A Single-Base Substitution in the Seed Region of miR-184 Causes EDICT Syndrome

Benjamin W. Iliff, S. Amer Riazuddin, and John D. Gottsch

PURPOSE. To investigate the cause of the syndrome characterized by endothelial dystrophy, iris hypoplasia, congenital cataract, and stromal thinning (EDICT).

METHODS. Previously a multigenerational family was reported that comprised 10 individuals affected by syndromal anterior segment dysgenesis. Blood samples were re-collected from eight affected and two unaffected individuals, and genomic DNA was extracted. A total of 24 candidate genes and 4 microRNAs residing within the critical interval were sequenced bidirectionally. In silico analyses were performed to examine the effect of the causal variant on the stability of the pre-microRNA structure.

RESULTS. Bidirectional sequencing identified the single-base substitution +57C>T in miR-184. This variation segregated with the disease phenotype and was absent in the 1000 Genomes project, 1130 control chromosomes, and 28 nonhuman vertebrates.

CONCLUSIONS. The single-base-pair substitution in the seed region of miR-184 is responsible for the disease phenotype observed in EDICT syndrome. (*Invest Ophthalmol Vis Sci.* 2012; 53:348–353) DOI:10.1167/iovs.11-8783

An autosomal dominant syndrome of anterior polar cataract and endothelial dystrophy was first described in a large Swedish family in 1951.¹ The affected individuals developed anterior polar cataracts in early childhood and corneal guttae as early as age 10, with corneal disease progressing with age. The severity of the disease varied between family members: while the majority of affected individuals developed both corneal guttae and cataracts, one individual had guttae and no cataract. A similar disease phenotype was later reported in an American family of Scandinavian descent. Through at least five generations, this family inherited autosomal dominant anterior polar cataract with corneal guttae.² Histologic examination of the cornea of the affected proband revealed epithelial edema, normal stroma, and thickening of the Descemet membrane with focal excrescences. A third instance of a dominant syndrome of cataract and corneal dystrophy was reported in a large Northern Irish family in 2003.³ Similar to the previous reports, affected members of this family developed anterior polar cataract in childhood; however, in contrast to previously described cases, the corneal dystrophy in this family was described as keratoconus rather than as cornea guttata.

From the Center for Corneal Genetics, The Wilmer Eye Institute, Johns Hopkins University School of Medicine, Baltimore, Maryland.

Supported by National Eye Institute Grant R01EY016835 (JDG) and by the Kwok Research Fund (JDG).

Submitted for publication October 11, 2011; revised November 15, 2011; accepted November 15, 2011.

Disclosure: **B.W. Iliff**, None; **S.A. Riazuddin**, None; **J.D. Gottsch**, None

Corresponding author: John D. Gottsch, The Wilmer Eye Institute, The Johns Hopkins Hospital, 600 N. Wolfe Street, Baltimore, MD 21287; jgottsch@jhmi.edu.

We have described EDICT syndrome, an autosomal dominant syndromal anterior segment dysgenesis characterized by endothelial dystrophy, iris hypoplasia, congenital cataract, and stromal thinning.^{4,5} The locus for keratoconus with cataract was mapped to a 5.5-Mb region within the previous linkage interval for EDICT syndrome, suggesting a possible common causative mutation.^{3,4,6} The common linkage interval harbors 75 annotated genes and 4 microRNAs.^{7,8}

MicroRNAs are short RNA molecules that bind to messenger RNA, downregulating gene expression by inhibiting translation and triggering degradation of mRNA by the Argonaute protein.^{9–12} MicroRNA is first produced as primary microRNA, which is cleaved in successive steps by the RNase III enzymes Drosha and Dicer to produce the mature microRNA that is incorporated into the RNA-induced silencing complex (RISC).^{13,14} The region from bases 2 to 7 of the mature microRNA, known as the seed region, is especially important for recognition of target mRNA and proper microRNA regulation of protein expression.¹⁵ Indeed, a mutation in the seed region of miR-96 has been implicated in hereditary hearing loss.¹⁶ We report a causal mutation for EDICT syndrome found in the seed region of miR-184.

MATERIALS AND METHODS

Recruitment and Sample Collection

After examination of the proband, affected and unaffected family members were invited to participate in a study to determine the genetic basis for EDICT syndrome, as previously described.^{4,5} Informed consent was obtained from all individuals included in the study. The study protocol was approved by the Johns Hopkins Medicine Institutional Review Board and adhered to the tenets of the Declaration of Helsinki and the regulations of the Health Insurance Portability and Accountability Act.

Venous blood (17 mL) had been collected from 14 family members, and samples were re-collected from 10 of those individuals for this study. Genomic DNA was extracted from blood samples (QIAamp DNA Blood Maxi Kit; Qiagen, Valencia, CA) and frozen at -20°C .

PCR Amplification and Sanger Sequencing

Candidate genes and pre-microRNAs were amplified by polymerase chain reaction (PCR). Reactions were performed in a volume of 25 μL , composed of 50 ng of genomic DNA, 10 picomoles of forward and reverse primers, 2.5 μL of 10 $\times$ PCR buffer, 2.5 μL of 2 mM dNTPs, 2.5 μL of 5 M betaine, and 0.15 units *Taq* DNA polymerase. Sequencing of the PCR product was accomplished using dye termination chemistry according to the manufacturer's instructions (BigDye Terminator, ver. 3.1; Life Technologies Corporation, Carlsbad, CA). After purification by ethanol precipitation, samples were commercially sequenced (ABI 3730xl sequencer; Life Technologies Corporation, Carlsbad, CA; performed by Eurofins MWG Operon, Huntsville, AL). Sequences were viewed using commercially available sequence analysis software (Sequencher 5.0; Gene Codes Corp., Ann Arbor, MI).

FIGURE 1. Candidate genes and microRNAs. *Black*: gene; *red*: microRNA; *blue bar*: critical interval of EDICT syndrome; and *green bar*: critical interval of autosomal dominant keratoconus with cataract (KC+C).

In Silico Analyses

The Vienna RNAfold algorithm was used to predict the effect of miR-184(+57C>T) on the secondary structure of pre-miR-184.^{17,18} The MC-fold/MC-sym pipeline was then used to predict the tertiary structure of the wild-type and miR-184(+57C>T) pre-miR-184 and to calculate the minimum free energy of the calculated secondary and tertiary structures.¹⁹ Subsequently, DIANA ver. 3.0 software was used to predict targets of wild-type miR-184 and miR-184(+57C>T).²⁰

RESULTS

In earlier work, we localized the critical interval of EDICT syndrome to the long arm of chromosome 15 (Fig. 1).⁴ In this study, we first analyzed 24 candidate genes involved in transcriptional regulation, early development, cell migration, cell-cell interaction, and extracellular matrix structure, all of which have shown expression in human corneal endothelial cells in a serial analysis of gene expression (SAGE) library.²¹ However, we did not identify a causal lesion in the coding regions of these candidate genes.

Next, we sequenced the four microRNAs that met two criteria: first, they were located within 100 kb of the linkage interval, and second, they had been reported as expressed in the eye (*MIR184* and *MIR422A*) or were paralogous to microRNAs expressed in the eye (*MIR7-2*, and *MIR9-3*).^{22–25} We identified a single base substitution, miR-184(+57C>T), which lies in the seed region at the fifth base of the mature miR-184. The substitution segregates with the disease phenotype in the family: all eight affected individuals are heterozygous for the variant, whereas the two unaffected individuals are homozy-

FIGURE 2. (A) Pedigree of the affected family. *Squares*: males; *circles*: females; *filled symbols*: affected individuals; *open symbols*: unaffected individuals; *black text*: sequenced base; and *red text*: base inferred from previously determined haplotype⁴; *diagonal line* through symbol: deceased; and *arrow*: proband. (B) Bidirectional sequencing chromatograms of the heterozygous mutation identified in EDICT-affected individuals: forward (Bi) and reverse (Bii) sequence of affected individual and forward (Biii) and reverse (Biv) sequence of unaffected individual.

gous for the reference allele (Fig. 2). No variations were identified in sequences of *MIR7-2*, *MIR9-3*, or *MIR422A*.

To assess the possibility that the identified substitution is a common variant, we searched the 1000 Genomes database and sequenced *MIR184* in two control cohorts: 282 Fuchs corneal dystrophy patients and 283 control individuals without corneal dystrophy. No variations in the mature miR-184 sequence were identified in the 1000 Genomes database or in 1130 chromosomes from individuals without EDICT syndrome. Two non-causal substitutions in pre-miR-184 were identified in our control cohorts: miR-184(+39G>T), previously identified as rs41280052, was present in 11 individuals (four Fuchs-affected, seven unaffected controls), and miR-184(+52T>C) was present in one unaffected control individual.²⁶

Next, we investigated the evolutionary conservation of the miR-184 sequence by querying the UCSC Genome Browser (<http://genome.ucsc.edu/>; provided by the University of California at Santa Cruz). In 28 nonhuman vertebrates that have an ortholog of miR-184, the cytosine base at position 57 is fully conserved (Fig. 3). The mature miR-184 sequence is fully conserved in all 28 orthologous sequences that were examined, with the exception of a +64C>T substitution in platypus and a +74T>C substitution in the medaka fish (Fig. 3).⁸ Taken together, these data suggest that miR-184(+57C>T) is a rare variant that is exclusively present in individuals affected by EDICT syndrome.

To evaluate potential mechanisms underlying the pathogenicity of the miR-184(+57C>T) mutation, we used a computational approach to predict the secondary and tertiary structures of the wild-type and mutant pre-miR-184. The miR-184(+57C>T) substitution is predicted to stabilize the secondary structure of pre-miR-184 by partially closing an

internal loop, resulting in a change in Gibbs free energy of -2.39 kcal/mol (Fig. 4). Our analyses also predict that the substitution will change the tertiary conformation of the molecule, although the calculated free energy of the two conformations is nearly identical: -75.99 kcal/mol for the wild-type pre-miR-184 and -75.61 kcal/mol for the miR-184(+57C>T) variant (Fig. 4).

Finally, to gain insight into the predicted targets for the wild type and the mutant miR-184, we queried the DIANA ver. 3.0 database.^{20,27} Computations predicted 879 potential target sites for wild-type miR-184, located in 797 different genes. In contrast, a search using the miR-184(+57C>T) variant suggested 3560 potential target sites in 2781 genes. Of the 3560 putative targets of mutant miR-184, 3114 are located in genes that are not predicted as targets of wild-type miR-184.

DISCUSSION

Here, we report the mutation that is likely causal in EDICT syndrome: the single-base-pair substitution miR-184(+57C>T). Our data offer four lines of evidence implicating this variant in EDICT syndrome. First, the mutation segregates completely with the disease phenotype in the family affected by EDICT syndrome. Second, the variant is absent in 1130 control chromosomes and has not been identified in the 1000 Genomes database. Third, the variant is absent in 28 nonhuman vertebrates. Finally, in silico analyses suggest that the miR-184(+57C>T) substitution alters the stability of pre-miR-184 and thus may interfere with Dicer binding or cleavage. Taken together, these data strongly suggest that the miR-184(+57C>T) substitution causes EDICT syndrome.

Species	Mature miR-184 Sequence																											
Human	T	G	G	A	C	G	G	A	G	A	A	C	T	G	A	T	A	A	G	G	G	T						
Chimpanzee	T	G	G	A	C	G	G	A	G	A	A	C	T	G	A	T	A	A	G	G	G	T						
Orangutan	T	G	G	A	C	G	G	A	G	A	A	C	T	G	A	T	A	A	G	G	G	T						
Rhesus	T	G	G	A	C	G	G	A	G	A	A	C	T	G	A	T	A	A	G	G	G	T						
Baboon	T	G	G	A	C	G	G	A	G	A	A	C	T	G	A	T	A	A	G	G	G	T						
Marmoset	T	G	G	A	C	G	G	A	G	A	A	C	T	G	A	T	A	A	G	G	G	T						
Bushbaby	T	G	G	A	C	G	G	A	G	A	A	C	T	G	A	T	A	A	G	G	G	T						
Tree shrew	T	G	G	A	C	G	G	A	G	A	A	C	T	G	A	T	A	A	G	G	G	T						
Mouse	T	G	G	A	C	G	G	A	G	A	A	C	T	G	A	T	A	A	G	G	G	T						
Rat	T	G	G	A	C	G	G	A	G	A	A	C	T	G	A	T	A	A	G	G	G	T						
Guinea Pig	T	G	G	A	C	G	G	A	G	A	A	C	T	G	A	T	A	A	G	G	G	T						
Squirrel	T	G	G	A	C	G	G	A	G	A	A	C	T	G	A	T	A	A	G	G	G	T						
Rabbit	T	G	G	A	C	G	G	A	G	A	A	C	T	G	A	T	A	A	G	G	G	T						
Pika	T	G	G	A	C	G	G	A	G	A	A	C	T	G	A	T	A	A	G	C	G	T						
Alpaca	T	G	G	A	C	G	G	A	G	A	A	C	T	G	A	T	A	A	G	G	G	T						
Dolphin	T	G	G	A	C	G	G	A	G	A	A	C	T	G	A	T	A	A	G	A	G	T						
Cow	T	G	G	A	C	G	G	A	G	A	A	C	T	G	A	T	A	A	G	G	G	T						
Horse	T	G	G	A	C	G	G	A	G	A	A	C	T	G	A	T	A	A	G	G	G	T						
Cat	T	G	G	A	C	G	G	A	G	A	A	C	T	G	A	T	A	A	G	G	G	T						
Dog	T	G	G	A	C	G	G	A	G	A	A	C	T	G	A	T	A	A	G	G	G	T						
Megabat	T	G	G	A	C	G	G	A	G	A	A	C	T	G	A	T	A	A	G	G	G	T						
Hedgehog	T	G	G	A	C	G	G	A	G	A	A	C	T	G	A	T	A	A	G	G	G	T						
Rock hyrax	T	G	G	A	C	G	G	A	G	A	A	C	T	G	A	T	A	A	G	G	G	T						
Elephant	T	G	G	A	C	G	G	A	G	A	A	C	T	G	A	T	A	A	G	G	G	T						
Tenrec	T	G	G	A	C	G	G	A	G	A	A	C	T	G	A	T	A	A	G	G	G	T						
Wallaby	T	G	G	A	C	G	G	A	G	A	A	C	T	G	A	T	A	A	G	G	G	T						
Opossum	T	G	G	A	C	G	G	A	G	A	A	C	T	G	A	T	A	A	G	G	G	T						
Platypus	T	G	G	A	C	G	G	A	G	A	A	T	T	G	A	T	A	A	G	G	G	T						
Medaka	T	G	G	A	C	G	G	A	G	A	A	C	T	G	A	T	A	A	G	G	G	C						
Human (EDICT)	T	G	G	A	T	G	G	A	G	A	A	C	T	G	A	T	A	A	G	G	G	T						

FIGURE 3. Evolutionary conservation of miR-184 sequence in 28 nonhuman vertebrates. Blue text: primates; purple text: placental mammals; red text: non-placental vertebrates; blue box: conservation of EDICT base in nonhuman vertebrates; orange background: miR-184(+57C>T) substitution. Green background: nonconserved bases in nonplacental vertebrates.

FIGURE 4. (A) The Vienna RNAfold algorithm predicted secondary structure of wild-type pre-miR-184, (B) secondary structure of miR-184(+57C>T), (C) MC-sym predicted tertiary structure of wild-type pre-miR-184, and (D) tertiary structure of miR-184(+57C>T). For secondary structures, *green*: pre-miR-184; *blue*: mature miR-184; *orange*: seed region; *red*: miR-184(+57C>T) substitution. For tertiary structures, see key at *bottom right* of (C). Tertiary structures rendered by PyMol ver. 1.4.1.^{17–19,45}

Recently, the miR-184(+57C>T) substitution was implicated in a syndrome of keratoconus and early-onset anterior polar cataracts.²⁸ The EDICT syndrome also includes congenital cataracts, but does not demonstrate a keratoconus phenotype. Keratoconus is a noninflammatory ectatic corneal disorder characterized by progressive stromal thinning with an unaffected endothelium and Descemet membrane. The stromal thinning noted in the EDICT syndrome, however, is uniform and nonectatic. Histologic analysis reveals that the corneal endothelium is attenuated and the Descemet membrane is thickened with marked posterior nodularity suggestive of Fuchs corneal dystrophy. Furthermore, intracellular aggregates of small-diameter filaments stain positively for cytokeratin, suggestive of posterior polymorphous dystrophy. Besides abnormalities of the lens and cornea, additional prominent phenotypic features of the EDICT syndromes are iris abnormalities, including small eccentric pupils, iris defects, and ectropion pupillae, features that were not described in the family with keratoconus and cataract.^{3–6,28}

In addition to pathology of the anterior segment, EDICT patients also have an increased incidence of retinal problems. Of 10 affected individuals, 4 developed retinal pathology. Three individuals experienced retinal detachments, and one developed macular chorioretinitis (JDG, unpublished data, 2011). Although we cannot rule out the possibility that this increase in retinal disease is a result of cataract extraction, the prospect of a retinal phenotype in EDICT syndrome merits further study. Although miR-184 has been studied most extensively in the anterior segment, miR-184 is also expressed in the retina, and miR-184 expression is significantly reduced after

retinal ischemia and in choroidal neovascularization, suggesting that miR-184 may play a role in normal retinal function.^{29,30}

Our computational analyses suggest several potential mechanisms for pathogenesis by the miR-184(+57C>T) mutation. The substitution is predicted to strengthen binding at the 5' end of the mature miRNA, a region that is critical for Dicer binding and RISC assembly.^{31,32} This prediction suggests that miR-184(+57C>T) could reduce the expression of mature miR-184 by interfering with Dicer cleavage, or that it could reduce the activity of mature miR-184 by preventing assembly of the RISC. The 5' binding affinity also plays an integral role in selection of pre-microRNA cleavage sites by Dicer, raising the possibility that miR-184(+57C>T) alters Dicer cleavage, resulting in production of a dramatically different mature microRNA.³³

In silico structure modeling offers conflicting data regarding the thermodynamic stability of the miR-184(+57C>T) variant. Although calculations of secondary structure suggest that miR-184(+57C>T) stabilizes pre-miR-184, predictions of the tertiary structure show little or no difference in free energy. Thus, it seems unlikely that a change in overall thermodynamic stability of pre-miR-184 is the main causal factor in the pathogenesis of EDICT syndrome.

In vitro, miR-184 has been shown to directly inhibit Akt2 in cultured human glioma and neuroblastoma cell lines, and overexpression of miR-184 suppresses cell viability and proliferation in those cell types.^{34–36} Of importance, the Akt pathway is involved in epithelial-mesenchymal transition, and defects in this process have been associated with Fuchs corneal dystrophy and posterior polymorphous dystrophy.^{37–41} However,

data are mixed regarding the effect of underexpression of miR-184. Inhibition of miR-184 suppresses proliferation in squamous cell carcinoma cells with endogenous overexpression of miR-184, but knockdown of miR-184 in neuroblastoma cells results in increased cell growth.^{35,42}

Hughes et al.²⁸ demonstrated that transfection with an miR-184 mimic, but not an miR-184(+57C>T) mimic, is sufficient to inhibit miR-205-mediated knockdown of the integrin ITGB4 and the lipid phosphatase SHIP2 (also known as INPPL1) in HeLa cells. ITGB4 is a major component of corneal hemidesmosomes in the form of the integrin $\alpha 6 \beta 4$ heterodimer, and miR-184 has previously been shown to disinhibit the production of SHIP2, which inhibits the Akt pathway.^{43,44}

The findings that miR-184 modulates the Akt signaling pathway and that the miR-184(+57C>T) variant is less effective at regulating the upstream Akt regulator SHIP2 offer a promising possibility for the pathogenesis of the corneal disease in EDICT syndrome. Defects in epithelial-mesenchymal transition could represent a common disease mechanism among Fuchs corneal dystrophy, posterior polymorphous dystrophy, and the endothelial pathology observed in EDICT syndrome. Future functional experiments will be essential in elucidating the mechanism by which the miR-184(+57C>T) mutation causes EDICT syndrome.

Acknowledgments

The authors thank all the participating members of the family.

References

- Dohlman CH. Familial congenital cornea guttata in association with anterior polar cataract. *Acta Ophthalmol.* 1951;29:445-473.
- Traboulsi EI, Weinberg RJ. Familial congenital cornea guttata with anterior polar cataracts. *Am J Ophthalmol.* 1989;108:123-125.
- Hughes AE, Dash DP, Jackson AJ, Frazer DG, Silvestri G. Familial keratoconus with cataract: linkage to the long arm of chromosome 15 and exclusion of candidate genes. *Invest Ophthalmol Vis Sci.* 2003;44:5063-5066.
- Jun AS, Broman KW, Do DV, Akpek EK, Stark WJ, Gottsch JD. Endothelial dystrophy, iris hypoplasia, congenital cataract, and stromal thinning (EDICT) syndrome maps to chromosome 15q22.1-q25.3. *Am J Ophthalmol.* 2002;134:172-176.
- Akpek EK, Jun AS, Goodman DF, Green WR, Gottsch JD. Clinical and ultrastructural features of a novel hereditary anterior segment dysgenesis. *Ophthalmology.* 2002;109:513-519.
- Dash DP, Silvestri G, Hughes AE. Fine mapping of the keratoconus with cataract locus on chromosome 15q and candidate gene analysis. *Mol Vis.* 2006;12:499-505.
- Kozomara A, Griffiths-Jones S. miRBase: integrating microRNA annotation and deep-sequencing data. *Nucleic Acids Res.* 2011;39:D152-D157.
- Fujita PA, Rhead B, Zweig AS, et al. The UCSC Genome Browser database: update 2011. *Nucleic Acids Res.* Published online November 15, 2011.
- Lim LP, Lau NC, Garrett-Engle P, et al. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. *Nature.* 2005;433:769-773.
- Mathonnet G, Fabian MR, Svitkin YV, et al. MicroRNA inhibition of translation initiation in vitro by targeting the cap-binding complex eIF4F. *Science.* 2007;317:1764-1767.
- Pillai RS, Bhattacharyya SN, Artus CG, et al. Inhibition of translation initiation by Let-7 microRNA in human cells. *Science.* 2005;309:1573-1576.
- Bagga S, Bracht J, Hunter S, et al. Regulation by let-7 and lin-4 miRNAs results in target mRNA degradation. *Cell.* 2005;122:553-563.
- He L, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. *Nat Rev Genet.* 2004;5:522-531.
- Kloosterman WP, Plasterk RHA. The diverse functions of microRNAs in animal development and disease. *Dev Cell.* 2006;11:441-450.
- Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell.* 2005;120:15-20.
- Mencia A, Modamio-Hoybjør S, Redshaw N, et al. Mutations in the seed region of human miR-96 are responsible for nonsyndromic progressive hearing loss. *Nat Genet.* 2009;41:609-613.
- Gruber AR, Lorenz R, Bernhart SH, Neuböck R, Hofacker IL. The Vienna RNA Websuite. *Nucleic Acids Res.* 2008;36:W70-W74.
- Hofacker IL, Fontana W, Stadler PF, Bonhoeffer LS, Tacker M, Schuster P. Fast folding and comparison of RNA secondary structures. *Monatsh Chem.* 1994;125:167-188.
- Parisien M, Major F. The MC-Fold and MC-Sym pipeline infers RNA structure from sequence data. *Nature.* 2008;452:51-55.
- Maragkakis M, Reczko M, Simossis VA, et al. DIANA-microT web server: elucidating microRNA functions through target prediction. *Nucleic Acids Res.* 2009;37:W273-W276.
- Gottsch JD, Bowers AL, Margulies EH, et al. Serial analysis of gene expression in the corneal endothelium of Fuchs dystrophy. *Invest Ophthalmol Vis Sci.* 2003;44:594-599.
- Karali M, Peluso I, Marigo V, Banfi S. Identification and characterization of microRNAs expressed in the mouse eye. *Invest Ophthalmol Vis Sci.* 2007;48:509-515.
- Li Y, Piatigorsky J. Targeted deletion of Dicer disrupts lens morphogenesis, corneal epithelium stratification, and whole eye development. *Dev Dyn.* 2009;238:2388-2400.
- Ryan DG, Oliveira-Fernandes M, Lavker RM. MicroRNAs of the mammalian eye display distinct and overlapping tissue specificity. *Mol Vis.* 2006;12:1175-1184.
- Huang K, Dentichev T, Stambolian D. MiRNA expression in the eye. *Mamm Genome.* 2008;19:510-516.
- A map of human genome variation from population-scale sequencing. *Nature.* 2010;467:1061-1073.
- Maragkakis M, Alexiou P, Papadopoulos G, et al. Accurate microRNA target prediction correlates with protein repression levels. *BMC Bioinform.* 2009;10:295.
- Hughes A, Bradley D, Campbell M, et al. Mutation altering the miR-184 seed region causes familial keratoconus with cataract. *Am J Hum Genet.* 2011;89:628-633.
- Kovacs B, Lumayag S, Cowan C, Xu S. microRNAs in Early diabetic retinopathy in streptozotocin-induced diabetic rats. *Invest Ophthalmol Vis Sci.* 2011;52:4402-4409.
- Shen J, Yang X, Xie B, et al. MicroRNAs regulate ocular neovascularization. *Mol Ther.* 2008;16:1208-1216.
- Khvorova A, Reynolds A, Jayasena SD. Functional siRNAs and miRNAs exhibit strand bias. *Cell.* 2003;115:209-216.
- Krol J, Sobczak K, Wilczynska U, et al. Structural Features of MicroRNA (miRNA) precursors and their relevance to miRNA biogenesis and small interfering RNA/short hairpin RNA design. *J Biol Chem.* 2004;279:42230-42239.
- Schwarz DS, Hutvagner GR, Du T, Xu Z, Aronin N, Zamore PD. Asymmetry in the assembly of the RNAi enzyme complex. *Cell.* 2003;115:199-208.
- Chen Y, Stallings RL. Differential patterns of microRNA expression in neuroblastoma are correlated with prognosis, differentiation, and apoptosis. *Cancer Res.* 2007;67:976-983.
- Foley N, Bray I, Tivnan A, et al. MicroRNA-184 inhibits neuroblastoma cell survival through targeting the serine/threonine kinase AKT2. *Mol Cancer.* 2010;9:83.
- Malzkorn B, Wolter M, Liesenberg F, et al. Identification and functional characterization of microRNAs involved in the malignant progression of gliomas. *Brain Pathol.* 2010;20:539-550.
- Larue L, Bellacosa A. Epithelial-mesenchymal transition in development and cancer: role of phosphatidylinositol 3' kinase/AKT pathways. *Oncogene.* 2005;24:7443-7454.
- Wright AF, Dhillon B. Major progress in Fuchs's corneal dystrophy. *N Engl J Med.* 2010;363:1072-1075.
- Krafchak CM, Pawar H, Moroi SE, et al. Mutations in TCF8 cause posterior polymorphous corneal dystrophy and ectopic expression of COL4A3 by corneal endothelial cells. *Am J Hum Genet.* 2005;77:694-708.
- Mehta JS, Vithana EN, Tan DTH, et al. Analysis of the posterior polymorphous corneal dystrophy 3 gene, TCF8, in late-onset Fuchs

- endothelial corneal dystrophy. *Invest Ophthalmol Vis Sci.* 2008; 49:184–188.
41. Riazuddin SA, Zaghoul NA, Al-Saif A, et al. Missense mutations in TCF8 cause late-onset Fuchs corneal dystrophy and interact with FCD4 on chromosome 9p. *Am J Hum Genet.* 2010;86:45–53.
42. Wong TS, Liu XB, Wong BY-H, Ng RW-M, Yuen AP-W, Wei WL. Mature miR-184 as potential oncogenic microRNA of squamous cell carcinoma of tongue. *Clin Cancer Res.* 2008;14:2588–2592.
43. Stepp MA, Spurr-Michaud S, Tisdale A, Elwell J, Gipson IK. Alpha 6 beta 4 integrin heterodimer is a component of hemidesmosomes. *Proc Natl Acad Sci U S A.* 1990;87:8970–8974.
44. Yu J, Ryan DG, Getsios S, Oliveira-Fernandes M, Fatima A, Lavker RM. MicroRNA-184 antagonizes microRNA-205 to maintain SHIP2 levels in epithelia. *Proc Natl Acad Sci U S A.* 2008;105:19300–19305.
45. The PyMOL Molecular Graphics System, Version 1.4. 2011.