Inhibition of miR-10a-5p suppresses cholangiocarcinoma cell growth through downregulation of Akt pathway

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Backgrounds: Cholangiocarcinoma (CCA) is epithelial cell malignancy with very poor prognosis. A lot of patients were diagnosed at advanced stage of CCA and no risk factors were identified. There are limited treatment options available for the management of CCA patients. It is urgent to develop effective targeted therapies for the treatment of CCA. miRNAs are small noncoding RNAs that negatively regulate the target genes. In this study, we investigated the role and mechanism of miR-10a-5p in CCA.

Methods: Human CCA cell lines (CCLP1 and SG-231) were transfected with miR-10a-5p mimic or miR-10a-5p inhibitor. qRT-PCR was performed to detect the miR-10a-5p level. Proliferation, colony formation, and apoptosis were analyzed. Luciferase reporter assay was used to explore the targeting of miR-10a-5p on PTEN. For in vivo tumorigenesis assay, CCLP1 cells with stable knockdown of miR-10a-5p or control CCLP1 cells were injected subcutaneously into the flank of the SCID mice and animals were monitored for tumor growth.

Results: miR-10a-5p expression was significantly upregulated in human CCA cell lines (CCLP1 and SG-231). Inhibition of miR-10a-5p significantly suppressed the proliferation and induced apoptosis in CCLP1 and SG-231. PTEN is a direct target of miR-10a-5p in CCA cells.

Conclusion: Inhibition of miR-10a-5p can decrease CCA cells growth by downregulation of Akt pathway. These results indicate that miR-10a-5p may serve as a potential target for the treatment of CCA and help to develop effective therapeutic strategies.

Keywords: miR-10a-5p, cholangiocarcinoma, PTEN, Akt, liver, proliferation

Introduction
Cholangiocarcinoma (CCA) is the second most common primary liver malignancy.1 CCA represents a diverse group of epithelial cell malignancy that develops along the biliary tract.2,3 CCA are classified into intrahepatic CCA (iCCA), perihilar CCA (pCCA), and distal CCA (dCCA) depending on their site of origin.4 Different types of CCA have different features and require specific treatments. Primary sclerosing cholangitis is considered to be the principal risk factor for CCA.5 Other risk factors include hepatitis C virus, human immunodeficiency virus, liver cirrhosis, and diabetes.6 However, in most CCAs, no risk factors are identified. The incidence of ICC in the US continues to rise. Between 1973 and 2012, the reported US incidence of ICC increased from 0.44 to 1.18 cases per 100,000.7 Patients with CCA often present symptoms with biliary obstruction or non-specific abdominal pain, a high proportion of patients were diagnosed at advanced stage of CCA.8 At early stage, curative options are available in the form of surgical resection and/or liver transplantation.9 The most frequently used treatment modality is chemotherapy. Due to high rate of recurrence after liver...
transplantation, distant metastasis and invasion, as well as the chemoresistance, CCA patients represent a very poor prognosis. The average 5-year survival rate for CCA patients is 5%–10%. It is urgent to develop new specific effective targeted therapies for the treatment of CCA.

miRNAs are small noncoding RNAs which are short single-stranded molecules about 21–23 nucleotides in length. miRNAs regulate gene expression at post transcriptional level. miRNAs inhibit the target genes expression by binding to 3′ untranslated regions (3′UTRs) of target mRNAs which cause mRNA degradation and destabilization. miRNAs play important roles in a broad range of biological processes, such as embryonic development, apoptosis, stem cell differentiation, hematopoiesis, and immune response. Dysregulation of miRNA expression has been reported in cancer, including CCA. For example, miR-29a has emerged as a tumor suppressor, miR-29a level was found significantly decreased in both CCA tissues and tumor cell lines. miR-34a was rhythmically expressed in CCA cells. Inhibition of miR-34a decreased proliferation, migration, and invasion in CCA cells. miR-21 and miR-221 levels significantly upregulated in CCA serum. Circulating plasma levels of miR-21 and miR-221 can serve as a diagnostic and prognostic biomarkers for CCA.

miR-10 family including miR-10a and miR-10b has attracted attention because of its conservation and the position of the miR-10 genes within the Homeobox (HOX) clusters. Hox genes are a group of evolutionarily conserved genes that encode a class of important transcription factors that regulate early developmental morphogenetic processes and continue to be expressed into adulthood. Hox genes organized into four distinct clusters. These clusters, labeled HOXA, HOXB, HOXC, and HOXD, are located on chromosomes 7p14, 17q21, 12q13, and 2q31. miR-10a was located within the HOX B cluster on 17q21 and miR-10b was located at HOX D cluster 2q31. miR-10 family members are deregulated in numerous types of cancers including uterine sarcomas, breast cancer, and hepatocellular carcinoma (HCC). miR-10a has been reported to be associated with liver regeneration, regulates human mesangial cells proliferation and chemokine expression by targeting IL-8. Plasma miR-10a levels were decreased in patients with coronary artery disease (CAD) and negatively associated with the presence and severity of CAD. miR-10a serves as a switch to control miR-10a-NF-κB regulatory circuit that promotes the excessive secretion of NF-κB-mediated inflammatory cytokines and the proliferation and migration of fibroblast-like synoviocytes of rheumatoid arthritis (RA). miR-10a-5p is overexpressed in human pancreatic ductal adenocarcinoma (PDAC) and acts as an oncogene to promote the metastatic behavior of PDAC cells. Abnormal high expression of miR-10a was also found in patients with acute myeloid leukemia (AML). miR-10a promotes proliferation of immature blood progenitors and repression of mature blood cell differentiation and maturation in AML.

The expression of miR-10 was upregulated in CCA. However, the function of miR-10a-5p in CCA is largely unknown. In the present study, we explored the role of miR-10a-5p in CCA. We found that PTEN is a direct target of miR-10a-5p in CCA cell lines. Inhibition of miR-10a-5p suppressed proliferation and promoted apoptosis in CCA cells through downregulation Akt pathway.

Methods

Cell culture

Human intrahepatic bile duct epithelial cell line HIBEC was obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). Human CCA cell lines CCLP1 and SG-231 were obtained from Cell Bank of Chinese Academy of Sciences (Shanghai, People’s Republic of China). Cells were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) containing 10% FBS, L-glutamine, and antibiotics (100 units/mL penicillin and 100 µg/mL streptomycin). All cells were maintained in a 37°C humidified incubator with 5% CO₂.

Transfections

CCLP1 and SG-231 cells were seeded in six-well plate and transfected with scramble control or miR-10a-5p mimic or miR-10a-5p inhibitor GenePharma (Shanghai, People’s Republic of China) using Oligofectamine reagent (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer’s instructions. Final concentration of scramble or miR-10a-5p mimic or miR-10a-5p inhibitor is 100 nM. At the indicated time point, cells samples were collected.

qRT-PCR

Total RNA was extracted from cells using Trizol (Thermo Fisher Scientific). Reverse transcription was performed using the miScript RT Kit (TaKaRa, Dalian, People’s Republic of China). qRT-PCR was performed using miScript SYBR Green PCR Kit (Qiagen NV, Venlo, the Netherlands) on a C1000 thermal cycler (Bio-Rad Laboratories Inc., Hercules, CA, USA). The primers of miR-10a-5p and U6 were obtained from Qiagen NV. U6 was used as an internal control.
Cell proliferation assay

Proliferation assays were conducted using WST-1 assay (Beyotime, Shanghai, People’s Republic of China). After CCLP1 and SG-231 cells were transfected with miR-10a-5p mimic or miR-10a-5p inhibitor or scramble control for 6 hours, cells were seeded in 96-well plates (2,000 cells/well). At 0, 24, 48, and 72 hours, culture medium was removed and 100 µL fresh medium containing 10 µL of WST-1 reagents was added into the wells. After 2–3 hours, the absorbance was measured at 450 nm by using ELISA Microplate Reader (Biocompare, San Francisco, CA, USA).

Western blot analysis

Total protein was extracted from cells using a protein extraction kit (Beyotime). Protein concentrations were measured using the BCA Protein Assay Kit (Beyotime). Protein fractions were separated on SDS-PAGE gel electrophoresis (Bio-Rad Laboratories Inc.) and transferred to a nitrocellulose membrane (Bio-Rad Laboratories Inc.). After blocking in 5% skim milk in PBS for 1 hour at room temperature, the membranes were incubated overnight at 4°C with primary antibodies. Primary antibodies against PARP, cleaved caspase-3, PTEN, p-Akt (ser473), and Akt were obtained from Cell Signaling Technology (Danvers, MA, USA). Primary antibody against β-actin was obtained from Abcam (Cambridge, MA, USA). Secondary antibodies IRDye800CW Goat anti-Mouse IgG and IRDye800CW Goat anti-Rabbit IgG were obtained from LI-COR (LI-COR Biosciences, Lincoln, NE, USA). Western bolt images were detecting by using LI-COR Odyssey 9120 Imaging System (LI-COR Biosciences).

Luciferase reporter assays

PTEN 3′-UTR was obtained from GeneCopoeia (Rockville, MD, USA). We mutated two nucleotides of the PTEN 3′-UTR by using Site-Directed Mutagenesis kit (Stratagene, Shanghai, People’s Republic of China). These vectors also express the Renilla luciferase serving as internal controls for the dual-luciferase assay. CCLP1 and SG231 were co-transfected with miR-10a-5p mimic (100 nM) or scramble (100 nM) with PTEN 3′-UTR or its mutant (Mut) using lipofectamine 2000 transfection reagent (Thermo Fisher Scientific). After 48 hours of transfection, the luciferase activity was measured using the dual luciferase reporter assay kit (Promega Corporation, Madison, WI, USA).

Colony formation assay

Lentiviral plasmid vector expresses miR-10a-5p inhibitor (LV-miR-10-5p-inhibitor) and scramble control lentivirus vector (LV-con) were obtained from ABM Industries Inc. (New York, NY, USA). We established CCLP1 cell line with stable knockdown of miR-10a-5p by transfecting cells with LV-miR-10-5p-inhibitor. The control CCLP1 cells were transfected with LV-con. Cells were seeded in 10 cm dishes at 2,000 cells/dish and cultured for 14 days. After fixation with methanol for 20 minutes, the colonies were stained with 0.1% crystal violet.

Mouse xenograft model

For tumorigenesis assays, 6 weeks old, male SCID mice were purchased from Wei Tong Li Hua Experimental Animal Technology Co., Ltd (Beijing, People’s Republic of China) (n=3). In total, 1 × 10⁶ miR-10a-5p stable knockdown CCLP1 cells (LV-miR-10a-5p-inhibitor) or control CCLP1 cells (LV-con) were injected subcutaneously into the flank of the mice. Mice were observed for 30 days for tumor formation. Tumor diameters are measured with digital calipers, and the tumor volume in mm³ is calculated by the formula: Volume = (width)² × length + 2. All animal studies were approved by the Ethics Committee of Fudan University Pudong Medical Center. The handling of the mice and all experimental procedures were carried out in strict accordance with Fudan University Guidelines for the Care and Use of Laboratory Animals.

Statistical analysis

Data represent the mean ± SD. Experiments were repeated at least three times. Statistical analysis was performed using GraphPad Prism (version 5.0, GraphPad Software, Inc., La Jolla, CA, USA). One-way ANOVA along with Bonferroni adjustment and Student’s t-test were used to evaluate the differences between groups. A P-value < 0.05 was considered statistically significant.

Results

Inhibition of miR-10a-5p suppresses proliferation and promotes apoptosis in CCA cells

We evaluated the expression of miR-10a-5p in human intrahepatic bile duct epithelial cell line HIBEC and human CCA cell lines (CCLP1 and SG-231) by qRT-PCR analysis. Results showed that miR-10a-5p was upregulated significantly in CCA cells compared with HIBEC (Figure 1A). To evaluate the role of miR-10a-5p on CCA cells growth, human CCA cell lines CCLP1 and SG-231 were transfected with miR-10a-5p mimic or miR-10a-5p inhibitor or scramble control. The expression of miR-10a-5p was determined by qRT-PCR. As shown in Figure 1B, compared with scramble control,
transfection of miR-10a-5p mimic for 72 hours led to a dramatic increase expression of miR-10a-5p in both CCLP1 and SG231 cells, whereas transfection of miR-10a-5p inhibitor for 72 hours led to a significant inhibition of the miR-10a-5p level in these cells (Figure 1C). Cell viability was measured using WST-1 assay. As shown in Figure 1D, upregulated miR-10a-5p level by miR-10a-5p mimic significantly increased the proliferation in both of CCLP1 and SG-231 cells, whereas a significant decrease in cell viability was detected when cells transfected with miR-10a-5p inhibitor compared with scramble control. Western blot analysis revealed that the cleaved PARP and cleaved caspase-3 were significantly increased in CCLP1 and SG-231 cells transfected with miR-10a-5p inhibitor (Figure 1E). These results indicated that miR-10a-5p promoted CCA cells proliferation, while inhibition of miR-10a-5p suppressed cell growth and induced apoptosis in CCA cells.

PTEN is a direct target of miR-10a-5p in CCA cells

To explore the tumor suppressive mechanism of miR-10a-5p inhibition, the potential target genes of miR-10a-5p were analyzed using miRNA target prediction programs TargetScan (http://www.targetscan.org). There are 287 transcripts with conserved sites, containing a total of 302 conserved sites and 99 poorly conserved sites. The predicted targets of human miR-10a-5p are shown in Table S1. We found that there was a predicted miR-10a-5p binding site in the 3′-UTR of PTEN (PTEN, phosphatase, and tensin homologue deleted on chromosome ten) (Figure 2A). To determine whether PTEN was regulated by miR-10a-5p, CCLP1 and SG-231 cells were transfected with miR-10a-5p inhibitor, Western blot analysis showed that inhibition of miR-10a-5p significantly upregulated the protein levels of PTEN (Figure 2B) and decreased the expression of p-Akt (ser473) (Figure 2C). To further verify whether PTEN is a direct target of miR-10a-5p, we generated PTEN reporter construct containing 3′-UTR with mutations of miR-10a-5p binding site (indicated in Figure 2A). CCLP1 and SG-231 cells were transfected with wild type or Mut PTEN 3′-UTR and miR-10a-5p mimic, luciferase reporter assay showed that miR-10a-5p mimic remarkably decreased the 3′-UTR luciferase reporter activity of PTEN, this effect was abolished when miR-10a-5p binding site was mutated (Figure 2D). These findings
Figure 2 PTEN was a direct target of miR-10a-5p in CCA.

Notes: (A) The 3′-UTR of PTEN contained a predicted miR-10a-5p binding site. Mutations were generated on the two nucleotides of the PTEN 3′-UTR as indicated. (B) CCLP1 and SG-231 were transfected with miR-10a-5p inhibitor or scramble control for 48 hours, protein levels of PTEN were determined by Western blot analysis. Quantifications of relative protein levels are shown at the right panel. (C) Western blot analysis of p-Akt (ser473) and total Akt. Quantifications of relative protein levels are shown at the right panel. (D) Relative luciferase activity in CCLP1 and SG-231 cells co-transfected with WT or Mut PTEN 3′-UTR and miR-10a-5p mimic or scramble control. Red bar indicates statistical difference. Data were expressed as mean ± SD. *P < 0.05, **P < 0.01.

Abbreviations: CCA, cholangiocarcinoma; 3′-UTR, 3′-untranslated regions; Mut, mutation; WT, wild type.
suggested that PTEN was a direct target of miR-10a-5p in CCA cells.

**Inhibition of miR-10a-5p suppresses CCA growth in SCID mice**

To further evaluate the effects of miR-10a-5p on CCA growth in vivo, we generated CCLP1 cells with stable knockdown of miR-10a-5p. CCLP1 cells were transfected with LV-miR-10a-5p-inhibitor or LV-con. As shown in Figure 3A, the downregulation of miR-10a-5p was confirmed by qRT-PCR. Knockdown of miR-10a-5p led to a significantly decreased colony formation in CCLP1 cells compared with control cells (Figure 3B). CCLP1 cells with stable knockdown of miR-10a-5p and control cells were injected subcutaneously into the flank of SCID mice to establish a xenograft model. Compared with the control group, knockdown of miR-10a-5p resulted in a significant reduction of tumor size and tumor volume (Figure 3C). Western blot analysis of the tumor tissues confirmed upregulated PTEN and decreased p-Akt (ser473) in miR-10a-5p knockdown tumors (Figure 3D). Taken together, these results suggested that inhibition of miR-10a-5p played an important role suppressed CCA cell proliferation.

**Discussion**

CCA is an aggressive tumor with very poor prognosis. The majority of patients present with unresectable disease and have a survival of less than 12 months following diagnosis.\(^\text{35}\) It is crucial to understand the pathogenesis of CCA, find out the effective, targeted, individualized therapies, and improve the quality of patient’s life. In our study, we investigated the effect of miR-10a-5p on CCA cells proliferation in vitro and in vivo. We found that overexpression of miR-10a-5p promoted CCA cells proliferation, whereas inhibition of miR-10a-5p suppressed proliferation and induced apoptosis in CCA cells. In a mouse xenograft model, inhibition of
miR-10a-5p significantly suppressed tumorigenicity. PTEN is a direct target of miR-10a-5p in CCA cells. Inhibition of miR-10a-5p led to the downregulation of Akt pathway. miRNA expression has been reported to be involved in tumor progression and prognosis, including CCA.36 It has been reported that overexpression of miR-10a-5p promoted the migration and invasion of human HCC cell lines (QGY-7703 and HepG2) in vitro but suppressed metastasis in vivo.37 EphA4 (Eph tyrosine kinase receptor) was identified as the direct target of miR-10a. miR-10a promotes HCC cell migration and invasion through targeting EphA4, thereby regulating epithelial–mesenchymal transition and cell adhesion.37 Downregulation of miR-10a-5p has been shown to promote proliferation and restricts apoptosis via targeting T-box transcription factor 5 (TBX5) in inflamed synovocytes.38 In our study, we found that inhibition of miR-10a-5p suppressed CCA cells proliferation through regulating PTEN-Akt pathway.

Akt pathway has been well established as an important signaling intermediate controlling cell survival, growth, proliferation, and other cellular processes.39 Activation of Akt pathway is an important survival pathway activated in cancer. Increased activation of AKT signaling was reproducibly observed in both CCA cell lines and CCA tissues.40 PTEN is a tumor suppressor and is a major negative regulator of the Akt signaling pathway. PTEN can be regulated by posttranslational modifications that include oxidation, acetylation, phosphorylation, ubiquitination, and proteolytic cleavage and by protein–protein interactions.41 PTEN can also be regulated by miRNAs. miRNAs may function as either oncogenes or tumor suppressors depending on their downstream targets.42 For example, miR-21 contributes both HCC and CCA growth by targeting PTEN.43,44 miR-22145 and miR-17-92 cluster46 promote CCA growth by targeting PTEN. In our study, we found that PTEN is a direct target of miR-10a-5p in CCA cells. Inhibition of miR-10a-5p promotes apoptosis in CCA cells through regulating PTEN.

Increasing evidences have shown that miRNAs are potential targets for human cancer treatment.47 Our study provided insight into the mechanism of inhibition of miR-10a-5p-suppressed CCA cells proliferation. miR-10a-5p may be serve as a potential target for CCA. These findings may help to better understand the tumorigenesis of CCA and develop effective therapeutic strategies.

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Disclosure
The authors report no conflicts of interest in this work.

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## Supplementary material

### Table S1 Predicted targets of human miR-10a-5p

| Target gene | Representative transcript | Gene name |
|-------------|----------------------------|-----------|
| BDNF        | ENST00000439476.2          | Brain-derived neurotrophic factor |
| AR5         | ENST00000315366.7          | Arylsulfatase family, member J   |
| CRLF3       | ENST00000324238.6          | Cytokine receptor-like factor 3  |
| HOXA3       | ENST00000396352.4          | Homeobox A3                        |
| VWC2L       | ENST00000427124.1          | von Willebrand factor C domain containing protein 2-like |
| RNF1B6      | ENST00000375121.2          | Ring finger protein 186            |
| SOBP        | ENST00000317357.5          | Sine oculis binding protein homolog (Drosophila) |
| TFRC        | ENST00000540528.1          | Transferrin receptor               |
| SMAP1       | ENST00000370452.3          | Small ArfGAP 1                     |
| KPNA5       | ENST00000368564.1          | Karyopherin alpha 5 (importin alpha 6) |
| HCN1        | ENST00000303230.4          | Hyperpolarization-activated cyclic nucleotide-gated potassium channel 1 |
| FIGN        | ENST00000333129.3          | Fidgetin                           |
| DAZAP1      | ENST00000336761.6          | DAZ-associated protein 1           |
| HOXB3       | ENST00000470495.1          | Homeobox B3                        |
| NR6A1       | ENST00000487099.2          | Nuclear receptor subfamily 6, group A, member 1 |
| KLHL29      | ENST00000486442.1          | Kelch-like family member 29       |
| NCOR2       | ENST00000405201.1          | Nuclear receptor corepressor 2     |
| ERGIC2      | ENST00000360150.4          | ERGIC and golgi 2                  |
| ELOVL2      | ENST00000354666.3          | ELOVL fatty acid elongase 2        |
| USP46       | ENST00000441222.3          | Ubiquitin-specific peptidease 46   |
| RPRD1A      | ENST00000399022.4          | Regulation of nuclear pre-mRNA domain containing 1A |
| FLJ20373    | ENST00000414004.2          |                                       |
| SDC1        | ENST00000254351.4          | Syndecan 1                          |
| KCNA6       | ENST00000343855.1          | Potassium voltage-gated channel, shaker-related subfamily, member 6 |
| CADM2       | ENST00000383699.3          | Cell adhesion molecule 2           |
| FLRT2       | ENST00000330753.4          | Fibronectin leucine-rich transmembrane protein 2 |
| LIK1        | ENST00000369083.3          | Lix1 homolog (mouse)-like          |
| RORB        | ENST00000376896.3          | RAR-related orphan receptor B      |
| RORA        | ENST00000335670.6          | RAR-related orphan receptor A      |
| CYTH1       | ENST00000585509.1          | Cytohesin 1                        |
| SMTNL2      | ENST00000338859.4          | Smoothelin-like 2                  |
| GOLGA3      | ENST00000204726.3          | Golgin A3                          |
| ATCAY       | ENST00000450849.2          | Ataxia, cerebellar, Cayman type    |
| MAP3K7      | ENST00000369323.3          | Mitogen-activated protein kinase kinase kinase 7 |
| UBE2J       | ENST00000355803.4          | Ubiquitin-conjugating enzyme E2    |
| TMEM183A    | ENST00000367242.3          | Transmembrane protein 183A         |
| ER13        | ENST00000372259.5          | Er11 exoribonuclease family member 3 |
| ATXN2       | ENST00000550104.1          | Ataxin 2                           |
| XRN1        | ENST00000264951.4          | 5′-3′ exoribonuclease 1            |
| LRRC8B      | ENST00000330947.2          | Leucine-rich repeat containing 8 family, member B |
| GABRB2      | ENST00000393959.1          | Gamma-aminobutyric acid (GABA) A receptor, beta 2 |
| CNNM4       | ENST00000450667.1          | Cyclin M4                          |
| IL1RAPL1    | ENST00000379893.1          | Interleukin 1 receptor accessory protein-like 1 |
| ZMYND11     | ENST00000381591.1          | Zinc finger, MYND-type containing 11 |
| IGDC4       | ENST00000352385.2          | Immunoglobulin superfamily, DCC subclass, member 4 |
| ALPL        | ENST00000374840.3          | Alkaline phosphatase, liver/bone/kidney |
| KLF7        | ENST00000423015.1          | Kruppel-like factor 7 (ubiquitous) |
| NPSA3       | ENST00000346562.2          | Neuronal PAS domain protein 3      |
| CECR6       | ENST00000399875.1          | Cat eye syndrome chromosome region, candidate 6 |
| SSX2IP      | ENST00000342203.3          | Synovial sarcoma, X breakpoint 2 interacting protein |
| ZNF367      | ENST00000375256.4          | Zinc finger protein 367            |
| EZF7        | ENST00000416496.2          | E2F transcription factor 7         |
| CELF2       | ENST00000379261.4          | CUGBP, Elav-like family member 2   |
| SNX18       | ENST00000343017.6          | Sorting nexin 18                   |
| ONECUT1     | ENST00000560699.2          | One cut homeobox 1                 |

(Continued)
Table S1 (Continued)

| Target gene | Representative transcript | Gene name |
|-------------|---------------------------|-----------|
| CTD-2267D19.3 | ENST00000578774.1 | Uncharacterized protein |
| PRKAA2 | ENST00000371244.4 | Protein kinase, AMP-activated, alpha 2 catalytic subunit |
| ELOVL6 | ENST00000394607.3 | ELOVL fatty acid elongase 6 |
| H3F3C | ENST00000340398.3 | H3 histone, family 3C |
| H3F3B | ENST00000591890.1 | H3 histone, family 3B (H3.3B) |
| ESRG | ENST00000361525.3 | Estrogen-related receptor gamma |
| BAZ1B | ENST00000337954.4 | Bromodomain adjacent to zinc finger domain, 1B |
| FNBP1L | ENST00000370253.2 | Formin binding protein 1-like |
| PAPD5 | ENST00000357464.3 | PAP-associated domain containing 5 |
| TBX5 | ENST00000349716.5 | T-box 5 |
| CRRN3 | ENST00000314499.7 | Cysteine-serine-rich nuclear protein 3 |
| BBX | ENST00000415149.2 | Bobby sox homolog (Drosophila) |
| FAM196A | ENST00000522781.1 | Family with sequence similarity 196, member A |
| PRR3 | ENST00000412055.1 | Proline-rich transmembrane protein 3 |
| IGFBP1 | ENST00000370900.1 | Immunoglobulin superfamily, member 1 |
| ACTG1 | ENST00000331925.2 | Actin, gamma 1 |
| EPHA2 | ENST00000358432.5 | EPH receptor A2 |
| KIAA0247 | ENST0000034745.4 | KIAA0247 |
| MDGA2 | ENST00000349988.3 | MAM domain-containing glycosylphosphatidylinositol anchor protein 2 |
| HNRNPK | ENST00000376281.4 | Heterogeneous nuclear ribonucleoprotein K |
| JARID2 | ENST0000034776.2 | Jumonji, AT-rich interactive domain 2 |
| KCTD16 | ENST00000507359.3 | Potassium channel tetramerization domain containing 16 |
| PALM2 | ENST0000044854.2 | Paralentin |
| MMC2 | ENST00000403733.3 | WW and C2 domain containing 2 |
| NCA4 | ENST00000330847.1 | Nuclear receptor subfamily 4, group A, member 3 |
| NEDD4 | ENST00000338963.2 | Neural precursor cell expressed, developmentally downregulated 4, E3 ubiquitin protein ligase |
| BCL6 | ENST00000406870.2 | B-cell CLL/lymphoma 6 |
| RP6-24A23.6 | ENST00000563887.1 | Uncharacterized protein |
| CTNB1 | ENST00000372263.1 | Catenin, beta-interacting protein 1 |
| WBP | ENST0000061167.2 | WW domain binding protein 11 |
| TRIM2 | ENST00000348700.5 | Tripartite motif containing 2 |
| ZFAND5 | ENST0000027937.3 | Zinc finger, AN1-type domain 5 |
| ANX7 | ENST00000372921.5 | Annexin A7 |
| CAMK2B | ENST00000457475.1 | Calcium/calmodulin-dependent protein kinase II beta |
| MTMR3 | ENST0000033027.3 | Myotubularin-related protein 3 |
| CTDSPL | ENST00000443503.2 | CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase-like |
| EPHA5 | ENST00000273854.3 | EPH receptor A5 |
| SVOP | ENST0000029931.4 | SV2-related protein homolog (rat) |
| ST6GALNAC6 | ENST00000373146.1 | ST6 (alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-N-acetylgalactosaminide alpha-2,6-sialyltransferase 6 |
| RYBP | ENST00000477973.2 | RING1 and YY1 binding protein |
| ELAVL2 | ENST00000380110.4 | ELAV like neuron-specific RNA-binding protein 2 |
| KIAA1429 | ENST00000347199.1 | KIAA1429 |
| NR2C2 | ENST00000425241.1 | Nuclear receptor subfamily 2, group C, member 2 |
| TMEM167B | ENST00000332872.2 | Transmembrane protein 167B |
| KLHDC10 | ENST00000335420.5 | Kelch domain containing 10 |
| GATA3 | ENST00000379328.3 | GATA-binding protein 3 |
| PRR3L | ENST00000300557.2 | Proline-rich 3-like |
| SH3D19 | ENST0000049598.4 | SH3 domain containing 19 |
| ITSN1 | ENST00000379960.5 | Intersectin 1 (SH3 domain containing 2) |
| CLASP2 | ENST00000359981.1 | Cytoplasmic linker-associated protein 2 |
| FXR2 | ENST00000250113.7 | Fragile X mental retardation, autosomal homolog 2 |
| ANKyr1 | ENST00000341657.4 | Ankyrin repeat and FYVE domain containing 1 |
| E2F3 | ENST00000346618.3 | E2F transcription factor 3 |
| SNX12 | ENST00000374274.3 | Sorting nexin 12 |
Table S1 (Continued)

| Target gene | Representative transcript | Gene name |
|-------------|---------------------------|-----------|
| MTF2        | ENST00000370298.4         | Metal response element binding transcription factor 2 |
| SERTAD4     | ENST00000367012.3         | SERTA domain containing 4 |
| TMEM168     | ENST00000312814.6         | Transmembrane protein 168 |
| SHANK3      | ENST00000414786.2         | SH3 and multiple ankyrin repeat domains 3 |
| ZNF280C     | ENST00000370978.4         | Zinc finger protein 280C |
| HOXA1       | ENST00000355633.5         | Homeobox A1 |
| PDE7A       | ENST00000401827.3         | Phosphodiesterase 7A |
| DPF2        | ENST00000528416.1         | D4, zinc and double PHD fingers family 2 |
| CDK6        | ENST00000265734.4         | Cyclin-dependent kinase 6 |
| CRK         | ENST00000398970.5         | v-crk avian sarcoma virus CT10 oncogene homolog |
| EB2         | ENST00000355458.1         | Early B-cell factor 2 |
| LPNHI       | ENST0000036736.6          | Latrophilin 1 |
| TBC1D22B    | ENST00000373491.3         | TBC1 domain family, member 22B |
| NFIX        | ENST00000361015.4         | Nuclear factor I/X (CCAAAT-binding transcription factor) |
| BLZF1       | ENST00000367080.3         | Basic leucine zipper nuclear factor 1 |
| CBX5        | ENST00000209875.4         | Chromobox homolog 5 |
| CCNK        | ENST00000389879.5         | Cyclin K |
| PDE12       | ENST00000341180.8         | Phosphodiesterase 12 |
| FAM76A      | ENST00000373954.6         | Family with sequence similarity 76, member A |
| BMP2K       | ENST0000035016.5          | BMP2 inducible kinase |
| GPCPD1      | ENST00000379019.4         | Glycerophosphocholine phosphodiesterase GDE1 homolog (S. cerevisiae) |
| MTF1        | ENST00000373036.4         | Metal-regulatory transcription factor 1 |
| MAP3K13     | ENST00000448876.1         | Mitogen-activated protein kinase kinase kinase 13 |
| ANK1        | ENST00000289734.7         | Ankyrin 1, erythrocytic |
| PTEN        | ENST00000371593.3         | Phosphatase and tensin homolog |
| MANEAL      | ENST00000397631.3         | Mannosidase, endo-alpha-like |
| LANCL1      | ENST00000443314.1         | LanC lantibiotic synthetase component C-like 1 (bacterial) |
| SLC6A5      | ENST00000352748.1         | Solute carrier family 6 (neurotransmitter transporter), member 5 |
| ARH2        | ENST00000356401.4         | Ariadne RBR E3 ubiquitin protein ligase 2 |
| FOSL2       | ENST00000379691.1         | FOS-like antigen 2 |
| NRS5A2      | ENST00000367362.3         | Nuclear receptor subfamily 5, group A, member 2 |
| TRIM66      | ENST00000295550.6         | Tripartite motif containing 66 |
| GPR61       | ENST0000027748.1          | G protein-coupled receptor 61 |
| KLC2        | ENST00000390494.2         | Kinesin light chain 2 |
| MAPKBP1     | ENST00000457542.2         | Mitogen-activated protein kinase binding protein 1 |
| BAZZB       | ENST00000392782.1         | Bromodomain adjacent to zinc finger domain, 2B |
| FBXO30      | ENST00000237281.4         | F-box protein 30 |
| SLC38A2     | ENST00000356689.5         | Solute carrier family 38, member 2 |
| NUP50       | ENST00000347635.4         | Nucleoporin 50 kDa |
| PEA15       | ENST00000360472.4         | Phosphoprotein enriched in astrocytes 15 |
| TSPAN9      | ENST00000537971.1         | Tetraspanin 9 |
| CREB1       | ENST00000432329.2         | cAMP responsive element binding protein 1 |
| GCLM        | ENST00000370238.3         | Glutamate-cysteine ligase, modifier subunit |
| AAK1        | ENST00000409085.4         | AP2 associated kinase 1 |
| ARRD3C      | ENST00000265138.3         | Arrestin domain containing 3 |
| SRSF1       | ENST00000373071.3         | Serine/arginine-rich splicing factor 1 |
| CNOT4       | ENST00000541284.1         | CCR4-Not transactivation complex, subunit 4 |
| MTSSl       | ENST00000338779.6         | Metastasis suppressor 1-like |
| PDE4A       | ENST00000380702.2         | Phosphodiesterase 4A, cAMP-specific |
| PEX5L       | ENST00000467460.1         | Peroxisomal biogenesis factor 5-like |
| IFFO2       | ENST00000455833.2         | Intermediate filament family orphan 2 |
| KIA1A1462   | ENST00000375377.1         | KIAA1462 |
| NFE2L1      | ENST00000585291.1         | Nuclear factor, erythroid 2-like 1 |
| MYT1L       | ENST00000399161.2         | Myelin transcription factor 1-like |
| MIEF1       | ENST00000325301.2         | Mitochondrial elongation factor 1 |
| NCOA6       | ENST00000374796.2         | Nuclear receptor coactivator 6 |
| RNF180      | ENST00000389100.4         | Ring finger protein 180 |

(Continued)
| Target gene | Representative transcript | Gene name |
|-------------|---------------------------|-----------|
| FRS2        | ENST00000550389.1         | Fibroblast growth factor receptor substrate 2 |
| RASAL2      | ENST00000448150.3         | RAS protein activator like 2 |
| TENM2       | ENST00000519204.1         | Teneurin transmembrane protein 2 |
| ZNF608      | ENST00000504926.1         | Zinc finger protein 608 |
| FZD2        | ENST00000315323.3         | Frizzled family receptor 2 |
| ARHGEF12    | ENST00000397843.2         | Rho guanine nucleotide exchange factor (GEF) 12 |
| MYCBP       | ENST00000397572.2         | MYC binding protein |
| BACH2       | ENST00000257749.4         | BTB and CNC homology 1, basic leucine zipper transcription factor 2 |
| MLLT6       | ENST00000325718.7         | Myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 6 |
| TBL1X       | ENST00000407597.2         | Transducin (beta)-like 1X-linked |
| ATF2        | ENST00000487334.2         | Activating transcription factor 2 |
| GINS2       | ENST00000253462.3         | GINS complex subunit 2 (Psf2 homolog) |
| FLT1        | ENST00000282397.4         | fms-related tyrosine kinase 1 |
| CEP85L      | ENST00000368491.3         | Centrosomal protein 85 kDa-like |
| BEND3       | ENST00000369042.1         | BEN domain containing 3 |
| SPAG9       | ENST00000262013.7         | Sperm-associated antigen 9 |
| KCTD17      | ENST00000402077.3         | Potassium channel tetramerization domain containing 17 |
| USF2        | ENST00000594064.1         | Upstream transcription factor 2, c-fos interacting |
| LGALS1      | ENST00000409537.2         | Lectin, galactoside-binding-like |
| TPP2        | ENST00000376052.3         | Tripeptidyl peptidase II |
| DLGAP2      | ENST00000421627.2         | Discs, large (Drosophila) homolog-associated protein 2 |
| TMEM170B    | ENST00000379426.1         | Transmembrane protein 170B |
| ZBTB43      | ENST00000449886.5         | Zinc finger and BTB domain containing 43 |
| L3MBTL3     | ENST00000529410.1         | l(3)mbt-like 3 (Drosophila) |
| KIAA1549    | ENST00000440172.1         | KIAA1549 |
| TNRC6B      | ENST00000335727.9         | Trinucleotide repeat containing 6B |
| SAMD14      | ENST00000430175.4         | Sterile alpha motif domain containing 14 |
| INO80D      | ENST00000403263.1         | INO80 complex subunit D |
| CALC R      | ENST00000359558.2         | Calcitonin receptor |
| TACOLN2     | ENST00000377386.3         | Trans-golgi network protein 2 |
| TET2        | ENST00000545826.1         | Set methylcytosine dioxygenase 2 |
| SUFU        | ENST00000369902.3         | Suppressor of fused homolog (Drosophila) |
| PADS3       | ENST00000540820.1         | Fatty acid desaturase 3 |
| LEPRE1      | ENST00000236040.4         | Leucine proline-enriched proteoglycan (leprecan) 1 |
| CAMKK2G     | ENST00000351293.3         | Calcium/calmodulin-dependent protein kinase II gamma |
| NFAT5       | ENST00000354436.2         | Nuclear factor of activated T-cells 5, tonicity-responsive |
| MED1        | ENST00000300651.6         | Mediator complex subunit 1 |
| CNOT6       | ENST000004393356.1        | CCR4-NOT transcription complex, subunit 6 |
| RP11-766F14.2 | ENST00000511828.1       | Protein LOC285556 |
| STAR D13    | ENST00000336934.5         | Star-related lipid transfer (START) domain containing 13 |
| LCOR        | ENST00000371103.3         | Ligand-dependent nuclear receptor corepressor |
| HDAC4       | ENST00000345617.3         | Histone deacetylase 4 |
| CCCD71L     | ENST00000523505.1         | Coiled-coil domain containing 71-like |
| ST8SIA1     | ENST00000396037.4         | ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 1 |
| MBDS4       | ENST00000407073.1         | Methyl-CpG binding domain protein 5 |
| RBMS3       | ENST00000396583.3         | RNA binding motif, single stranded interacting protein 3 |
| THRA        | ENST00000450525.2         | Thyroid hormone receptor, alpha |
| BCL2L11      | ENST00000393256.3         | BCL2-like 11 (apoptosis facilitator) |
| FBXO28      | ENST00000424254.2         | F-box protein 28 |
| NT5DC1      | ENST00000319500.4         | 5'-nucleotidase domain containing 1 |
| POLR3H      | ENST00000396504.4         | Polymerase (RNA) III (DNA directed) polypeptide H (22.9 kD) |
| ANK3        | ENST00000280772.2         | Ankyrin 3, node of Ranvier (ankyrin G) |
| BICD2       | ENST00000356884.6         | Bicaudal D homolog 2 (Drosophila) |
| PAPOLA      | ENST00000557471.1         | Poly(A) polymerase alpha |
| ZXDC        | ENST00000389709.3         | ZXD family zinc finger C |
| DLGS        | ENST00000372391.2         | Discs, large homolog 5 (Drosophila) |
Table S1 (Continued)

| Target gene | Representative transcript | Gene name |
|-------------|----------------------------|-----------|
| CCDC88A     | ENST00000336838.6          | Coiled-coil domain containing 88A |
| LYZ1        | ENST00000256178.3          | Lymphatic vessel endothelial hyaluronan receptor 1 |
| PURB        | ENST00000395699.2          | Purine-rich element binding protein B |
| GJA9        | ENST00000454994.2          | Gap junction protein, alpha 9, 59 kDa |
| KCNH5       | ENST00000322893.7          | Potassium voltage-gated channel, subfamily H (eag-related), member 5 |
| WNK3        | ENST00000375169.3          | WNK lysine deficient protein kinase 3 |
| STRN        | ENST00000263918.4          | Striatin, calmodulin binding protein |
| UNC5B       | ENST00000335350.6          | unc-5 homolog B (C. elegans) |
| FKB15       | ENST00000238256.3          | FK506 binding protein 15, 133 kDa |
| SHISA7      | ENST00000376325.4          | Shisa family member 7 |
| AGO3        | ENST00000373191.4          | Argonaute RISC catalytic component 3 |
| CELF6       | ENST00000287202.5          | CLGGBP, Elav-like family member 6 |
| MAP3K2      | ENST00000409941.7          | Mitogen-activated protein kinase kinase kinase 2 |
| TIAM1       | ENST00000286827.3          | T-cell lymphoma invasion and metastasis 1 |
| SCN3A       | ENST00000360093.3          | Sodium channel, voltage-gated, type III, alpha subunit |
| ZDHHC18     | ENST00000374142.4          | Zinc finger, DHHC-type containing 18 |
| ONECUT2     | ENST00000491143.2          | One cut homeobox 2 |
| SPTY2D1     | ENST00000336349.5          | SPT2, Suppressor of T domain containing 1 (S. cerevisiae) |
| CHD6        | ENST00000373233.3          | Chromodomain helicase DNA binding protein 6 |
| AKA2        | ENST00000374525.1          | A kinase (PKA) anchor protein 2 |
| BTRC        | ENST00000370187.3          | Beta-transducin repeat containing E3 ubiquitin protein ligase |
| SMURF1      | ENST00000363168.2          | SMAD-specific E3 ubiquitin protein ligase 1 |
| EPHA4       | ENST00000281821.2          | EPH receptor A4 |
| WDR26       | ENST00000414432.3          | WD repeat domain 26 |
| GATA2A       | ENST00000360315.3          | GATA zinc finger domain containing 2A |
| RIM52       | ENST00000507740.1          | Regulating synaptic membrane exocytosis 2 |
| PURG        | ENST00000475541.1          | Purine-rich element binding protein G |
| PALM2-AKAP2 | ENST00000374530.3          | PALM2-AKAP2 read through |
| NAFSC       | ENST00000401399.1          | Neurofascin |
| ELAVL3      | ENST00000359227.3          | ELAV like neuron-specific RNA binding protein 3 |
| LHPL4       | ENST00000287585.6          | Lipoma HMGIC fusion partner-4 |
| ARNT        | ENST00000358595.5          | Aryl hydrocarbon receptor nuclear translocator |
| STK35       | ENST00000381482.3          | Serine/threonine kinase 35 |
| CEPS30       | ENST00000367607.3          | Centrosomal protein 350 kDa |
| ZBTB16      | ENST00000335953.4          | Zinc finger and BTB domain containing 16 |
| NUFP2       | ENST00000225388.4          | Nuclear fragile X mental retardation protein interacting protein 2 |
| CLCN5       | ENST00000376088.3          | Chloride channel, voltage-sensitive 5 |
| C3orf14     | ENST00000494481.1          | Chromosome 3 open reading frame 14 |
| TFA2A       | ENST00000379613.3          | Transcription factor AP-2 alpha (activating enhancer binding protein 2 alpha) |
| CSMD1       | ENST00000400186.3          | CUB and Sushi multiple domains 1 |
| MAPRE1      | ENST00000375571.5          | Microtubule-associated protein, RP/Eβ family, member 1 |
| UBXN7       | ENST00000296328.4          | UBX domain protein 7 |
| WAPAL       | ENST00000298767.5          | Wings apart-like homolog (Drosophila) |
| SLC9A7      | ENST00000328306.4          | Solute carrier family 9, subfamily A (NHE7, cation proton antiporter 7), member 7 |
| ZDHHC21     | ENST00000380916.4          | Zinc finger, DHHC-type containing 21 |
| RBCC1       | ENST00000025008.5          | RBI-inducible coiled-coil 1 |
| DVL3        | ENST00000313143.3          | Dishevelled segment polarity protein 3 |
| SMAD2       | ENST00000262160.6          | SMAD family member 2 |
| MCMBP       | ENST00000360003.3          | Minichromosome maintenance complex binding protein |
| OTUD7A      | ENST00000307050.4          | OTU domain containing 7A |
| AFF4        | ENST00000263343.5          | AF4/FMR2 family, member 4 |
| KCNC3       | ENST00000376959.2          | Potassium voltage-gated channel, Shaw-related subfamily, member 3 |
| SLC25A1      | ENST00000215882.5          | Solute carrier family 25 (mitochondrial carrier; citrate transporter), member 1 |
| C1orf28     | ENST00000325192.3          | Chromosome 14 open reading frame 28 |
| SESN3       | ENST00000536441.1          | Sessin 3 |
| SCARB2      | ENST00000264896.2          | Scavenger receptor class B, member 2 |
| ZNF202      | ENST00000336139.4          | Zinc finger protein 202 |

(Continued)
Table S1 (Continued)

| Target gene | Representative transcript | Gene name |
|-------------|---------------------------|-----------|
| SLC35G1     | ENST00000371408.3          | Solute carrier family 35, member G1 |
| IRS1        | ENST00000305123.5          | Insulin receptor substrate 1 |
| AHSA2       | ENST00000394457.3          | AHA1, activator of heat shock 90 kDa protein ATPase homolog 2 (yeast) |
| CADM1       | ENST00000452722.3          | Cell adhesion molecule 1 |
| HTT         | ENST00000355072.5          | Huntingtin |
|CNTNAP5     | ENST00000431078.1          | Contactin associated protein-like 5 |
|ZNF827      | ENST00000379448.4          | Zinc finger protein 827 |
|CDH19       | ENST00000540086.1          | Cadherin 19, type 2 |