Proteases and Dry Eye Disease

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Dry eye disease (DED) is a multifactorial disorder that leads to ocular discomfort, visual disturbance, and tear film instability. DED is accompanied by an increase in tear osmolarity and ocular surface inflammation. The diagnosis and treatment of DED still present significant challenges.

dry eye inflammation ocular surface proteases

1. Introduction

Dry eye disease (DED), defined by the Tear Film and Ocular Society (TFOS) in the Dry Eye Workshop II (DEWS) report, is a multifactorial disease of the ocular surface defined by the disruption of homeostasis of the tear film. The ocular signs accompanying DED are tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities [1]. Consequently, some symptoms patients describe are dryness, itching, redness, visual disturbance, and ocular fatigue. The impact of DED on the quality of life increases with the disease severity [2]. In general, DED affects the ability to perform daily activities and work, and in more severe cases, it can instigate mood alterations and depression [3]. The prevalence of DED ranges from 5% to 50%, depending on the studied population [4]. However, prevalence studies can differ due to a lack of heterogeneity in the description of DED and whether the study is based on the symptoms or the signs of the patients [5]. Risk factors for DED include sex and race [6]. Furthermore, the constant use of screens, wear of contact lenses, environmental conditions, and use of medication are also considered risk factors [7]. Thus, with the current general population lifestyle, the prevalence is expected to increase in the following years. For instance, during the recent COVID-19 pandemic, a rise in patients with DED symptoms was described. This increase is correlated to wearing a face mask, also known as mask-associated dry eye. The misplacement of the face mask potentially displaces the air around the eyes and increases the evaporation of tears [8][9].

DED patients can be broadly divided into evaporative dry eye (EDE) and aqueous deficient dry eye (ADDE) [10]. They are not exclusive; thus, patients can present characteristics of the two simultaneously. EDE is associated with the dysregulation of the lipid layer of the tear film. This leads to excessive evaporation of the aqueous layer, causing hyperosmolarity and inflammation of the ocular surface [11]. This DED type is commonly associated with meibomian gland dysfunction (MGD). The meibomian glands are responsible for segregating lipids towards the eyelid margin to form the tear film lipid layer. In patients with MGD, the quantity and quality of the lipids are decreased [12]. Thus, MGD can lead to DED. On the contrary, ADDE is correlated with reduced aqueous production by the lacrimal system or the accessory glands [13]. ADDE, in itself, can be divided into Sjögren syndrome dry eye (SSDE) and non-Sjögren syndrome dry eye (NSSDE) [1]. Sjögren syndrome is an autoimmune disease affecting

the exocrine glands, specifically the salivary and lacrimal glands, resulting in dryness of mucosal surfaces [14]. The DED pathophysiology can be described by the vicious circle of dry eye [15]. The ocular surface disruption leads to osmotic stress. Then, hyperosmolarity initiates stress-related signaling pathways and the release of inflammatory cells and cytokines [16]. Among the activated signaling pathways, there are the nuclear factor kappa beta (NFκB), the mitogen-activated protein kinase (MAPK), and the c-Jun N-terminal kinase (JNK) [17].

2. Proteases and Dry Eye Disease

Proteases play pivotal roles in inflammation and are considered therapeutic targets and biomarkers for different pathologies. Different reviews can be found on this subject [18][19][20]. The correlation between DED and inflammation and the involvement of proteases in inflammatory events show the potential for proteases to become new drug targets or biomarkers for DED. Researchers summarize their role in inflammation and immunity and their involvement in various pathologies, specifically in DED (**Figure 1**).

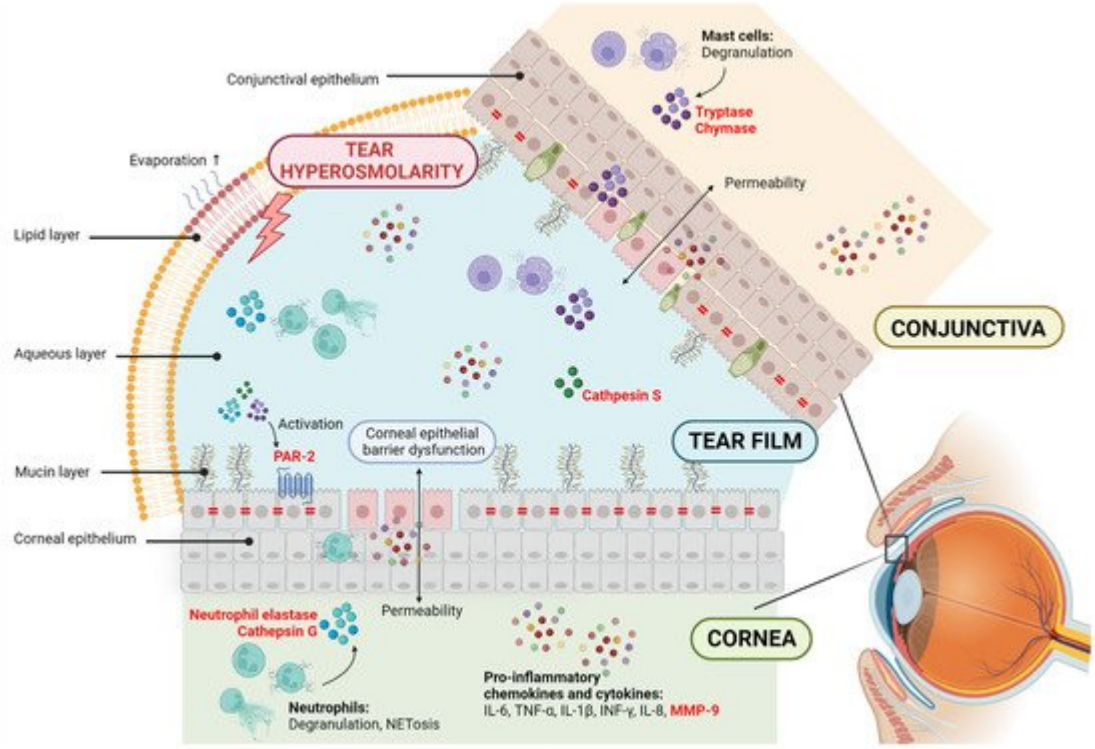

Figure 1. Potential contribution of proteases in dry eye disease (DED). The disruption of homeostasis of the tear film is accompanied by tear instability and hyperosmolarity and ocular surface inflammation and damage. DED is related to corneal epithelial barrier dysfunction, allowing permeability and cell circulation to the tear film. This elevates the production of proinflammatory chemokines and cytokines, including the metalloprotease MMP-9. Neutrophils and mast cells are innate immune cells in the cornea and conjunctiva that, upon degranulation, release biological mediators to the environment, including serine proteases. Cathepsin S is a cysteine protease found in the tears of Sjögren syndrome patients. Proteases are known to promote the expression and activation of proinflammatory cytokines and impact the degradation of extracellular matrix components and the loss of epithelial barrier function. Proteases are also known for activating protease-activated receptors (PARs) and starting

intracellular signaling. PAR-2 is expressed in corneal epithelial cells. In red, the proteases and protease-activated receptors are highlighted.

3. Protease-Activated Receptors and Dry Eye Disease

Proteases are also known for their role in activating PARs [21]. These belong to the seven-transmembrane G protein-coupled receptors (GPCRs). Thus, PARs are cell-surface proteins composed of seven transmembrane domains and three extracellular and three intracellular loops [22]. PARs differ from other GPCR receptors due to their activation by proteases instead of activation by a ligand. Especially, serine proteases can cleave their N-terminal exodomain by proteolysis. The new N-terminal sequence, acting as a tethered ligand, interacts with extracellular loop-2, leading to a conformational change of the receptor [23]. After that, intracellular signaling starts (**Figure 2**). However, this can vary from the receptor subtype, the protease responsible for activation, and the activated pathway. Contrarily, some proteases can inactivate PARs by cleaving the N-terminus in a different position [24]. Currently, four subtypes of PARs are described from PAR-1 to PAR-4 [21]. The functions and proteases that can cleave and activate them differ from subtypes [25]. This section focuses on PAR-2 since only studies on this receptor could be found with interest in the ocular surface.

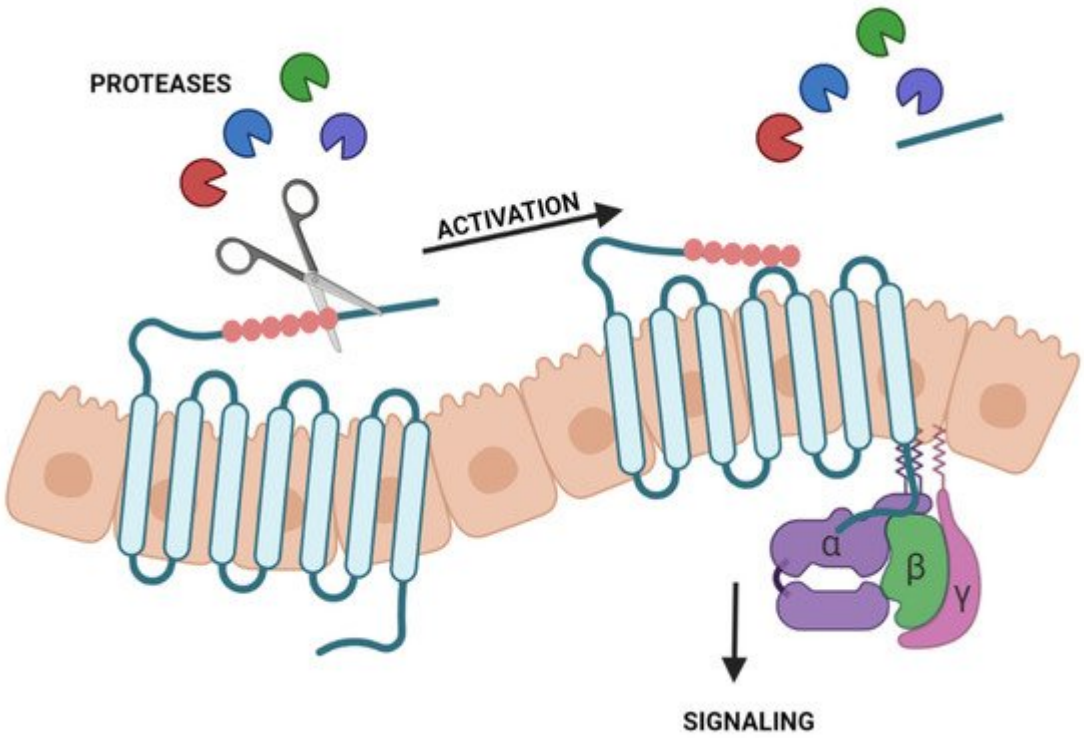

Figure 2. Activation of protease-activated receptors (PARs) by proteases. Proteases cleave the N-terminal exodomain of a specific PAR. The new N-terminal acting as a tethered ligand interacts with the extracellular loop-2 starting intracellular signaling.

The canonical activation of PAR-2 can be performed by several trypsin-like serine proteases, including trypsin, thrombin, tryptase, matriptase, plasmin and some kallikreins. After activation, PAR-2 couples with $G_{\alpha q/11}$, which

leads to the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2), and the Ca^{2+}/inositol 1,4,5-triphosphate (IP_3)/PKC signaling pathway is initiated [26]. This pathway leads to the activation of NFκB, which induces NFκB-dependent genes' induction of proinflammatory cytokines and intracellular adhesion molecule-1 (ICAM-1) [27]. The canonical activation of PAR-2 has also been related to MAPK signaling and subsequent inflammatory response. Other proteases can activate PAR-2 in a biased manner, initiating different signaling pathways [28]. For instance, studies demonstrated that biased activation by neutrophil elastase activates MAPK [29]. The activation of MAPK and NFκB is also associated with DED inflammatory response [30].

PARs have been widely studied and demonstrated to be potential drug targets and valuable biomarkers for many pathologies. For instance, PAR-2 is correlated with visceral hypersensitivity in irritable bowel syndrome [31]; in the cardiovascular system, it is associated with vascular inflammation [32], and airway proteases and PARs have been proposed as therapeutic targets for various lung diseases [33].

While the presence of PARs has been widely studied in diverse human tissues and pathologies, not much is known about their expression and function in the different human eye tissues. The involvement of PARs in DED was never mentioned until recently when Joossen et al. described a significant increase in PAR-2 expression on the corneal tissue of untreated dry eye rats [34]. Several studies indicate the expression of PAR-1 and PAR-2 in HCE [35][36][37]. Lang et al. also demonstrated that a specific activation of PAR-2 by trypsin and thrombin increased the production of the proinflammatory cytokines IL-6, IL-8, and TNF-α by HCE [36]. These cytokines are also found on the tear film of DED patients. Li et al. postulated that tryptase activates PAR-2 in HCE, compromising the barrier function and triggering the expression of MMP-9 [35], another DED biomarker. Cathepsin S can activate PAR-2 in a distinctive site compared with the serine proteases, which proved to cause inflammation and neuropathic pain [38]. Klinngam et al. showed that the increased secretion of IL-6, IL-8, TNF-α, IL-1β, and MMP-9 in HCE treated with cathepsin S is correlated with PAR-2 expression [39].

Although little is known about the function of PAR-2 in DED, studies demonstrating their presence in corneal epithelial cells and their ability to increase the levels of proinflammatory cytokines support the hypothesis of the participation of these receptors in DED inflammatory responses.

4. Protease Inhibitors and Dry Eye Disease

Only a limited number of papers on this subject are reported. However, with evidence that proteases play a role in the pathophysiology of DED, protease inhibitors could potentially be new therapeutics. This section summarizes the studies and results with protease inhibitors in DED animal models (**Table 1**).

Table 1. Protease inhibitors and the effect they have in a specific experimental setting.

Inhibitor	Target	Experimental Setting	Effect [a]	Ref.
PES_103	MMP-9	Dry eye mice model Transdermal scopolamine	↑ Tear production	[40]

Inhibitor	Target	Experimental Setting	Effect [a]	Ref.
		patches		
Divalent PAMAM	MMP-9	Dry eye rabbit model Atropine sulfate	↑ Tear production ↓ Corneal damage	[41]
RSH-12	MMP-9	Dry eye rabbit model Atropine sulfate	↑ Tear volume ↓ Tear breakup time	[42]
SERPINA3K	Serine proteases	Dry eye mice model BAC induced	↓ Epithelial damage ↓ TNF-α	[43]
PEDF	Serine protease	Dry eye mice model Controlled environment chamber	↓ DCs, Th17 ↓ Proinflammatory cytokines ↓ Fluorescein score	[44] [45]
UAMC-00050	Serine proteases	Dry eye rat model Surgical removal exorbital lacrimal gland	↓ IL-1α, TNF-α, MMP-9 ↓ CD3+, CD45+	[34]

[a] An up-facing arrow (↑) represents an increase, whereas a down-facing arrow (↓) corresponds to a reduction.

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