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

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Received: January 17, 2022
Accepted: April 11, 2022
Published: April 27, 2022

Citation: Kim YJ, Yeon Y, Lee WJ, et al. Analysis of microRNA expression in tears of patients with herpes epithelial keratitis: A preliminary study. *Invest Ophthalmol Vis Sci.* 2022;63(4):21. https://doi.org/10.1167/iovs.63.4.21

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.[1,2] 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%.[3,4] Each year, approximately 1 million people worldwide suffer from new or recurrent HEK, which may significantly impair their quality of life.[5,6]

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.[7] Numerous studies have implicated many cellular processes in host responses after viral infection, such as apoptosis,[8,9] inflammation,[10,11] angiogenesis,[12–14] and lymphogenesis[13,14]; 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.[15,16] MiRNAs play significant roles in many cellular processes, including cell differentiation, proliferation, and apoptosis.[17,18] Tears are complex biological mixtures that serve as a first line of defense against pathogens.[19] MiRNAs are also present in tears and have been found to be very stable[20,21]; 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, complementary 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 Primer 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). Quantitative real-time PCR (RT-qPCR) reactions were carried out using the Roche LightCycler 480 SYBR Green platform (Roche Diagnostics, Basel, Switzerland), following the manufacturer's protocols.

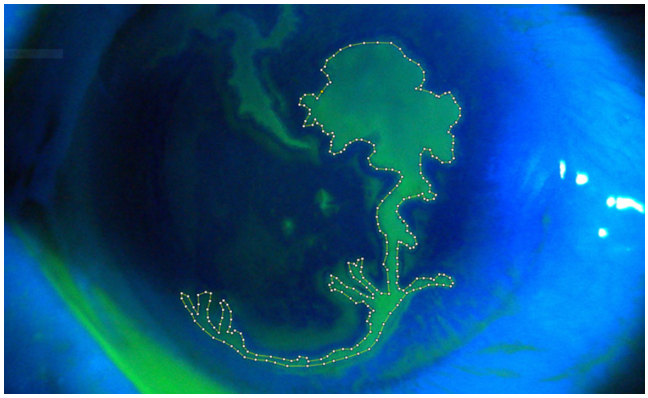

Figure 1. Representative example of dendritic or geographic corneal ulcer area measurements using ImageJ software.

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.[22] 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 C_T ($\Delta\Delta C_T$) method. Fold-changes were calculated using the $2^{-\Delta\Delta CT}$ method.[23,24]

Statistical Analysis

Data are presented as means ± SD. Differences between controls and patients with HEK and between patients with HEK with 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	63	M	Unilateral	HTN, ITP	—	0.7	13	Unknown
3	53	M	Unilateral	HTN	—	0.6	11	10
4	60	M	Unilateral	HTN	Glaucoma (betaxolol, tafluprost), 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-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 (Fig. 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

Table 2. Expression of Tear MicroRNAs in Patients with HEK Compared with Control Group

MicroRNA	ΔC_T† (Mean ± SD) HEK	Control	Fold-Change	*P*
miR-15b-5p	2.93 ± 2.08	7.32 ± 1.57	20.92	0.003*
miR-16-5p	1.96 ± 2.95	6.45 ± 1.03	22.54	0.011*
miR-17-5p	7.66 ± 5.71	9.15 ± 1.41	2.82	0.118
miR-20a-5p	5.64 ± 5.51	7.40 ± 1.36	3.39	0.105
miR-20b-5p	7.97 ± 5.49	13.01 ± 3.41	32.92	0.045*
miR-21-5p	−3.71 ± 2.35	0.69 ± 1.89	21.17	0.003*
miR-23b-3p	2.04 ± 2.25	4.58 ± 1.19	5.81	0.049*
miR-25-3p	3.11 ± 2.25	6.90 ± 1.60	13.79	0.008*
miR-29a-3p	1.87 ± 2.38	5.10 ± 1.36	9.40	0.011*
miR-30a-3p	7.29 ± 3.93	9.38 ± 1.68	4.26	0.037*
miR-30b-5p	5.86 ± 5.03	6.43 ± 1.21	1.49	0.165
miR-30c-5p	6.29 ± 5.14	7.91 ± 1.95	3.07	0.247
miR-30d-5p	4.69 ± 2.41	6.93 ± 1.28	4.72	0.049*
miR-34a-5p	6.31 ± 4.77	9.43 ± 4.22	8.71	0.183
miR-92a-3p	2.05 ± 2.27	4.92 ± 1.39	7.30	0.028*
miR-93-5p	6.73 ± 4.88	8.80 ± 1.21	4.21	0.083
miR-99a-5p	7.45 ± 5.77	7.79 ± 1.27	1.27	0.132
miR-100-5p	7.30 ± 5.81	7.62 ± 1.22	1.25	0.105
miR-103a-3p	10.38 ± 5.62	11.98 ± 3.26	3.03	0.48
miR-124-3p	7.26 ± 1.37	9.40 ± 0.99	4.41	0.008*
miR-125b-5p	3.19 ± 3.13	5.15 ± 1.11	3.90	0.105
miR-127-3p	4.15 ± 1.96	6.83 ± 1.06	6.40	0.028*
miR-132-3p	6.77 ± 4.36	10.60 ± 2.87	14.14	0.032*
miR-134-5p	13.62 ± 4.01	16.51 ± 0.00	7.39	0.082
miR-137	16.51 ± 0.00	16.51 ± 0.00	1.00	1
miR-142-3p	5.77 ± 4.84	13.66 ± 3.87	237.35	0.007*
miR-142-5p	13.32 ± 6.05	14.55 ± 3.42	2.34	0.882
miR-143-3p	12.61 ± 6.19	14.87 ± 2.81	4.81	0.63
miR-145-5p	4.73 ± 2.31	7.55 ± 1.18	7.09	0.021*
miR-146a-5p	2.80 ± 2.37	5.66 ± 2.15	7.23	0.028*
miR-146b-5p	7.81 ± 5.66	13.20 ± 4.22	41.98	0.042*
miR-152-3p	8.18 ± 5.68	8.48 ± 1.27	1.23	0.417
miR-155-5p	9.85 ± 4.49	14.99 ± 2.61	35.32	0.020*
miR-182-5p	7.82 ± 4.00	12.63 ± 3.88	28.15	0.023*
miR-183-5p	6.93 ± 4.51	10.53 ± 3.36	12.13	0.043*
miR-203a-3p	1.65 ± 3.73	3.91 ± 1.83	4.79	0.132
miR-221-3p	4.93 ± 5.33	7.79 ± 1.30	7.26	0.049*
miR-223-3p	−1.86 ± 3.08	4.53 ± 1.16	83.89	0.003*
miR-302d-5p	14.74 ± 3.53	14.95 ± 2.81	1.16	1
miR-338-5p	4.67 ± 2.51	6.73 ± 1.22	4.16	0.049*
miR-365b-3p	6.14 ± 4.98	7.38 ± 1.71	2.36	0.165
miR-374c-5p	12.2 ± 5.98	14.25 ± 3.91	4.12	0.449
miR-487b-3p	15.91 ± 1.70	16.51 ± 0.00	1.52	0.35

* $P < 0.05$.
† Where $\Delta C_T = C_T$ (gene of interest) – C_T (housekeeping gene).

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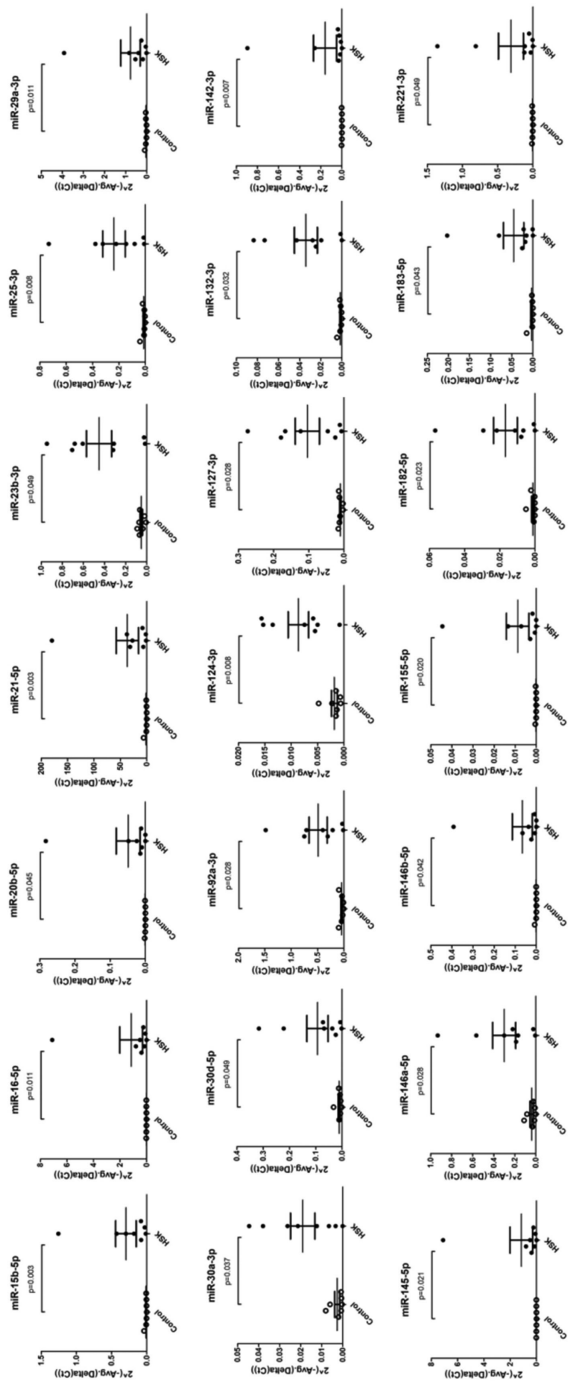

Figure 2. Highly expressed microRNAs in tears of patients with HEK compared to normal controls.

Table 3. Comparison of Tear MicroRNAs in Patients with HEK with Dendritic Ulcers and Geographic Ulcers

MicroRNA	ΔC_T[†] (Mean ± SD) HEK Dendritic Ulcer	HEK Geographic Ulcer	*P*
miR-15b-5p	2.29 ± 2.27	3.84 ± 2.05	0.480
miR-16-5p	0.28 ± 2.81	3.63 ± 2.75	0.15
miR-17-5p	3.69 ± 2.35	9.60 ± 4.59	0.074
miR-20a-5p	1.96 ± 2.28	8.09 ± 5.11	0.077
miR-20b-5p	4.19 ± 2.28	9.77 ± 4.37	0.074
miR-21-5p	–4.16 ± 1.25	–2.43 ± 2.17	0.480
miR-23b-3p	0.97 ± 0.63	3.33 ± 2.62	0.289
miR-25-3p	1.90 ± 1.60	4.45 ± 2.31	0.157
miR-29a-3p	–0.12 ± 1.69	3.60 ± 1.74	0.034[*]
miR-30a-3p	5.76 ± 1.39	8.33 ± 3.67	0.289
miR-30b-5p	2.92 ± 2.16	7.87 ± 4.69	0.157
miR-30c-5p	3.48 ± 3.30	8.00 ± 4.97	0.289
miR-30d-5p	3.07 ± 2.01	6.12 ± 2.29	0.157
miR-34a-5p	3.61 ± 1.59	8.42 ± 4.13	0.077
miR-92a-3p	0.71 ± 1.40	3.25 ± 2.62	0.289
miR-93-5p	3.52 ± 3.23	8.82 ± 3.89	0.157
miR-99a-5p	3.72 ± 2.28	9.36 ± 4.81	0.154
miR-100-5p	3.57 ± 1.88	9.27 ± 4.90	0.154
miR-103a-3p	7.09 ± 5.65	11.51 ± 2.83	0.271
miR-124-3p	7.52 ± 0.11	7.11 ± 2.06	0.289
miR-125b-5p	1.64 ± 0.97	4.96 ± 3.69	0.480
miR-127-3p	5.39 ± 0.98	3.50 ± 2.40	0.289
miR-132-3p	4.86 ± 1.12	8.21 ± 4.20	0.480
miR-134-5p	11.60 ± 3.32	12.54 ± 1.97	0.569
miR-137	13.52 ± 0.00	13.52 ± 0.00	1.000
miR-142-3p	2.90 ± 3.32	7.14 ± 4.26	0.289
miR-142-5p	7.00 ± 6.16	13.52 ± 0.00	0.078
miR-143-3p	6.62 ± 6.52	13.13 ± 0.77	0.172
miR-145-5p	3.03 ± 2.02	5.39 ± 2.06	0.157
miR-146a-5p	1.93 ± 0.96	4.13 ± 2.63	0.480
miR-146b-5p	4.35 ± 2.82	9.88 ± 4.26	0.074
miR-152-3p	4.52 ± 0.38	10.65 ± 4.29	0.154
miR-155-5p	7.32 ± 2.47	11.15 ± 3.05	0.154
miR-182-5p	6.41 ± 0.86	8.81 ± 4.10	0.480
miR-183-5p	5.78 ± 0.26	8.20 ± 4.55	1.000
miR-203a-3p	0.51 ± 1.29	3.25 ± 4.86	1.000
miR-221-3p	1.63 ± 2.96	7.10 ± 4.70	0.157
miR-223-3p	–2.46 ± 3.12	–0.79 ± 3.42	0.724
miR-302d-5p	13.52 ± 0.00	11.48 ± 3.09	0.186
miR-338-5p	3.82 ± 0.74	5.35 ± 3.61	1.000
miR-365b-3p	4.84 ± 1.97	7.34 ± 5.40	0.724
miR-374c-5p	7.55 ± 5.24	11.63 ± 3.79	0.172
miR-487b-3p	13.52 ± 0.00	13.07 ± 0.91	0.386

[*] $P < 0.05$.
[†] Where $\Delta C_T = C_T$ (gene of interest) – C_T (housekeeping gene).

Table 4. Correlation Between Dendritic or Geographic Ulcer Area and MicroRNA Expression in Patients with HEK

MicroRNA	Spearman's Rank-Order Correlation Coefficient (r_s)	*P*
miR-15b-5p	–0.429	0.397
miR-16-5p	0.029	0.957
miR-20b-5p	0.232	0.658
miR-21-5p	–0.200	0.704
miR-23b-3p	0.200	0.704
miR-25-3p	–0.029	0.957
miR-29a-3p	0.429	0.397
miR-30a-3p	–0.029	0.957
miR-30d-5p	–0.086	0.872
miR-92a-3p	–0.257	0.623
miR-124-3p	–0.771	0.072
miR-127-3p	–0.543	0.266
miR-132-3p	–0.371	0.468
miR-142-3p	0.257	0.623
miR-145-5p	–0.086	0.872
miR-146a-5p	–0.086	0.872
miR-146b-5p	0.232	0.658
miR-155-5p	0.638	0.173
miR-182-5p	–0.200	0.704
miR-183-5p	–0.429	0.397
miR-221-3p	–0.086	0.872
miR-223-3p	–0.543	0.266
miR-338-5p	–0.486	0.329

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-γ (*IFN-*γ), 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.[25] ACKR1 is a minor blood group antigen expressed in red blood cells, capillaries, and post-capillary venular endothelial cells,[26] functioning as a chemokine receptor.[27] Lee et al.[26] 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.[10] 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.[28] Commencing at 7 days post-infection, CD4 T cells, which are major drivers of the progression to stromal keratitis, reach the cornea.[10] CD4 T cells produce various cytokines such as IFN-γ and interleukin (IL)-17.[29,30] Additional pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α are major drivers of corneal inflammation.[31–33]

NK cells are innate lymphocytes that are critical mediators of host immunity to infection, allowing pathogen elimination or limiting viral spread.[34,35] 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

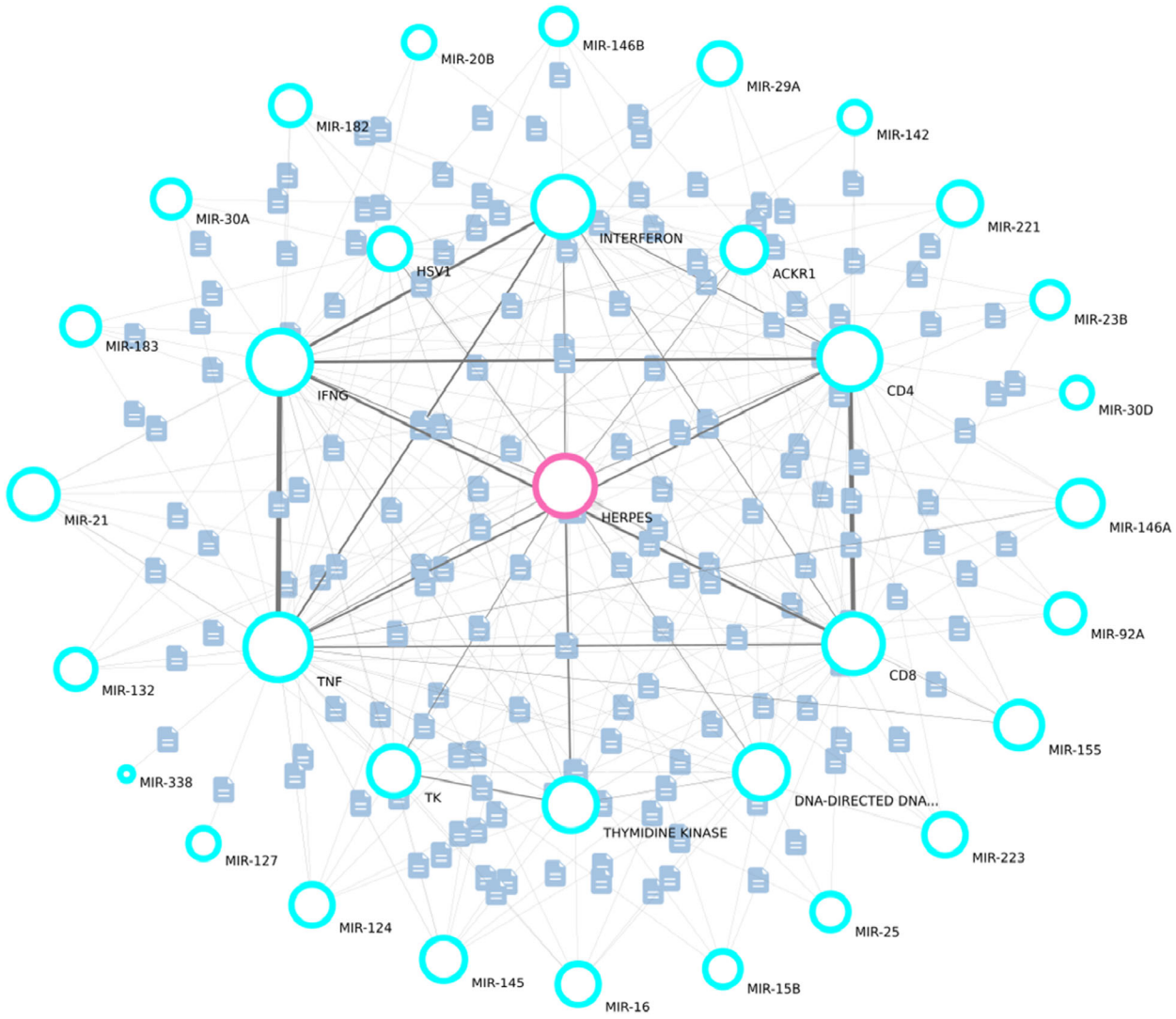

Figure 3. Network of miRNAs and their target genes related to herpes keratitis pathogenesis using bioinformatic analysis.

the most relevant roles in the recognition and killing of infected cells.[36,37]

Several studies have reported that global loss of miRNAs has a significant effect on NK cell activation and effector function.[38,39] Bezman et al.[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.[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-21, 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.[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.[43] Bhela et al.[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.[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.[7] Yang et al.[46] reported that miR-29a inhibits influenza A virus infection, probably via the frizzled five receptors. also, Patel et al.[47] 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.

Acknowledgments

Supported by the Basic Science Research Program through the National Research Foundation of Korea and funded by the Ministry of Science, ICT and Future Planning (2020R1C1C1011696).

Disclosure: **Y.J. Kim**, None; **Y. Yeon**, None; **W.J. Lee**, None; **Y.U. Shin**, None; **H. Cho**, None; **H.W. Lim**, None; **M.H. Kang**, None

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