, primarily affecting the small Aδ and C nerve fibers. This condition leads to structural abnormalities and reduced corneal innervation, which causes corneal epithelial breakdown, delayed wound healing, and progression to corneal ulceration, melting and perforation (5,45).

Corneal confocal microscopy has become the standard method of assessing the cornea at a cellular level *in vivo* (46). Studies using this technique have shown that corneal nerve parameters, including subbasal nerve fiber density, fiber length and number of fibers, are reduced in patients with T1DM or T2DM, and exhibit an association with diabetic peripheral neuropathy (47-51). Furthermore, corneal sensitivity in patients with diabetes is significantly lower compared with that in non-diabetic controls (52). Notably, these changes are inversely associated with the duration of DM (53).

Diabetic corneal stroma lesions. DM can cause both structural and functional changes in the corneal stroma, leading to a loss of corneal transparency and threatening vision (54). The accumulation of advanced glycosylation end products (AGEs) promotes non-enzymatic crosslinking between collagen molecules and proteoglycans, resulting in corneal sclerosis and thickening. The levels of collagen I and III in the corneal stroma of patients with DM have been observed to be significantly increased compared with those in healthy controls, which is consistent with fibrosis and disruption of the matrix structure, leading to thickening and scarring (55). In addition, Ossabaw mini pigs with T2DM exhibit disorganized stromal collagen fibrils, with decreased levels of collagen IV and upregulated expression levels of MMP-9 (56). In addition, Kalteniece *et al* (57) found that anterior, mid and posterior stromal keratocyte density was significantly reduced in diabetic patients with and without diabetic peripheral neuropathy compared with those in non-diabetic controls, suggesting an association with corneal sub-basal plexus nerve damage in patients with DM. However, Gad *et al* (58) observed downregulations in corneal nerve fiber density and anterior and mid-stromal keratocyte densities in children with T1DM, but no correlation between nerve and keratocyte loss.

Diabetic corneal endotheliopathy. The corneal endothelium plays a critical role in the maintenance of appropriate stromal hydration via tight junctions and Na^+/K^+-ATPase pump activity (59). In diabetes, structural and functional impairments in the corneal endothelium reduce endothelial pump efficiency,

leading to stromal edema, which can be detected clinically as increased central corneal thickness (CCT). These impairments are exacerbated with disease progression, and can manifest as corneal haze and decreased vision (60,61). Numerous studies on the effects of DM on the corneal endothelium have reported that patients with DM exhibit a higher CCT, reduced corneal endothelial cell density (ECD), decreased percentages of hexagonal cells, and increased cell area variation coefficients compared with those in controls (62-67). Furthermore, a multivariate analysis of patients with DM demonstrated that a low ECD is significant associated with elevated glycated hemoglobin levels, longer DM duration and more advanced DR (68).

DK results from prolonged hyperglycemia, which induces severe morphological and functional alterations in the cornea. Chronic hyperglycemia activates multiple pathological mechanisms, such as the accumulation of AGEs, oxidative stress, activation of the polyol pathway and protein kinase C pathways, chronic inflammation, immune cell activation, and reduced neurotrophic innervation (9). These mechanisms complement each other and jointly promote the development of DK.

4. NPs

NPs are proteins that localize to the cell nucleus, and include structural proteins, transcription factors and other functional proteins. They are synthesized in the cytoplasm and are transported into the nucleus through NPCs (69). This transport relies on NLSs and nuclear export signals within the primary structure of the NP. The NPC, composed of ~30 different protein components called nucleoporins, regulates the movement of molecules across the nuclear envelope (70). Small molecules such as ions, metabolites and <40-kDa proteins can diffuse freely though the NPC, whereas larger molecules require specific carrier proteins for transport into and out of the nucleus (69).

There are numerous regulatory NPs that interact with DNA, RNA, nucleosomes and other proteins to form biomolecular condensates, thereby functionally impacting gene expression. These NPs include DNA- and RNA-binding proteins, as well as transcription factors (14). Deoxyribonucleoproteins are involved in the regulation of DNA replication and transcription (14), whereas ribonucleoproteins participate in post-transcriptional regulatory processes (71). Some NPs also mediate post-translational modifications, such as acetylation and methylation, which regulate the physiological activities of cells and influence cell transformation and progression (72). For example, EZH2, a histone methyltransferase of the polycomb repressive complex 2 (PRC2) complex, promotes the trimethylation of histone H3 at lysine 27 (H3K27me3), which alters the chromatin structure and represses gene transcription (73,74). PEST-containing nuclear protein (PCNP) is a small NP of only 178 amino acids that contains two PEST sequences, rich in proline, glutamic acid, serine and threonine. PCNP is involved in vital cellular processes such as cell proliferation and has been implicated in tumorigenesis (75).

5. Role of NPs in DK

In the cell nuclei of the cornea, NPs regulate gene expression and modulate cellular responses. DK is associated with the dysregulation of NPs, leading to molecular and cellular alterations. Certain NPs have been well studied, including PPAR, HMGB1, EZH2, phosphatase and tensin homolog (PTEN) and sirtuin 1 (SIRT1); however, these proteins have not been systematically analyzed in relation to DK in previous reviews.

PPARs. PPARs are a group of ligand-activated transcription factors belonging to the nuclear hormone receptor family (76). The PPAR family comprises three isotypes: PPARα, PPARγ and PPARδ (also known as PPARβ or PPARβ/δ), with genes located on human chromosomes 22, 3 and 6, respectively (77,78). PPARs play crucial roles in glucose and lipid metabolism, and are involved in cell proliferation and differentiation, inflammation, angiogenesis and insulin sensitivity (79). PPARs have been investigated as therapeutic targets, and PPAR agonists represent a promising treatment approach for metabolic diseases such as diabetes, diabetic complications and neurodegeneration (80-82).

Within the eye, PPARα and PPARγ are expressed in the cornea, conjunctiva, retina, meibomian glands and lacrimal glands, whereas PPARδ is located in the cornea, retina and lacrimal glands (15). Matlock *et al* (83) demonstrated that in both human diabetic corneas and the corneas of diabetic rats, the expression of PPARα was significantly downregulated. In addition, experiments using PPARα$^{-/-}$ mice revealed a marked decline in corneal nerve densities, accompanied by an increased incidence of epithelial lesions in the central cornea. These findings suggest that PPARα plays a protective role against diabetes-induced corneal nerve degeneration, potentially by maintaining neuronal integrity and modulating pathological processes associated with diabetes (83). The PPARα agonist fenofibrate has been shown to alleviate mitochondrial dysfunction and promote corneal wound healing in diabetic mice (84). Furthermore, a study of 30 patients with T2DM revealed that oral treatment with fenofibrate for 30 days significantly improved corneal nerve regeneration, reduced nerve edema, and improved the density and width of corneal nerve fibers (85). In addition, both topical and oral fenofibrate are able to ameliorate DCN in diabetic mice, with topical fenofibrate significantly reducing neuroinflammation (86). These findings highlight the therapeutic potential of PPARα agonists in the treatment of DK.

Selective agonists to PPARγ, including thiazolidinedione class drugs such as troglitazone, can decrease insulin resistance and inhibit inflammation, fibrosis and corneal scarring (87). PPARγ downregulates the expression of TGF-β1-induced connective tissue growth factor, thereby exerting anti-fibrogenic effects that inhibit the development of corneal scarring (88). Rosiglitazone, a PPARγ agonist, was shown to alleviate ocular surface damage, enhance corneal sensitivity and increase tear production in a mouse model of diabetes-related dry eye. These effects were accompanied by the upregulation of antioxidant enzyme expression, indicating that oxidative stress was reduced in the lacrimal glands of the diabetic mice (89). Furthermore, the PPARβ/δ agonist GW501516 was reported to suppress inflammatory cytokine release, promote neovascularization by promoting the expression of VEGF-α, and increase the infiltration of M2 macrophages and vascular endothelial cells into the corneal wound area (90).

HMGB1. A ubiquitously expressed and evolutionarily conserved chromosomal protein, HMGB1 plays diverse roles in the mediation of inflammation. It is the most abundant and well-studied member of the HMG superfamily. HMGB1 was first identified in bovine thymus in 1973 by Goodwin and Johns (91), and its name reflects its high migration ability in polyacrylamide gel electrophoresis. It is mainly located in the nucleus, wherein it binds to DNA and regulates chromatin remodeling, gene transcription and DNA damage repair (92). The HMGB1 protein comprises a single polypeptide chain of 215 amino acids, which contains binding sites for DNA as well as for Toll-like receptor 4 (TLR4) and receptor for AGEs (RAGE), which promote inflammation (93). HMGB1 can be passively released by necrotic cells or actively secreted by activated immune cells into the extracellular milieu (94). By interacting with its receptors, RAGE and TLR, HMGB1 ultimately activates NF-κB and stimulates the production of proinflammatory cytokines, including IL-6, IL-1β and TNF-α, while also inducing its own expression and that of its receptors, creating a positive feedback loop of inflammation (95). HMGB1 has been implicated in numerous diseases, including cancer, arthritis (96), diabetes and autoimmune diseases (97,98).

It has been reported that HMGB1 levels are associated with diabetic complications, with increased levels of HMGB1 being observed in both diabetic patients and animal models of diabetes (99,100). HMGB1 promotes insulin resistance and inflammation by upregulating the expression of RAGE, activating the TLR4/JNK/NF-κB pathway and reducing activation of the insulin receptor substrate-1 signaling pathway (101). Studies have also demonstrated the involvement of HMGB1 in the occurrence and development of DR (102,103). Furthermore, HMGB1 exacerbates neuronal apoptosis and autophagy defects in DN by binding to TLR4, with increased HMGB1 and TLR4 levels promoting apoptosis and impairing autophagy (104). Hou *et al* (17) reported that the corneas of diabetic mice with DK exhibit significantly upregulated protein expression levels of HMGB1 and its receptors, indicating that HMGB1 and its receptors play a key role in the development of DK. In another study, a dipotassium glycyrrhizinate-based micelle formulation encapsulating genistein blocked HMGB1 signaling, which promoted diabetic corneal and nerve wound healing (105). These studies suggest that targeting HMGB1 may be a promising therapeutic strategy to enhance corneal and nerve repair in patients with diabetes.

EZH2. EZH2 is a protein that contributes to the regulation of gene activity in cells. The EZH2 gene is located on chromosome 7q35 and comprises 20 exons, encoding a protein comprising 746 amino acids (106). Acting as a catalytic subunit of PRC2, EZH2 promotes transcriptional silencing through H3K27me3 (73,74). EZH2 also forms complexes with transcriptions factors or directly binds to the promoters of target genes to regulate gene transcription. As a key regulator in DNA damage repair, the cell cycle, cell differentiation, autophagy, apoptosis and immunological modulation, EZH2 has been implicated in a variety of diseases, including cancer and ocular disorders (18). Notably, the upregulation of EZH2 expression has been reported in ocular tumors (107), corneal injury (108), cataracts (109) and DR (110).

Studies suggest that EZH2 is actively involved in the pathogenesis of DK. Myofibroblast transdifferentiation induced by TGF-β plays an important role in corneal wound healing but can cause cornea scarring fibrosis when excessively activated (111). In a mouse model of corneal injury, EZH2 expression was upregulated, and the EZH2 inhibitor EPZ-6438 suppressed corneal myofibroblast activation and ECM protein synthesis (112). Corneal neovascularization is another key process in wound healing. In a mouse model of alkali burn-induced corneal neovascularization, the EZH2 inhibitor 3-deazaneplanocin A reduced EZH2 expression and the release of proangiogenic factors, thereby alleviating oxidative stress and abnormal neovascularization in the cornea (113). These findings suggest that EZH2 promotes corneal injury and may serve as a novel and valuable therapeutic target. In addition, in human corneal endothelial cells cultured *in vitro*, EZH2 promoted apoptosis by mediating the H3K27me3-dependent suppression of heme oxygenase-1 gene transcription, further supporting the potential of EZH2 as a target for the treatment of corneal apoptosis (108).

EZH2 has also been implicated in DR. Upregulated expression of EZH2 has been detected in HG-induced human retinal endothelial cells and in diabetic animal models, and the inhibition of EZH2 activity has been shown to reduce MMP-9 and VEGF levels both *in vitro* and *in vivo* (110,114).

PTEN. PTEN is a tumor suppressor gene located at 10q23. It encodes a 403-amino acid protein containing five main functional domains: An N-terminal phosphatidyl-inositol-4,5-diphosphate (PIP_2)-binding domain, a phosphatase domain, a membrane-targeting C2 domain, a C-terminal tail, and a PDZ binding motif (115). PTEN is normally present in the nucleus and cytoplasm of cells in normal tissue, but absent from neoplastic tissues (115). Nuclear PTEN contributes to genome stability by regulating damage repair, cell-cycle progression, DNA replication and chromatin organization (116).

PTEN was the first tumor suppressor gene with phosphatase function identified in humans, and plays a role in tumor inhibition by regulating cell-cycle progression, apoptosis and cell migration (117). The PTEN protein exhibits both lipid- and protein-phosphatase activity. Its lipid-phosphatase function dephosphorylates phosphatidyl-inositol-3,4,5-triphosphate (PIP_3) to form PIP_2, which antagonizes PI3K signaling and blocks the activation of AKT to inhibit tumorigenesis, while its protein-phosphatase activity dephosphorylates various protein substrates, influencing various signaling pathways (118,119). It has been identified that PTEN has three alternative translational isoforms: PTENα, PTENβ and PTENε, which each have tumor-suppressive functions (118). Consequently, the dysfunction of PTEN due to genetic mutation, epigenetic silencing or post-translational modifications contributes to the development and progression of numerous cancers.

The PI3K/Akt signaling pathway is a major network activated in response to insulin or insulin-like growth factor 1 (120). As a negative regulator of this pathway, PTEN also serves as an inhibitor of insulin signaling. It dephosphorylates PIP_3 to PIP_2, which suppresses insulin-induced PI3K signaling, ultimately leading to decreased insulin sensitivity and insulin resistance, a key pathogenic process in T2DM (117). The

tissue-specific deletion of PTEN improves insulin sensitivity and protects against systemic insulin resistance, suggesting a potential strategy for therapeutic intervention in both cancer and DM (117).

During the development of DR, hyperglycemia induces the dysregulation of mitophagy, which causes the accumulation of dysfunctional mitochondria and activation of the inflammasome, processes that are mainly regulated by the PTEN-induced putative kinase protein 1/parkin pathway (121).

The role of PTEN in keratopathy has been investigated in a number of studies. One study demonstrated that in hyperglycemia-induced diabetic mice, neuropeptide FF (NPFF) levels were significantly reduced, while the application of NPFF promoted corneal nerve injury recovery and epithelial wound healing, and downregulated PTEN expression (122). Another study found that PTEN mRNA and protein expression levels were upregulated in diabetic cornea, which hindered corneal epithelial regeneration and Akt activation, while the inhibition of PTEN by the topical administration of dipotassium bisperoxo(picolinato)oxovanadate (V) dihydrate [bpV(pic)] or potassium bisperoxo (1,10-phenanthroline) oxovanadate (V) trihydrate [bpV(phen)] facilitated corneal epithelial regeneration by reactivating Akt signaling. These treatments also improved the recovery of corneal nerve fiber density and sensitivity (123). Similarly, Zhang *et al* (124) demonstrated that the intracameral injection of bpV(pic) promoted the proliferation and migration of corneal endothelial cells, and enhanced corneal endothelial wound healing in a rat model. Furthermore, human umbilical cord mesenchymal stem cell-derived small extracellular vesicles overexpressing miR-21 were shown to enhance the recovery of corneal epithelial wounds by suppressing PTEN expression (125).

SIRTs. SIRTs are a family of NAD^+-dependent lysine deacetylases that act on histones and other proteins, thereby regulating diverse cellular processes such as proliferation, apoptosis and metabolism (126). Seven subtypes of SIRTs, namely SIRT1-7, have been identified in mammals (127). They are involved in numerous physiological and pathological processes, including energy metabolism, oxidative stress responses, inflammation and carcinogenesis (126). Accordingly, the SIRT family has emerged as a potential therapeutic target for various types of pathologies, including cancer, cardiovascular disease and respiratory disease (128). SIRT1 and SIRT2 exist in the cell nucleus and cytoplasm, while SIRT3-5 are predominantly mitochondrial, and SIRT6 and SIRT7 are primarily located in the cell nucleus (129). SIRT1 is the most well studied member of the SIRT family.

Numerous studies have reported that SIRTs, particularly SIRT1-6, are associated with biological processes involved in the development and progression of DM (130). SIRTs participate in the onset and development of DM by regulating glucose metabolism and maintaining insulin homeostasis. Most evidence suggests that SIRT1-4 and SIRT6 have protective effects against the development of DM, while SIRT5 promotes DM progression. However, downregulation of SIRT1 and SIRT2 has been reported to improve DM in some contexts, likely due to cell- and tissue-specific effects (128).

In terms of DM complications, SIRT1 and SIRT3 have been shown to exert protective effects against DKD (131), DR (132), DN (133) and diabetic cardiomyopathy (134). Mechanistically, studies indicate that SIRT1 mediates its protective effects by inhibiting endoplasmic reticulum stress (ERS) and suppressing the NF-κB inflammatory signaling pathway (135). Furthermore, SIRT1 activators have been demonstrated to exert a beneficial impact by reversing T2DM-related complications and promoting diabetic wound healing, underscoring their therapeutic potential in T2DM (136,137).

In DR, SIRT1, SIRT3, SIRT5 and SIRT6 play important roles by regulating insulin sensitivity, inflammatory responses and glucose metabolism (138). More specifically, in DK, Wei *et al* (139) reported that SIRT1 alleviates disease progression by regulating ERS and decreasing the apoptosis of corneal epithelial cells in diabetic rat corneal tissues. Furthermore, another study found that SIRT1 expression was downregulated in the trigeminal sensory neurons of diabetic mice. However, the overexpression of SIRT1 led to the upregulation of its downstream effector miR-182, which mitigated the harmful effects of hyperglycemia by stimulating diabetic corneal nerve regeneration. These findings suggest that targeting SIRT1 may be a potential therapeutic approach for diabetic sensory nerve regeneration and DK (140). Similarly, Hu *et al* (141) demonstrated that HG (25 mM D-glucose) reduced SIRT3 expression and impaired mitophagy in both TKE2 cells and corneal tissues from $Ins2^{Akita/+}$mice. Conversely, the overexpression of SIRT3 has been shown to promote wound healing by upregulating mitophagy under HG conditions (142). These results indicate that SIRT3 may positively impact corneal repair in DK (Table I).

6. Conclusions and prospects

DM is a global public health problem, and its complications seriously impact quality of life. Although DK has a high prevalence it has long been overlooked (2). Clinically, it is characterized by dry eye, corneal epithelial erosions, superficial punctate keratopathy and delayed epithelial regeneration, which in severe cases may result in impaired vision or permanent vision loss.

NPs regulate gene expression and cellular physiological activities in the cell nucleus, and their dysregulation is closely associated with the development of multiple diseases. In DK, the aberrant expression or dysfunction of NPs such as PPARs, HMGB1, EZH2, PTEN and SIRT1 has been implicated in the pathological process of keratopathy. PPAR agonists have shown therapeutic potential in DK by ameliorating corneal neuropathy and enhancing wound healing, while the blockade of HMGB1 suppresses inflammatory responses and promotes corneal healing. EZH2 contributes to corneal scar formation, and its inhibition may prevent corneal fibrosis. PTEN negative regulates the PI3K/Akt pathway, and its inhibition helps to promote corneal epithelial regeneration and nerve repair. Conversely, SIRT1 exerts protective effects by attenuating ERS and inflammation. Collectively, these findings suggest novel targets and strategies for the treatment of DK.

Table I. Nuclear proteins involved in diabetic keratopathy.

Nuclear proteins	Protein class	Isotypes	Genes	Biological effects	Agonists and inhibitors
PPARs	Nuclear receptors, transcription factors (77)	PPARα, PPARγ, PPARδ (77)	PPARA (22q13.31), PPARG (3p25.2), PPARD (6p21.31) (78)	Protects the corneal nerve (83); anti-fibrogenic (87)	Fenofibrate[a] (84-86), thiazolidinedione class drugs[a] (87,89), GW501516[a] (90)
HMGB1	Chromosomal protein (92,93)	-	HMGB1 (93)	Promotes the development of diabetic keratopathy (17) and diabetic neuropathy	Dipotassium glycyrrhizinate-based micelle formulation encapsulating active agents[b] (105)
EZH2	Catalytic subunit of polycomb repressive complex 2 (73,74)	-	EZH2 (7q35) (106)	Promotes corneal scarring and fibrosis (111,112), corneal oxidative stress and neovascularization (113) and human corneal endothelial cell apoptosis (108)	EPZ-6438[b] (112), 3-deazaneplanocin A[b] (113)
PTEN	Phosphatase and tumor suppressor gene (115,117)	PTENα, PTENβ, PTENε (118)	PTEN (10q23) (115)	Decreases insulin sensitivity and causes insulin resistance (117); inhibits corneal epithelium and nerve regeneration (122,123)	BpV(pic)[b] and BpV(Phen)[b] (123); BpV(Pic)[b] (124); HUMSC-sEVs[b] (125)
SIRTs	NAD^+-dependent lysine deacetylases (126)	SIRT1, SIRT2, SIRT3, SIRT4, SIRT5, SIRT6, SIRT7 (128)	SIRT1 (10q21.3), SIRT2 (19q13.3), SIRT3 (11p15.5), SIRT4 (12q24.31), SIRT5 (6p23), SIRT6 (19p13.3), SIRT7 (17q25.3) (127)	Regulates endoplasmic reticulum stress and decreases corneal epithelial cell apoptosis (139); stimulates diabetic corneal nerve regeneration (140); promotes wound healing (141,142)	-

[a]Agonists and [b]inhibitors. PPAR, peroxisome proliferator-activated receptor; HMGB1, high-mobility group box 1; EZH2, enhancer of zeste homolog 2; PTEN, phosphatase and tension homologue; bpV(pic), dipotassium bisperoxo(picolinato)oxovanadate (V) dihydrate; bpV(phen), potassium bisperoxo(1,10-phenanthroline) oxovanadate (V) trihydrate; HUMSC-sEVs, human umbilical cord mesenchymal stem cell-derived small extracellular vesicles; SIRT, sirtuin.

While other reviews have broadly summarized the corneal complications of DM, the present review is the first to specifically examine the functional interplay between specific NPs, namely PPARs, EZH2 and SIRTs, in all corneal layers in DK. However, research on NPs in DK is at an early stage, and numerous questions remain to be addressed. For example, the specific molecular mechanisms underlying the functions of NPs in DK have not yet been fully elucidated, and their interactions with other cell signaling pathways merit further exploration. In addition, the clinical application of NPs as potential biomarkers or therapeutic targets requires validation in additional clinical studies to establish their safety and efficacy.

In conclusion, NPs play complex and diverse roles in DK. Investigating these mechanisms contributes to an in-depth understanding of the pathophysiological process of DK, and also provides an important theoretical basis for the development of novel diagnostic and therapeutic strategies. Continued research in this field may be expected to provide more effective solutions for DK and improve the quality of life of diabetic patients.

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Availability of data and materials

Not applicable.

Authors' contributions

HX and ZJ were responsible for conceptualization, methodology, investigation, formal analysis and writing the original draft of the manuscript. YW collected and collated clinical literature on DK phenotypes and classification, assisted in analyzing clinical evidence of corneal confocal microscopy for DK, and verifying key clinical data such as DK prevalence to ensure the review's clinical conclusions were evidence-based. XH and WD made contributed to data analysis and interpretation by designing key figures, conducting statistical analysis of visualized data, drafting the "Structure and Function of the Cornea" section and figure legends, and critically revising the manuscript. YC and QZ contributed to data acquisition by curating and verifying experimental resources and to data interpretation by optimizing figure layouts and cross-validating visualized data, while also revising figure descriptions and approving the final version. XJ, SJ and YD contributed to conceptualization, resources, supervision, funding acquisition, and the review and editing of the manuscript. Data authentication is not applicable. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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