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Lack of MIR143, MIR145, MIR184, MIR1224, and MIR29b1 mutations in keratoconus pathogenesis

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To the Editor,

Keratoconus (KC) is a noninflammatory disease, characterized by progressive thinning of the corneal stroma. Normal corneal curvature is deformed and optical quality decreases due to stromal thinning. Incidence of KC in the general population is 1 in 2000 (1). The disease begins at 15–20 years of age, but it may also be seen at earlier ages. Despite several studies on the pathogenesis of KC, the biochemical mechanism of the disease has not been clarified yet. The most commonly accepted idea about KC is that both genetic and environmental factors have a joint effect on the pathogenesis of the disease. Despite positive findings in genetic research and the recent discovery of several mutations in KC patients, no mutations are directly related with KC at present (1–11). Absence of any mutations in KC, despite the expected genetic background, lead us to consider epigenetic aberrations in the pathogenesis.

Noncoding RNAs are promising as emerging regulatory molecules that play an important role in epigenetic transmission. MicroRNAs (miRNAs) are noncoding RNAs, transcribed from DNA and posttranscriptionally modified. Their mature single-stranded form, about 22 nucleotides in length, inhibits the translation of other target mRNAs. Single miRNA can target hundreds of different mRNAs, and the translation of mRNA can also be inhibited by dozens of different miRNAs. This is a complicated regulatory pathway of cellular metabolism, and KC is a complicated disease. Other genetic diseases caused by miRNA-related pathologies have been described (12). A miRNA mutation that causes familial KC together with congenital cataracts has been defined (13–15). In light of this knowledge, we aimed to identify miRNA mutations that may play a role in the pathogenesis of KC. The miRNAs to be analyzed were chosen with the following approach. First, chromosomal regions shown to be related to KC disease in the literature were listed. Then

messenger RNAs (mRNAs) related to proteins and genes shown to be overexpressed in KC epithelial cells were listed (14–19). The miRNAs that show strong relations with both the above lists were searched in silico to find miRNAs targeting the KC-related mRNAs. This analysis yielded 5 different miRNAs, whose gene mutations may have a potential causal relationship with KC. Those miRNA genes are MIR143/145, MIR184, MIR1224, and MIR29b1. These miRNAs target mRNAs that are shown to be overexpressed in KC (16–21). Additionally, MIR184 (not shown in the Figure) was included in the list, because a mutation related with KC has been documented (13–15).

The hsa-miR143 and has-miR-145 genes are located in 5q32, and their pre-miRNA is transcribed as one molecule. It has been shown that miR143/145 downregulates Matrix Metalloproteinase 13 MMP13 and Plasminogen Activator Inhibitor-1 PAI-1 translation. MMP13 is a matrix metalloprotease that has been shown to be overexpressed in KC. Thus, MMP13 protein overexpression may be due to a malfunction of the miR-143/145 inhibition process. hsa-miR-1224 and miR29b1 have a similar story, and its target SP-1 is a transcription factor, upregulating other genes that are overexpressed in KC.

The miRNA gene regions were multiplied by PCR using the primers. The reaction conditions are listed in the Table.

Patients diagnosed with KC according to biomicroscopy and corneal topography findings were included in the study. Peripheral blood samples were obtained from patients (99 males and 108 females, between 19 and 33 years of age) with their informed consent. Blood samples were kept in EDTA-containing blood sample tubes at 4 °C. Samples were screened for the presence of any mutations with the Sanger sequencing technique using a genetic analyzer (Applied Biosystems 3130) (22). All samples were collected with the approval of the Non-Drug Clinical Research Ethics

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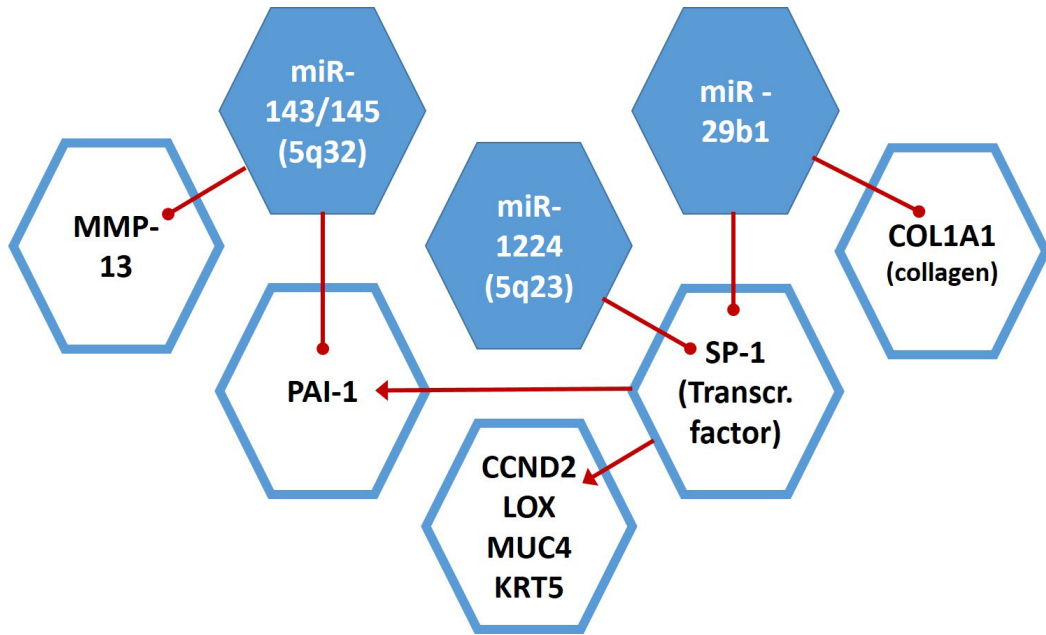


Figure. Chosen miRNAs and their mRNA targets shown by KC relations. All proteins given are shown to be overexpressed in KC.

Table. List of primers.

Primer name of miRNA amplification	Primer sequence 5'–3'	Hybridization conditions of PCR
Hsa-miR-143	F: CCTCTAACACCCCTTCTCC	57 °C for 30 s
	R: CTTCAAGAATGGATGCCTGG	
Hsa-miR-145	F: CCCCAATCTTATTCATCTCACC	58 °C for 30 s
	R: AACCTCATCCTGTGAGCC	
Hsa-miR-184	F: CCGGGAAATCAAACGTCC	52 °C for 30 s
	R: CAGGTTTTCCCATCACG	
Hsa-miR-1224	F: ACCAAAATGGCAACTCCAAG	57 °C for 30 s
	R: GCGGAAGGAGCCTGGTG	
Hsa-miR-29b1	F: AGACCTGACTGCCATTTGTGA	58 °C for 30 s
	R: AGAATAAGGGAGTCCCAGGCA	

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After the analysis, no mutations were detected in the investigated miRNA genes. Two single nucleotide polymorphisms, rs1701 and rs116464056, were found in 17 of the patients.

Although these miRNA genes are directly related to the chromosome regions of KC-related genes and mRNAs are overexpressed in KC patients' corneal tissue, we could not find any genetic abnormalities related to KC in our research. In contrast to previous studies on miR184 mutation in familial KC associated with cataracts (15–17),

no mutations in miR184 were detected in our patient group. Mutations in miR184 were absent in another study screening 134 KC patients (23). Considering the complex and still unexplained etiology of KC, miRNA profiling of KC corneas and its comparison with normal corneas should be performed to demonstrate miRNAs that are differentially expressed in KC. Then mutation screening for these miRNAs could be performed to find mutations related with KC. However, these analyses are quite expensive. Nevertheless, the search strategy defined in this study utilized causally interconnected data from various databases to find specific targets, instead of screening the

entire miRNAome. This strategy enabled us to study 207 patients with an economic budget. Although we could not achieve positive results, this search strategy may be used as a model for investigating the etiologies of other genetic or epigenetic diseases.

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