Biomarkers in the Occurrence and Development of Pterygium

Siyling He  Zhaoxia Wu
Clinical Lab, Jinhua Hospital of Zhejiang University, Jinhua, China

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Abstract
Pterygium is a kind of common conjunctival degeneration. The pathogenesis of pterygium is complex, and various biomarkers provide new targets for treatment and prognosis. Currently, the most common treatment for pterygium is surgical excision, but it is invasive risk and has a high recurrence rate. Since the development of sequencing, gene chip technology, and proteomics technologies has been rapid, research on the internal mechanism of disease has been facilitated. This review focuses on recent advances in the discovery of biomarkers from the fields of genetics, proteomics, and epigenetics and their likely functional mechanisms and clinical applications in pterygium.

Introduction
Pterygium is a common ocular disease. Epidemiological studies worldwide indicate that the prevalence rates of pterygium range from 0.3% to 37.46% [1, 2]. Pterygium is characterized by hyperplastic, centripetally directed growth of altered limbal epithelial cells accompanied by Bowman’s layer dissolution and epithelial-mesenchymal transition (EMT) [3]. Pterygium is usually located on the nasal side, affecting patients’ appearance, generating a foreign body sensation, and even causing blindness [4].

Pterygium is widely considered a chronic inflammation and is associated with prolonged exposure to outdoor ultraviolet (UV) light and dust [5]. Records of pterygium date back to 770 BC, and the disease is derived from the syndrome of wind-heat and damp-heat [6]. Pterygium is widely considered to be caused by joint genetic and environmental factors [7], such as human papillomavirus [8], allergens [9], and cholesterol metabolism [10]. A brief history of hypotheses on the etiology of pterygium is presented in Figure 1.

Currently, the most common treatment for pterygium is surgical excision with conjunctival autografts [11]. However, postoperative use of adjuvant agents, such as mitomycin C and 5-fluorouracil, can effectively reduce the recurrence rate [12]. Nonetheless, there are still many difficulties associated with therapy, such as operative risks, postoperative scarring, and a high postoperative recurrence rate [13, 14]. These problems have led many clinicians to devote themselves to the study of the pathogenesis and treatment of both primary and recurrent pterygium. Therefore, the identification of biomarkers for the occurrence and development of primary pterygium is important to deepen the understanding of pterygium pathogenesis and provide new biomarkers for therapeutics and recurrence prevention.
Genetic Biomarkers of Pterygium

Both gene mutations and single nucleotide polymorphisms involve changes in gene structure or expression levels. The use of next-generation sequencing and DNA microarrays has provided new strategies for research on the mechanism of pterygium.

The first genetic biomarker of pterygium, heparin binding-epidermal growth factor (HB-EGF), was identified in 2003; its mRNA was elevated 6.8-fold at 6 h after irradiation [15]. HB-EGF is a potent mitogen that is significantly induced by UV radiation in pterygium-derived epithelial cells, suggesting that HB-EGF is positively correlated with the progression of pterygium and that UV irradiation might participate in inducing pterygium pathogenesis.

Early research has shown that both the expression and function of p53 are dysregulated in pterygium [16, 17]. Mutations within exons 4–8 in p53 are detected in the pterygium epithelium, but only 15.7% (8 of 51) of pterygium have p53 mutations [17]. Mutation in the p53 gene leads to increased protein stability, allowing more pronounced immunohistochemical detection [18]. UV light activates survivin to suppress the biological function of p53, leading to abnormal cell cycle, apoptosis, and DNA repair [19]; therefore, the possibility of restoring p53 function offers hope for more effective pterygium treatment.

Unlike p53, Kirsten rat sarcoma viral oncogene homologue (K-ras) is one of the most frequently mutated oncogenes in cancer. Mutated K-ras leads to continual K-ras activation, and disordered signal transduction results in cell proliferation. The K-ras mutation rate at codon 12 is significantly higher in patients with recurrent pterygium and young patients [20], while the K-ras codon 61 mutation frequency is significantly higher in primary and bilateral pterygium tissues [21]. However, the number of specimens in both studies was too small to obtain a conclusion with certainty, but it may be speculated that K-ras is associated with a recurrent risk of pterygium.

Differentially expressed genes were first identified from the DNA microarray and further characterized by real-time polymerase chain reaction, western blot, and immunohistochemistry. Thus, neutrophil gelatinase-associated lipocalin and matrix metalloproteinase 9 (MMP9) were upregulated, while insulin-like growth factor-binding protein 3 was downregulated in pterygium [22, 23]. Neutrophil gelatinase-associated lipocalin and MMP9 account for extracellular matrix (ECM) accumulation and a chronic inflammatory reaction, and low expression of insulin-like growth factor-binding protein 3 leads to uncontrolled cell proliferation.

Fig. 1. The history of hypotheses for the etiology of pterygium. EMT, epithelial-mesenchymal transition; HPV, human papillomavirus; Pax6, paired box protein 6.
There is a restriction fragment length polymorphism in intron 16 of angiotensin-converting enzyme (ACE) based on its presence (insertion I) or absence (deletion D) that leads to three genotypes: DD homozygote, II homozygote, and ID heterozygote. Detection of the ACE polymorphism genotype has been performed in Sardinian patients, and the D and DD genotypes are associated with a higher risk of developing pterygium, while the I allele plays a protective role in pterygium occurrence [24]; the DD genotype activates more circulating ACE to promote vasoconstriction, cell proliferation, and inflammatory responses.

A missense variant in cysteine-rich transmembrane bone morphogenetic protein regulator 1 (CRIM1) (NM_016441.2: c.1235A>C (H412P)) has been identified in a family affected by pterygium [25]. CRIM1 is a widely expressed gene in the eye and plays a significant role in cell migration, differentiation, and angiogenesis. Mutated CRIM1 loses its activation in extracellular single-regulated kinase phosphorylation, leading to excessive cell proliferation and inhibition of apoptosis. Although the CRIM1 mutation was discovered in an Irish family, larger sample sizes are needed to verify the hypothesis that CRIM1 may serve as a predictive biomarker for evaluating the pterygium recurrence rate.

The SNP (rs1060043) in the SLC1A5 gene was significantly associated with conjunctival UV autofluorescence [26]. The SLC1 gene family has been widely detected in the human cornea, so the role of SLC1A5 in pterygium deserves further study.

A recent study showed that allelic frequency analysis of MMP9 rs3918242 showed no significant difference in the distribution of allelic frequencies between the pterygium and control groups [27]. Therefore, the role of the MMP9 SNP in determining personal susceptibility to pterygium is still open to debate.

With the development of science and technology, weighted gene coexpression network analysis has been used to provide novel insights into the potential regulatory mechanisms of pterygium. Among the differentially expressed genes, 200 upregulated genes in pterygium were mainly involved in two biological functions: ECM and cell adhesion or migration. The 139 downregulated genes were enriched in inflammation pathways [28]. These differentially expressed genes are mainly involved in ECM-receptor interactions, the phosphoinositide 3-kinase (PI3K)-protein kinase B (Akt) signaling pathway, and an endoplasmic reticulum-related pathway.

Table 1. The changes of DNA methylation in pterygium

| Gene     | DNA methylation | Phenotypes affected | Initiator   |
|----------|-----------------|---------------------|-------------|
| p16      | ↑               | Proliferation       | DNMT3b      |
| E-cadherin| ↑               | EMT                 | DNMT3b      |
| TGM2     | ↑               | ECM remodeling      | –           |
| MMP2     | ↓               | ECM remodeling      | –           |
| CD24     | ↓               | ECM remodeling      | –           |
| MDM2     | ↑               | Proliferation       | DNMT3a      |
| LAT51/LAT52| ↑         | Apoptosis           | –           |

CD24, cluster of differentiation 24; DNMT3a/3b, DNA methyltransferase 3a/3b; ECM, extracellular matrix; EMT, epithelial-mesenchymal transition; LAT51/2, large tumor suppressor kinase 1/2; MDM2, mouse double minute 2; MMP2, matrix metalloproteinase 2; TGM2, transglutaminase 2.

Epigenetics, including DNA methylation, histone modification, nucleosome localization, and noncoding RNA editing, is based on the genetic changes in gene expression but without changes in nucleotide sequences. Abnormal changes have been identified in DNA methylation and microRNA (miRNA) expression in pterygium.

DNA Methylation Biomarkers of Pterygium

DNA methylation refers to the covalent binding of a methyl group at the 5’ C site of the cytosine in genomic CpG dinucleotides due to the action of DNA methyltransferases. In general, CpG dinucleotides in “useless” sequences are relatively rare and always in a methylated state, while most coding genes are always unmethylated [29]. Changes in DNA methylation in pterygium reported to date are shown in Table 1.

The protein expression of p16 is downregulated in pterygium, which is due to the high methylation of the p16 promoter in pterygium caused by DNA methyltransferase 3b [30]. As a tumor suppressor, p16 participates in the regulation of the cell cycle. Staining results have shown that p16 is limited to the nuclei of the epithelial layer, indicating that high methylation of the p16 promoter participates in initiating the development of pterygium.

In 2011, researchers detected the methylation level of 29 genes encoding ECM proteins in pterygium [31]. Transglutaminase 2 was highly methylated in pterygium, while matrix metalloproteinase 2 (MMP2) and cluster of differentiation 24 (CD24) had low methylation levels. Decreased transglutaminase 2 attenuated cell adhesion and...
facilitated the migration of pterygium tissue, while MMP2 and CD24 participated in ECM remodeling and EMT promotion. Furthermore, CD24 has been verified to play a role in neovascularization [32]. However, E-cadherin, a surface marker of epithelial cells, has a hypermethylated promoter region, suggesting the presence of EMT in pterygium [33].

Previous studies have demonstrated that p53 is differentially expressed in pterygium [16, 17], and mouse double minute 2 (MDM2) acts as an E3 ubiquitin protein ligase in the degradation of p53 [34]. Compared to that of control conjunctiva, the methylation rate of the MDM2 promoter in pterygium is significantly lower [34, 35]. It is controversial whether MDM2 suppresses p53 transcriptional activity despite abundant p53 in pterygium.

Early studies have demonstrated that large tumor suppressor kinases 1 and 2 (LATS1 and LATS2) are common tumor suppressor genes in the DNA damage response pathway induced by UV radiation [36]. Najafi et al. [36] found that the LATS1 and LATS2 methylation levels are too high to resist the effects of UV DNA damage. Thus, methylation levels of LATS1 and LATS2 are related to the risk of recurrence.

Histone Modification Biomarkers of Pterygium

Histone modification refers to the process by which histones undergo methylation, acetylation, adenylation, ubiquitination, or ADP ribosylation through related enzymes [37]. Among the histone modifications, methylation is the most stable, while acetylation is highly dynamic, and the modification level correlates with the degree of gene silencing and activation. Thus far, no research has confirmed the actual role of histone modification in the development of pterygium, but some researchers have speculated that pterygium may be related to histone modification.

Koga et al. [38] found that butyrate and phenylbutyrate may be used as histone deacetylase inhibitors to inhibit the expression of fibrosis-related proteins in pterygium, such as α-smooth muscle actin, collagen I/III, and matrix metalloproteinase 1. This research suggests that histone acetylation may be involved in the occurrence and development of pterygium. However, the specific biomarkers involved require further study and exploration.

Biomarkers from Noncoding RNA Regulation in Pterygium

Noncoding RNAs are a type of RNA that includes miRNAs, long noncoding RNAs (lncRNA), and circular RNAs (circRNA), which do not encode proteins but regulate translation. Noncoding RNAs have also been extensively studied in pterygium. The abnormal expression of noncoding RNAs in pterygium is shown in Table 2.

miRNA Biomarkers in Pterygium

miRNAs are a class of small noncoding RNAs that are approximately 22 nt in length and are known to be powerful regulators of gene expression. miRNAs bind to the 3′ untranslated region of target genes and induce gene silencing or degradation.

Some miRNAs Are Associated with Cell Proliferation or Apoptosis in Pterygium. Compared to control conjunctival tissues, miR-221 expression is upregulated, and miR-215 expression is downregulated in pterygium tissues. miR-221 suppresses the expression of the cyclin-dependent kinase inhibitor p27 kinase protein 1 to drive the transformation from G1 phase to S phase [39]. Furthermore, miR-221 levels were significantly higher in the fleshy and intermediate groups than in the atrophic group, indicating that miR-221 is positively related to pterygium safety. miR-215 targets minichromosome maintenance protein 10 and cell division cycle 25A, and both of these genes promote cell proliferation by participating in G1/S and G2/M phase transitions [40]. For this reason, it was speculated that miR-215 could be used as a therapeutic target in pterygium in the future.

miR-21 is reported to activate the Akt signaling pathway in various cancers by inhibiting phosphatase and tensin homologue [41, 42]. Li et al. [43] have found that the miR-21 inhibitor suppresses pterygium fibroblast proliferation and promotes apoptosis via phosphatase and tensin homologue-PI3K/Akt signaling, indicating that an inhibitor of miR-21 may be a novel agent used for treatment.

miR-145 has been found to be significantly downregulated in the head part of the pterygium [44]. p53 plays an agonist role in triggering the transcription of various apoptosis proteins, while MDM2 binds to p53 to initiate the ubiquitin-mediated degradation of p53 [45]. Therefore, the miR-145-MDM2-p53 axis may serve as a potential target pathway for pterygium therapy.

The expression of miR-122 decreases in pterygium, whereas the expression of the targeted gene, B-cell lymphoma-w (Bcl-w), is upregulated [46]. miR-122 functions as a tumor suppressor by targeting Bcl-w, and overexpressed miR-122 arrests the cell cycle at the G1 phase and activates the caspase-3/7 signaling pathway [47]. Oleic acid-based nanoparticles successfully transfer miR-122 into hepatocytes in mice to effectively suppress Bcl-w ex-
expression [48], which indicates the feasibility of miR-122 in pterygium treatment.

Some miRNAs Are Associated with Cell Migration or EMT of Pterygium. In a recent study, upregulated miR-3175 was identified in patients with pterygium and directly inhibited the expression of Drosophila mothers against decapentaplegic protein 7 to promote proliferation, migration, invasion, and EMT in human conjunctival epithelial cell lines [49]. miR-3175 promotes pathological phenotypes of pterygium in normal conjunctival cells, which might be used in clinical therapy.

Aberrant epidermal growth factor receptor (EGFR) expression or function has been detected in several eye diseases, including angiogenesis, ECM disorder, and EMT [50, 51]. In pterygium epithelial cells, miR-218-5p suppresses the expression of EGFR and inhibits the downstream PI3K/Akt/mTOR pathway to promote cell migration and proliferation [52]. This research shows the significance of EGFR in EMT of pterygium and suggests a possible therapeutic candidate, as agents against EGFR have been developed in various cancers [53].

miR-200a is significantly lower in pterygium than in conjunctiva controls, and further tests have shown that the expression level of miR-200a is negatively associated with zinc finger E-box binding homeobox (ZEB) 1/2. ZEB1 and ZEB2 are classified as the main regulators of EMT by inhibiting p53 [54, 55], and p53 is the downstream molecule of both miR-200a and miR-145.

Additionally, Some miRNAs Are Associated with Pterygium Severity. The severity grade of pterygium is based on a comprehensive assessment of three aspects of slit-lamp images: extension, vascularity, and thickness of the pathological tissues. In a study by Chien et al. [56], miR-145 expression was negatively correlated with pterygium severity and was significantly higher in primary pterygium tissues than in recurrent tissues, which suggests the prognostic value of miR-145.

miRNA and mRNA microarray results revealed the important regulatory capacity of the miR-200 family in pterygium [57]. Members of the miR-200 family are all downregulated in pterygium, while the predicted targets of the miR-200 family, including a large number of ECM-related proteins, are upregulated [57], suggesting that the miR-200 family initiates EMT in pterygium. Lee et al. [58] suggested that, compared to normal fibroblasts, miR-143a-3p, miR-181a-2-3p, miR-377-5p, and miR-411a-5p are overexpressed in pterygium fibroblasts. However, these studies lacked further exploration, so their clinical applications require further research.
LncRNA Biomarkers in Pterygium

LncRNAs are a class of noncoding RNAs with a length greater than 200 nucleotides. Studies have shown that lncRNAs play important roles in many physiological activities, such as cell cycle and differentiation regulation, and have become a research hotspot in epigenetics.

The results of lncRNA microarrays can provide a new focus for pterygium. Researchers have found that lncRNA-polled intersex syndrome regulated transcript 1, LOC283761, and lncRNA-forkhead box d2 adjacent opposite strand RNA 1 are upregulated in pterygium and promote cell proliferation, while lncRNA-lipoprotein(a) like 2 and lncRNA-small nucleolar RNA host gene 1 are downregulated in pterygium and suppress cell apoptosis [59]. These lncRNAs hold great promise for clinical application.

A novel lncRNA, linc-9432, is upregulated in pterygium and may regulate 30 differentiation-related genes. In pterygium fibroblasts, linc-9432 participates in inhibiting differentiation-induced cell death, promoting the expression of stem cell and EMT markers [60]. Linc-9432 is positively related to the process of cell differentiation in pterygium, which may be used as an indicator of progression.

CircRNA Biomarkers in Pterygium

CircRNAs are a special class of noncoding RNA molecules and are the most recent research focus in the RNA field. A circRNA molecule has a closed and stably expressed ring structure that protects it from RNA exonucleases. CircRNAs are rich in miRNA binding sites and act as miRNA sponges to block the effects of the miRNAs; this is called the competitive endogenous RNA mechanism.

CircRNA microarrays have indicated that circular RNA-lysosomal associated protein transmembrane 4b (circ-LAPTM4B) is upregulated in pterygium. Inhibiting the expression of circ-LAPTM4B in pterygium fibroblasts and epithelial cells suppresses cell viability and proliferation but promotes apoptosis [61]. Thus, circ-LAPTM4B may be a valuable new therapeutic agent that is more stable.

Biomarkers from Proteomic Analysis in Pterygium

Proteomics refers to the large-scale study of protein characteristics, including protein expression level, post-translational modification, and protein-protein interaction, and therefore provides a comprehensive understanding of disease at the protein level. Many studies have investigated the protein profile of pterygium using proteomic technology.

Overexpressed peroxiredoxin-2 (PRDX2) has been found in pterygium using a proteomic approach [62]. PRDX2 is a cytoplasmic enzyme involved in decreasing intracellular reactive oxygen species levels and protecting cells from oxidative stress induced by UV irradiation. Upregulated PRDX2 reduces peroxide-induced apoptosis and necrosis and leads to aberrant cell proliferation and EMT. The PRDX2 expression level indicates the cause of the disease in that excessive UV exposure promotes the occurrence of pterygium.

Among the 230 differentially expressed proteins in pterygium, aldehyde dehydrogenase 3 family member A1, protein disulfide isomerase A3, and PRDX2 were significantly overexpressed and even increased in recurrent pterygium. These three proteins are protective factors that protect against UV-induced oxidative stress, such as apoptosis suppression and over-proliferation [63]. These three biomarkers demonstrate the predominance of oxidative stress in pterygium formation and may be used to evaluate prognosis.

Based on primary pterygium and conjunctival fibroblasts, a collection of differentially expressed proteins has identified that a large part of the 433 detected proteins are ECM and fibroblast-secreted proteins [64]. The collagen family is overexpressed in the pterygium, and the over-sedimentary collagen produced by pterygium fibroblasts facilitates pterygium growth. The upregulation of fibulin 1 and the downregulation of serpin family E member 1 assemble elastic matrix fibers and inactivate tissue plasminogen activator, leading to ECM changes.

In a recent study, Linghu et al. [65] identified 156 proteins differently expressed between pterygium and normal conjunctival tissues; among these genes, matrix metalloproteinase 10 (MMP10) and cluster of differentiation 34 (CD34) were significantly upregulated in pterygium. MMP10 is a significant factor in denaturing basement membrane components to participate in pterygium invasion, while CD34 is recognized as a surface biomarker of hematopoietic progenitor cells and stem cells to evaluate angiogenesis. MMP10 and CD34 may regulate invasion and neovascularization in pterygium and may be used for prognosis evaluation.

Discussion

In the period of DNA sequencing development, genetics has become a hotspot in the study of disease biomarkers. With advancements in chip technology, RNA sequencing, mass spectrometry, epigenetics, and pro-
Biomarkers have gradually become the core of mechanistic research. All the mentioned biomarkers in our review have been listed in Table 3.

Pterygium is a multifactorial disease with a high incidence and recurrence rate. The most common treatment for pterygium is surgical excision, and many adjunctive agents may be used, such as doxycycline, mitomycin C, and 5-fluorouracil [66]. However, these agents have toxicity and side effects and are only used postoperatively.

Table 3. The disordered source and phenotypes affected of biomarkers

| Biomarker | Disordered source | Expression | Phenotypes affected |
|-----------|------------------|------------|---------------------|
| HB-EGF    | Genomics         | ↑          | Proliferation       |
| p53       |                  | ↑          | Proliferation and apoptosis |
| K-ras     |                  | ↑          | Proliferation       |
| NGAL      |                  | ↑          | ECM remodeling      |
| MMP9      |                  | ↑          | Inflammatory        |
| IGFBP3    |                  | ↓          | Proliferation       |
| ACE       |                  | ↑          | Vasoconstriction    |
| CRIM1     |                  | ↓          | Differentiation     |
| SLC1A5    |                  | ↑          | Further study       |
| p16       | Epigenetics      | ↑          | Proliferation       |
| E-cadherin|                  | ↑          | EMT                 |
| TGM2      |                  | ↑          | ECM remodeling      |
| MMP2      |                  | ↓          | ECM remodeling      |
| CD24      |                  | ↓          | ECM remodeling      |
| MDM2      |                  | ↓          | Proliferation       |
| LAT51/LAT52|                | ↑          | Proliferation       |
| miR-221-p27Kip1 |       | ↑          | Proliferation       |
| miR-215-Cdc25A/ |               | ↓          | Proliferation       |
| MCM10     |                  | ↑          | Proliferation and apoptosis |
| miR-21-PTEN|                | ↑          | Proliferation and apoptosis |
| miR-145-MDM2 |              | Progression related | Proliferation and apoptosis |
| miR-122-Bcl-w |            | ↓          | Apoptosis           |
| miR-3175  |                  | ↑          | EMT                 |
| miR-218-5p-EGFR |          | ↓          | Migration and proliferation |
| miR-200a-ZEB1/ZEB2 |  | ↓          | EMT                 |
| linc-9432 |                  | ↑          | Differentiation     |
| circ-LAPTM4B |            | ↑          | Proliferation and apoptosis |
| PRDX2     | Proteomics       | ↑          | Necrosis and EMT    |
| ALDH3A1   |                  | ↑          | Apoptosis           |
| PDLA3     |                  | ↑          | Apoptosis           |
| FN1       |                  | ↑          | ECM remodeling      |
| SERPINE1  |                  | ↓          | ECM remodeling      |
| MMP10     |                  | ↑          | Invasion            |
| CD34      |                  | ↑          | Neovascularization  |

ALDH3A1, aldehyde dehydrogenase 3 family member A1; Bcl-w, B-cell lymphoma-w; CD24, cluster of differentiation 24; CD34, cluster of differentiation 34; Cdc25A, cell division cycle 25A; circ-LAPTM4B, circular RNA-lysosomal associated protein transmembrane 4B; ECM, extracellular matrix; EGFR, epidermal growth factor receptor; EMT, epithelial-mesenchymal transition; FN1, fibulin 1; LAT51/2, large tumor suppressor kinase 1/2; MCM10, minichromosome maintenance protein 10; MDM2, mouse double minute 2; MMP2, matrix metalloproteinase 2; MMP10, matrix metalloproteinase 10; p27Kip1, p27 kinase protein 1; PDLA3, protein disulfide isomerase A3; PISRT1, long noncoding RNA-pulled intersex syndrome regulated transcript 1; PRDX2, peroxiredoxin-2; PTEN, phosphatase and tensin homologue; SERPINE1, serpin family 1; TGM2, transglutaminase 2; ZEB1/2, zinc finger E-box binding homeobox 1; NGAL, neutrophil gelatinase-associated lipocalin; IGFBP3, insulin-like growth factor-binding protein 3.
Epigenetics links genetics to the environment; thus, the importance of epigenetics in environmental responses should be well established in pterygium to better prevent the occurrence of disease. Primary pterygium pathogenesis is mainly associated with UV light exposure, and some biomarkers support this conclusion. LATS1 and LATS2 act as effectors against UV-induced DNA damage and participate in regulating the cell cycle and apoptosis [67]. In addition, aberrant methylation of p16 is speculated to be associated with UV exposure [30].

As a neoplasm-like disease, cancer includes changes similar to those in pterygium. p16 and p53 are both tumor suppressors that induce growth arrest or apoptosis; p16 acts as a stabilizer of p53 to interact with the E3 ubiquitin-protein ligase MDM2 [68]. Both p16 and p53 share a common function in controlling the G1 phase of the cell cycle. In contrast, K-ras possesses intrinsic GTPase activity, and mutated K-ras induces transcriptional silencing of tumor suppressor genes [69]. Therefore, some anticancer therapies targeting cancer genes may also be feasible for pterygium treatment. For example, siRNA or shRNAs can knockdown mutant p53 to restore activity, gentamicin can induce readthroughs of premature termination codons, and CDB3 can restore sequence-specific DNA binding and transcriptional activity [70]. In addition, MDM2 small-molecule inhibitors have advanced into human clinical trials [71], which may also be taken into consideration for therapy in the future.

It is worth noting that p53 is ectopically expressed in pterygium. miR-145 is predominantly expressed in the basal pterygium epithelium, whereas oncogene MDM2 expression is abundant in pterygium epithelium andstroma [44]. Ectopic expression of miR-145 in pterygium cells induces G1 arrest, downregulates MDM2 and elevates p53 expression.

In addition to the tumor-related proteins, many ECM proteins are abnormally expressed in pterygium. The most notable result from the WGCNA was the identification of fibulin 1, one of the five hub genes that serves as a potential regulator of epithelial cell migration, ECM deposition, and EMT and has also been verified in proteomic findings [28, 64]. Members of the MMP family, including MMP2, MMP9, and MMP10, are overexpressed in the epithelial layer of the pterygium to activate fibroblasts and promote EMT [22, 32, 65]. These profiling findings point to the core role of ECM remodeling in the disease progression, suggesting an important research prospect for ECM remodeling and reversal of EMT in pterygium treatment. Biomarkers identified in genetics, epigenetics, and proteomics studies were classified as potential mechanisms and shown in a network (Fig. 2).

Some core molecules are observed in the complicated biomarker network. The first central point is p53, whose expression levels and functions are regulated by various factors [16, 17, 19]. First, the mutation of p53 is partly regulated by UV light [19]. Second, levels of MDM2 methylation are also involved in p53 expression because MDM2 acts as an E3 ubiquitin protein ligase to degrade p53 [72]. Third, miR-145 can regulate p53 via the miR-145-MDM2-p53 axis [44]. Furthermore, p16 is a protective factor for p53, whereas abnormally activated K-ras inhibits p53 expression. Teng et al. [44] found that miR-145 expression is lower in the head of pterygium than in the body, whereas Chien et al. [56] indicated that miR-145 is negatively correlated with pterygium severity. To the best of our knowledge, these two conclusions are not contradictory. The head of pterygium is affected at the beginning of disease development; at this stage, the low expression of miR-145 in cells promotes rapid cell proliferation. An abnormal increase in cell proliferation leads to increased disease severity and a poor prognosis. The above research demonstrated that the miR-145-MDM2-p53 axis may be a new target for prognosis or therapy.

miRNA microarrays have been used to identify new study targets. The latest study performed by Liu et al. [73] mainly focused on the expression patterns of mRNAs, IncRNAs, and circRNAs. However, much of the information was listed rather than verified. Based on research using these microarrays, miR-143-3p lies at the intersection [40, 46, 57, 58]. miR-143-3p has been reported as a tumor suppressor that suppresses proliferation, migration, and invasion, and promotes apoptosis in various tumors [74, 75]. The use of a miR-143-3p mimic has provided a new strategy for pterygium therapy.

Due to their invertibility, epigenetic biomarkers may be more applicable in clinical settings. Small molecule drugs for DNA methyltransferases and histone deacetylases are in the developmental stage and have made some achievements in tumor treatment. Furthermore, the use of the miR-122 antagonist in patients with hepatitis C has entered phase III clinical trials [76]. This application indicates the prospect of miRNA antagonists and activators being used in the clinic and provides a possibility for noninvasive treatment of pterygium. The first studies using proteomics in pterygium were performed in 2006, and their study specimens were tears from patients with pterygium [77]. Although many genetic biomarkers and proteomic biomarkers lack deep exploration, noninva-
Review Based on Genetic, Epigenetic, and Proteomic Biomarkers in Pterygium

**Fig. 2.** The mechanism network of biomarkers in pterygium. EMT, epithelial-mesenchymal transition; "others" include neovascularization, cell differentiation, and fibrosis.

**Fig. 3.** Clinical applications of biomarkers in pterygium.

Clinical applications

- Upregulated: ACE, MMP2, CD24, PRDX2, ALDH3A1, PDIA3, fibulin 1, MMP10, CD34
- Downregulated: miR-145, TGM2, E-cadherin, SERPINE1

Prognosis prediction

- Severity
  - Upregulated: HB-EGF, K-ras, NGAL, MMP9, Linc-9432, VEGFA, VEGFC
  - Downregulated: MDM2, miR-3175, miR-21, miR-221, circ-LAPTM4B, Semaphorin 7a

- Recurrence
  - Upregulated: CRIM1, IGFBP3, LATS1/LATS2
  - Downregulated: p53, p16, miR-218-5p, miR-145, miR-122, miR-210, miR-215, FGF21

Therapeutic development
sive detection may be used to evaluate prognosis in the future. The probable clinical applications of these biomarkers are shown in Figure 3.

In addition to biomarkers from genetics, epigenetics, and proteomics, other biomarkers may also be used clinically. A recent study demonstrated that serum fibroblast growth factor 21, which is a target for neovascularization reduction, is lower in patients with pterygium [78]. Furthermore, fibroblast growth factor 21 is negatively related to cholesterol, triglyceride, and LDL levels; therefore, im paired lipid metabolism should be considered for therapy. Essential biomarkers in tears, including interleukin-6, interleukin-8, and vascular endothelial growth factor (VEGF), are positively correlated with disease severity, and may also be used for postoperative monitoring [79]. Han et al. [80] found that semaphorin 7a is upregulated in pterygium tissues, and the level in recurrent pterygium is even higher than that in primary pterygium. Semaphorin 7a shows a positive association with some angiogenic factors, such as VEGFA and VEGFR, and migration-promoting factors, such as β1-integrin. Based on the malignant nature of semaphorin 7a in pterygium development and recurrence, its inhibitor may act as a new target for prognosis.

In summary, this review introduced multiple biomarkers in pterygium identified from genetics, epigenetics, and proteomics studies. It was concluded that these biomarkers might play critical roles in severity degree judgement, recurrence risk prediction, and adjuvant therapy in pterygium. Among these biomarkers, it is noteworthy that p53 is involved in pterygium by abnormal changes in various pathways and even has different phenotypes in different development stages. The abnormal changes of the cell cycle dominated by p53 may be a new target for pterygium therapy.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Siying He contributed to the conception and collected the data for the work; while Zhaoxia Wu drafted the work and revised it.

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