Analysis of MicroRNA Expression in Tears of Patients with Herpes Epithelial Keratitis: A Preliminary Study

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PURPOSE. Herpes epithelial keratitis (HEK) is the most common form of herpes simplex virus (HSV) eye involvement, and understanding the molecular mechanisms underlying HEK is important. We investigated the expression of microRNAs (miRNAs) in the tears of patients with HEK.

METHODS. Tear samples from eight patients with HEK and seven age-matched controls were evaluated. Clinical ophthalmologic evaluation was performed, and an anterior segment photograph was obtained after fluorescence staining. Dendritic or geographic ulcer areas were measured using ImageJ software. The expression of 43 different miRNAs in tears was measured using real-time polymerase chain reaction and compared between patients with HEK and controls. Differences in miRNA expression between the dendritic and geographic ulcer groups and correlations involving miRNA expression and ulcer area were evaluated.

RESULTS. Of the 43 miRNAs, 23 were upregulated in patients with HEK compared to normal controls. MiR-15b-5p, miR-16-5p, miR-20b-5p, miR-21-5p, miR-23b-3p, miR-25-3p, miR-29a-3p, miR-30a-3p, miR-30d-5p, miR-92a-3p, miR-124-3p, miR-127-3p, miR-132-3p, miR-142-3p, miR-145-5p, miR-146a-5p, miR-146b-5p, miR-155-5p, miR-182-5p, miR-183-5p, miR-221-3p, miR-223-3p, and miR-338-5p were significantly upregulated in patients with HEK. MiR-29a-3p exhibited significant differences between the dendritic and geographic ulcer groups. All 23 miRNAs with significant differences between patients with HEK and the control group were not significantly correlated with ulcer area.

CONCLUSIONS. Twenty-three miRNAs were significantly upregulated in the tears of patients with HEK, and the expression of miRNAs may play important roles in herpes infection in relation to host immunity.

Keywords: biomarkers, herpes epithelial keratitis, microRNAs, miRNAs, tears

Herpes simplex keratitis, a major cause of visual impairment globally, is caused by herpes simplex virus (HSV) infection. Based on anatomical location, HSV keratitis is subdivided into epithelial, stromal, and endothelial keratitis. In the Herpetic Eye Disease Study, HSV epithelial keratitis (HEK) was the most common HSV eye involvement, with reported prevalence of 79%. Each year, approximately 1 million people worldwide suffer from new or recurrent HEK, which may significantly impair their quality of life.

Despite the availability of antiviral drugs in clinical practice, many patients experience repeated recurrence and suffer from visual loss of varying extent induced by neovascularization or scar formation. Numerous studies have implicated many cellular processes in host responses after viral infection, such as apoptosis, inflammation, angiogenesis, and lymphogenesis; however, the molecular mechanisms underlying this disease remain poorly understood.

MicroRNAs (miRNAs) are a group of endogenous, small, non-coding RNAs, approximately 20 to 25 nucleotides in length, that regulate gene expression post-transcriptionally. MiRNAs play significant roles in many cellular processes, including cell differentiation, proliferation, and apoptosis. Tears are complex biological mixtures that serve as a first line of defense against pathogens. MiRNAs are also present in tears and have been found to be very stable; however, miRNA expression profiles in human tears have not yet been well established, and little research has been performed to determine such miRNA expression profiles in patients with HEK.

Therefore, understanding the regulation of HEK by miRNAs is of great importance to investigate host responses to viral infection and to facilitate the development of novel strategies for the treatment of HEK. To our knowledge, this is the first report of miRNA analysis in the tears of patients with HEK. In this study, we compared the expression of miRNAs between patients with HEK and normal subjects and
examined the correlation between miRNA expression and ulcer area.

**Materials and Methods**

This was a prospective cross-sectional study. The study protocol was approved by the Institutional Review Board of Hanyang University Hospital (no. HYUH 2018-10-013-003). Written informed consent was obtained from all subjects. The study design followed the tenets of the Declaration of Helsinki for biomedical research.

**Patient Enrollment**

Fifteen tear samples were collected from normal participants (seven eyes) and patients with HEK (eight eyes) at the Hanyang University Hospital. HEK is usually diagnosed based on characteristic clinical features including typical patterns of dendritic and geographic ulcers with terminal bulbs, as well as a history of recurrent herpes simplex keratitis, and was confirmed by response to acyclovir treatment after enrollment in this study. Patients with herpes stromal keratitis, endothelitis, and pseudodendritic lesions were excluded. Participants in this study did not use any eye drops or antiviral agents. Careful history-taking and clinical ophthalmologic assessments were performed. When both eyes were involved, the eye with the most severe lesion was selected. After fluorescein staining, anterior segment photography was performed, and the area of the dendritic or geographic ulcer to be fluorescently stained was measured using ImageJ software (National Institutes of Health, Bethesda, MD, USA) (Fig. 1). Each ulcer was measured three times using ImageJ, and the average value of these areas was used. For multiple lesions, the sum of the areas was calculated.

**Sample Collection**

Tear samples were acquired non-traumatically from the inferior tear meniscus of the affected eye using micropipettes (Eppendorf, Hamburg, Germany) before clinical examination, especially fluorescein delivery. Care was taken to avoid touching corneal and conjunctival surfaces. Tear samples were placed in microtubes and stored at −80°C until further examination, including total RNA extraction, complementarily DNA (cDNA) synthesis, pre-amplification, and real-time polymerase chain reaction (PCR). Tear samples were homogenized in QIAzol reagent (QIAGEN, Hilden, Germany). Total RNA, including small RNAs and miRNAs, was isolated from tear samples using the QIAGEN miRNeasy Serum/Plasma Kit and miScript Primer Assay according to the manufacturer’s instructions. RNA samples were stored at −80°C until cDNA synthesis. The isolated RNA was reverse-transcribed into cDNA using the QIAGEN miScript II RT Kit. Prior to PCR, cDNA samples were pre-amplified using the QIAGEN miScript PreAMP PCR Kit and miScript PreAMP Mix. All reactions were performed in accordance with the manufacturer’s protocol. The miRNA expression profiling was performed using a customized miScript miRNA PCR array of the selected 43 miRNAs of interest (see Table 2).

**Normalization and Relative Quantification of Tear miRNA Expression**

To eliminate the normalization issue for miRNA expression in tears, in the absence of stable RNA we used the global mean normalization method for normalizing serum/plasma miRNA expression. The global mean normalization of the miRNA RT-qPCR data was performed using the QIAGEN GeneGlobe Data Analysis Center. The relative expression of miRNAs was calculated using the comparative Ct (ΔΔCt) method. Fold-changes were calculated using the $2^{-\Delta\Delta Ct}$ method.

**Statistical Analysis**

Data are presented as means ± SD. Differences between controls and patients with HEK and between patients with HEK and dendritic and geographic ulcers were estimated using a two-tailed Mann-Whitney U test. Only candidate miRNAs that showed significant differences between the control group and patients with HEK ($P < 0.05$) were selected for correlation analysis. Spearman correlation analysis was used to study correlations involving miRNA values and ulcer area. All analyses were performed using SPSS Statistics 17.0 (IBM, Chicago, IL, USA). Statistical significance was set at $P < 0.05$.

**Results**

**Clinical Characteristics of Subjects**

For this study, eight patients with HEK and seven controls were included. The mean ages of patients with HEK and the control subjects were $56.25 ± 7.63$ years and $48.29 ± 9.98$ years, respectively. There were no significant differences in age distribution between patients with HEK and controls ($P = 0.0703$). All eight patients had underlying systemic diseases, such as hypertension, diabetes mellitus, and rheumatic diseases (Table 1). There were two cases of bilateral invasion and six cases of unilateral invasion. Two patients with bilateral invasion were taking prednisolone for rheumatic disease, and one of them was taking tenofovir for chronic hepatitis B. The average time from onset of...
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**Table 1.** Demographic and Clinical Data for Patients with HEK

| Case | Age | Sex | Laterality | Systemic Disease | Ocular History | VA | IOP | Time After Symptom Onset (Days) |
|------|-----|-----|------------|------------------|--------------|----|-----|---------------------------------|
| 1    | 42  | F   | Bilateral  | RA, chronic hepatitis B | —            | 0.4| 15  | 3                               |
| 2    | 65  | M   | Unilateral | HTN, ITP          | —            | 0.7| 13  | Unknown                         |
| 3    | 53  | M   | Unilateral | HTN              | —            | 0.6| 11  | 10                              |
| 4    | 60  | M   | Unilateral | HTN              | Glaucoma (betaaxol, tafloprost), cataract surgery | 0.5| 10  | 1                               |
| 5    | 53  | F   | Unilateral | HTN, thyroid papillary cancer | —            | 0.2| 16  | 10                              |
| 6    | 63  | M   | Unilateral | DM, unstable angina | —            | 0.05| Not check | 1                              |
| 7    | 65  | M   | Unilateral | Behçet's disease | Uveitis       | 0.2| 15  | Unknown                         |
| 8    | 52  | F   | Bilateral  | Dermatomyositis   | —            | 0.5| 16  | 21                              |

VA, visual acuity; IOP, intraocular pressure; F, female; M, male; RA, rheumatoid arthritis; HTN, hypertension; ITP, idiopathic thrombocytopenic purpura; DM, diabetes mellitus.

Symptoms to the visit was 7.67 ± 7.74 days. Participant demographic and clinical data are summarized in Table 1.

**Differential Expression of miRNAs in Patients with HEK and Controls**

Of the 43 miRNAs, 23 were upregulated in patients with HEK compared to those in the normal controls (Table 2). The 23 miRNAs upregulated in patients with HEK were miR-15b-5p, miR-16-5p, miR-223-3p, miR-25-3p, miR-29a-3p, miR-30a-3p, miR-30d-5p, miR-92a-3p, miR-124-3p, miR-137, miR-138-5p, miR-142-5p, miR-145-5p, miR-146a-5p, miR-146b-5p, miR-155-5p, miR-182-5p, miR-183-5p, miR-221-3p, miR-223-3p, and miR-338-5p. We used an efficient miR-146b-5p, miR-155-5p, miR-182-5p, miR-183-5p, miR-132-3p, miR-142-3p, miR-145-5p, miR-146a-5p, miR-146b-5p, miR-155-5p, miR-182-5p, miR-183-5p, miR-221-3p, miR-223-3p, and miR-338-5p. A total of 23 miRNAs was increased in the tears of patients with HEK compared to the control group: miR-15b-5p, miR-16-5p, miR-24-3p, miR-20b-5p, miR-21-5p, miR-23b-3p, miR-25-3p, miR-29a-3p, miR-30a-3p, miR-30d-5p, miR-92a-3p, miR-124-3p, miR-127-3p, miR-132-3p, miR-142-3p, miR-145-5p, miR-146a-5p, miR-146b-5p, miR-155-5p, miR-182-5p, miR-183-5p, miR-221-3p, miR-223-3p, and miR-338-5p. We used an efficient method to determine HEK severity. The correlation between ulcer severity and the level of miRNAs that was relatively significant for determining HEK severity is presented in Table 2.

**Comparison of miRNAs in Patients with HEK with Dendritic and Geographic Ulcers**

HEK initially appears as small vesicles or punctate epithelial keratopathies and progresses to dendritic and geographic ulcers. The subjects in this study had no punctate epithelial keratopathy and had three dendritic ulcers and five geographic ulcers. We compared miRNA expression in the dendritic and geographic ulcer groups. Moreover, miR-29a-3p was significantly increased in the dendritic ulcer group compared to that in the geographic ulcer group (Table 3).

**Correlation Between Ulcer Area and miRNA Levels in Patients with HEK**

The areas of dendritic or geographic ulcers were measured to determine HEK severity. The correlation between the ulcer area and the level of miRNAs that was relatively significant in the HEK group was analyzed. None of the miRNAs was significantly correlated with ulcer areas (Table 4).

**Discussion**

In this study, we found that the expression of the following 23 miRNAs was increased in the tears of patients with HEK compared to the control group: miR-15b-5p, miR-16-5p, miR-20b-5p, miR-21-5p, miR-23b-3p, miR-25-3p, miR-29a-3p, miR-30a-3p, miR-30d-5p, miR-92a-3p, miR-124-3p, miR-127-3p, miR-132-3p, miR-142-3p, miR-145-5p, miR-146a-5p, miR-146b-5p, miR-155-5p, miR-182-5p, miR-183-5p, miR-221-3p, miR-223-3p, and miR-338-5p. We used an efficient method to determine HEK severity. The correlation between ulcer severity and the level of miRNAs that was relatively significant for determining HEK severity is presented in Table 2.

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FIGURE 2. Highly expressed microRNAs in tears of patients with HEK compared to normal controls.
bioinformatics approach to systematically analyze the regulatory roles of differentially expressed miRNAs. Using this method, we determined that genes such as CD4, CD8, tumor necrosis factor (TNF), interferon-α (IFN-α), thymidine kinase, and atypical chemokine receptor1 (ACKR1) are involved in HEK (Fig. 3). These bioinformatic results showed the involvement of immune cells and inflammatory cytokines in HEKs. All subjects responded well to treatment, without exhibiting drug resistance, and it can be predicted that thymine kinase, which activates the antiviral agent from the pro-drug, was well expressed. ACKR1 is a minor blood group antigen expressed in red blood cells, capillaries, and post-capillary venular endothelial cells, functioning as a chemokine receptor. Lee et al. concluded that ACKR1 has a role in enhancing leukocyte recruitment to sites of inflammation by facilitating movement of chemokines across the endothelium. We inferred that ACKR1, derived from endothelial cells of the conjunctival or limbal vessel, played a role in promoting the influx of inflammatory cells in HEK. However, further studies are needed regarding the association between ACKR1 and HEK.

In HSV keratitis, HSV infects host corneal epithelial cells, replicates, remains latent in the trigeminal ganglion, and then reactivates. Many factors have been implicated in the activation of recurrent HSV keratitis; in particular, the immune system should be the core of regulation in infections and reactivation of latent infection. Virus replication in the cornea triggers innate immune signaling through the production of cytokines and chemokines in epithelial and stromal corneal cells. In the early stages of infection, there is an influx of inflammatory cells such as neutrophils, natural killer (NK) cells, dendritic cells, and macrophages. Type I interferons (IFN-α and IFN-β), which can be produced by most cell types, including corneal epithelial cells, limit viral spread within tissues. Commencing at 7 days post-infection, CD4 T cells, which are major drivers of the progression to stromal keratitis, reach the cornea. CD4 T cells produce various cytokines such as IFN-γ and interleukin (IL)-17. Additional pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α are major drivers of corneal inflammation.

NK cells are innate lymphocytes that are critical mediators of host immunity to infection, allowing pathogen elimination or limiting viral spread. Humans lacking functional NK cells exhibit increased susceptibility to a variety of viral pathogens, especially herpes viruses, including HSV. Their rapid responses mainly rely on the expression of multiple germ-line-encoded activating receptors that play
the most relevant roles in the recognition and killing of infected cells.\cite{36,37}

Several studies have reported that global loss of miRNAs has a significant effect on NK cell activation and effector function.\cite{36,39} Bezman et al.\cite{39} showed that global depletion of either Dicer1 or Dcgr8 in adult mice impaired the ability of NK cells to degranulate or produce IFN-γ. Therefore, it is inferred that the expression of various miRNAs was increased in our study to activate NK cells to remove infected cells during HSV infection. Beaulieu et al.\cite{40} reported the 14 most highly expressed miRNAs in NK cells. Six out of 43 candidate miRNAs were included in those identified in that report. In our study, levels of five (miR-15b, miR-16, miR-29a, miR-23b, and miR-29a) of these six miRNAs were significantly increased, which was thought to be due to NK cell action against HSV infection.

NK cells are recognized to share many common features with CD8 T cells, most notably, their shared ability to kill infected cells through direct cytotoxic mechanisms. NK cell supplementation enhances the function of wild-type anti-HSV CD8 T cells.\cite{41,42} Several studies have reported a significant overlap in specific miRNAs expressed by NK and CD8 T cells. In this study, miR-15b, miR-16, miR-29a, miR-142-3p, and miR-146 levels were elevated, possibly due to the synergistic action of NK and CD8 T cells.

Several studies have reported an association involving miR-155 and miR-132 in the HSV keratitis mouse model.\cite{43} Bhela et al.\cite{44} reported that the expression of miR-155 was increased in the cornea of HSV1-infected mice, which plays an important role in herpetic keratitis by regulating the immune system. Additionally, Mulik et al.\cite{45} reported that miR-132 is increased in HSV1-infected corneas and promotes abnormal angiogenesis by targeting Ras-specific GTPase-activating proteins (RasGAPs). The high expression of miR-155-5p and miR-132-3p identified in this study supports previous studies and showed that they are expressed in human tears in patients with herpetic keratitis, as well as in animal models.

This study had certain limitations. First, the sample size was rather small. Second, this study included several patients with underlying medical conditions. Moreover, there was a lack of clinical data related to disease severity and HSV1 real-time PCR analysis or inflammation-related data, such as tear cytokine levels. However, all patients showed the
typical clinical features characteristic of HEK and exhibited appropriate responses to treatment. Further studies regarding miRNA analysis in patients with a chronic clinical course or herpes stromal keratitis are needed.

In our study, miR-29a-3p was significantly increased in the dendritic ulcer group compared to the geographic ulcer group. The mechanisms behind the formation of typical dendritic or geographic HSV lesions are not fully understood, but geographic corneal ulcers are recognized as progressed form of dendritic ulcers.²⁷ Yang et al.⁶² reported that miR-29a inhibits influenza A virus infection, probably via the frizzled five receptors. also, Patel et al.³⁷ reported that miR-29a expression correlated inversely with active HIV-1 replication. According to previous reports, miR–29a may be a negative regulator of active virus replication. Mir-29a could also inhibit HSV replication, which may suggest the possibility of increased expression in the less severe form of dendritic ulcers, but further studies are needed.

Nevertheless, this study is meaningful in that it is the first to analyze miRNA expression in the tears of patients with HEK, and it is in accordance with the results of previous animal studies. We believe that further research will be helpful in understanding the pathophysiology of herpes keratitis and in the development of new therapeutic targets.

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References

1. Liesegang TJ. Herpes simplex virus epidemiology and ocular importance. Cornea. 2001;20:1–13.
2. Farooq AV, Shukla D. Herpes simplex epithelial and stromal keratitis: an epidemiologic update. Surv Ophthalmol. 2012;57:448–462.
3. Herpetic Eye Disease Study Group. Predictors of recurrent herpes simplex virus keratitis. Cornea. 2001;20:123–128.
4. Young RC, Hodge DO, Liesegang TJ, Baratz KH. Incidence, recurrence, and outcomes of herpes simplex virus eye disease in Olmsted County, Minnesota, 1976-2007: the effect of oral antiviral prophylaxis. Arch Ophthalmol. 2010;128:1178–1183.
5. Wilhelmus KR. Antiviral treatment and other therapeutic interventions for herpes simplex virus epithelial keratitis. Cochrane Database Syst Rev. 2015;1:CD002898.
6. Reynaud C, Rousseau A, Kaswin G, M’garrech M, Barreau E, Labetoulle M. Persistent impairment of quality of life in patients with herpes simplex keratitis. Ophthalmology. 2017;124:160–169.
7. Hill GM, Ku ES, Dwarkanathan S. Herpes simplex keratitis. Dis Mon. 2014;60:239–246.
8. Nguyen ML, Blaho JA. Apoptosis during herpes simplex virus infection. Adv Virus Res. 2007;69:67–97.
9. Goodkin ML, Morton ER, Blaho JA. Herpes simplex virus infection and apoptosis. Int Rev Immunol. 2004;23:141–172.
10. Lobo AM, Agelidis AM, Shukla D. Pathogenesis of herpes simplex keratitis: the host cell response and ocular surface sequelae to infection and inflammation. Ocul Surf. 2019;17:40–49.
11. Wang L, Wang R, Xu C, Zhou H. Pathogenesis of herpes stromal keratitis: immune inflammatory response mediated by inflammatory regulators. Front Immunol. 2020;11:766.
12. Gimenez F, Suryawanshi A, Rouse BT. Pathogenesis of herpes stromal keratitis—a focus on corneal neurovascularization. Prog Retin Eye Res. 2013;33:1–9.
13. Wuest TR, Carr DJ. VEGF-A expression by HSV-1-infected cells drives corneal lymphangiogenesis. J Exp Med. 2010;207:101–115.
14. Koujah L, Suryawanshi RK, Shukla D. Pathological processes activated by herpes simplex virus-1 (HSV-1) infection in the cornea. Cell Mol Life Sci. 2019;76:405–419.
15. Peters L, Meister G. Argonaute proteins: mediators of RNA silencing. Mol Cell. 2007;26:611–623.
16. Chen K, Rajewsky N. The evolution of gene regulation by transcription factors and microRNAs. Nat Rev Genet. 2007;8:93–103.
17. Brennecke J, Hipfner DR, Stark A, Russell RB, Cohen SM. bantam encodes a developmentally regulated microRNA that controls cell proliferation and regulates the proapoptotic gene hid in Drosophila. Cell. 2003;113:25–36.
18. Li X, Carthew RW. A microRNA mediates EGF receptor signaling and promotes photoreceptor differentiation in the Drosophila eye. Cell. 2005;123:1267–1277.
19. Hohenstein-Blau NVU, Funke S, Grus FH. Tears as a source of biomarkers for ocular and systemic diseases. Exp Eye Res. 2013;117:126–137.
20. Turchinovich A, Tonevitsky AG, Burwinkel B. Extracellular miRNA: a collision of two paradigms. Trends Biochem Sci. 2016;41:883–892.
21. Weber JA, Baxter DH, Zhang S, et al. The microRNA spectrum in 12 body fluids. Clin Chem. 2010;56:1733–1741.
22. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 2001;29:e45.
23. Mestdagh P, Van Vlierberghe P, De Weer A, et al. A novel and universal method for microRNA RT-qPCR data normalization. Genome Biol. 2009;10:R64.
24. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the (-Delta Delta C(T)) method. Methods. 2001;25:402–408.
25. Huang C-Y, Yao H-W, Wang L-C, Hsu S-M, Chen S-H. Thymidine kinase-negative herpes simplex virus 1 can efficiently establish persistent infection in neural tissues of nude mice. J Virol. 2017;91:e01979–16.
26. Lee JS, Frevert CW, Wurfel MM, et al. Duffy antigen receptor for chemokines: structural analysis and expression. J Immunol. 2003;170:5244–5251.
27. Horuk R, Martin A, Hesselgesser J, et al. The Duffy antigen receptor for chemokines: structural analysis and expression in the brain. J Leukoc Biol. 1996;59:29–38.
28. Hendricks RL, Weber PC, Taylor JL, Kounbis A, Tumpey TM, Glorioso JC. Endogenously produced interferon alpha protects mice from herpes simplex virus type 1 corneal disease. J Gen Virol. 1991;72:1601–1610.
29. Schoenborn JR, Dorschner MO, Sekimata M, et al. Comprehensive epigenetic profiling identifies multiple distal regulatory elements directing transcription of the gene encoding interferon-gamma. Nat Immunol. 2007;8:732–742.
30. Molesworth-Kenyon SJ, Yin R, Oakes JE, Lausch RN. IL-17 receptor signaling influences virus-induced corneal inflammation. J Leukoc Biol. 2008;83:401–408.
31. Biswas PS, Banerjee K, Kim B, Rouse BT. Mice transgenic for IL-1 receptor antagonist protein are resistant to herpetic stromal keratitis: possible role for IL-1 in herpetic stromal keratitis pathogenesis. J Immunol. 2004;172:3736–3744.

32. Fenton RR, Molesworth-Kenyon S, Oakes JE, Lausch RN. Linkage of IL-6 with neutrophil chemoattractant expression in virus-induced ocular inflammation. Invest Ophthalmol Vis Sci. 2002;43:737–743.

33. Keadle TL, Usui N, Laycock KA, Miller JK, Pepose JS, Stuart PM. IL-1 and TNF-alpha are important factors in the pathogenesis of murine recurrent herpetic stromal keratitis. Invest Ophthalmol Vis Sci. 2000;41:96–102.

34. Vivier E, Raulet DH, Moretta A, et al. Innate or adaptive immunity? The example of natural killer cells. Science. 2011;331:44–49.

35. Della Chiesa M, Sivori S, Carlomagno S, Moretta L, Moretta A. Activating KIRs and NKG2C in viral infections: toward NK cell memory. Front Immunol. 2015;6:573.

36. Vidal SM, Khakoo SI, Biron CA. Adaptive immune responses mediated by natural killer cells. Immunol Rev. 2010;235:286–296.

37. Lanier LL. NK cell recognition. Annu Rev Immunol. 2005;23:225–274.

38. Sullivan RP, Leong JW, Schneider SE, et al. MicroRNA-deficient NK cells exhibit decreased survival but enhanced function. J Immunol. 2012;188:3019–3030.

39. Bezman NA, Cedars E, Steiner DF, Blelloch R, Hesslein DGT, Lanier LL. Distinct requirements of microRNAs in NK cell activation, survival, and function. J Immunol. 2010;185:3855–3864.

40. Beaulieu AM, Bezman NA, Lee JE, Matloubian M, Sun JC, Lanier LL. MicroRNA function in NK-cell biology. Immunol Rev. 2013;253:40–52.

41. Paust S, Senman B, von Andrian UH. Adaptive immune responses mediated by natural killer cells. Immunol Rev. 2010;235:286–296.

42. Sun JC, Lanier LL. NK cell development, homeostasis and function: parallels with CD8(+) T cells. Nat Rev Immunol. 2011;11:645–657.

43. Xu S, Hazlett LD. MicroRNAs in ocular infection. Microorganisms. 2019;7:359.

44. Bhela S, Mulik S, Gimenez F, et al. Role of miR-155 in the pathogenesis of herpetic stromal keratitis. Am J Pathol. 2015;185:1073–1084.

45. Mulik S, Xu J, Reddy PB, et al. Role of miR-132 in angiogenesis after ocular infection with herpes simplex virus. Am J Pathol. 2012;181:525–534.

46. Yang X, Liang Y, Ramunuarachchi G, et al. miR-29a is a negative regulator of influenza virus infection through targeting of the frizzled 5 receptor. Arch Virol. 2021;166:363–373.

47. Patel P, Ansari MY, Bapat S, Thakar M, Gangakhedkar R, Jameel S. The microRNA miR-29a is associated with human immunodeficiency virus latency. Retrovirology. 2014;11:108.