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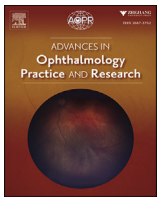

Review

The alterations of ocular surface metabolism and the related immunity inflammation in dry eye

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ABSTRACT

Background: Dry eye disease (DED) stands as a prominent ocular condition of global prevalence, emerging as a growing concern within public health. However, the underlying mechanisms involved in its pathogenesis remain largely unknown. In recent years, with the development of metabolomics, numerous studies have reported alterations in ocular surface metabolism in DED and offered fresh perspectives on the development of DED.
Main text: The metabolic changes of the ocular surface of DED patients are closely intertwined with the cellular metabolism process and immune inflammation changes. This article expounds upon the correlation between ocular surface metabolism and immune inflammation alterations in DED in terms of glycolysis, lipid metabolism, amino acid metabolism, cellular signaling pathways, and immune inflammation regulation.
Conclusions: The alterations in ocular surface metabolism of patients with dry eye are closely associated with their inflammatory status. Our work contributes novel insights into the pathogenesis of dry eye diseases and offers innovative molecular targets for diagnosing, detecting, and managing DED patients.

1. Introduction

Dry Eye Disease (DED) is a prevalent ocular surface ailment frequently encountered in clinical settings, with a global incidence rate ranging from 5% to 50%.[1] In the Chinese population aged 5–89 years, the prevalence of DED is 31.40%.[2] In 2017, the Dry Eye Workshop II proposed that the fundamental mechanisms underlying DED encompass hyperosmolarity of the tear film, tear film instability, inflammation and damage of the ocular surface, as well as aberrant sensory nerve function.[3] The typical clinical manifestations experienced by patients diagnosed with DED comprise dryness, grittiness, foreign body perception, photophobia, burning sensation, and Vision fluctuation.[4] These symptoms profoundly impact the living quality of individuals affected and result in substantial economic and medical burdens for society as a whole.

The alteration of the microenvironment in DED can lead to ocular surface metabolism changes. Researchers have indicated variations in tear metabolomic characteristics between DED patients and healthy individuals, with significantly elevated levels of tear metabolites including glycolytic enzymes, lactate dehydrogenase, and glucose.[5,6] One of the fundamental mechanisms associated with dry eye syndrome is inflammation, which requires a substantial amount of energy to fulfill the needs of immune cells. Metabolic reprogramming transforms cells from a quiescent metabolic state to an active one, which is pivotal in supplying immune cells with ATP and metabolic intermediates necessary for synthesizing pro-inflammatory cytokines.[7]

In recent years, immune metabolism as a pivotal element in regulating immune responses has garnered much attention, thereby providing a novel perspective for understanding the impact of metabolic states on immunological processes in health and disease. The activity of intracellular and extracellular signaling pathways regulates the coupling of cell growth and survival requirements with metabolic mechanisms that regulate the generation of critical products to meet these demands.[8] Intracellular metabolic pathways are widely recognized as important regulatory factors in immune differentiation and activation, which influence immune responses directly. However, there is limited research on the immunometabolism mechanisms of dry eye disease. This review discusses the metabolic pathways underlying DED and the related immune inflammatory response. Our comprehensive review of the immunometabolism in DED will contribute to better comprehending the pathogenesis of dry eye and help develop emerging targeted therapies for DED.

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2. Alterations of ocular surface metabolism in DED

2.1. Glycolysis and DED

The glycolytic pathway, also known as glycolysis, is a crucial metabolic pathway that involves the catalysis of glucose by various enzymes to produce metabolic products and ATP, thereby providing energy to the cell[9]. When transported into the cytoplasm, glucose then undergoes glycolysis, with the resulting pyruvate predominantly utilized for oxidative phosphorylation (OXPHOS) after the tricarboxylic acid (TCA) cycle, thereby facilitating the production of ATP. Glucose-6-phosphate (G-6-P) and 3-phosphoglycerate, conducted as intermediates within glycolysis, are closely related to the pentose phosphate pathway and the biosynthesis of amino acids. Glycolytic intermediates play a crucial role not only in energy provision but also in bolstering the immune cellular response to extrinsic stimuli.[10] Despite its relative efficiency in ATP generation compared to the TCA cycle and OXPHOS, glycolysis stands as a significant metabolic route for the activation of immune cells. In the period of active inflammation, immune cells heavily rely on glycolysis as their principal metabolic pathway to fulfill the exigencies of the inflammatory process, transitioning back to OXPHOS as inflammation subsides.[11] Dysregulation of glycolysis has been identified as a contributing factor to a range of medical conditions, including type 2 diabetes, rheumatoid arthritis, and cancer.[12]

Researcher findings have demonstrated notably elevated concentrations of glucose, lactate, and creatine in tears of DED patients, implying a potential state of heightened energy metabolism.[13] Jiang et al. observed a significant increase in levels of malic acid, L-lactic acid, pyruvate, D-glucose, L-proline, and citric acid, as well as their metabolic pathways, indicating a substantial enhancement in glycolysis/gluconeogenesis.[14] Perumal et al. illustrated, via proteomic analysis of tears from aqueous-deficient and evaporative dry eye patients, an upregulation in the expression of glycolytic enzymes ENO1, PGK1, and ALDOA.[15] Furthermore, Chen et al. elucidated a notable enrichment in amino acid biosynthesis, glycolysis/gluconeogenesis, and glutathione metabolism through proteomic and metabolomic analysis of tears from DED patients, among which six glycolysis-related proteins were upregulated, namely ENO1, PRDX6, PGAM1, PKM, ALDOA, and GAPDH.[16] Moreover, a study conducted by Qingfeng Ni et al. suggested that lactate dehydrogenase A, ENO1, and PKM may serve as potential targets for treating meibomian gland dysfunction.[17]

These findings indicate that the upregulation of glycolysis plays a significant role in the ocular surface metabolic changes of DED patients. As the control of glycolytic flux primarily depends on the activity of three key rate-limiting enzymes, namely hexokinases (HKs), phosphofructokinase-1 (PFK-1), and pyruvate kinases (PKs), we would further elaborate on the glycolytic process in dry eye patients focusing on the alterations in these three enzymes and their effects (Fig. 1).

2.1.1. HKs

HKs serve as the initial crucial enzyme in the glycolytic pathway, facilitating the phosphorylation of glucose into glucose-6-phosphate. There are five subtypes of HKs in mammalian tissues, namely HK1, HK2, HK3, HK4, and HK5, with different predominant distributions in various body tissues. Among them, HK1 and HK2[18] have been extensively studied. HK1 is constitutively expressed in nearly all cells. Previous

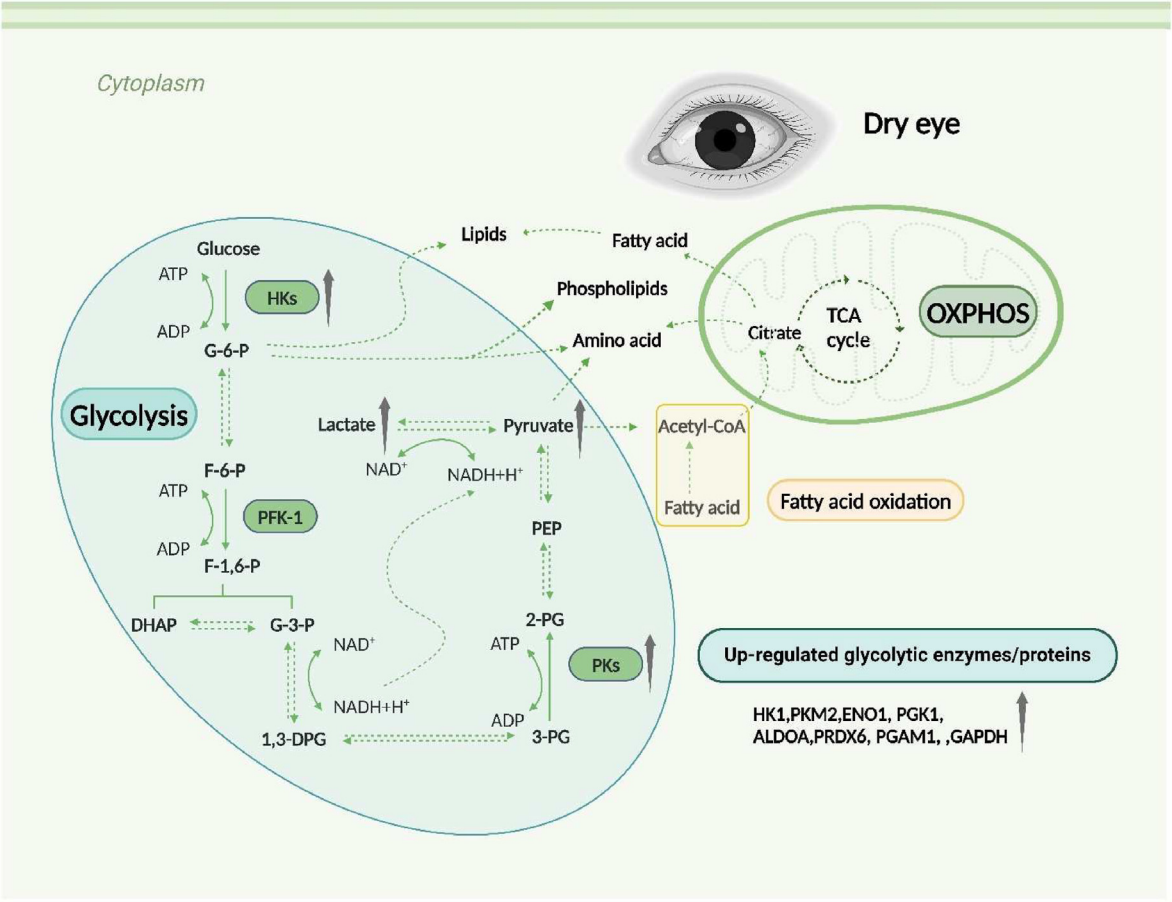

Fig. 1. Changes of glycolysis and key metabolic enzymes in patients with dry eye. HKs, hexokinases; G-6-P, glucose-6-phosphate; F-6-P, fructose-6-phosphate; F-1,6-P, fructose-1,6-biphosphate; PFK-1, phosphofructokinase-1; DHAP, dihydroxyacetone phosphate; G-3-P, glyceraldehyde-3-phosphate; 1,3-DPG, 1,3-diphosphoglycerate; 3-PG, 3-phosphoglycerate; 2-PG, 2-phosphoglycerate; PEP, phosphoenolpyruvate; TCA, tricarboxylic acid; OXPHOS, oxidative phosphorylation.

studies have indicated that upregulation of cytoplasmic HK1 expression can enhance macrophage-mediated production of inflammatory cytokines, which are associated with inflammation related to diabetes and aging.[19,20] On the contrary, HK2 exhibits broad expression in insulin-sensitive tissues such as muscle and adipose tissue and has been shown to have significant involvement in inflammasome activation[21] and virus-induced inflammation.[22] These results collectively underscore the essential significance of HK-mediated glycolysis in the activation of inflammatory cells. What's more, our most recent research has revealed a notable increase in HK1 gene expression within the tears of DED patients as well as hypertonicity-induced human corneal epithelial cells (HCECs).[6] These evidences highlight the crucial role of HK-dependent glycolysis in DED.

2.1.2. PFK-1

PFK-1, acting as the secondary pivotal rate-limiting enzyme in glycolysis, catalyzes the transfer of phosphate groups from ATP to fructose-6-phosphate (F-6-P), ultimately generating fructose-1,6-bisphosphate (F-1,6-BP), which represents as a crucial control point for regulating the flux of glycolysis. Notably, fructose 2,6-bisphosphate (F-2,6-BP) is the strongest allosteric effector of PFK-1, whose level is closely associated with phosphofructokinase-2/fructose-2,6-bisphosphatase 3 (PFK-2, PFKFB).[23] By regulating the levels of intracellular F-2,6-BP, PFKFB effectively governs the glycolytic flux.[24] Of the four isoforms of PFKFB, PFKFB3 is the isoform with the highest kinase activity among them and can significantly enhance the rate of glycolysis. The expression of PFKFB3 can respond to mitosis, inflammation, and hypoxia stimulation, and is upregulated during the DNA synthesis phase of the cell cycle.[25] Liu et al. demonstrate that inhibition of PFKFB3-mediated glycolysis and activation in macrophages can attenuate the pathologic neovascularization in laser-induced choroidal neovascularization.[26] Zhang et al. revealed that the suppression of the TLR4/NF-κB/PFKFB3 pathway could rectify glucose metabolism reprogramming and pyroptosis process induced by NLRP3 inflammasome.[27] Based on the close association of PFKFB3 with macrophage activation and inflammation, the effect of PFKFB3-driven glycolysis on dry eye is worth further exploring.

2.1.3. PKs

PKs are the third crucial rate-limiting enzyme in glycolysis, which facilitates the transformation of phosphoenolpyruvate (PEP) into pyruvate and is activated by the upstream metabolite F-1,6-BP. Pyruvate kinase has four isoforms: PKM1 (skeletal muscle, heart, and brain), PKM2, PKL (liver), and PKR (red blood cells). PKM2 predominates as the sole detectable isoform during embryonic development and is present in diverse differentiated adult tissues.[28] Researchers have indicated that PKM2 facilitated the expression of pro-IL-1β through a positive feedback loop by interacting with hypoxia-inducible factor-1α (HIF-1α), thereby triggering the activation of NLRP3 and AIM2 inflammasomes.[29] Similarly, conditional knockout of PKM2 in bone marrow cells has been shown to confer protection against septicemia caused by activation of NLRP3 and AIM2 inflammasomes in mice.[29] Our previous studies have revealed a substantial up-regulation in the expression of glycolysis and pyroptosis-related genes, such as PKM2 and GSDMD in clinical samples of DED and in the in vitro indirect coculture model of macrophages and HCECs.[6] Suppression of glycolysis can improve macrophage pyroptosis and subsequent inflammation, suggesting the feasibility of PKM2 as a potential glycolysis intervention target in dry eye inflammation.

2.2. Lipid metabolism and DED

Lipids in tears constitute a vital component of the tear film, primarily deriving from the meibomian glands. Research has substantiated that wax esters, cholesterol esters, triglycerides, diglycerides, and free fatty acids are the primary lipid constituents found in meibum secretion.[30] These lipids play a crucial role in upholding the surface tension, viscosity, elasticity, and osmotic pressure of the tear film, contributing to maintaining the integrity of the tear film on the ocular surface and reducing tear evaporation.[31] Variations in the lipid profile, both terms of quality and quantity, are indicative of alterations in tear composition.

Previous studies have indicated that levels of triglycerides are heightened in the tears of DED patients and are susceptible to reactive oxygen species (ROS). Biomarkers associated with lipid peroxidation, including HNE and malondialdehyde, are increased in patients diagnosed with meibomian gland dysfunction (MGD).[30] Borchman et al. conducted a principal component analysis of infrared spectra of meibum samples from MGD patients and healthy individuals, and they found that MGD patients had relatively lower levels of cholesterol esters compared to normal individuals.[32] These studies suggest that alterations in tear composition and meibomian gland lipid metabolism occur throughout the progression of DED and could potentially serve as vital factors in its pathophysiology. Delving into lipid research within DED has the potential to unveil novel biomarkers and therapeutic targets essential for the identification and management of DED patients.

Lipid metabolism is closely associated with ocular inflammation in dry eye (Fig. 2). Bioactive lipids generated from the precursors of omega-6 or omega-3 essential polyunsaturated fatty acids (PUFA), including classical eicosanoids, specialized pro-resolving mediators (SPM), sphingolipids, and endocannabinoids, not only play a role in the initiation and amplification of inflammation but also contribute to the resolution and termination of inflammatory responses, exerting synergistic effects at different stages of the inflammatory process.[33] The classic twenty-carbon fatty acids exhibit potent pro-inflammatory properties, while SPMs promote the resolution of inflammation by inhibiting pro-inflammatory cytokines and inducing the production of anti-inflammatory mediators.[34] On the contrary, omega-3 PUFAs undergo enzymatic conversion into SPMs, thereby exerting anti-inflammatory effects.

Ceramide (Cer), sphingosine 1-phosphate (S1P), ceramide 1-phosphate (C1P), and their precursor molecule sphingomyelin (SM) are currently among the most extensively researched sphingolipids, playing pivotal roles in cellular apoptosis and inflammation processes.[35] Ceramide serves as a critical component in SPL metabolism, directly or indirectly associated with inflammation through its downstream metabolites C1P and S1P. Enhanced sphingolipid metabolism is a significantly altered metabolic pathway in the peripheral cornea and aqueous humor of DED.[36] Robciuc et al. reported that sphingomyelin (SM) is one of the most important sphingolipids and is crucial for maintaining homeostasis in the pathological physiological processes of the anterior segment of the eye.[37] Ham et al. studied tear samples from normal and dry eyes of New Zealand female white rabbits and found that the types and concentrations of SM molecules in DED tears were significantly higher than those in the control group.[38] Xiaoniao Chen et al. demonstrated that SM is upregulated in the peripheral cornea and aqueous humor of DED patients, and the hydrolysis of sphingomyelin leads to an accumulation of ceramides in corneal epithelial cells of DED patients.[36] Given the significant role of sphingolipids in regulating inflammatory responses, it is reasonable to speculate that elevated levels of SM and Cer are involved in the occurrence of inflammation in patients with dry eye diseases.

2.3. Amino acid metabolism and DED

Amino acids are essential nutrients in organisms and serve as the building blocks for protein synthesis. Free amino acids in tissues participate in various biological processes and help maintain organ and tissue homeostasis.[39] The tear film contains multiple amino acids, and studies have reported significant differences in amino acid metabolism between DED patients and healthy individuals. Amino acid profiling has the potential to function as a sensitive biomarker for detecting ocular inflammation, offering a novel perspective on comprehending the pathophysiologic mechanism of various ocular surface diseases.[39]

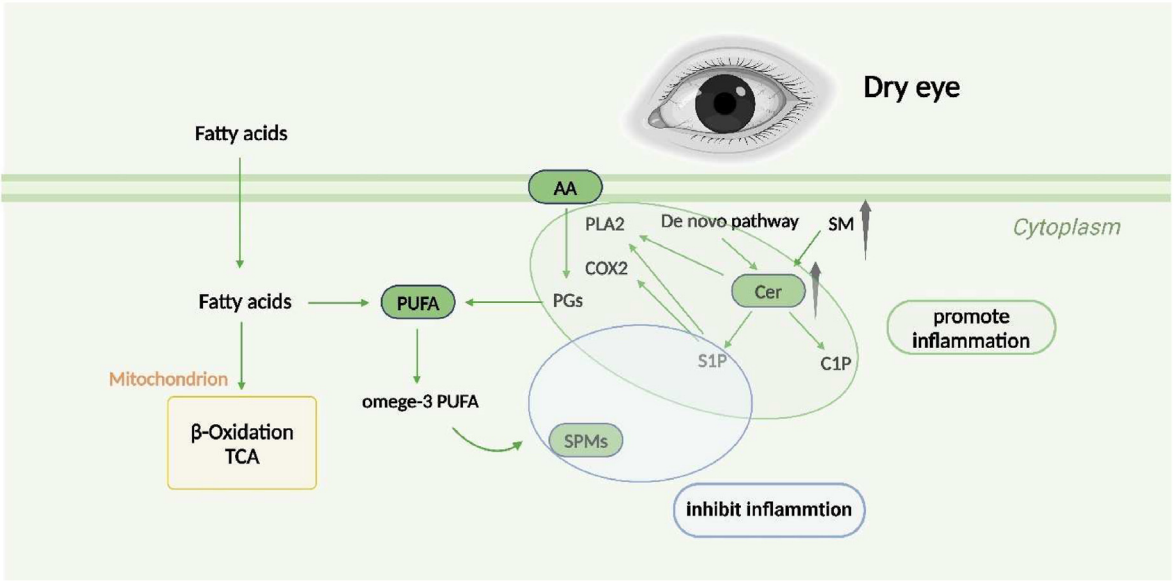

Fig. 2. Changes of lipid metabolism in patients with dry eye. PUFA, polyunsaturated fatty acids; AA, Arachidonic acid; PLA2, phospholipase A2; COX2, cyclooxygenase; SPMs, specialized pro-resolving mediators; Cer, ceramide; S1P, sphingosine 1-phosphate; C1P, ceramide 1-phosphate; SM, sphingomyelin; TCA, tricarboxylic acid.

2.3.1. Tau

Taurine, an abundant amino acid found in the cornea, retina, and lens, is a sulfonated β-amino acid that is synthesized from cysteine and methionine metabolism.[40] It plays a crucial role in various biological functions, including the regulation of inflammatory responses.[41] Its actions in mammals include membrane stabilization, antioxidant activity, maintenance of calcium homeostasis, and osmotic regulation.[42] Recent research has underscored the significance of Tau in maintaining epithelial barrier function, with studies revealing a notable 4.1-fold increase in taurine uptake in HCECs exposure to a 450mOsm culture medium.[42] Notably, Claudio Bucolo et al. have demonstrated the antioxidant capabilities in corneal epithelial cells, showcasing its potential in ameliorating ocular surface damage in a rabbit model of atropine-induced dry eye.[43]

2.3.2. Betaine

Betaine, a key metabolite involved in glycine, serine, and threonine metabolism, serves as an osmoprotectant in plants to mitigate salt and temperature-induced stress. Studies by Xiaoniao Chen et al. have revealed heightened expression of betaine in the cornea compared to the conjunctiva, particularly notable in DED patients.[36] Furthermore, Garrett et al. have underscored the role of betaine in maintaining cell volume and preventing apoptosis in HCECs under conditions of high osmotic stress.[44] Treatment with betaine in DED mice has been shown to ameliorate corneal damage and lower the expression of inflammatory factors TNF-α, IL-1β, IL-6, and IL-8.[45]

2.3.3. Arg

Arginine exhibits anti-inflammatory properties and is detectable in the tears and aqueous humor of healthy individuals. Metabolomic analysis of the tear composition in patients with severe ocular surface diseases indicates a decline in Arg levels in comparison to healthy individuals.[39] Additionally, studies have shown that DED patients exhibit a diminished presence of Arg in both tears and aqueous humor, suggesting a potential correlation between reduced Arg and inflammation in DED.[46]

3. Metabolic disorders and related signaling pathways

The intricate interplay between ocular surface metabolic disorder and the induction of ocular inflammation in DED patients is inseparable. The core mechanisms of DED encompass elevated osmolarity of the tear film and inflammatory reactions. The increased osmotic pressure triggers a cascade of processes within ocular epithelial cells, implicating signaling pathways like MAPK and NF-κB, along with the release of inflammatory factors, such as IL-1β, IL-6, and TNF-α as well as proteases like MMP9. These components activate inflammatory cells and recruit them to the ocular surface, acting as supplementary origins of inflammatory mediators.[47] The heightened metabolic state of corneal epithelial cells is closely involved in the development of ocular inflammation in DED. In this section, we will provide an overview of the signaling pathways implicated in the immune metabolism of DED. The relationship between key metabolites and related signaling pathways and their impacts on ocular inflammation are summarized as follows (Table 1).

3.1. MAPK signal pathway

The mitogen-activated protein kinase (MAPK) is a crucial cellular signaling mediator involved in the regulation of ocular inflammation and immune responses. MAPK consists of three main subtypes: c-Jun N-terminal kinases (JNK), extracellular signal-regulated kinases (ERK), and p38 mitogen-activated protein kinase (p38 MAPK).[58] Among these, MAPK activated protein kinase 2 (MK2) and functions as a key checkpoint kinase in the cell cycle. Phosphorylation of the p38α MAPK subtype and its formation into a complex with MK2 facilitate transcription, protein synthesis, changes in cell surface receptor expression, and alterations in cell cytoskeletal structure, ultimately impacting cell survival and apoptosis.

Novellasdemunt et al. demonstrated that stress stimuli, such as NaCl, can phosphorylate MK2 and promote the transcription and conformational activation of PFKFB3 via the p38 MAPK/MK2 pathway, leading to enhanced activation of PFK1 and subsequent increase in glycolytic rate.[48] In an experimental DED mouse model, the activation of the MAPK signaling pathway led to a notable increase in phosphorylated forms of ERK, JNK, and p38 within corneal and conjunctival epithelial cells.[59] Additionally, there was a marked upregulation of pigment epithelium-derived factor (PEDF) expression in tears of DED patients. Furthermore, the administration of recombinant PEDF showed promising therapeutic effects by suppressing the production of inflammatory cytokines such as IL-β, IL-6, TNF-α, and IL-17a, through the inhibition of the

Table 1
Correlations between key metabolites and related signaling pathways and their impacts on ocular inflammation.

Metabolites	Signaling pathway	Association between metabolites and signaling pathway	Impact on ocular surface inflammation
PFKFB3	MARK	PFKFB3 activation via p38 MAPK/MK2 pathway to increase glycolysis rate under hyperosmotic stress[48]	Activate MAPK signaling pathway could increase the expression of inflammatory cytokines IL-β, IL-6, TNF-α, and IL-17a[51]
	PI3K/AKT	Decreased level of PFKFB3 activate PI3K/AKT pathway[49]	Restore PI3K/AKT signal reduced inflammatory cytokines TNF-α, IL-1β.[52]
	JAK/STAT	JAK/STAT pathway inhibitor tofacitinib reduced the expression of PFKFB3[50]	Decreased the levels of inflammatory factors such as IL-1β and IL-6[53]
Lactate	cGAS-STING	Lactate induced mt DNA leakage and activation of the cGAS-STING pathway[54]	Lactate scavenger inhibited inflammation both in vivo and in vitro[54]
PKM2	PI3K/AKT	Decreased level of PKM2 activated PI3K/AKT pathway[52]	Restore PI3K/AKT signal reduced inflammatory cytokines TNF-α, IL-1β.[52]
PUFA	NF-kB MARK	Supplementation with PUFAs reduced expression of the inflammatory factor, NF-κB, and MAPK[55]	Omega-3 PUFA inhibited MAPKs and NF-κB pathway induced by TNF-α and exerted anti-inflammatory effect[55]
Betaine	AMPK	Omega-3 PUFA enhanced AMPK activity in skeletal muscle[56] and intestinal epithelial cells[57]	omega-3 PUFAs underwent enzymatic conversion into SPMs, thereby exerting anti-inflammatory effects[34]
	AMPK	Endogenous activator of AMPK	Down-regulation of inflammatory factors TNF-α, IL-1β, IL-6, and IL-8[34]

MAPK p38 and JNK signaling pathways, thereby effectively treating DED mice.[51] Su Li et al. have developed a highly efficient treatment for DED by developing an eye drop formulation of Losmapimod, a p38 MAPK inhibitor with dual anti-inflammatory and antioxidant effects, coupled with ROS scavenger Tempo in cationic peptide micelles to break the vicious cycle of dry eye inflammation.[60] Inhibiting the MAPK signaling pathway to alleviate ocular inflammation in dry eyes is an effective strategy.

3.2. NF-κB signaling pathway

Nuclear factor kappa B (NF-κB) is a group of widely distributed gene pleiotropic transcription factors that exist in eukaryotic cells. It is composed of NF-κB1 (p50, its precursor p105), NF-κB2 (p52, its precursor p100), RelA (p65), RelB, and C-Rel, forming homologous or heterologous dimeric protein complexes.[61] Different combinations of NF-κB and Rel proteins form different NF-κB complexes, with p50/p65 being the most widely distributed and active, also known as NF-κB. Typically, NF-κB remains in the cytoplasm by binding to its inhibitory factor (IκB) protein family members or by associating with its precursors, p100 or p105, and therefore lacks transcriptional activity.[62] Among them, IκBα is the most important regulatory protein that plays a significant role in masking the nuclear localization signal of NF-κB, thereby impeding its translocation into the nucleus for the regulation of gene transcription.

Upon cellular stimulation, IκBα undergoes phosphorylation and subsequent degradation by IκB kinase (IKK), resulting in the dissociation of NF-κB, exposure of the nuclear localization signal, and subsequent translocation into the nucleus to regulate the transcription of target genes.[63] Furthermore, NF-κB can bind to κB binding sites present in gene promoters responsible for encoding cytokines such as TNF-α, IL-1β, and MMP-9, thereby regulating the expression of these genes. The classical NF-κB pathway is predominantly triggered by receptors for pro-inflammatory cytokines and pattern recognition receptors. This pathway involves the activation of the IKK complex, which comprises NF-κB essential modulator, leading to the degradation of IκBα and the nuclear translocation of dimers containing p50, p65, and c-Rel. Subsequently, these dimers initiate the transcription of pro-inflammatory genes.

The NF-κB signaling pathway is a vital target in the innate and adaptive immune response in DED. Hyperosmolarity of tear film, as the primary pathogenic factor in DED, triggers the phosphorylation and translocation of NF-κB p65 to the nucleus, resulting in the regulation of various pro-inflammatory genes such as IL-6,[64] TNF-α,[65] IL-1β.[66] Research by Joo Youn Oh et al. has shown that targeting the HSPB4/TLR2/NF-κB axis in macrophages holds promise for therapeutic interventions in DED.[67] Chang He et al. discovered that in cases of DED related to chronic graft-versus-host disease, there was an activation of the TLR2-mediated NF-κB signaling pathway, which led to an inflammatory state.[68] Donghui Yu et al. reported that PM2.5, as a risk factor of DED, caused the activation of p65 and downstream molecules, leading to DED-related inflammatory response.[69] It is worth mentioning that *Tisochrysis lutea*, which is rich in PUFA, has been proven to improve DED symptoms as a functional food supplement by inhibiting the degradation of IκB-α and activation of NF-κB.[55] These indicated that the NF-κB signaling pathway is a pivotal pathway and viable therapeutic target in DED inflammation.

3.3. PI3K/AKT/mTOR signaling pathway

Phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/AKT (also known as protein kinase B, PKB) signaling pathway is essential for the regulation of various cellular processes within corneal epithelial homeostasis, including cell proliferation, apoptosis, glucose metabolism, and protein synthesis. Growth factors such as epidermal growth factor, insulin-like growth factor 1, substance P/neurokinin 1, and insulin trigger the phosphorylation of PI3K by binding to their respective membrane receptors, and activating the PI3K/AKT signaling pathway, resulting in promoting corneal epithelial cell proliferation, migration, anti-apoptosis, anti-inflammatory response, and wound healing functions.[70] The mammalian target of rapamycin (mTOR) is an atypical serine/threonine protein kinase and acts as a sensor for ATP, amino acids, and hormones within the cellular environment, which regulates cell growth, autophagy, and metabolism.[71] AKT primarily regulates cell growth by stimulating synthetic routes and inhibiting degradation processes like apoptosis and autophagy via the mTOR1 pathway.[72] PTEN activates the PI3K/AKT signaling pathway to suppress glycolysis by diminishing the levels of PFKFB3 and PKM2.[49]

In DED, the hyperosmolar conditions induced oxidative stress and an excessive accumulation of ROS, thereby inhibiting the PI3K/AKT pathway, and culminating in corneal inflammation, and tissue damage. Consequently, reinvigorating the PI3K/AKT pathway may be a promising therapeutic strategy for DED. Astaxanthin, a carotenoid, has been shown to restore the activity of the PI3K/AKT pathway and notably reduced the expression of high mobility group box 1 (HMGB1) as well as inflammatory cytokines TNF-α and IL-1β in a dose-dependent manner, potentially serving as a treatment for DED.[52] Moreover, the immunosuppressant cyclosporine A (CsA) impedes the expression of TNF-α, p-NF-κB p65, and Bax, consequently mitigating cell apoptosis and inflammation by reviving the PI3K/AKT pathway in corneal epithelial cells in vitro.[73]

3.4. AMPK signaling pathway

Adenosine 5′-monophosphate-activated protein kinase (AMPK)

serves as a crucial cellular energy sensor responding to signals of energy consumption, particularly stimulated by higher levels of the AMP/ATP ratio.[74] Its regulatory functions extend to diverse metabolic processes encompassing mTORC1, fatty acid oxidation, glycolysis, and maintenance of mitochondrial homeostasis. AMPK suppresses synthetic metabolism to reduce ATP consumption, while simultaneously enhancing catabolic metabolism to generate ATP. This pivotal function of AMPK significantly impacts cellular functions including cell growth, proliferation, and autophagy. In addition to its role in metabolic processes, AMPK is recognized as a key anti-inflammatory target. Acting as a crucial inducer of macrophage polarization, the activation of AMPK suppresses M1 polarization of macrophage by upregulating the expression of SIRT1 and CREB to inhibit the NF-κB pathway. Conversely, AMPK activation facilitates M2 polarization of macrophage and exerts anti-inflammatory effects by upregulating the expression of STAT6 and PPARγ.

Numerous studies have reported the significant involvement of AMPK in the regulation of inflammation process and immune reactions. Sung et al. discovered that phosphorylated AMPK is decreased in conjunctival tissues, and the activation of AMPK significantly improves clinical symptoms and reduces ocular inflammation in DED mice.[75] Besides various pharmacological activators of AMPK, research has demonstrated that omega-3 polyunsaturated fatty acids (ω3-PUFA) can enhance AMPK activity in skeletal muscle[56] and intestinal epithelial cells.[57] Moreover, as an endogenous activator of AMPK, betaine could promote the improvement of corneal damage and down-regulation of inflammatory factors TNF-α, IL-1β, IL-6, and IL-8.[45] This finding holds promising implications for the improvement of dry eye conditions utilizing regulating the AMPK signaling pathway.

3.5. JAK/STAT signaling pathway

The JAK/STAT signaling pathway is composed of three key components, namely, the tyrosine kinase-associated receptors, the transcription factor STAT, and the tyrosine kinase JAK.[76] Upon the binding of ligands to cytokine receptors, the JAK/STAT pathway can regulate the expression of target genes.[76] Research has highlighted the role of the JAK-STAT pathway in Sjögren's syndrome. Patients with primary Sjögren's syndrome (pSS) exhibited reduced autophagy levels in salivary glands, resulting in elevated levels of pro-inflammatory mediators and activation of the JAK-STAT pathway.[77] Furthermore, JAK inhibitor tofacitinib can reverse the overexpression of IL-6 caused by autophagy defects and exert anti-inflammatory effects.[78]

Inhibiting the JAK-STAT pathway has been proven to have therapeutic effects in DED models. Studies have shown that local treatment with tofacitinib improves corneal fluorescein staining, reduces inflammatory cell infiltration, and decreases pro-inflammatory cytokine levels in an experimental DED model.[79] Furthermore, elevated levels of phosphorylated STAT3 have been observed in three DED models induced by benzalkonium chloride exposure, lacrimal gland ablation as well as meibomian gland dysfunction. Local administration of the STAT3 inhibitor S3I-201 has downregulated the expression of MMP-3/9 and inflammatory cytokine IL-1β and IL-6. It also prevented apoptosis in corneal and conjunctival epithelial cells, enhanced the corneal epithelial barrier function, and promoted increased tear production and conjunctival goblet cell density.[53] Particularly, previous research has provided evidence of the interaction between JAK-STAT signaling transduction and metabolic pathways. Tofacitinib has been discovered to inhibit the expression of key enzymes PFKFB and HK2 in glycolysis and suppress the expression of pro-inflammatory mediators IL-6, IL-8, and IL-1β in Rheumatoid Arthritis.[50]

3.6. cGAS-STING signaling pathway

The cGAS-STING pathway represents a recently discovered inflammatory pathway instigated by the recognition of cytoplasmic double-stranded DNA (dsDNA), which is closely related to metabolic disorders including obesity, nonalcoholic fatty liver disease, insulin resistance, and cardiovascular diseases.[80] Under diverse stress conditions, the release of DNA from the cell nucleus and mitochondria leads to the activation of the STING pathway, with cytoplasmic dsDNA recognized by cGAS. Consequently, the activation of TBK1 and IRF3 is initiated, facilitating the secretion of downstream inflammatory mediators like IFN-α/β and CXCL10.[81]

Studies have demonstrated the accumulation of dsDNA in the tears of DED patients, accompanied by a decrease in DNAse levels.[82] Additionally, increased expression of cGAS and STING proteins have been noted in experimental dry eye animal models and hyperosmotic stress-induced HCECs,[83,84] revealing a correlation between dry eye and activation of the cGAS-STING pathway. Researchers have also found that the damage to the ocular surface caused by BAC could be suppressed in mice through genetic or pharmacological inhibition of STING, indicating the potential therapeutic value of targeting the cGAS-STING pathway in dry eyes. It is noteworthy that lactate-induced mitochondrial DNA (mtDNA) damage in salivary gland epithelial cells of patients with pSS, leading to its leakage and subsequent activation of the cGAS-STING pathway.[54] Sodium dichloroacetate (DCA), a lactate scavenger, has displayed effectiveness in inhibiting inflammation both in vivo and in vitro.[54] This mechanism significantly contributes to understanding the involvement of the cGAS-STING pathway in dry eye.

4. Immune inflammation reactions in DED

The dysregulation of ocular surface metabolism is intricately linked to the inflammatory responses and immune processes underlying DED. Previous discussions have delved into the alterations of ocular surface metabolism in DED and the regulatory signaling pathways involved. Subsequently, further elucidation will be provided regarding immune-inflammatory changes associated with dry eye.

4.1. Pyroptosis

Pyroptosis is characterized as a proinflammatory form of programmed cell death mediated by gastrin. The initiation of inflammasome activation triggers the caspase-mediated cleavage of gasdermin D (GSDMD), the pore-forming protein, which consequently facilitates the maturation and secretion of inflammatory cytokines via GSDMD membrane pores.[85] The current studies have demonstrated that inflammasome-induced pyroptosis, dependent on GSDMD, is heightened in DED patients. This is manifested by increased expression levels of caspase-1, the active cleavage of GSDMD (N-terminal domain, N-GSDMD), as well as IL-18, IL-1β, and LDH.

Researchers have demonstrated that the expression of myocardial infarction-associated transcript (MIAT) is observably involved in the induction of pyroptosis and apoptosis under conditions of hyperosmotic stress. Notably, the silence of MIAT has been found to enhance pyroptosis and apoptosis, while impeding the migration and proliferation of HCECs.[86] Moreover, the administration of Calcitriol effectively alleviated hyperosmotic stress-stimulated pyroptosis in HCECs via NLRP3-ASC-caspase-1-GSDMD pathway, with pyroptosis cells remarkably decreasing by 41.6% after calcitriol treatment.[87] The activation of NLRP12 and NLRC4 inflammasomes-induced GSDMD-mediated pyroptosis, along with IL-33 release,[88] has also been identified in DED. Our research also confirmed the increased levels of NLRP3, cleaved-caspase-1, cleaved-GSDMD, and cleaved-IL-1β proteins upon the indirect co-culturing model of THP-1 macrophages and HCECs.[6] This upregulation can be modulated by the glycolysis inhibitor 2-DG, linking the NLRP3-driven pyroptosis of macrophages to the glycolysis of corneal epithelial cells in DED. The Pyroptosis pathways involved in DED are summarized in Fig. 3.

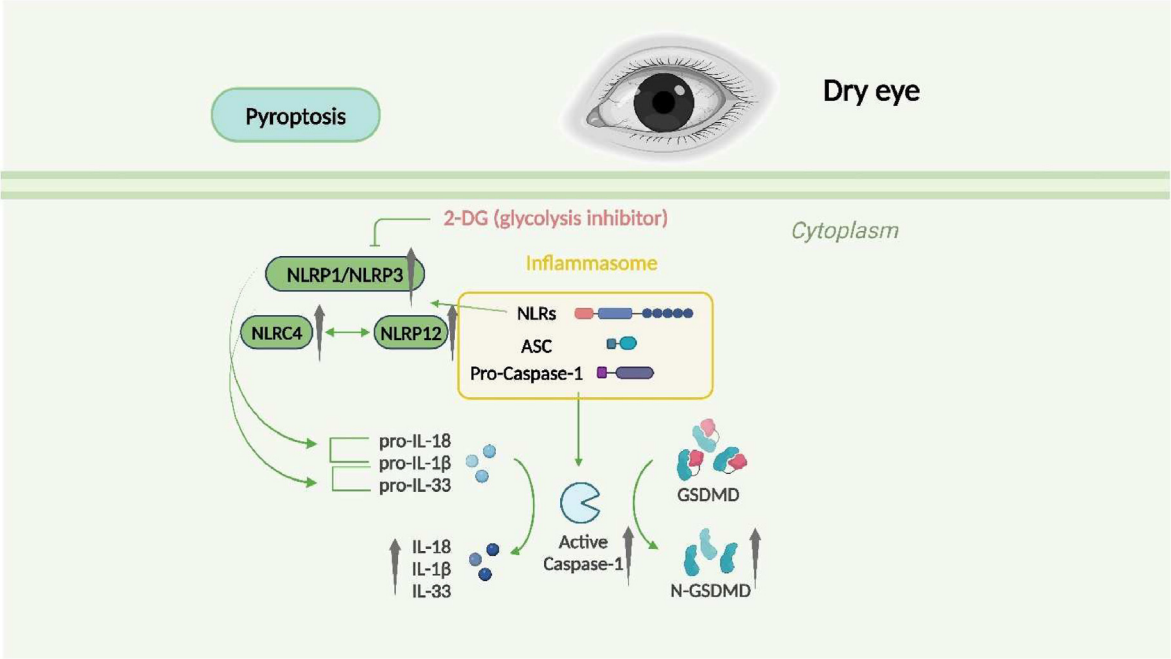

Fig. 3. The Pyroptosis pathways involved in patients with dry eye. NLRs, NOD-like receptors; ASC, apoptosis-associated speck-like protein containing a caspase recruitment domain; GSDMD, gasdermin D. Several pyroptosis pathways are up-regulated in DED. Particularlly, the NLRP3-caspase-1-GSDMD pathway can be suppressed by 2-DG, a glycolysis inhibitor.

4.2. Activation of inflammasomes

The activation of inflammasomes represents a critical step in the initiation and progression of innate immunity and inflammatory conditions. The innate immune system initiates its functions by recognizing exogenous pathogen-associated molecular patterns (PAMP) and endogenous damage-related molecular patterns (DAMP) through pattern-recognition receptors (PRRs). Among these activities, the activation of inflammasomes is of paramount importance. Inflammasomes are protein complexes primarily comprising NLRs (NOD-like receptors, types of PRRs), adaptor protein ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain (CARD)), and pro-caspase-1. These components regulate the activation of caspase-1 and stimulate the maturation and secretion of cytokines pro-IL-1β and pro-IL-18, triggering tissue inflammation and the cellular demise known as pyroptosis.[89]

Importantly, changes in metabolic pathways substantially influence the activation of the inflammasome. Research has shown that choline and its metabolic processes could promote the stimulation of NLRP3 inflammasome and subsequent inflammation responses. Activation of Toll-like receptor (TLR) enhanced the choline uptake by macrophages and microglia through the induction of choline transporter protein CTL1 and further facilitated the activation of NLRP3 inflammasome and the secretion of IL-1β and IL-18 in stimulated macrophages.[90] Additionally, inhibiting glycolysis could decrease the NLRP3 inflammasome activation triggered by triggering receptors expressed on myeloid cells-1 (TREM-1) in macrophages.[91] Therefore, we summarized several inflammasomes associated with the development of DED.

4.2.1. NLRP1

Nucleotide-binding domain and leucine-rich repeat pyrin-domain containing protein 1 (NLRP1) is recognized as the inaugural member of the NLR family to be assembled into an inflammasome. The assembled inflammasome initiates the activation of pro-caspase-1 into its active form, which subsequently activates pro-IL-1β. Mutations in NLRP1 have been associated with autoinflammatory diseases with corneal and mucosal dyskeratosis.[92,93] Further investigations have demonstrated that Val-boroPro induced pyroptosis by activating NLRP1 in primary human corneal epithelial cells.[94] These findings suggest a potential involvement of NLRP1 in the pathogenesis of DED or other ocular surface inflammation.

4.2.2. NLRP3

Extensive research has explored the involvement of NLRP3 in the development of inflammasomes in DED. Niu et al. have revealed the up-regulated expressions of the mRNA and protein of NLRP3 in DED patients, along with the inflammatory factors caspase-1, IL-1β, and IL-18.[95] In murine models of DED, desiccating stress-induced ROS accumulation triggers the formation of NLRP3 inflammation and the release of bioactive IL-1β.[96] Furthermore, ROS inhibition has been shown to attenuate dry eye symptoms by down-regulation of NLRP3 inflammasomes and IL-1β.[96] Huang et al. indicated that local administration of mitochondria-targeted SkQ1 nanoparticles could significantly reduce mitochondrial DNA oxidation and the activation of NLRP3 and improve therapeutic outcomes for DED.[97] Changes in lipid metabolism in dry eye models, especially the increase of sphingolipids and ceramides,[36] were also believed to be associated with the inflammatory process of dry eye, which was likely linked to the mechanism of NLRP3 activation by ceramides and deserves further investigation.[98]

4.2.3. NLRC4 and NLRP12

The activation of NLR family caspase-associated recruitment domain-containing protein 4 (NLRC4) inflammasome is essential in the innate immune response to bacterial infections and autoinflammatory diseases.[99] A recent study has confirmed that NLRP12, collaborating with NLRC4 inflammasomes, initiated GSDMD-mediated pyroptosis and exacerbated DED, accompanied by IL-33 secretion.[88] To better understand the distinct roles of these components, experiments involving NLRP12 knockout mice and the administration of NLRC4 siRNA via subconjunctival injection have been conducted. This study indicated that the activation of TLR4 elicited GSDMD-mediated pyroptosis via caspase 8 signaling.[88]

4.3. Alterations of inflammatory factors

Previous studies have demonstrated the elevated levels of proinflammatory cytokines in tears, such as IL-1, IL-6, IL-8, IL-10, TNF-α, and IFN-γ, which contributed to ocular surface inflammation and amplified immune response. Increased levels of inflammatory mediators IL-18, IL-22, IL-23, and MCP-1 have also been detected in the conjunctiva and cornea of DED mice induced by PM2.5 exposure.[100] Additionally, many lipids exhibit pro-inflammatory properties and trigger the release of inflammatory factors in DED. Ceramides, as mentioned above, promoted inflammation by stimulating the release of IL-1β, and induced cell apoptosis through the activation of PKCζ and PP2A.[98] De novo synthesis of ceramides has been linked to the release of CCL2, IL-6, and IL-8, which further recruited macrophages and triggered the subsequent inflammatory pathway.[101] On the contrary, certain amino acid constituents exhibited inhibitory effects on the release of inflammatory mediators as aforementioned in the preceding text. In conclusion, we summarize the main inflammatory factors involved in DED, along with specific metabolites and inhibitors that influence them (Fig. 4).

4.3.1. IL-1

IL-1α/β and IL-18, prominent members of the IL-1 family, are recognized as highly proinflammatory cytokines that are counteracted by natural antagonist proteins IL-Ra and IL-18 binding protein. Solomon et al. found a significant increase in proinflammatory forms of IL-1 (IL-1α and mature IL-1β), and decreased levels of the biologically inactive precursor IL-1β in the tears and conjunctiva of DED patients.[102] The activation of inflammasomes in response to danger signals led to the processing and release of IL-1β and IL-18 by caspase-1, contributing to the development of dry eye. Moreover, there existed a caspase-1-independent mechanism for IL-1β activation through heightened matrix metalloproteinase (MMP) activity, with MMP9 being identified as a particularly potent factor.[103] Notably, a combination therapy involving taurine and sodium hyaluronate has demonstrated significant efficacy in mitigating corneal damage induced by atropine in rabbits and reducing MMP9 levels in tears.[43] Experimental models of induced dry eye have validated that local administration of IL-1 receptor antagonist (IL-1Ra) and a specific MMP9 inhibitor known as RSH-12 effectively improve clinical symptoms and suppress inflammation.[104] These findings suggest that the topical application of IL-1Ra and selective MMP9 inhibitors could represent novel therapeutic approaches for managing DED patients.

4.3.2. PGs

The emergence of lipid droplets in hyperosmolarity-induced HCECs served as a pivotal effector in inflammation, notably through the function of cyclooxygenase (COX) 2 situated within these lipid droplets.[105] Prostaglandins (PGs) were crucial inflammatory agents derived from arachidonic acid (AA) through the enzymatic actions of COX and PG synthase. Exception from various cytokines and chemokines, the level of PGs increased in tears under hyperosmotic conditions. Shim et al. revealed a significant positive correlation between the ratio of PGE2 to PGD2 levels in tears and dry eye symptom scores, along with an upregulation of COX-2 mRNA in corneas and lacrimal glands of DED mice.[106] Furthermore, a study by Ji et al. showed that the COX-2/PGE2/EP2 axis probably contributed to the migration of CCR7+CD11b + cells to lymph nodes in dry eye and topical treatment of COX-2/EP2 suppressed this migration and the associated Th17 immune response in DED.[107] In conclusion, COX-2 and EP2 may be feasible targets for dry eye treatment.

4.3.3. IL-33

IL-33, another important member of the IL-1 family, is activated upon binding to its receptor, suppression of tumorigenicity 2 (ST2), expressed on diverse immune cells, thereby regulating immune and inflammatory responses. The activation of IL-33 triggered NF-κB and MAPK pathways in mast cells, promoting the secretion of Th2-associated cytokines IL-4, IL-5, and IL-13.[108] Luo et al. proved a significant correlation between elevated tear levels of IL-33 and Th2-related cytokines, namely IL-4 and IL-5, and the clinical severity of DED patients.[109] In addition, Wang et al. studied the IL-33/ST2 pathway in human conjunctival epithelial cells under hyperosmotic conditions, revealing an upregulation of these proteins alongside increased concentrations of IL-13 and IL-5 released from activated Th2 cells, which were closely correlated with disease severity.[110] These findings underscore the pivotal role of L-33/ST2 in the inflammatory damage of the ocular surface through the activation of Th2 responses in DED.

Further research has delved into the role of IL-33 within the pyroptosis pathway. Researchers discovered that the expression levels of bioactive IL-33 decreased upon genetic deletion of GSDMD, selective knockdown of NLRP12 or NLRC4, and pharmacological inhibition of caspase-1. Conversely, heightened caspase-1 activity has been found to trigger the maturation of IL-33[88]. It indicated that the processing and release of IL-33 were regulated by the activation of caspase-1 in the context of NLRP12- and NLRC4-mediated pyroptosis.

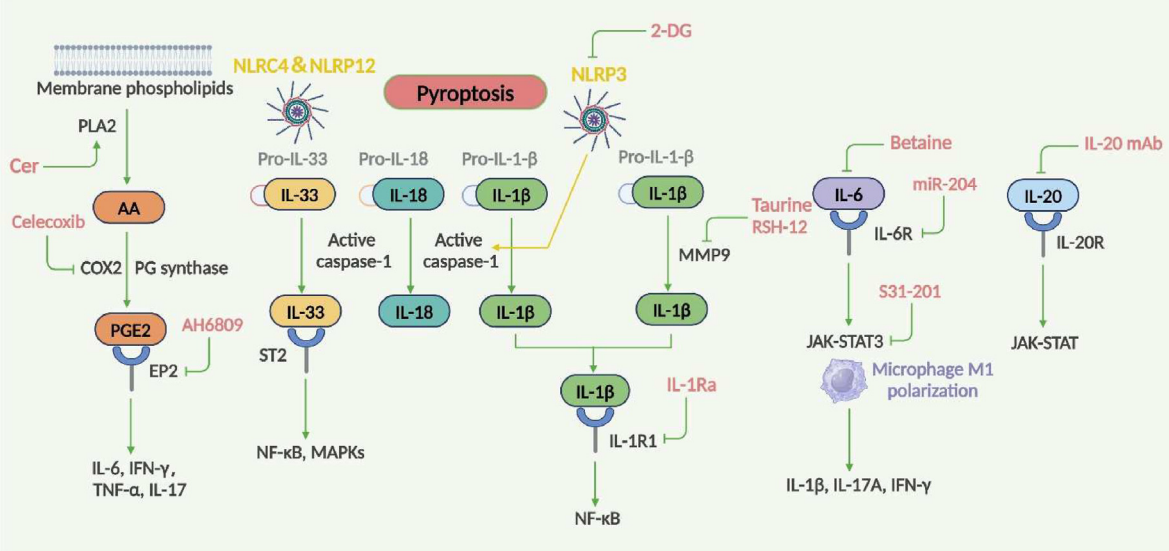

Fig. 4. Inflammatory factors involved in DED, along with specific metabolites and inhibitors regulating them. PLA2, phospholipase A2; Cer, ceramide; AA, arachidonic acid; COX2, cyclooxygenase; PG, Prostaglandin; EP2, eicosanoid-prostanoid receptor 2; ST2, suppression of tumorigenicity 2.

4.3.4. IL-6

The key functions of IL-6 in the ocular context encompassed the initiation of ocular inflammation and facilitation of angiogenesis. Research has demonstrated that elevated IL-6 levels were detectable in the tears of DED patients, showing a correlation with disease severity and various tear films and ocular surface parameters.[111] IL-6, in conjunction with TGF-β, preferentially stimulated the differentiation of Th17 cells that secreted IL-17, while concurrently inhibiting TGF-β-induced Treg differentiation. This process led to the up-regulation of the Th17/Treg ratio, which substantially contributed to the autoimmune damage in DED.[112,113] Moreover, the binding of IL-6 to specific receptors triggered the activation of the JAK/STAT pathway, induced apoptosis under hyperosmotic conditions, and regulated the polarization of pro-inflammatory M1 macrophages and anti-inflammatory M2 macrophages.[114]

STAT3 inhibition by S31-201 improved tear production and reduced the levels of inflammatory cytokines IL-1β, IL-6, IL-17A, and IFN-γ in DED mice.[53] Similarly, miR-204 was found to suppress the IL-6/IL-6R/STAT3 pathway by specifically inhibition the IL-6 receptor (IL-6R), resulting in the reprogramming of M1 macrophages into the M2 phenotype on the ocular surface, thus ameliorated the clinical symptoms of patients with GVHD-associated DED.[115] Moreover, prophylactic administration with betaine in DED mice reduced corneal staining and the expression of inflammatory factors, including TNF-α, IL-1β, IL-6, and IL-17[45].

4.3.5. IL-20

Recent studies have revealed a novel therapeutic objective for the treatment of dry eye. The study identified an increase in the expression of the pro-inflammatory cytokine IL-20, a member of the IL-10 family, in the tears of DED patients and animal models, which was associated with the infiltration of macrophages and the initiation of a strong inflammatory response.[116] A subsequent study demonstrated that the use of a monoclonal antibody targeting IL-20 could inhibit the activation and polarization towards M1-type macrophage, protect corneal epithelial cells from hyperosmolar stress-induced cell apoptosis, and alleviate ocular surface damage in three murine models of dry eye.[116]

4.4. Autophagy

Autophagy is a highly conserved self-degradation pathway mediated by autophagosomes and lysosomes that effectively facilitates orderly degradation and recycling of cellular components,[117] which are essential to maintain cell homeostasis and quality control. It was reported that autophagy activation was a late response to hypertonic stress and interacted with inflammatory factors in DED. Inflammation was usually accompanied by the destruction of organelles and the accumulation of damaged substances, which could be cleared by activated autophagy. In other words, autophagy had a resistant effect on inflammatory reaction.[118] Remarkably, under hyperosmotic conditions, key autophagy markers such as Berclin1, AGT5, AGT7, and LC3B in HCECs exhibited notable increases primarily within 24–48 h, while proinflammatory cytokines such as TNF-α, IL-1β, IL-6, and chemokines like IL-8 showed significant elevation within 24 h but tend to decrease moderately post-24 h.[119] In vitro DED models, the application of Rapamycin (mTOR1 inhibitor) not only enhanced autophagy activity but also diminished the levels of inflammatory cytokine TNF-α, IL-1β, IL-6, IL-8, thereby promoting cell viability.[119] It suggested that autophagy triggered by dry eyes was beneficial to its prognosis.

Researchers have observed a connection between autophagy and pyroptosis in DED. A study by Liao et al. demonstrated an increase in the expression of potassium channel protein KCNK5 in both experimental and cellular models of dry eye. Overexpression of KCNK5 in vitro led to potassium efflux, which not only activated NLRP3 inflammasome-mediated pyroptosis but also impaired autophagy as a consequence of TNFSF10 downregulation.[120] Supplementation of TNFSF10 could promote autophagy and alleviate cell pyroptosis in DED.

5. Conclusions

Metabolomics in ophthalmology has garnered considerable attention over the past decade, particularly with the growing number of studies focusing on tear composition. Through the examination of tear metabolomics, researchers have shed light on the intricate connection between metabolism and immune inflammation, offering fresh perspectives on the development of DED. This review provides a comprehensive overview of the pertinent research regarding ocular surface metabolism and immune inflammation pathways in DED. By emphasizing these specific molecular targets, new possibilities have emerged for the identification, monitoring, and treatment of DED. Moreover, the association between the metabolic processes of the ocular surface and immune-mediated inflammation in DED offers a compelling direction for prospective research endeavors.

Study approval

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Author contributions

XW: conceptualization, design, writing original draft and revision; YZ: conceptualization, design, writing original draft; KZ & YM: writing original draft; XJ & XH: conceptualization, supervision, manuscript review, and editing. All authors reviewed the results and approved the final version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations

DED	Dry eye disease
OXPHOS	Oxidative phosphorylation
TCA	Tricarboxylic acid
G-6-P	Glucose-6-phosphate
HKs	Hexokinases
PFK-1	Phosphofructokinase-1
PKs	Pyruvate kinases
HCECs	Human corneal epithelial cells
F-6-P	Fructose-6-phosphate
F-1,6-BP	Fructose-1,6-bisphosphate
F-2,6-BP	Fructose-2,6-bisphosphate
PFK-2, PFKFB	Phosphofructokinase-2/Fructose-2,6-bisphosphatase 3
PEP	Phosphoenolpyruvate
HIF-1α	Hypoxia-inducible factor-1α
ROS	Reactive oxygen species
MGD	Meibomian gland dysfunction
PUFA	Polyunsaturated fatty acids

SPM	Specialized pro-resolving mediators
Cer	Ceramide
S1P	Sphingosine 1-phosphate
C1P	Ceramide 1-phosphate
SM	Sphingomyelin
MARK	Mitogen-activated protein kinase
JNK	c-Jun N-terminal kinases
ERK	Extracellular signal-regulated kinases
MK2	MAPK activated protein kinase 2
PEDF	Pigment epithelium-derived factor
NF-κB	Nuclear factor kappa B
PI3K	Phosphatidylinositol-4,5-bisphosphate 3-kinase
mTOR	Mammalian target of rapamycin
pSS	primary Sjögren's syndrome
AMPK	Adenosine 5′-monophosphate-activated protein kinase
dsDNA	Cytoplasmic double-stranded DNA
GSDMD	Gasdermin D
MIAT	Myocardial infarction-associated transcript
PAMP	Pathogen-associated molecular patterns
DAMP	Damage-related molecular patterns
PRRs	Pattern-recognition receptors
NLRs	(NOD-like receptors
CARD	Caspase recruitment domain
MMP	Metalloproteinase
IL-1Ra	IL-1 receptor antagonist
PGs	Prostaglandins
AA	Arachidonic acid
PLA2	Phospholipase A2
COX	Cyclooxygenase
ST2	Suppression of Tumorigenicity2
IL-6R	IL-6 receptor
CFTR	Cystic fibrosis transmembrane conductance regulator

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