Drug Design, Development and Therapy

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Hsa-microRNA-181a is a regulator of a number of cancer genes and a biomarker for endometrial carcinoma in patients: a bioinformatic and clinical study and the therapeutic implication

This article was published in the following Dove Press journal:
Drug Design, Development and Therapy
18 February 2015
Number of times this article has been viewed

Shuming He[1]
Shumei Zeng[1]
Zhi-Wei Zhou[2,3]
Zhi-Xu He[3]
Shu-Feng Zhou[2,3]

[1]Department of Obstetrics and Gynecology, Xiaolan People's Hospital affiliated to Southern Medical University, Zhongshan, Guangdong, People's Republic of China; [2]Department of Pharmaceutical Sciences, College of Pharmacy, University of South Florida, Tampa, FL, USA; [3]Guizhou Provincial Key Laboratory for Regenerative Medicine, Stem Cell and Tissue Engineering Research Center and Sino-US Joint Laboratory for Medical Sciences, Guiyang Medical University, Guiyang, Guizhou, People's Republic of China

Correspondence: Shuming He
Department of Obstetrics and Gynecology, Xiaolan People's Hospital affiliated to Southern Medical University, Xiaolan, Zhongshan 528415, Guangdong, People's Republic of China
Tel +86 760 8862 6236
Fax +86 760 2283 6688
Email 13703036288@139.com

Shu-Feng Zhou
Department of Pharmaceutical Sciences, College of Pharmacy, University of South Florida, 12901 Bruce B. Downs Blvd., MDC 30, Tampa, FL 33612, USA
Tel +1 813 974 6276
Fax +1 813 905 9885
Email szhou@health.usf.edu

Abstract: The aberrant expression of human microRNA-181a-1 (hsa-miR-181a) has been implicated in the pathogenesis of various cancers, serving as an oncogene or a tumor suppressor. However, the role of hsa-miR-181a in the pathogenesis of endometrial carcinoma (EC) and its clinical significance are unclear. This study aimed to search for the molecular targets of hsa-miR-181a using bioinformatic tools and then determine the expression levels of hsa-miR-181a in normal, hyperplasia, and EC samples from humans. To predict the targets of hsa-miR-181a, ten different algorithms were used, including miRanda-mirSVR, DIANA microT v5.0, miRDB, RNA22 v2, TargetMiner, TargetScan 6.2, PicTar, MicroCosm Targets v5, and miRWALK. Two algorithms, TarBase 6.0 and miRTarBase, were used to identify the validated targets of hsa-miR-181a-5p (a mature product of hsa-miR-181a), and the web-based Database for Annotation, Visualization and Integrated Discovery (DAVID) 6.7 was used to provide biological functional interpretation of the validated targets of hsa-miR-181a-5p. A total of 78 formalin-fixed, paraffin-embedded tissue specimens from 65 patients and 13 healthy subjects were collected and examined, including normal endometrium (n=13), endometrial hyperplasia (n=18), and EC (37 type I and 10 type II EC cases). Our bioinformatic studies have showed that hsa-miR-181a might regulate a large number of target genes that are important in the regulation of critical cell processes, such as cell fate, cell survival, metabolism, and cell death. To date, 313 targets of hsa-miR-181a have been validated, and 22 of these targets are cancer genes. The precision of predictions by all the algorithms for hsa-miR-181a-1's targets was low. Many of these genes are involved in tumorigenesis of various cancers, including EC, based on the DAVID and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. In comparison with normal endometrial tissue, the expression level of hsa-miR-181a was significantly increased in type I and type II EC ($P<0.05$), and type II EC exhibited a significant higher expression level of hsa-miR-181a than that in type I EC ($P<0.05$). In addition, there was a significant increase in the expression level of hsa-miR-181a in type II EC compared with endometrial hyperplasia ($P<0.05$). Taken together, these results suggest that hsa-miR-181a may serve as an oncogene in endometrial tumorigenesis and that hsa-miR-181a might be used as a new biomarker in the prediction of prognosis of EC in clinical practice. More functional and mechanistic studies are needed to validate the role of hsa-miR-181a in the development, progression, and metastasis of EC.

Keywords: RT-PCR, endometrial cancer, development, progression

Introduction

microRNAs (miRNAs) are a large group of noncoding small RNAs with 20–25 nucleotides that have a capability of regulating gene expression at posttranscriptional levels by antisense complementarily to specific target messenger (m)RNAs.[1,2] Based

on miRBase version 21 released in June 2014 (http://www.mirbase.org/), there are 1,881 miRNA precursors and 2,588 mature miRNAs in humans. miRNAs are transcribed as ~70 nucleotide stem-loop precursors and subsequently processed by the cytoplasmic RNase III-type enzyme Dicer to generate ~22 nucleotide mature products that can target and modulate protein expression by inhibiting translation and/or inducing degradation of target mRNAs. The mature miRNA is incorporated into a RNA-induced silencing complex (RISC), which recognizes target mRNAs through imperfect base pairing with the miRNA. miRNAs act as adaptors that employ a silencing complex to target mRNAs by selective base-pairing, primarily in the 3′-untranslated region (3′-UTR). Target interaction does not require perfect complementarity between microRNA and mRNA sequences, although near-perfect base-pairing in a small region in the 5′-end (positions 2–8) of the microRNA (sometimes termed "seed") appears to be one of the key determinants of target recognition. miRNAs regulate almost every signaling pathway and play crucial roles in diverse biological processes, such as development, differentiation, apoptosis, and proliferation.[1–3] It has been shown that aberrant expression of miRNAs is involved in the development and progression of many types of cancer through regulation of functional proteins and the network of signaling pathways related to cell proliferation, cell migration and invasion, programmed cell death, and cell survival.[3–7] It has been proposed that miRNAs can function as tumor suppressors or oncogenes, targeting other oncogenes and/or tumor-suppressors to modulate cancer development, progression, and metastasis.[1,5–8]

An extremely large number of potential target sites exist for any given miRNA, and the process of validating a potential miRNA target in the laboratory is time consuming and costly. A computational approach to prediction of miRNA targets facilitates the process of narrowing down potential target sites for experimental validation, which is a critical initial step in identifying miRNA–target interactions. Several useful algorithms/tools provide microRNA target predictions based on sequence complementarity to target sites, with emphasis on perfect or near-perfect base-pairing in the seed region and sequence conservation.[9,10] These tools for miRNA target prediction, encompassing a range of different computational approaches, from the modeling of physical interactions to the incorporation of machine learning, are mostly based on seed match, conservation, free energy, and site accessibility.[10]

Endometrial cancer (EC) is the sixth most common cancer in women worldwide, with at least 320,000 new cases being diagnosed and 74,000 women who die from this disease every year.[11] In the United States, there was an estimation of 52,630 new cases and 8,590 deaths due to EC in 2014.[12] In the United Kingdom, there were 8,474 women diagnosed with EC and 1,914 deaths from EC in 2011.[11] In the People's Republic of China, the incidence of EC is much lower than Western countries. It contributes about 1% of the world's new EC cases.[11]

There are two types of EC, type I and type II, with different molecular expression profiles and histopathological and clinical behaviors.[13,14] Type I EC, accounting for 75% of EC, is estrogen-dependent with endometrioid morphology, and the 5-year survival rate is 80%−85%.[15] Type II EC exhibits poorly differentiated endometrioid and serous histological alterations with myometrial invasion and extrauterine spread, and the 5-year survival rate is about 35%. Type I EC is related to hyperestrogenism, associated with endometrial hyperplasia, frequent expression of estrogen and progesterone receptors (ER and PR), and younger age, whereas type II EC is unrelated to estrogen and is associated with atrophic endometrium, frequent lack of ER and PR, and older age. The morphologic differences in type I and type II EC are mirrored in their molecular genetic profile, with type I showing defects in DNA-mismatch repair and mutations in phosphatase and tensin homolog (PTEN), phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit α (PIK3CA), V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS), and β-catenin/CTNNB1, and type II showing chromosomal instability and aneuploidy, p53 mutations, and human epidermal growth factor receptor 2 (HER2)/neu (erbB-2) amplification.[16,17] Type I EC is associated with chronic exposure to unopposed estrogen and is often preceded by complex atypical hyperplasia. Current therapies for EC include surgery, chemotherapy, radiation therapy, immunotherapy, and biological therapy.[18] Most cases of EC are diagnosed at an early stage, which has a 5-year survival rate of over 91%.[11] However, the prognosis of EC does not meet the long-term survival expectation due to tumor metastasis, lack of effective treatment, and rarity of valid biomarkers to precisely predict therapeutic outcome.[19] There are lines of evidence that a number of genetic and epigenetic factors have been implicated in the pathogenesis of EC, including abnormality in oncogenes, tumor suppressors, and miRNAs and related signaling pathways.[13] Alterations in the expression profiles of oncogenes and tumor suppressor genes are the major contributing factors to the initiation, development, progression, and metastasis of EC. However, the association between such alterations and the clinical phenotypes of EC has not been conclusively established yet, and the underlying mechanism for EC etiology remains elusive.

There is an increased interest in the discovery and identification of miRNAs as novel epigenetic biomarkers

for early detection and prediction of therapeutic outcomes in cancer therapy.[20–24] So far, there are a number of human miRNAs that have been identified to have a potential role in the development and progression of EC, including hsa-miR-181a, hsa-miR-185, hsa-miR-106a, hsa-miR-210, hsa-miR-423, hsa-miR-107, hsa-miR-let7c, and hsa-miR-221.[3,4] hsa-miR-181a is one of the many miRNAs conserved among the vertebrates that is preferentially expressed in B lymphocytes of bone marrow, and its ectopic expression in hematopoietic stem/progenitor cells modulates blood cell development.[25] hsa-miR-181a has been reported to be a key modulatory factor in the regulation of cell proliferation and differentiation at transcriptional and posttranscriptional levels in gastric cancer, acute myeloid leukemia, and other types of cancer,[26,27] and hsa-miR-181a overexpression resulted in promotion of cell proliferation and migration but inhibition of apoptosis in colorectal cancer.[28] RalA, one of the Ral family small G proteins, is directly regulated by hsa-miR-181a and plays an important role in the development chronic myelogenous leukemia.[29] hsa-miR-181a and hsa-miR-181b act as tumor suppressors by inducing cell growth inhibition, apoptosis, and repression of invasion in glioma cells.[30] So far, there have been only scattered reports about the role of a form of miR-181a in EC.[4] Panda et al[31] showed that the expression level of miR-181a-1 was higher in EC than in normal endometrial tissues. However, there is limited information on the association between the expression profile of miR-181a and the development and progression of EC. In this regard, we conducted a comprehensive bioinformatic study to predict the targets of miR-181a-1 and then validate these targets based on published experimental evidence. Finally, we examined the expression levels of miR-181a-1 in different types of EC and the association with the clinical progression of EC.

Material and methods

Gene nomenclature

The miR-181 family includes four members, namely miR-181a, miR-181b, miR-181c, and miR-181d. They are expressed in at least 70 species and various human cancers and are highly conserved in the seed-region sequence and RNA secondary structure. hsa-miR-181a-1 was retrieved from miRBase 21 (http://www.mirbase.org/). The *hsa-miR-181a-1* gene (*MIR181A1*) has been mapped to 1q32.1. miRBase was established in 2002 as the public and central online repository for all published miRNA sequences and associated annotations, and the latest miRBase release of v21 (released in June 2014) contains 28,645 miRNA loci from 223 species (of which 1,881 precursors and 2,588 mature miRNAs are for humans), processed to produce 35,828 mature miRNAs. As compared with miRBase v20, a total of 4,196 new hairpin sequences and 5,441 novel mature products, mainly for bat, horse, goat, cobra, and salmon, have been added, with 72 dubious and misannotated entries removed from version 21. miRBase provides a user-friendly web interface for miRNA data, allowing the user to search using key words or sequences, trace links to the primary literature referencing the miRNA discoveries, analyze genomic coordinates and context, and mine relationships between miRNA sequences.[32–34] Clusters of miRNA sequences in the genome are highlighted and can be defined and retrieved with any inter-miRNA distance. The mature forms of hsa-miR-181a include hsa-miR-181a-3p and hsa-miR-181a-5p (Table 1).

Prediction of the targets of hsa-miR-181a using various computational algorithms

Before starting the bench and clinical work, we conducted a bioinformatic study to predict the target genes regulated by hsa-miR-181a-3p and hsa-miR-181a-5p, using ten different algorithms, including miRanda-mirSVR (http://www.microrna.org/), DIANA microT v5.0 (http://diana.cslab.ece.ntua.gr/microT/), miRDB (http://mirdb.org/miRDB/), RNA22 v2 (https://cm.jefferson.edu/rna22v2.0/), TargetMiner (http://www.isical.ac.in/~bioinfo_miu/targetminer20.htm), TargetScan 6.2 (http://www.targetscan.org/), PicTar (http://pictar.mdc-berlin.de/), MicroCosm Targets v5 (http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets/v5/), and miRWALK (http://www.umm.uni-heidelberg.de/apps/zmf/mirwalk/index.html).

miRanda-mirSVR (http://www.microrna.org/) is an online tool that can be used to predict targets and score them.[35] Unlike most miRNA target predictors, miRanda considers matching along the entire miRNA sequence, and it takes the seed region into account by weighting matches in the seed region more heavily; free energy is calculated by predicting the folding of the miRNA:mRNA hybrid, using the Vienna package. DIANA microT v5.0 currently hosts miRNA target predictions for *Homo sapiens*, *Mus musculus*, *Drosophila melanogaster*, and *Caenorhabditis elegans*, based on data from Ensembl release 69 and miRBase version 18.[36,37] miRDB, a web-based database and tool, can predict miRNAs and their targets.[38] All the targets are predicted by the bioinformatic tool MirTarget2, which has been developed by analyzing thousands of genes impacted by miRNAs, using a support vector machine (SVM) learning machine. RNA22 v2 can be used to predict the targets of miRNAs in

Table 1 Basic information on hsa-miR-181a-1 gene (*MIR181A1*) retrieved from miRBase 21

Gene	Accession number	Previous IDs	Sequence	Number of nucleotides	Predicted targets	Validated targets
hsa-miR-181a-1	MI0000289	hsa-miR-213	UGAGUUUUGAGGUUGCUUCAGUGAACAUUCAA CGCUGUCGGUGAGUUUGGAAUUAAAAUCAAA ACCAUCGACCGUUGAUUGUACCCUAUGGCUAA CCAUCAUC UACUCCA	109	–	–
hsa-miR-181a-5p	MIMAT0000256	hsa-miR-181a	24-AACAUUCAACGCUGUCGGUGAGU-46	23	DIANA microT v3.0, miRanda-mirSVR (microRNA.org), miRDB, RNA22 v2, TargetMiner, TargetScan6.2, PicTar, MicroCosm, and miRWALK	miRTarBase and TarBase
hsa-miR-181a-3p	MIMAT0000270	hsa-miR-213 and hsa-miR-181a*	64-ACCAUCGACCGUUGAUUGUACC-85	22	DIANA microT v3.0, miRanda-mirSVR (microRNA.org), miRDB, RNA22 v2, TargetMiner, MicroCosm, and miRWALK	–

human, mouse, roundworm, and fruit fly.[39] It allows users to visualize the predictions within a complementary (c)DNA map and also find transcripts where multiple miRNAs of interest target.

TargetMiner is a robust tool for microRNA target prediction with systematic identification of negative examples.[40] In this algorithm, ~300 tissue-specific negative examples have been identified, using a novel approach that involves expression profiling of miRNAs and mRNAs, miRNA–mRNA structural interactions, and seed-site conservation. TargetScan 6.2 can predict biological targets of miRNAs by searching for the presence of conserved 8-mer and 7-mer sites matching the seed region of each miRNA,[41] with non-conserved sites being predicted as well. TargetScan is the first computational method used for human miRNA target prediction, that uses mouse, rat, and fish genomes for conservation analysis. In mammals, the prediction is ranked based on the predicted efficacy of targeting as calculated using the context+ scores of the sites.[42] The context score for a specific site is the sum of the contribution of four features: site-type contribution, 3′-pairing contribution, local nucleobases adenine and uracil contribution, and position contribution. In the current work, the sum of the context scores for each miRNA was calculated, and the most favorable (lowest) was shown. PicTar is an algorithm for the identification of miRNA targets.[43] In addition, MicroCosm Targets v5 was used to predict the targets that might be regulated by hsa-miR-181a. In this tool, there are 851 miRNAs (711 native) with 34,788 targets for humans.

miRWALK is a comprehensive database that provides information on miRNAs from the human, mouse, and rat, on their predicted as well as validated binding sites on their target genes (http://www.umm.uni-heidelberg.de/apps/zmf/mirwalk/index.html).[44] miRWALK predicts miRNA binding sites on the complete sequence of all known genes, including all transcripts and mitochondrial genes of the human, mouse, and rat, based on a comparison of identified miRNA binding sites with ten established miRNA-target prediction programs: miRWALK, DIANA-microT v3.0, miRanda, miRDB, PicTar 4 and PicTar 5, PITA (http://genie.weizmann.ac.il/pubs/mir07/mir07_prediction.html), RNA22, RNAhybrid (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/submission.html), and TargetScan. In addition, it provides predicted miRNA binding sites on genes associated with 449 human biological pathways and 2,356 Online Mendelian Inheritance in Man (OMIM) disorders.[44] This algorithm also presents information on experimentally validated miRNA interaction information associated with

genes, pathways, diseases, organs, OMIM disorders, cell lines, and literature on miRNAs.

Validated targets of hsa-miR-181a based on TarBase 6.0 and miRTarBase 4.0

Two algorithms were used to identify the validated targets of miR-181a-5p: TarBase 6.0 and miRTarBase 4.0. TarBase 6.0 is a database that houses a manually curated collection of experimentally supported miRNA targets in 21 species, including human, rat, mouse, virus, *Caenorhabditis elegans*, *Danio rerio* (zebrafish), *Drosophila*, and plant (http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=tarbase/index).[45] TarBase 6.0 is the largest available manually curated target database, indexing more than 65,000 miRNA-gene interactions, 16.5- to 175-fold more than any other available implementation. The database includes targets derived from specific as well as high-throughput experiments, such as microarrays and proteomics. Specific attention was paid in the inclusion of targets derived from sequencing experiments, such as high-throughput sequencing of RNA isolated by crosslinking immunoprecipitation (HITS-CLIP) and photoactivatable-ribonucleoside-enhanced crosslinking and immunoprecipitation (PAR-CLIP). TarBase 6.0 hosts data derived from three CLIP-Seq and 12 Degradome-Seq studies, significantly more than any other available database. DIANA TarBase 6.0 offers a significant amount of crucial information to the user, including detailed description of the involved genes and miRNAs, a list of publications supporting each interaction, and the experimental methods used for validations, along with their outcomes. The database also provides links to related Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, as well as to other external databases, such as Ensembl, Uniprot, and RefSeq. It is also equipped with powerful searching and filtering capabilities.

miRTarBase 4.0 has collected 51,460 miRNA–target interactions from 18 species having experimental evidence (http://mirtarbase.mbc.nctu.edu.tw/).[46] Generally, the collected miRNA–target interactions are validated experimentally by reporter assay, Western blotting, microarray, and next-generation sequencing experiments. There are 4,572 miRNA–target interactions validated by reporter assays or Western blotting experiments.

Cancer genes are defined as "mutated genes that are causally implicated in oncogenesis" based on the criteria by Futreal et al[47] (Table S1 gives the full list of cancer genes). The proteins that are encoded by cancer genes normally regulate cell proliferation, cell differentiation, and cell death. Mutations underlying oncogenesis also occur in genes that mediate DNA-repair processes. Currently, more than 1% of all human genes have been implicated, via mutation, in cancer. Of these, approximately 90% have been implicated in somatic mutations in cancer, 20% bear germline mutations that predispose to cancer, and 10% show both somatic and germline mutations.[48]

Pathway analysis by the Database for Annotation, Visualization and Integrated Discovery (DAVID)

The web-based DAVID 6.7 (http://david.abcc.ncifcrf.gov/)[49,50] was used to provide biological functional interpretation of the validated targets of hsa-miR-181a-5p, based on TarBase 6.0 and miRTarBase 4.0. DAVID 6.7 systematically maps a large number of interesting genes in a list to associated Gene Ontology (GO) terms, and then statistically highlights the most overrepresented (enriched) GO terms out of a list of hundreds or thousands of terms.[47,48] The DAVID Knowledgebase has comprehensively integrated more than 20 types of major gene/protein identifiers and more than 40 well-known functional annotation categories from dozens of public databases, to address the enriched and redundant relationships among many genes to many terms. The protein IDs of the validated targets of hsa-miR-181a-5p from the National Center for Biotechnology Information (NCBI), Protein Information Resource (PIR), and UniProtKB were converted into gene lists, using the Gene ID Conversion Tool in DAVID. By doing so, interesting genes derived from one identifier system can be quickly translated to other gene identifier types preferred by a given annotation resource. The DAVID database adds biological function annotation including GO terms, protein–protein interactions, protein functional domains, disease associations, gene clustering, biopathways, sequence general features, homologies, gene functional summaries, and gene tissue expressions in a network context.[47,48] The genes of interest were visualized using BioCarta and KEGG pathway maps. The highest classification stringency was selected for functional annotation clustering. Enrichment scores and Fisher's exact test *P*-values (and corresponding false discovery rate [FDR]) were then calculated to identify which functionally related gene groups are significantly enriched in the target list.

Reagents and antibodies

The RNA inhibitor and Moloney murine leukemia virus reverse transcriptase were obtained from Promega Inc. (Madison, WI, USA). The antigen retrieval solution (ethylenediaminetetraacetic acid [EDTA] method) and Dolichos

biflorus agglutinin kit were purchased from Maixin Biological Co. Ltd., Fuzhou, Fujian, People's Republic of China. Human monoclonal primary antibodies against (ER, PR), and horseradish peroxidase-conjugated secondary antibodies were bought from Linked-Biotech Pathology Co. Ltd. (Guangzhou, Guangdong, People's Republic of China). MiREasy FFPE Kit and Syber® Green PCR mix were purchased from QIAGEN Inc. (Venlo, the Netherlands).

Sample collection

Fresh tissue samples were obtained from 65 patients who received uterusectomy and 13 healthy subjects at Xiaolan People's Hospital or Zhongshan People's Hospital, Zhongshan, Guangdong, or at Nanfang Hospital of Southern Medical University, Guangzhou, Guangdong, People's Republic of China, with a mean age of 48 years (range 24–69). We collected the endometrial samples from 47 patients with EC, 18 patients with endometrial hyperplasia, and 13 healthy subjects. There was no preoperative radiotherapy, chemotherapy, or endocrine therapy performed in any of the recruited participants. All samples were fixed with formalin and embedded with paraffin. International Federation of Gynecology and Obstetrics (FIGO) staging was performed according to the FIGO classification.[15,51] Histological classification of tissue samples was performed according to the World Health Organization (WHO) criteria (www.iarc.fr/en/.../BB2.pdf), and samples were classified as G1 (well differentiated), G2 (moderately differentiated), or G3 (poorly differentiated). The study design was approved by the Ethics Committees of Xiaolan People's Hospital, Zhongshan People's Hospital, and Nanfang Hospital. Written informed consent was obtained from each participant.

Immunohistochemistry

Dewaxed and dehydrated sections were first washed with phosphate-buffered saline (PBS) and then incubated with 3% peroxyl in methanol for 15 minutes to terminate the activity of endogenous peroxidases. The sections were washed with PBS, and antigen retrieval was performed. The sections were immersed into boiled citrate-buffered solution for 10 minutes and blocked with 5% bovine serum albumin in PBS for 20 minutes at room temperature. Thereafter, the sections were probed with primary antibody against ER or PR overnight in a humidified chamber at 4°C. On the following day, sections were incubated with biotinylated anti-Rabbit antibody (Boster Biotechnology Ltd., Wuhan, Hubei, People's Republic of China) for 30 minutes at room temperature and then coupled with diaminobenzidine to visualize the expression of the targeted proteins. After all sections were counterstained with hematoxylin, they were dehydrated in ascending ethanol and then mounted using neutral resins. Samples with both ER-positive and PR-positive were classified as type I EC, while samples with both ER-negative and PR-negative were classified as type II EC.[4,25]

Primer design

The sequences of target gene were retrieved from GenBank (http://www.ncbi.nlm.nih.gov/genbank/) and miRBase (http://www.mirbase.org/). The primers were designed using Primer Designer 2.0, and the sequences are shown in Table 2. All primers were synthesized by Beijing Liu He Synthetic Genomics Ltd., Beijing, People's Republic of China. *U6* was used as the internal control.

Total RNA extraction

A series of sections of thickness 10 μm was obtained, and the paraffin was dissolved by xylene treatment. Ten slides were prepared for each sample. Sections with cell content more than 50% of the area were selected for total RNA extraction. Briefly, sections were washed twice with ethanol in a 1.5 mL centrifuge tube to remove residual xylene. Total RNA was extracted, and the purity and integrity of the total RNA were examined using a miRNeasy FFPE kit according to the manufacturer's instruction. The purity of the total RNA was tested using an ultraviolet (UV) spectrophotometer. The ratio of A260/A280 between 1.8 and 2.1 was considered as high purity. Electrophoresis was performed to detect the RNA integrity in 1.0% agarose denaturing gel.

Quantitative real-time polymerase chain reaction (qRT-PCR) analysis

RNA was reversely transcribed into cDNA, using a RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific Inc., Waltham, MA, USA) according to the manufacturer's instruction. The resultant cDNA was subject to qRT-PCR analysis using a Bio-Rad Real-time PCR System (Bio-Rad Laboratories

Table 2 Sequences of the primers for the determination of hsa-miR-181a and U6

Gene	Forward primer	Reverse primer
hsa-miR-181a	GTCGTATCCAGTGCGTGTCGTGGAGTCG	GCAATTGCACTGGATACGACACTCAC
U6	GTCGTATCCAGTGCGTGTCGTGGAGTCGG	CAATTGCACTGGATACGACAAAATATG

Inc., Hercules, CA, USA) and Syber green PCR mix. The conditions for RT-PCR were 95°C for 6 minutes and then 50 cycles of 95°C for 10 seconds, 55°C for 10 seconds, and 72°C for 30 seconds. The dissolution curve was analyzed to determine the specificity of the real-time PCR amplification. The relative expression level of *hsa-miR-181a* was calculated by the comparative cycle threshold method, with *U6* as the internal reference and expressed as the percentage change relative to untreated controls. Quantification of the relative expression levels of *hsa-miR-181a* was achieved by the following formula: $2^{-\Delta\Delta Ct}$, where ΔΔCt equals (Ct of *hsa-miR-181a*- Ct of *U6*)$_{experiment}$ minus (Ct of *hsa-miR-181a*- Ct of *U6*)$_{control}$. $2^{-\Delta\Delta Ct}$ was presented as the relative change of *hsa-miR-181a* expression.

Statistical analysis

Data are expressed as the mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison to determine statistical significance among multiple groups. Student's *t*-test was used to compare the difference between two groups when appropriate. $P<0.05$ was considered as statistically significant.

Results

Predicted targets of hsa-miR-181a-5p and hsa-miR-181a-3p using various algorithms

Predicted targets of hsa-miR-181a-5p

Using DIANA microT v3.0, hsa-miR-181a-5p was predicted to regulate 522 targets when the threshold was set to 0.7, including *ATP1B1*, *BHLHE40*, *CLASP1*, *CREBRF*, *FBXO33*, *GLS*, *KLHL5*, *LRBA*, *MAP1B*, *MTF2*, *NMT2*, *PAPD5*, *REPS2*, *RLF*, *SESN3*, *SLC2A3*, *SLITRK1*, *TMEM131*, *TRIM2*, *ZFP90*, *ZNF268*, *ZNF441*, *ZNF527*, *ZNF570*, *ZNF704*, *ZNF717*, *ZNF781*, *ZNF788*, *ZNF799*, *ZNF846*, etc (Table 3 and Table S2). Many of these targets regulate a number of important cellular processes, such as cell proliferation, cell death, cell division, mitosis, metabolism of glucose, lipids, nuclear acids, and amino acids, and transport. Among the predicted targets, 30 of the predicted targets (5.74%) were cancer genes, including *ABI1*, *ARID2*, *ATM*, *BCL11B*, *BCL6*, *CARD11*, *CCDC6*, *CHN1*, *CREB1*, *GNAQ*, *HMGA2*, *LCP1*, *MAF*, *MAP2K1*, *MLLT10*, *MSI2*, *NOTCH2*, *NR4A3*, *PAX5*, *PBX1*, *PHOX2B*, *PMS1*, *PRDM1*, *PTEN*, *SS18L1*, *STAG2*, *TRIM33*, *WHSC1*, *WIF1*, and *XPO1* (Table 3 and Table S2).

miRanda-mirSVR predicted that hsa-miR-181a-5p could regulate 7,847 transcripts/targets (Table 3 and Table S3). These included *ZNF527*, *ZNF439*, *ZNF781*, *ZNF559*, *ZNF204P*, *BAZ2B*, *ZNF844*, *C15orf29*, *FBXO34*, *C5orf41*, *KIAA0528*, *ZNF594*, *EIF4A2*, *ZNF833*, *GATM*, *ZNF440*, *MARK1*, *OSBPL3*, *AP1G1*, *GABRA1*, *DDX3X*, etc. Most of these genes regulate a number of important cellular processes, such as cell proliferation, cell death, division, mitosis, metabolism of glucose, lipids, nuclear acids, and amino acids, and transport. Notably, 223 genes from these predicted targets were cancer genes (2.84%) (Table S4). These included *ABI1*, *ABL1*, *ABL2*, *AKAP9*, *AKT2*, *APC*, *ARHGEF12*, *ARID2*, *ARNT*, *ATF1*, *ATM*, *ATP2B3*, *ATRX*, *BAP1*, *BCL2*, *BCL6*, *BCL7A*, *BCL9*, *BCOR*, *BCR*, *BRCA1*, *BTG1*, *C16orf75*, *CALR*, *CAMTA1*, *CANT1*, *CARD11*, *CASP8*, *CBFA2T3*, *CBL*, *CBLB*, *CCDC6*, *CCNE1*, *CD274*, *CDC73*, *CDH1*, *CDK6*, *CDX2*, *CEBPA*, *CHCHD7*, *CHN1*, *CLTC*, *CREB1*, *CREB3L2*, *CREBBP*, *CRTC3*, *CTNNB1*, *CYLD*, *DAXX*, *DDX10*, *DDX5*, *DEK*, *DICER1*, *DNM2*, *ECT2L*, *EGFR*, *EIF4A2*, *EML4*, *EPS15*, *ERG*, *ETV1*, *ETV6*, *EXT2*, *EZH2*, *FAM46C*, *FANCA*, *FANCD2*, *FANCF*, *FANCG*, *FAS*, *FBXO11*, *FGFR1*, *FGFR1OP*, *FGFR2*, *FGFR3*, *FLT3*, *FNBP1*, *FOXP1*, *FSTL3*, *FUS*, *GAS7*, *GATA2*, *GNAS*, *GOPC*, *H3F3B*, *HERPUD1*, *HEY1*, *HLF*, *HMGA2*, *HNRNPA2B1*, *HOOK3*, *HOXA11*, *HOXC11*, *HOXC13*, *IDH1*, *IGL@*, *IL2*, *IL21R*, *IL6ST*, *IL7R*, *IRF4*, *JAK2*, *JAZF1*, *JUN*, *KCNJ5*, *KDM5A*, *KDM5C*, *KDM6A*, *KDR*, *KIAA1549*, *KLK2*, *KRAS*, *LASP1*, *LCP1*, *LIFR*, *LMO1*, *LPP*, *MAF*, *MAFB*, *MALAT1*, *MALT1*, *MAP2K1*, *MAP2K4*, *MDM2*, *MDM4*, *MDS2*, *MET*, *MITF*, *MKL1*, *MLF1*, *MLH1*, *MLL*, *MLL3*, *MLLT10*, *MLLT3*, *MLLT4*, *MLLT6*, *MN1*, *MPL*, *MSH2*, *MSI2*, *MYB*, *MYCN*, *MYH11*, *NCOA1*, *NCOA2*, *NF1*, *NF2*, *NFE2L2*, *NFIB*, *NIN*, *NOTCH2*, *NPM1*, *NR4A3*, *NRAS*, *NSD1*, *NT5C2*, *NUP98*, *PAFAH1B2*, *PAX3*, *PAX5*, *PAX7*, *PBRM1*, *PBX1*, *PDE4DIP*, *PDGFRA*, *PDGFRB*, *PHF6*, *PHOX2B*, *PICALM*, *PIK3R1*, *PLAG1*, *PML*, *PMS1*, *PPARG*, *PRDM1*, *PTEN*, *PTPRC*, *RAC1*, *RAD21*, *RALGDS*, *RANBP17*, *RAP1GDS1*, *RB1*, *RNF43*, *RPL5*, *RUNX1*, *SDC4*, *SETBP1*, *SF3B1*, *SH2B3*, *SLC34A2*, *SMARCA4*, *SMARCE1*, *SRGAP3*, *SS18L1*, *STAT3*, *SUFU*, *SUZ12*, *SYK*, *TAF15*, *TAL2*, *TBL1XR1*, *TCF7L2*, *TCL6*, *TET2*, *TFRC*, *TMPRSS2*, *TOP1*, *TPM3*, *TRA@*, *TRIM27*, *TRIM33*, *TRRAP*, *TSC1*, *TSHR*, *U2AF1*, *UBR5*, *VTI1A*, *WHSC1*, *WIF1*, *YWHAE*, and *ZNF521* (Table S4).

miRDB predicted that 1,065 targets/transcripts were possibly regulated by hsa-miR-181a-5p (Table 3 and Table S5). These included *PDE5A*, *ZNF439*, *PRTG*, *BRWD1*, *ZNF549*, *NFAT5*, *SH3TC2*, *OSBPL3*, *GFPT1*, *ZNF781*, *TNPO1*, *PAPD5*, *FIGN*, *S1PR1*, *TMEM87B*, *DDX3X*, *ZNF559*, *ZNF844*, *CLMN*, *GPR26*, *CTDSPL*, *ANKRD13C*, *FUT9*,

Table 3 Predicted targets of hsa-miR-181a-5p by various predicting tools

Tool	Website (URL)	Number of predicted targets/transcripts	Examples of predicted targets	Cancer genes
DIANA microT v5.0	http://diana.cslab.ece.ntua.gr/microT/	522 (threshold set at 0.7)	*ATP1B1*, *BHLHE40*, *CLASP1*, *CREBRF*, *FBXO33*, *GLS*, *KLHL5*, *LRBA*, *MAP1B*, *MTF2*, *NMT2*, *PAPD5*, *REPS2*, *RLF*, *SESN3*, *SLC2A3*, *SLITRK1*, *TMEM131*, *TRIM2*, *ZFP90*, *ZNF268*, *ZNF441*, *ZNF527*, *ZNF570*, *ZNF704*, *ZNF717*, *ZNF781*, *ZNF788*, *ZNF799*, *ZNF846*, etc	*ABI1*, *ARID2*, *ATM*, *BCL11B*, *BCL6*, *CARD11*, *CCDC6*, *CHN1*, *CREB1*, *GNAQ*, *HMGA2*, *LCP1*, *MAF*, *MAP2K1*, *MLLT10*, *MSI2*, *NOTCH2*, *NR4A3*, *PAX5*, *PBX1*, *PHOX2B*, *PMS1*, *PRDM1*, *PTEN*, *SS18L1*, *STAG2*, *TRIM33*, *WHSC1*, *WIF1*, and *XPO1* (n=30)
miRanda-mirSVR	http://www.microrna.org/	7,847	*ZNF527*, *ZNF439*, *ZNF781*, *ZNF559*, *ZNF204P*, *BAZ2B*, *ZNF844*, *C15orf29*, *FBXO34*, *C5orf41*, *KIAA0528*, *ZNF594*, *EIF4A2*, *ZNF833*, *GATM*, *ZNF440*, *MARK1*, *OSBPL3*, *AP1G1*, *GABRA1*, *DDX3X*, *HCN1*, *CPOX*, *TMEM87B*, *RPE65*, *BIRC6*, *NOVA1*, *LOC442421*, *ZNF780A*, etc	*ABI1*, *ABL1*, *ABL2*, *AKAP9*, *AKT2*, *APC*, *ARHGEF12*, *ARID2*, *ARNT*, *ATF1*, *ATM*, *ATP2B3*, *ATRX*, *BAP1*, *BCL2*, *BCL6*, *BCL7A*, *BCL9*, *BCOR*, *BCR*, *BRCA1*, *BTG1*, *C16orf75*, *CALR*, *CAMTA1*, *CANT1*, *CARD11*, *CASP8*, *CBFA2T3*, *CBL*, *CBLB*, *CCDC6*, *CCNE1*, *CD274*, *CDC73*, *CDH1*, *CDK6*, *CDX2*, *CEBPA*, *CHCHD7*, *CHN1*, *CLTC*, *CREB1*, *CREB3L2*, *CREBBP*, *CRTC3*, *CTNNB1*, *CYLD*, *DAXX*, *DDX10*, *DDX5*, *DEK*, *DICER1*, *DNM2*, *ECT2L*, *EGFR*, *EIF4A2*, *EML4*, *EPS15*, *ERG*, *ETV1*, *ETV6*, *EXT2*, *EZH2*, *FAM46C*, *FANCA*, *FANCD2*, *FANCF*, *FANCG*, *FAS*, *FBXO11*, *FGFR1*, *FGFR1OP*, *FGFR2*, *FGFR3*, *FLT3*, *FNBP1*, *FOXP1*, *FSTL3*, *FUS*, *GAS7*, *GATA2*, *GNAS*, *GOPC*, *H3F3B*, *HERPUD1*, *HEY1*, *HLF*, *HMGA2*, *HNRNPA2B1*, *HOOK3*, *HOXA11*, *HOXC11*, *HOXC13*, *IDH1*, *IGL@*, *IL2*, *IL21R*, *IL6ST*, *IL7R*, *IRF4*, *JAK2*, *JAZF1*, *JUN*, *KCNJ5*, *KDM5A*, *KDM5C*, *KDM6A*, *KDR*, *KIAA1549*, *KLK2*, *KRAS*, *LASP1*, *LCP1*, *LIFR*, *LMO1*, *LPP*, *MAF*, *MAFB*, *MALAT1*, *MALT1*, *MAP2K1*, *MAP2K4*, *MDM2*, *MDM4*, *MDS2*, *MET*, *MITF*, *MKL1*, *MLF1*, *MLH1*, *MLL*, *MLL3*, *MLLT10*, *MLLT3*, *MLLT4*, *MLLT6*, *MN1*, *MPL*, *MSH2*, *MSI2*, *MYB*, *MYCN*, *MYH11*, *NCOA1*, *NCOA2*, *NF1*, *NF2*, *NFE2L2*, *NFIB*, *NIN*, *NOTCH2*, *NPM1*, *NR4A3*, *NRAS*, *NSD1*, *NT5C2*, *NUP98*, *PAFAH1B2*, *PAX3*, *PAX5*, *PAX7*, *PBRM1*, *PBX1*, *PDE4DIP*, *PDGFRA*, *PDGFRB*, *PHF6*, *PHOX2B*, *PICALM*, *PIK3R1*, *PLAG1*, *PML*, *PMS1*, *PPARG*, *PRDM1*, *PTEN*, *PTPRC*, *RAC1*, *RAD21*, *RALGDS*, *RANBP17*, *RAP1GDS1*, *RB1*, *RNF43*, *RPL5*, *RUNX1*, *SDC4*, *SETBP1*, *SF3B1*, *SH2B3*, *SLC34A2*, *SMARCA4*, *SMARCE1*, *SRGAP3*, *SS18L1*, *STAT3*, *SUFU*, *SUZ12*, *SYK*, *TAF15*, *TAL2*, *TBL1XR1*, *TCF7L2*, *TCL6*, *TET2*, *TFRC*, *TMPRSS2*, *TOP1*, *TPM3*, *TRA@*, *TRIM27*, *TRIM33*, *TRRAP*, *TSC1*, *TSHR*, *U2AF1*, *UBR5*, *VTI1A*, *WHSC1*, *WIF1*, *YWHAE*, and *ZNF521*
miRDB	http://mirdb.org/miRDB/	1,065	*PDE5A*, *ZNF439*, *PRTG*, *BRWD1*, *ZNF549*, *NFAT5*, *SH3TC2*, *OSBPL3*, *GFPT1*, *ZNF781*, *TNPO1*, *PAPD5*, *FIGN*, *S1PR1*, *TMEM87B*, *DDX3X*, *ZNF559*, *ZNF844*, *CLMN*, *GPR26*, *CTDSPL*, *ANKRD13C*, *FUT9*, *RAB3IP*, *DLGAP2*, *BIRC6*, *ZNF268*, *C5orf41*, *PAM*, *KIAA0528*, *ARHGEF3*, etc	*ABI1*, *ATF1*, *ATM*, *BCL11A*, *CBLB*, *CREB1*, *EIF4A2*, *ETV6*, *FAS*, *FOXP1*, *GAS7*, *HLF*, *HOOK3*, *HOXA11*, *IL2*, *JAZF1*, *KDM5A*, *LIFR*, *LPP*, *MAP2K1*, *MAP2K4*, *MDM4*, *MET*, *MLL*, *MLL3*, *MLLT10*, *NCOA2*, *NFIB*, *NOTCH2*, *NR4A3*, *NRAS*, *PBX1*, *PDGFRA*, *PLAG1*, *RAD21*, *SH2B3*, *TCF7L2*, *TET2*, *TFRC*, *TMPRSS2*, *VHL*, *VTI1A*, and *WIF1*

RNA22 v2	https://cm.jefferson.edu/rna22v2.0/	7,042	*NIPAL3*, *PAX7*, *METTL13*, *FMO1*, *CLCN6*, *CLCA1*, *CLCA1*, *ATP1A2*, *KPNA6*, *SLAMF7*, *ZZZ3*, *USH2A*, *TRIT1*, *TPR*, *VPS13D*, *PER3*, *COL9A2*, *HHAT*, *TNFRSF9*, *KIF1B*, *ATP2B4*, *ZC3H11A*, *ZC3H11A*, *TMEM48*, *TARBP1*, *DDX20*, *GNAI3*, *SPEN*, *TIE1*, *IARS2*, *KCNAB2*, *TGFBR3*, etc	*AKAP9*, *ARHGEF12*, *ARID2*, *ARNT*, *ASXL1*, *ATM*, *ATP1A1*, *ATRX*, *AXIN1*, *BAP1*, *BCL11A*, *BCL11B*, *BCL3*, *BCL6*, *BCOR*, *BCR*, *BLM*, *BMPR1A*, *BRAF*, *BRCA1*, *BRD4*, *BTG1*, *BUB1B*, *C15orf55*, *C2orf44*, *CAMTA1*, *CARD11*, *CARS*, *CBLB*, *CCND1*, *CD74*, *CDH1*, *CDK4*, *CHCHD7*, *CHEK2*, *CHN1*, *CIITA*, *CLTC*, *COL1A1*, *CREB1*, *CREB3L2*, *CRTC3*, *CTNNB1*, *CYLD*, *DDX5*, *DICER1*, *DNM2*, *DNMT3A*, *ECT2L*, *EGFR*, *ELF4*, *ELL*, *ERBB2*, *ERCC2*, *ETV5*, *EXT1*, *EZH2*, *FANCA*, *FANCD2*, *FBXO11*, *FBXW7*, *FGFR1*, *FGFR1OP*, *FGFR2*, *FHIT*, *FNBP1*, *FOXP1*, *GAS7*, *GATA2*, *GATA3*, *GNAQ*, *GNAS*, *GOLGA5*, *GPHN*, *HERPUD1*, *HIP1*, *HMGA1*, *HMGA2*, *HRAS*, *IDH1*, *IDH2*, *IL6ST*, *IL7R*, *ITK*, *JAK3*, *JAZF1*, *JUN*, *KCNJ5*, *KDM5A*, *KIAA1549*, *KIT*, *KRAS*, *LASP1*, *LCP1*, *LIFR*, *LPP*, *MAF*, *MALT1*, *MAML2*, *MAP2K1*, *MAX*, *MDM2*, *MDM4*, *MED12*, *MITF*, *MKL1*, *MLF1*, *MLH1*, *MLL*, *MLL3*, *MLLT10*, *MLLT3*, *MLLT4*, *MLLT6*, *MN1*, *MSH6*, *MSI2*, *MSN*, *MYD88*, *MYH11*, *MYST4*, *NACA*, *NCOA1*, *NDRG1*, *NF1*, *NF2*, *NIN*, *NONO*, *NOTCH1*, *NOTCH2*, *NT5C2*, *NTRK3*, *NUP214*, *NUP98*, *OLIG2*, *P2RY8*, *PAFAH1B2*, *PALB2*, *PAX5*, *PAX7*, *PBRM1*, *PBX1*, *PDE4DIP*, *PDGFRA*, *PDGFRB*, *PER1*, *PHF6*, *PHOX2B*, *PIK3CA*, *PIK3R1*, *PLAG1*, *PML*, *POU2AF1*, *PPARG*, *PRDM16*, *PTEN*, *PTPN11*, *PTPRC*, *RAD21*, *RAF1*, *RALGDS*, *RANBP17*, *RET*, *ROS1*, *RPN1*, *SBDS*, *SDC4*, *SDHD*, *SETBP1*, *SETD2*, *SFPQ*, *SLC45A3*, *SMO*, *SOX2*, *SRGAP3*, *SS18*, *SS18L1*, *SSX1*, *SSX2*, *SSX4*, *STAG2*, *STAT3*, *SUZ12*, *TAF15*, *TAL1*, *TBL1XR1*, *TCL1A*, *TERT*, *TET2*, *TFRC*, *THRAP3*, *TMPRSS2*, *TNFAIP3*, *TNFRSF14*, *TOP1*, *TRAF7*, *TRIM33*, *TRIP11*, *TRRAP*, *TSC1*, *TSC2*, *TSHR*, *UBR5*, *VHL*, *WHSC1*, *WHSC1L1*, *WRN*, *WWTR1*, *ZNF331*, *ZNF384*, and *ZNF521*
TargetMiner	http://www.isical.ac.in/~bioinfo_miu/targetminer20.htm	108	*GPD2*, *THRB*, *DIO2*, *GABRA4*, *KITLG*, *PGR*, *SMAD5*, *LDLRAD4*, *MFAP3L*, *MTX3*, *CREB5*, *KCNMA1*, *RAB3IP*, *AP1G1*, *FOXK1*, *GK5*, *CREBZF*, *CHIC1*, *PAPD5*, *CYLD*, *KDM5A*, *ACVR2B*, *LOC124389*, *CALCR*, *AFF2*, *ITGA2*, etc	*CYLD*, *KDM5A*, *KRAS*, *LIFR*, and *TET2*
TargetScan 6.2	http://www.targetscan.org/	1,194 transcripts (626 genes)	*ZNF780A*, *PPIP5K2*, *NUDT12*, *HOXC8*, *MARK1*, *TOM1L1*, *CLVS1*, *ZNF563*, *S1PR1*, *ZNF568*, *FLT1*, *BTBD3*, *TCERG1*, *CTDSPL*, *SLC25A37*, *DDX3Y*, *RPS6KB1*, *METAP1*, *FGD4*, *PBMUCL1*, *CDON*, *DDX3X*, *ZFP62*, *CLMN*, *TMEM165*, *PAPD5*, *ZFP82*, *CLIP1*, *SLC7A2*, etc	*ABL2*, *ARID2*, *ATP2B3*, *ATXN1*, *BCL2*, *BCL9*, *BCR*, *CALR*, *CBFA2T3*, *CBLB*, *CCDC6*, *CDC73*, *CEBPA*, *CREBL2*, *CYLD*, *EIF4A2*, *ERG*, *FGFR3*, *H3F3B*, *HLF*, *HOXA11*, *KDM5A*, *KIAA1549*, *LMO1*, *LPP*, *MAP2K1*, *NCOA2*, *NFIB*, *NOTCH2*, *PAFAH1B2*, *PBX1*, *PRDM1*, *SH2B3*, *SS18L1*, *TCF7L2*, and *WHSC1*
PicTar	http://pictar.mdc-berlin.de/	510 transcripts (399 genes)	KIAA0195, OSBPL3, CTDSPL, HIC2, GRIK2, *ATXN1*, *ADAM11*, *ZBTB4*, *KIAA0802*, *FBXO33*, *PIP3AP*, *EYA3*, *CBX7*, *TARSH*, *CPEB4*, *LRRC5*, *MMP14*, *RLF*, *AKAP7*, *ZIC2*, *CLASP1*, *ATP2B2*, *SEMA4G*, *YTHDF3*, *FLJ23548*, *ALS2CR3*, *HOXC8*, *RSN*, *SOX6*, *TCERG1*, *COPEB*, etc	*ATXN1*, *CARD11*, *CBFA2T3*, *CBLB*, *CHN1*, *COPEB*, *CREB1*, *EIF4A2*, *ETV6*, *FOXP1*, *HLF*, *HOXA11*, *JAZF1*, *KIT*, *LMO1*, *MYCN*, *NCOA2*, *NR4A3*, *PDGFRA*, *PHOX2B*, *PLAG1*, *RUNX1*, and *SS18L1*
MicroCosm Targets v5	http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets/v5/	1,104	*NR6A1*, *TMEM64*, *TMED8*, *TMED4*, *TADA1L*, *ZNF17*, *ZNF487*, *SLC3A1*, *CARD11*, *DOCK7*, *SLC10A7*, *THBS4*, *E2F5*, *PRDX3*, *PLCL2*, *RGMA*, *DHX29*, *FAM58A*, *DMRT3*, *MAB21L1*, *C19orf59*, *TGFBRAP1*, *DEPDC6*, *CARM1*, *WDR45L*, *MDH1B*, etc	*AKAP9*, *BCL11A*, *C16orf75*, *CAGE1*, *CARD11*, *CSF3R*, *EIF4A2*, *EXT2*, *FANCE*, *FAS*, *FGFR2*, *FOS*, *FOXP1*, *FVT1*, *HOOK3*, *IDH1*, *IL2*, *LMO1*, *MLF1*, *MRAS*, *MYBL1*, *PALB2*, *PHOX2B*, *RAB38*, *RALA*, *RASGRP4*, *RASIP1*, *RASSF1*, *RASSF6*, *SSX1*, *SSX2*, *STAG2*, *TAF15*, *TSG101*, *TUSC3*, *WIF1*, and *ZNF521*

RAB3IP, *DLGAP2*, *BIRC6*, *ZNF268*, *C5orf41*, *PAM*, *KIAA0528*, *ARHGEF3*, etc. Many of the predicted targets play a role in the regulation of cell proliferation, cell cycle, apoptosis, energy, nuclear acid, and protein metabolism, signaling transduction, and transport. Among these targets, 43 targets were cancer genes (4.04%). These included *ABI1*, *ATF1*, *ATM*, *BCL11A*, *CBLB*, *CREB1*, *EIF4A2*, *ETV6*, *FAS*, *FOXP1*, *GAS7*, *HLF*, *HOOK3*, *HOXA11*, *IL2*, *JAZF1*, *KDM5A*, *LIFR*, *LPP*, *MAP2K1*, *MAP2K4*, *MDM4*, *MET*, *MLL*, *MLL3*, *MLLT10*, *NCOA2*, *NFIB*, *NOTCH2*, *NR4A3*, *NRAS*, *PBX1*, *PDGFRA*, *PLAG1*, *RAD21*, *SH2B3*, *TCF7L2*, *TET2*, *TFRC*, *TMPRSS2*, *VHL*, *VTI1A*, and *WIF1* (Table 3 and Table S5).

Using RNA22 v2, 7,028 targets were predicted to be regulated by hsa-miR-181a-5p (Table 3 and Table S6). These included *NIPAL3*, *PAX7*, *METTL13*, *FMO1*, *CLCN6*, *CLCA1*, *CLCA1*, *ATP1A2*, *KPNA6*, *SLAMF7*, *ZZZ3*, *USH2A*, *TRIT1*, *TPR*, *VPS13D*, *PER3*, *COL9A2*, *HHAT*, *TNFRSF9*, *KIF1B*, *ATP2B4*, *ZC3H11A*, *ZC3H11A*, *TMEM48*, *TARBP1*, *DDX20*, *GNAI3*, *SPEN*, *TIE1*, *IARS2*, *KCNAB2*, *TGFBR3*, etc. Many of the predicted targets play a role in the regulation of cell proliferation, cell cycle, apoptosis, energy, nuclear acid and protein metabolism, signaling transduction, and transport. Among the predicted targets, 211 genes were cancer genes (3.00%) (Table S7). These included *AKAP9*, *ARHGEF12*, *ARID2*, *ARNT*, *ASXL1*, *ATM*, *ATP1A1*, *ATRX*, *AXIN1*, *BAP1*, *BCL11A*, *BCL11B*, *BCL3*, *BCL6*, *BCOR*, *BCR*, *BLM*, *BMPR1A*, *BRAF*, *BRCA1*, *BRD4*, *BTG1*, *BUB1B*, *C15orf55*, *C2orf44*, *CAMTA1*, *CARD11*, *CARS*, *CBLB*, *CCND1*, *CD74*, *CDH1*, *CDK4*, *CHCHD7*, *CHEK2*, *CHN1*, *CIITA*, *CLTC*, *COL1A1*, *CREB1*, *CREB3L2*, *CRTC3*, *CTNNB1*, *CYLD*, *DDX5*, *DICER1*, *DNM2*, *DNMT3A*, *ECT2L*, *EGFR*, *ELF4*, *ELL*, *ERBB2*, *ERCC2*, *ETV5*, *EXT1*, *EZH2*, *FANCA*, *FANCD2*, *FBXO11*, *FBXW7*, *FGFR1*, *FGFR1OP*, *FGFR2*, *FHIT*, *FNBP1*, *FOXP1*, *GAS7*, *GATA2*, *GATA3*, *GNAQ*, *GNAS*, *GOLGA5*, *GPHN*, *HERPUD1*, *HIP1*, *HMGA1*, *HMGA2*, *HRAS*, *IDH1*, *IDH2*, *IL6ST*, *IL7R*, *ITK*, *JAK3*, *JAZF1*, *JUN*, *KCNJ5*, *KDM5A*, *KIAA1549*, *KIT*, *KRAS*, *LASP1*, *LCP1*, *LIFR*, *LPP*, *MAF*, *MALT1*, *MAML2*, *MAP2K1*, *MAX*, *MDM2*, *MDM4*, *MED12*, *MITF*, *MKL1*, *MLF1*, *MLH1*, *MLL*, *MLL3*, *MLLT10*, *MLLT3*, *MLLT4*, *MLLT6*, *MN1*, *MSH6*, *MSI2*, *MSN*, *MYD88*, *MYH11*, *MYST4*, *NACA*, *NCOA1*, *NDRG1*, *NF1*, *NF2*, *NIN*, *NONO*, *NOTCH1*, *NOTCH2*, *NT5C2*, *NTRK3*, *NUP214*, *NUP98*, *OLIG2*, *P2RY8*, *PAFAH1B2*, *PALB2*, *PAX5*, *PAX7*, *PBRM1*, *PBX1*, *PDE4DIP*, *PDGFRA*, *PDGFRB*, *PER1*, *PHF6*, *PHOX2B*, *PIK3CA*, *PIK3R1*, *PLAG1*, *PML*, *POU2AF1*, *PPARG*, *PRDM16*, *PTEN*, *PTPN11*, *PTPRC*, *RAD21*, *RAF1*, *RALGDS*, *RANBP17*, *RET*, *ROS1*, *RPN1*, *SBDS*, *SDC4*, *SDHD*, *SETBP1*, *SETD2*, *SFPQ*, *SLC45A3*, *SMO*, *SOX2*, *SRGAP3*, *SS18*, *SS18L1*, *SSX1*, *SSX2*, *SSX4*, *STAG2*, *STAT3*, *SUZ12*, *TAF15*, *TAL1*, *TBL1XR1*, *TCL1A*, *TERT*, *TET2*, *TFRC*, *THRAP3*, *TMPRSS2*, *TNFAIP3*, *TNFRSF14*, *TOP1*, *TRAF7*, *TRIM33*, *TRIP11*, *TRRAP*, *TSC1*, *TSC2*, *TSHR*, *UBR5*, *VHL*, *WHSC1*, *WHSC1L1*, *WRN*, *WWTR1*, *ZNF331*, *ZNF384*, and *ZNF521* (Table S7).

TargetMiner predicted that 108 targets were regulated by hsa-miR-181a-5p, including *GPD2*, *THRB*, *DIO2*, *GABRA4*, *KITLG*, *PGR*, *SMAD5*, *LDLRAD4*, *MFAP3L*, *MTX3*, *CREB5*, *KCNMA1*, *RAB3IP*, *AP1G1*, *FOXK1*, *GK5*, *CREBZF*, *CHIC1*, *PAPD5*, *CYLD*, *KDM5A*, *ACVR2B*, *LOC124389*, *CALCR*, *AFF2*, *ITGA2*, etc (Table 3 and Table S8). Among these predicted targets, five were cancer genes, including *CYLD*, *KDM5A*, *KRAS*, *LIFR*, and *TET2* (4.63%) (Table 3 and Table S8).

TargetScan 6.2 only provided the predicted targets for the precursor hsa-miR-181a. It predicted that hsa-miR-181a could regulate 1,194 transcripts with conserved sites, with a total of 1,412 conserved sites and 626 poorly conserved sites (Table 3 and Table S9). Among these transcripts, 626 were functional genes. These included *ZNF780A*, *PPIP5K2*, *NUDT12*, *HOXC8*, *MARK1*, *TOM1L1*, *CLVS1*, *ZNF563*, *S1PR1*, *ZNF568*, *FLT1*, *BTBD3*, *TCERG1*, *CTDSPL*, *SLC25A37*, *DDX3Y*, *RPS6KB1*, *METAP1*, *FGD4*, *PBMUCL1*, *CDON*, *DDX3X*, *ZFP62*, *CLMN*, *TMEM165*, *PAPD5*, *ZFP82*, *CLIP1*, *SLC7A2*, etc. Many of the targets were involved in the regulation of cell proliferation, cell cycle, apoptosis, energy, nuclear acid, and protein metabolism, signaling transduction, and transport. Among the predicted targets, 36 were cancer genes (5.75%). These included *ABL2*, *ARID2*, *ATP2B3*, *ATXN1*, *BCL2*, *BCL9*, *BCR*, *CALR*, *CBFA2T3*, *CBLB*, *CCDC6*, *CDC73*, *CEBPA*, *CREBL2*, *CYLD*, *EIF4A2*, *ERG*, *FGFR3*, *H3F3B*, *HLF*, *HOXA11*, *KDM5A*, *KIAA1549*, *LMO1*, *LPP*, *MAP2K1*, *NCOA2*, *NFIB*, *NOTCH2*, *PAFAH1B2*, *PBX1*, *PRDM1*, *SH2B3*, *SS18L1*, *TCF7L2*, and *WHSC1* (Table 3 and Table S9).

PicTar only provided predicted targets for the precursor hsa-miR-181a. It predicted that hsa-miR-181a could regulate 510 transcripts with 399 genes, including *KIAA0195*, *OSBPL3*, *CTDSPL*, *HIC2*, *GRIK2*, *ATXN1*, *ADAM11*, *ZBTB4*, *KIAA0802*, *FBXO33*, *PIP3AP*, *EYA3*, *CBX7*, *TARSH*, *CPEB4*, *LRRC5*, *MMP14*, *RLF*, *AKAP7*, *ZIC2*, *CLASP1*, *ATP2B2*, *SEMA4G*, *YTHDF3*, *FLJ23548*,

ALS2CR3, *HOXC8*, *RSN*, *SOX6*, *TCERG1*, *COPEB*, etc (Table 3 and Table S10). Many of the predicted targets play a role in the regulation of cell proliferation, cell cycle, apoptosis, energy, nuclear acid, and protein metabolism, signaling transduction, and transport. Among the predicted targets, 23 (5.76%) were cancer genes involved in the initiation, growth, and development and metastasis of cancer, including *ATXN1*, *CARD11*, *CBFA2T3*, *CBLB*, *CHN1*, *COPEB*, *CREB1*, *EIF4A2*, *ETV6*, *FOXP1*, *HLF*, *HOXA11*, *JAZF1*, *KIT*, *LMO1*, *MYCN*, *NCOA2*, *NR4A3*, *PDGFRA*, *PHOX2B*, *PLAG1*, *RUNX1*, and *SS18L1* (Table 3 and Table S10).

MicroCosm Targets v5 predicted that 1,104 targets/transcripts were likely regulated by hsa-miR-181a-5p (Table 3 and Table S11). These included *NR6A1*, *TMEM64*, *TMED8*, *TMED4*, *TADA1L*, *ZNF17*, *ZNF487*, *SLC3A1*, *CARD11*, *DOCK7*, *SLC10A7*, *THBS4*, *E2F5*, *PRDX3*, *PLCL2*, *RGMA*, *DHX29*, *FAM58A*, *DMRT3*, *MAB21L1*, *C19orf59*, *TGFBRAP1*, *DEPDC6*, *CARM1*, *WDR45L*, *MDH1B*, etc. Among the predicted targets, 37 (3.35%) were cancer genes, including *AKAP9*, *BCL11A*, *C16orf75*, *CAGE1*, *CARD11*, *CSF3R*, *EIF4A2*, *EXT2*, *FANCE*, *FAS*, *FGFR2*, *FOS*, *FOXP1*, *FVT1*, *HOOK3*, *IDH1*, *IL2*, *LMO1*, *MLF1*, *MRAS*, *MYBL1*, *PALB2*, *PHOX2B*, *RAB38*, *RALA*, *RASGRP4*, *RASIP1*, *RASSF1*, *RASSF6*, *SSX1*, *SSX2*, *STAG2*, *TAF15*, *TSG101*, *TUSC3*, *WIF1*, and *ZNF521* (Table 3 and Table S11).

These results showed that the number of predicted targets of hsa-miR-181a-5p by the eight algorithms was very different, ranging from 108 to 7,847, with a mean of 2,424. Most of the predicted targets are involved in the regulation of cell proliferation, cell division, cell apoptosis, energy metabolism, amino acid, and nucleic acid metabolism, and transport, inflammation, redox homeostasis, and stress response. Many of the predicted targets are cancer genes, which participate in cancer initiation, development, growth, and metastasis. These cancer genes, including tumor suppressor genes and oncogenes, act as drivers or passengers in tumorigenesis. They are involved in various aspects of functions implicated in cancer initiation, development, and metastasis, including control of cell proliferation, apoptosis, signal transduction, transcription regulation, immunity, and defense.

Predicted targets of hsa-miR-181a-3p

Using DIANA microT v3.0, hsa-miR-181a-3p was predicted to regulate 249 transcripts/targets when the threshold was set at 0.45 (Table 4 and Table S12). These included *ETV1*, *GGCT*, *ODZ1*, *NUB1*, *CPS1*, *AGPAT4*, *TRIO*, *LMO3*, *COL9A2*, *ELN*, *HEBP2*, *CYFIP2*, *MCOLN3*, *RC3H2*, *FLYWCH1*, *COL11A1*, *MRPS35*, *DGKA*, *LAPTM4A*, *IFT80*, *CLEC2D*, *AFF4*, *MARK2*, *SENP1*, *STX7*, *PDS5B*, *NKAIN1*, *PSMC5*, *SLC26A4*, etc. Among these targets, 15 were cancer genes (6.12%), including *ASPSCR1*, *BCL11A*, *CACNA1D*, *CCND2*, *ELN*, *ETV1*, *EZH2*, *GATA3*, *HIP1*, *HRAS*, *MSI2*, *PIM1*, *TSHR*, *USP6*, and *WIF1* (Table 4 and Table S12).

miRanda-mirSVR predicted that hsa-miR-181-a-3p could regulate 1,873 targets (Table 4 and Table S13). These genes included *PMS2L2*, *COL27A1*, *SAE1*, *CNTNAP3B*, *FAM153B*, *GRIP2*, *NR4A1*, *RXRA*, *AES*, *POM121C*, *AFG3L1*, *XDH*, *XPA*, *MID1*, *AIRE*, *CTSK*, *HSD17B1*, *LOR*, *LTBP2*, *NEU1*, *AMPD3*, *AMT*, *APP*, *FUT2*, etc. Most of these genes regulate a number of important cellular processes, such as cell proliferation, cell death, division, mitosis, metabolism of glucose, lipids, nuclear acids, and amino acids, and transport. Among the predicted targets, 61 were cancer genes (3.26%). These included *ABI2*, *ATM*, *AXIN1*, *BCL11A*, *BCL2*, *BTG1*, *CACNA1D*, *CASP8*, *CDH1*, *CDK12*, *CEBPA*, *CHCHD7*, *CREB1*, *CRTC3*, *DDB2*, *DDX10*, *DNM2*, *DUX4*, *EBF1*, *EIF4A2*, *ELL*, *ETV6*, *EZH2*, *FOXP1*, *GAS7*, *GATA3*, *GNA11*, *GNAS*, *HIP1*, *LMO1*, *LPP*, *MALAT1*, *MAX*, *MKL1*, *MLL3*, *MLLT1*, *MN1*, *MSI2*, *NF1*, *PAFAH1B2*, *PAX5*, *PAX7*, *PDE4DIP*, *PER1*, *PIM1*, *PML*, *PTEN*, *RANBP17*, *RPL10*, *SEPT6*, *SET*, *SETBP1*, *SMARCE1*, *SS18L1*, *TAL1*, *TBL1XR1*, *TCL6*, *TET2*, *TP53*, *TSHR*, and *TTL* (Table 4 and Table S14).

miRDB predicted that 22 targets were possibly regulated by hsa-miR-181a-3p (Table 4 and Table S15). These included *ALDH18A1*, *ATP13A4*, *ALDH6A1*, *C16orf57*, *NIPA2*, *RIBC1*, *SLC20A2*, *C14orf28*, *CFL2*, *ZNF3*, *RHOBTB1*, *H1F0*, *AP1S3*, *ARL4A*, *RBM22*, *MIER1*, *ACTR3*, *CLEC2D*, *AFF2*, *ACAP2*, *RABGEF1*, and *KCTD12*. These targets play a role in the regulation of cell proliferation, cell cycle, apoptosis, energy, nuclear acid, and protein metabolism, signaling transduction, and transport. However, none of the predicted targets were cancer genes.

Using RNA22 v2, 5,142 transcripts/2,718 genes were predicted to be regulated by hsa-miR-181a-3p (Table 4 and Table S16). These included *CFH*, *TTC22*, *FMO1*, *ATP1A2*, *VPS13D*, *COL9A2*, *UTS2*, *LAMC2*, *PIGV*, *PTPRU*, *COL11A1*, *DDX20*, *WDR3*, *YBX1*, *ASPM*, *LRRC40*, *EPHA8*, *ARHGEF10L*, *RASAL2*, *PLXNA2*, *RAP1GAP*, *PPP1R12B*, *SDF4*, *TP73*, *NKAIN1*, *WDR47*, *OVGP1*, *SLC25A24*, *EPS15*, *POMGNT1*, etc. Many of the predicted targets play a role

Table 4 Predicted targets of hsa-miR-181a-3p by various predicting tools

Tool	Website (URL)	Number of predicted transcripts	Examples of predicted targets	Cancer genes
DIANA microT v5.0	http://diana.cslab.ece.ntua.gr/microT/	249 (threshold set at 0.45)	*ETV1*, *GGCT*, *ODZ1*, *NUB1*, *CPS1*, *AGPAT4*, *TRIO*, *LMO3*, *COL9A2*, *ELN*, *HEBP2*, *CYFIP2*, *MCOLN3*, *RC3H2*, *FLYWCH1*, *COL11A1*, *MRPS35*, *DGKA*, *LAPTM4A*, *IFT80*, *CLEC2D*, *AFF4*, *MARK2*, *SENP1*, *STX7*, *PDS5B*, *NKAIN1*, *PSMC5*, *SLC26A4*, etc	*ASPSCR1*, *BCL11A*, *CACNA1D*, *CCND2*, *ELN*, *ETV1*, *EZH2*, *GATA3*, *HIP1*, *HRAS*, *MSI2*, *PIM1*, *TSHR*, *USP6*, and *WIF1*
miRanda-mirSVR	http://www.microrna.org/	1,873	*PMS2L2*, *COL27A1*, *SAE1*, *CNTNAP3B*, *FAM153B*, *GRIP2*, *NR4A1*, *RXRA*, *AES*, *POM121C*, *AFG3L1*, *XDH*, *XPA*, *MID1*, *AIRE*, *CTSK*, *HSD17B1*, *LOR*, *LTBP2*, *NEU1*, *AMPD3*, *AMT*, *APP*, *FUT2*, etc	*ABI2*, *ATM*, *AXIN1*, *BCL11A*, *BCL2*, *BTG1*, *CACNA1D*, *CASP8*, *CDH1*, *CDK12*, *CEBPA*, *CHCHD7*, *CREB1*, *CRTC3*, *DDB2*, *DDX10*, *DNM2*, *DUX4*, *EBF1*, *EIF4A2*, *ELL*, *ETV6*, *EZH2*, *FOXP1*, *GAS7*, *GATA3*, *GNA11*, *GNAS*, *HIP1*, *LMO1*, *LPP*, *MALAT1*, *MAX*, *MKL1*, *MLL3*, *MLLT1*, *MN1*, *MSI2*, *NF1*, *PAFAH1B2*, *PAX5*, *PAX7*, *PDE4DIP*, *PER1*, *PIM1*, *PML*, *PTEN*, *RANBP17*, *RPL10*, *SEPT6*, *SET*, *SETBP1*, *SMARCE1*, *SS18L1*, *TAL1*, *TBL1XR1*, *TCL6*, *TET2*, *TP53*, *TSHR*, and *TTL*
miRDB	http://mirdb.org/miRDB/	22	*ALDH18A1*, *ATP13A4*, *ALDH6A1*, *C16orf57*, *NIPA2*, *RIBC1*, *SLC20A2*, *C14orf28*, *CFL2*, *ZNF3*, *RHOBTB1*, *H1F0*, *AP1S3*, *ARL4A*, *RBM22*, *MIER1*, *ACTR3*, *CLEC2D*, *AFF2*, *ACAP2*, *RABGEF1*, and *KCTD12*	None
RNA22 v2	https://cm.jefferson.edu/rna22v2.0/	5,142 (2,718 genes)	*CFH*, *TTC22*, *FMO1*, *ATP1A2*, *VPS13D*, *COL9A2*, *UTS2*, *LAMC2*, *PIGV*, *PTPRU*, *COL11A1*, *DDX20*, *WDR3*, *YBX1*, *ASPM*, *LRRC40*, *EPHA8*, *ARHGEF10L*, *RASAL2*, *PLXNA2*, *RAP1GAP*, *PPP1R12B*, *SDF4*, *TP73*, *NKAIN1*, *WDR47*, *OVGP1*, *SLC25A24*, *EPS15*, *POMGNT1*, etc	*ABL1*, *ABL2*, *AKAP9*, *AKT1*, *AKT2*, *ALDH2*, *ARID1A*, *ARID2*, *ASXL1*, *ATP1A1*, *AXIN1*, *BCL6*, *BCL7A*, *BCR*, *BRD3*, *CCNB1IP1*, *CCND2*, *CDK6*, *CHEK2*, *CREB1*, *CTNNB1*, *CYLD*, *DDB2*, *DNMT3A*, *ELK4*, *EPS15*, *FANCA*, *FANCC*, *FGFR2*, *FOXP1*, *FUS*, *GNAS*, *HIP1*, *HLF*, *HMGA2*, *HOXD11*, *IL7R*, *KCNJ5*, *KDR*, *KLF4*, *KTN1*, *LASP1*, *MAML2*, *MDM4*, *MED12*, *MET*, *MKL1*, *MLL3*, *MUTYH*, *MYB*, *MYH11*, *NACA*, *NCOA2*, *NFIB*, *NOTCH2*, *NSD1*, *NTRK3*, *PAX8*, *PBRM1*, *PDGFRB*, *POT1*, *POU2AF1*, *REL*, *RNF43*, *RPL10*, *RUNDC2A*, *RUNX1*, *SETBP1*, *SF3B1*, *SMARCA4*, *STAT3*, *SUFU*, *TAF15*, *TCEA1*, *TCF3*, *TFEB*, *TSC1*, *UBR5*, *USP6*, *VHL*, *WHSC1L1*, and *YWHAE*
TargetMiner	http://www.isical.ac.in/~bioinfo_miu/targetminer20.htm	13	*CD47*, *CELF2*, *CPNE3*, *FECH*, *FGF5*, *IKZF2*, *MIER1*, *NLGN1*, *NR2C2*, *RBM12B*, *SMAD2*, *SRSF8*, and *TLR4*	None
TargetScan 6.2	http://www.targetscan.org/	See Table 3		
PicTar	http://pictar.mdc-berlin.de/	See Table 3		
MicroCosm Targets v5	http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets/v5/	1,039	*JAG2*, *Q6ZWB7*, *PCDH11Y*, *KLF1*, *MAN1B1*, *TMED5*, *ONECUT1*, *OCIAD1*, *FAM84B*, *WDR69*, *ARFIP1*, *LRRC45*, *SLC1A7*, *ATP8A2*, *SPACA5*, *TTN*, *IER3*, *PPIL1*, *C12orf45*, *IL27*, *KRT33B*, *HYI*, *TSPAN32*, *TCHP*, *KDELR2*, *FOXA3*, *CPXM2*, etc	*AKT2*, *ALDH2*, *BCL2A1*, *BCL7C*, *CARS*, *CDK4*, *DAXX*, *DDB2*, *DLEU7*, *ERCC5*, *FUBP1*, *FUS*, *H3F3A*, *HOXA9*, *IRF4*, *JAK1*, *LCK*, *LMO1*, *MKL1*, *MSI2*, *NFIB*, *PAX3*, *RAC2*, *RAF1*, *RASIP1*, *SETBP1*, *SSX2*, *USP6*, and *WHSC1L1*

in the regulation of cell proliferation, cell cycle, apoptosis, energy, nuclear acid, and protein metabolism, signaling transduction, and transport. Among these targets, 82 were cancer genes (3.02%), including *ABL1*, *ABL2*, *AKAP9*, *AKT1*, *AKT2*, *ALDH2*, *ARID1A*, *ARID2*, *ASXL1*, *ATP1A1*, *AXIN1*, *BCL6*, *BCL7A*, *BCR*, *BRD3*, *CCNB1IP1*, *CCND2*, *CDK6*, *CHEK2*, *CREB1*, *CTNNB1*, *CYLD*, *DDB2*, *DNMT3A*, *ELK4*, *EPS15*, *FANCA*, *FANCC*, *FGFR2*, *FOXP1*, *FUS*, *GNAS*, *HIP1*, *HLF*, *HMGA2*, *HOXD11*, *IL7R*, *KCNJ5*, *KDR*, *KLF4*, *KTN1*, *LASP1*, *MAML2*, *MDM4*, *MED12*, *MET*, *MKL1*, *MLL3*, *MUTYH*, *MYB*, *MYH11*, *NACA*, *NCOA2*, *NFIB*, *NOTCH2*, *NSD1*, *NTRK3*, *PAX8*, *PBRM1*, *PDGFRB*, *POT1*, *POU2AF1*, *REL*, *RNF43*, *RPL10*, *RUNDC2A*, *RUNX1*, *SETBP1*, *SF3B1*, *SMARCA4*, *STAT3*, *SUFU*, *TAF15*, *TCEA1*, *TCF3*, *TFEB*, *TSC1*, *UBR5*, *USP6*, *VHL*, *WHSC1L1*, and *YWHAE* (Table 4 and Table S16).

TargetMiner predicted that 13 targets were regulated by hsa-miR-181a-3p, including *CD47*, *CELF2*, *CPNE3*, *FECH*, *FGF5*, *IKZF2*, *MIER1*, *NLGN1*, *NR2C2*, *RBM12B*, *SMAD2*, *SRSF8*, and *TLR4* (Table 4 and Table S17). None of the predicted targets was a cancer gene.

TargetScan 6.2 predicted that hsa-miR-181a could regulate 626 targets (Tables 3 and 4). In PicTar, only hsa-miR-181a could be searched. It probably regulated 510 transcripts with 399 genes (Tables 3 and 4).

MicroCosm Targets v5 predicted that 1,039 targets were likely regulated by hsa-miR-181a-3p (Table 4 and Table S18). These included *JAG2*, *Q6ZWB7*, *PCDH11Y*, *KLF1*, *MAN1B1*, *TMED5*, *ONECUT1*, *OCIAD1*, *FAM84B*, *WDR69*, *ARFIP1*, *LRRC45*, *SLC1A7*, *ATP8A2*, *SPACA5*, *TTN*, *IER3*, *PPIL1*, *C12orf45*, *IL27*, *KRT33B*, *HYI*, *TSPAN32*, *TCHP*, *KDELR2*, *FOXA3*, *CPXM2*, etc. Among the predicted targets, 29 were cancer genes (2.79%), including *AKT2*, *ALDH2*, *BCL2A1*, *BCL7C*, *CARS*, *CDK4*, *DAXX*, *DDB2*, *DLEU7*, *ERCC5*, *FUBP1*, *FUS*, *H3F3A*, *HOXA9*, *IRF4*, *JAK1*, *LCK*, *LMO1*, *MKL1*, *MSI2*, *NFIB*, *PAX3*, *RAC2*, *RAF1*, *RASIP1*, *SETBP1*, *SSX2*, *USP6*, and *WHSC1L1* (Table 4 and Table S18).

These results demonstrated that the number of predicted targets of hsa-miR-181a-3p by the eight tools was very different, ranging from 13 to 5,142, with a mean of 1,184. Most of the predicted targets are involved in the regulation of cell proliferation, cell division, cell apoptosis, energy metabolism, amino acid and nucleic acid metabolism, and transport, inflammation, redox homeostasis, and stress response. Many of the predicted targets are cancer genes which participate in cancer initiation, development, growth, and metastasis.

Predicted targets of hsa-miR-181a by miRWALK

miRWALK provides information on predicted targets from ten algorithms. When only miRWALK was chosen, where only one longest binding site was set per miRNA per mRNA, 3,762 transcripts were predicted to be regulated by hsa-miR-181a (Table S19). These included *AASS*, *ABCB11*, *ACN9*, *ACTA2*, *ADAM28*, *BAG2*, *BCL11A*, *BCL6B*, *BTRC*, *CARD11*, *CCR3*, *CDK8*, *CDKN3*, *CIT*, *CNOT1*, *DAD1*, *DCN*, *DKC1*, *DR1*, *EHF*, *ESCO2*, *FBN2*, *FUT1*, *GALNT3*, *GCS1*, *GPC5*, *HK2*, *HSPB3*, *IL2*, *IL25*, *IVD*, *KCNG3*, *KIF2C*, *LACE1*, *MAEL*, *MAOA*, *MLL5*, *NCL*, *NOVA1*, *OCA2*, *PAG1*, *PROCR*, *RAD21*, *RFC3*, *RNF6*, *SELT*, *SIX2*, *TAF15*, etc. Among these transcripts, 1,436 were functional genes (Table S20).

miRWALK also provided a summarized table that included all targets predicted to be regulated by hsa-miR-181a by the ten algorithms. In total, there were 17,395 transcripts that would be regulated by hsa-miR-181a (Table S21). Only 24 genes were predicted to be the targets of hsa-miR-181a by all the algorithms or at least nine algorithms. These included *RNF145*, *TCERG1*, *SIRT1*, *SS18L1*, *NR6A1*, *FOXP1*, *GLS*, *HOXA11*, *SMAD7*, *MAP1B*, *INOC1*, *CDKN2AIP*, *LRRN1*, *TGFBI*, *YWHAG*, *C17orf39*, *CHD9*, *NAT13*, *C6orf62*, *ACVR2A*, *DCLK1*, *NMT2*, *NPEPPS*, and *KIAA0195*. There were 102 targets that were predicted to be regulated by hsa-miR-181a by eight algorithms, including *CDH13*, *PDIA6*, *IPO8*, *IGF2BP2*, *SLITRK1*, *SSX2IP*, *COL16A1*, *ADM*, *ZNF800*, *ADAMTS18*, *DPYSL2*, *E2F5*, *EIF4A2*, *EN2*, *ETV6*, *ACSL1*, *FBN2*, *C7orf41*, *FKBP1A*, *BTBD3*, *KIAA0423*, *HIC2*, *KANK1*, *HISPPD1*, *SYNE1*, *ZNF281*, *FOS*, *LEMD3*, *BRD1*, *OSBPL3*, *EPC2*, *GAPVD1*, *GATA6*, *NPTN*, *ATP11C*, *KLF15*, *LRP12*, *BAZ2B*, *HLF*, *HOXA1*, *ID4*, *JARID2*, *KPNA1*, etc. There were 191, 778, 1,615, and 3,168 targets that were predicted to be regulated by hsa-miR-181a by seven, six, five, and four algorithms, respectively.

Validated targets of hsa-miR-181a-5p based on TarBase

Based on TarBase, 211 targets of hsa-miR-181a-5p have been validated with experimental evidence (Table 5). These included *ACOT12*, *ALG10B*, *AMMECR1*, *ANKRD1*, *ANKRD13C*, *ARF6*, *ARHGAP11A*, *ARL6IP1*, *ARL6IP6*, *ATF7IP2*, *ATG10*, *ATM*, *ATP6V0E1*, *BAG2*, *BCL2*, *BDNF*, *BPGM*, *BRIX1*, *BRMS1L*, *BTBD3*, *C1orf109*, *C1orf43*, *C1QTNF9*, *C8A*, *CBX3*, *CCDC6*, *CCDC82*, *CCND1*, *CCNG1*, etc. Among these validated targets, only eleven of them are cancer genes (5.21%), including *ATM*, *BCL2*,

Table 5 Targets of hsa-miR-181a-5p with experimental evidence based on TarBase 6.0

Gene symbol	Accession number	Full name	Alias	Function	Cancer gene
ACOT12	NM_130767	Acyl-CoA thioesterase 12	CACH-1, Cach, STARD15, THEAL	Hydrolyzes acetyl-CoA to acetate and CoA	
ALG10B	NM_001013620	α-1,2-Glucosyltransferase	ALG10, KCR1	Transfers glucose from dolichyl phosphate glucose onto the lipid-linked oligosaccharide Glc(2) Man(9)GlcNAc(2)-PP-Dol	
AMMECR1	NM_001025580	Alport syndrome, mental retardation, midface hypoplasia and elliptocytosis chromosomal region gene 1	RP13-360B22.1, AMMERC1		
ANKRD1	NM_014391	Ankyrin repeat domain 1 (cardiac muscle)	ALRP, C-193, CARP, CVARP, MCARP, bA320F15.2	Plays an important role in endothelial cell activation	
ANKRD13C	NM_030816	Ankyrin repeat domain 13C	RP4-677H15.5, dJ677H15.3		
ARF6	NM_001663	ADP-ribosylation factor 6		Involved in protein trafficking	
ARHGAP11A	NM_001286479	Rho GTPase activating protein 11A	RP11-1000B6.5, GAP (1–12)	GTPase activator activity	
ARL6IP1	NM_015161	ADP-ribosylation factor-like 6 interacting protein 1	AIP1, ARL6IP, ARMER, SPG61	May be involved in protein transport, membrane trafficking, or cell signaling during hematopoietic maturation	
ARL6IP6	NM_022989	ADP-ribosylation factor-like 6 interacting protein 6	RP23-265N10.1, 2310057C01Rik, 2610529A11Rik, Aip-6		
ATF7IP2	NM_001256160	Activating transcription factor 7 interacting protein 2	MCAF2	Recruiter that couples transcriptional factors to general transcription apparatus and thereby modulates transcription regulation and chromatin formation	
ATG10	NM_001131028	Autophagy related 10	PP12616, APG10, APG10L, pp12616	Plays a role in autophagy	
ATM	NM_000051	ATM serine/threonine kinase	AT1, ATA, ATC, ATD, ATDC, ATE, TEL1, TELO1	Serine/threonine protein kinase	Yes
ATP6V0E1	NM_003945	ATPase, H^+ transporting, lysosomal 9 kDa, V0 subunit e1	ATP6H, ATP6V0E, M9.2, Vma21, Vma21p	Vacuolar ATPase is responsible for acidifying a variety of intracellular compartments in eukaryotic cells	
BAG2	NM_004282	BCL2-associated athanogene 2	RP3-496N17.2, BAG-2, dJ417I1.2	Inhibits the chaperone activity of HSP70/HSC70 by promoting substrate release	
BCL2	NM_000633	B-cell CLL/lymphoma 2	Bcl-2, PPP1R50	Suppresses apoptosis	Yes
BDNF	NM_001143805	Brain-derived neurotrophic factor	ANON2, BULN2	Promotes the survival of neuronal populations	
BPGM	NM_001293085	2,3-Bisphosphoglycerate mutase	DPGM	Plays a major role in regulating hemoglobin oxygen affinity	
BRIX1	NM_018321	Biogenesis of ribosomes, homolog (*S. cerevisiae*)	BRIX, BXDC2	Required for biogenesis of the 60S ribosomal subunit	
BRMS1L	NM_032352	Breast cancer metastasis-suppressor 1-like	BRMS1	Involved in the HDAC1-dependent transcriptional repression activity	
BTBD3	NM_001282550	BTB (POZ) domain containing 3	RP4-742J24.3, dJ742J24.1	Acts as a key regulator of dendritic field orientation during development of sensory cortex	
C1orf109	NM_017850	Chromosome 1 open reading frame 109			
C1orf43	NM_001098616	Chromosome 1 open reading frame 43	HSPC012, NICE-3, NS5ATP4, S863-3		
C1QTNF9	NM_183175	C1q and tumor necrosis factor related protein 9	9130217G22Rik, CTRP9, Ciqtnf9	Activates AMPK, AKT, and p44/42 MAPK signaling pathways	

Table 5 (*Continued*)

Gene symbol	Accession number	Full name	Alias	Function	Cancer gene
C8A	NM_000562	Complement component 8, α polypeptide		C8 is a constituent of the membrane attack complex	
CBX3	NM_007276	Chromobox homolog 3	HECH, HP1-GAMMA, HP1Hs-γ	Involved in transcriptional silencing in heterochromatin-like complexes	
CCDC6	NM_005436	Coiled-coil domain containing 6	D10S170, H4, PTC, TPC, TST1	Functions as a tumor suppressor	Yes
CCDC82	NM_024725	Coiled-coil domain containing 82	HT025, HSPC048		
CCND1	NM_053056	Cyclin D1	BCL1, D11S287E, PRAD1, U21B31	Essential for the control of the cell cycle at the G1/S (start) transition	Yes
CCNG1	NM_004060	Cyclin G1	CCNG	May play a role in growth regulation	
CD46	NM_002389	CD46 molecule, complement regulatory protein	AHUS2, MCP, MIC10, TLX, TRA2.10	Acts as a cofactor for complement factor I	
CDKN1B	NM_004064	Cyclin-dependent kinase inhibitor 1B (p27, Kip1)	CDKN4, KIP1, MEN1B, MEN4, P27KIP1	Important regulator of cell cycle progression	
CDX2	NM_001265	Caudal type homeobox 2	CDX-3, CDX3	Involved in the transcriptional regulation of multiple genes expressed in the intestinal epithelium	Yes
CEP97	NM_024548	Centrosomal protein 97 kDa	2810403B08Rik, LRRIQ2	Collaborates with cep110, being involved in the suppression of a cilia assembly program	
CFI	NM_000204	Complement factor I	AHUS3, ARMD13, C3BINA, C3b-INA, FI, IF, KAF	Responsible for cleaving the α-chains of C4b and C3b in the presence of the cofactors C4-binding protein and factor H, respectively	
CHD1	NM_001270	Chromodomain helicase DNA binding protein 1		Sequence-selective DNA-binding protein	
CHL1	NM_001253387	Cell adhesion molecule L1-like	CALL, L1CAM2	Plays a role in nervous system development and in synaptic plasticity	
CHRFAM7A	NM_139320	CHRNA7 (cholinergic receptor, nicotinic, α 7, exons 5–10) and FAM7A (family with sequence similarity 7A, exons A-E) fusion	CHRNA7, CHRNA7-DR1, D-10	Extracellular ligand-gated ion channel activity	
CLUAP1	NM_015041	Clusterin associated protein 1	CFAP22, FAP22	May play a role in cell proliferation or apoptosis	
COL27A1	NM_032888	Collagen, type XXVII, α 1	RP11-82I1.1	Plays a role during the calcification of cartilage and the transition of cartilage to bone	
COPS2	NM_001143887	COP9 signalosome subunit 2	ALIEN, CSN2, SGN2, TRIP15	Involved in various cellular and developmental processes	
CST5	NM_001900	Cystatin D		Cysteine proteinase inhibitor	
CXorf1	NM_004709	Transmembrane protein 257	CXorf1		
D3R	NM_000796.5	DRD3	D3DR; ETM1; FET1	Associated with cognitive, emotional, and endocrine functions	
DCP2	NM_001242377	Decapping mRNA 2	NUDT20	Necessary for the degradation of mRNAs	
DCST1	NM_001143687	DC-STAMP domain containing 1	RP11-307C12.10-003	Protein and zinc ion binding	
DDIT4	NM_019058	DNA-damage-inducible transcript 4	RP11-442H21.1, Dig2, REDD-1, REDD1	Inhibits cell growth by regulating the frap1 pathway upstream of the tsc1-tsc2 complex and downstream of Akt1	
DNAJC7	NM_001144766	DnaJ (HSP40) homolog, subfamily C, member 7	DJ11, DJC7, TPR2, TTC2	Acts as co-chaperone regulating the molecular chaperones HSP70 and HSP90 in folding of steroid receptors	

(*Continued*)

Table 5 (*Continued*)

Gene symbol	Accession number	Full name	Alias	Function	Cancer gene
DSCR8	NM_032589	Down syndrome critical region gene 8	C21orf65, CT25.1a, CT25.1b, MMA-1, MMA-1a, MMA-1b, MMA1, MTAG2		
EIF1	NM_005801	Eukaryotic translation initiation factor 1	A121, EIF-1A, ISO1, SUI1, EIF1	Necessary for scanning and involved in initiation site selection	
EIF2C1	NM_012199	Argonaute RISC catalytic component 1	RP4-789D17.1, EIF2C, AGO1, GERP95, Q99	Required for RNA-mediated gene silencing	
EIF2C3	NM_024852	Argonaute RISC catalytic component 3	AGO3	Required for RNA-mediated gene silencing	
ELAVL1	NM_001419	ELAV like RNA binding protein 1	ELAV1, HUR, Hua, MelG	Binds avidly to the AU-rich element in FOS and IL3/ interleukin-3 mRNAs	
ENAH	NM_001008493	Enabled homolog	RP11-496N12.7, ENA, MENA, NDPP1	Ena/VASP proteins are actin-associated proteins involved in a range of processes dependent on cytoskeleton remodeling and cell polarity	
EP300	NM_001429	E1A binding protein p300	RP1-85F18.1, KAT3B, RSTS2, p300	Functions as HAT and regulates transcription via chromatin remodeling	Yes
EPHA5	NM_001281765	EPH receptor A5	CEK7, EHK-1, EHK1, EK7, HEK7, TYRO4	Receptor for members of the ephrin-A family	
ESR1	NM_000125	Estrogen receptor 1	RP1-130E4.1, ER, ESR, ESRA, ESTRR, Era, NR3A1	Nuclear hormone receptor	
EYA4	NM_001301012	EYA transcriptional coactivator and phosphatase 4	RP11-704J17.4, CMD1J, DFNA10	Tyrosine phosphatase that specifically dephosphorylates "Tyr-142" of histone H2AX (H2AXY142ph)	
FAM47B	NM_152631	Family with sequence similarity 47, member B	RP13-520K9.1		
FBXO34	NM_017943	F-box protein 34	CGI-301, Fbx34	Substrate-recognition component of the SCF E3 ubiquitin ligase complex	
FKBP10	NM_021939	FK506 binding protein 10	PSEC0056, FKBP65, OI11, OI6, PPIASE, hFKBP65	PPIases accelerate the folding of proteins during protein synthesis	
FKBP4	NM_002014	FK506 binding protein 4	FKBP51, FKBP52, FKBP59, HBI, Hsp56, PPIase, p52	May play a role in the intracellular trafficking of heterooligomeric forms of steroid hormone receptors	
FKBP7	NM_001135212	FK506 binding protein 7	UNQ670/PRO1304, FKBP23, PPIase	PPIases accelerate the folding of proteins during protein synthesis	
FRA10AC1	NM_145246	Fragile site, folic acid type, rare, fra(10)(q23.3) or fra(10)(q24.2) candidate 1	PRO2972, C10orf4, F26C11.1-like, FRA10A		
FSIP1	NM_152597	Fibrous sheath interacting protein 1	HSD10		
FXYD6	NM_001164831	FXYD domain containing ion transport regulator 6	UNQ521/PRO1056		
GADD45G	NM_006705	Growth arrest and DNA-damage-inducible, γ	RP11-260L6.1, CR6, DDIT2, GADD45γ, GRP17	Involved in the regulation of growth and apoptosis	
GATA6	NM_005257	GATA binding protein 6		Regulates terminal differentiation and/or proliferation	
GCNT1	NM_001097633	Glucosaminyl (*N*-acetyl) transferase 1, core 2	RP11-214N16.1, C2GNT, C2GNT-L, C2GNT1, G6NT, NACGT2, NAGCT2	Forms critical branches in O-glycans	
GNA13	NM_001282425	G protein, α 13	G13	Modulators or transducers in various transmembrane signaling systems	

Table 5 (*Continued*)

Gene symbol	Accession number	Full name	Alias	Function	Cancer gene
GNB1	NM_001282538	G protein, β polypeptide 1	RP1-283E3.7	A modulator or transducer in various transmembrane signaling systems	
GPR137B	NM_003272	G protein-coupled receptor 137B	RP5-985L19.1, TM7SF1		
GPR83	NM_016540	G protein-coupled receptor 83	GIR, GPR72	Orphan receptor. Could be a neuropeptide y receptor	
GSTM2	NM_000848	Glutathione *S*-transferase mu 2 (muscle)	GST4, GSTM-2, GTHMUS, GSTM2	Conjugation of reduced glutathione to a wide number of exogenous and endogenous hydrophobic electrophiles	
H1F0	NM_005318	H1 histone family, member 0	H10, H1FV	Histones H1 are necessary for the condensation of nucleosome chains into higher order structures	
HERC3	NM_001271602	HECT and RLD domain containing E3 ubiquitin protein ligase 3		E3 ubiquitin-protein ligase	
HEY2	NM_012259	Hes-related family bHLH transcription factor with YRPW motif 2	RP1-293L8.3, CHF1, GRIDLOCK, GRL, HERP1, HESR2, HRT2, bHLHb32	Downstream effector of Notch signaling which may be required for cardiovascular development	
HIPK2	NM_001113239	Homeodomain interacting protein kinase 2	PRO0593	Protein kinase acting as a corepressor of several transcription factors	
HMGB2	NM_001130688	High mobility group box 2	HMG2	Binds preferentially ssDNA and unwinds double-stranded DNA	
HNRPDL	NM_001207000	Heterogeneous nuclear ribonucleoprotein D-like	HNRNP, HNRPDL, JKTBP, JKTBP2, laAUF1	Acts as a transcriptional regulator	
HOXA10	NM_018951	Homeobox A10	HOX1, HOX1.8, HOX1H, PL	Sequence-specific transcription factor	
HOXA11	NM_005523	Homeobox A11	HOX1, HOX11	Sequence-specific transcription factor	Yes
HSD17B3	NM_000197	Hydroxysteroid (17-β) dehydrogenase 3	RP11-240L7.3, EDH17B3, SDR12C2	Favors the reduction of androstenedione to testosterone	
HSP90B1	NM_003299	Heat-shock protein 90 kDa β (Grp94), member 1	ECGP, GP96, GRP94, HEL-S-125m, HEL35, TRA1	Molecular chaperone that functions in the processing and transport of secreted proteins	
HSPA1B	NM_005346	Heat-shock 70 kDa protein 1B	DAAP-21F2.7, HSP70-1B, HSP70-2	Stabilizes preexistent proteins against aggregation and mediates the folding of newly translated polypeptides in the cytosol as well as within organelles	
ICMT	NM_012405	Isoprenylcysteine carboxyl methyltransferase	RP1-120G22.4, HSTE14, MST098, MSTP098, PCCMT, PCMT, PPMT	Catalyzes the posttranslational methylation of isoprenylated C-terminal cysteine residues	
IDS	NM_000202	Iduronate 2-sulfatase	MPS2, SIDS	Required for the lysosomal degradation of heparan sulfate and dermatan sulfate	
INCENP	NM_001040694	Inner centromere protein antigens		Component of the chromosomal passenger complex, a complex that acts as a key regulator of mitosis	
IQCG	NM_001134435	IQ motif containing G	CFAP122, DRC9		
KAT2B	NM_003884	K(lysine) acetyltransferase 2B	CAF, P/CAF, PCAF	Functions as a HAT to promote transcriptional activation	
KBTBD3	NM_152433	Kelch repeat and BTB (POZ) domain containing 3	BKLHD3		

(*Continued*)

Table 5 (*Continued*)

Gene symbol	Accession number	Full name	Alias	Function	Cancer gene
KBTBD7	NM_032138	Kelch repeat and BTB (POZ) domain containing 7			
KCTD3	NM_016121	Potassium channel tetramerization domain containing 3	RP11-5F19.1, NY-REN-45		
KIAA0101	NM_001029989	KIAA0101	L5, NS5ATP9, OEATC, OEATC-1, OEATC1, PAF, PAF15, p15(PAF), p15/PAF, p15PAF	May be involved in protection of cells from UV-induced cell death	
KIAA2026	NM_001017969	KIAA2026			
KLHL15	NM_030624	Kelch-like family member 15	HEL-S-305	Probable substrate-specific adapter of an E3 ubiquitin-protein ligase complex which mediates the ubiquitination and subsequent proteasomal degradation of target proteins	
KLRC4	NM_013431	Killer cell lectin-like receptor subfamily C, member 4	NKG2-F, NKG2F	May play a role as a receptor for the recognition of MHC class I HLA-E molecules by NK cells	
KRAS	NM_004985	Kirsten rat sarcoma viral oncogene homolog	C-K-RAS, CFC2, K-RAS2A, K-RAS2B, K-RAS4A, K-RAS4B, KI-RAS1, KRAS2, NS, NS3, RASK2, KRAS	Binds GDP/GTP and possesses intrinsic GTPase activity	Yes
LFNG	NM_001040167	LFNG *O*-fucosylpeptide 3-β-*N*-acetylglucosaminyltransferase	SCDO3	Glycosyltransferase	
LPGAT1	NM_014873	Lysophosphatidylglycerol acyltransferase 1	FAM34A, FAM34A1, NET8	Lysophoshatidylglycerol-specific acyltransferase	
LRRC17	NM_001031692	Leucine rich repeat containing 17	UNQ3076/PRO9909, P37NB	Involved in bone homeostasis, acting as a negative regulator of RANKL-induced osteoclast precursor differentiation from bone marrow precursors	
LRRN3	NM_001099658	Leucine rich repeat neuronal 3	Nbla10363, FIGLER5, NLRR-3, NLRR3		
LYSMD3	NM_001286812	LysM, putative peptidoglycan-binding, domain containing 3			
MAP1B	NM_005909	Microtubule-associated protein 1B	FUTSCH, MAP5, PPP1R102	May play a role in the cytoskeletal changes that accompany neurite extension	
METAP1	NM_015143	Methionyl aminopeptidase 1	MAP1A, MetAP1A	Removes the amino-terminal methionine from nascent proteins	
MFAP3	NM_001135037	Microfibrillar-associated protein 3		Component of the elastin-associated microfibrils	
MIF	NM_002415	Macrophage migration inhibitory factor (glycosylation-inhibiting factor)	GIF, GLIF, MMIF	The expression of MIF at sites of inflammation suggests a role for the mediator in regulating the function of macrophage in host defense. Also acts as a phenylpyruvate tautomerase	
MOB3B	NM_024761	MOB kinase activator 3B	C9orf35, MOB1D, MOBKL2B	May regulate the activity of kinases	
MRPS14	NM_022100	Mitochondrial ribosomal protein S14	DJ262D12.2, HSMRPS14, MRP-S14, S14mt		
MTMR12	NM_001040446	Myotubularin-related protein 12	3-PAP, PIP3AP	Inactive phosphatase that plays a role as an adapter for the phosphatase myotubularin to regulate myotubularin intracellular location	

Table 5 (*Continued*)

Gene symbol	Accession number	Full name	Alias	Function	Cancer gene
MTRR	NM_002454	5-Methyltetrahydrofolate-homocysteine methyltransferase reductase	MSR, cblE	Involved in the reductive regeneration of cob(I)alamin cofactor required for the maintenance of methionine synthase in a functional state	
MYO9A	NM_006901	Myosin IXA		Myosins are actin-based motor molecules with ATPase activity Unconventional myosins serve in intracellular movements	
NCAPG	NM_022346	Non-SMC condensin I complex, subunit G	CAPG, CHCG, NY-MEL-3, YCG1	Regulatory subunit of the condensin complex, a complex required for conversion of interphase chromatin into mitotic-like condense chromosomes	
NKX3-2	NM_001189	NK3 homeobox 2	BAPX1, NKX3.2, NKX3B, SMMD	Transcriptional repressor that acts as a negative regulator of chondrocyte maturation	
NLK	NM_016231	Nemo-like kinase		Role in cell fate determination, required for differentiation of bone marrow stromal cells	
NMRK2	NM_001289117	Nicotinamide riboside kinase 2	ITGB1BP3, MIBP, NRK2		
NOL4	NM_001198546	Nucleolar protein 4	HRIHFB2255, CT125, NOLP		
NUDT12	NM_001300741	Nudix-type motif 12		Hydrolyzes NAD(P)H to NMNH and AMP (2′,5′-ADP), and diadenosine diphosphate to AMP	
OAZ1	NM_001301020	Ornithine decarboxylase antizyme 1	AZI, OAZ	Binds to and destabilizes ornithine decarboxylase, which is then degraded. Also inhibits cellular uptake of polyamines by inactivating the polyamine uptake transporter	
OFCC1	NM_153003	Orofacial cleft 1 candidate 1	MRDS1		
OR11A1	NM_013937	Olfactory receptor, family 11, subfamily A, member 1	DAAP-34I1.2, 6M1-18, OR11A2, dJ994E9.6, hs6M1-18	Odorant receptor	
OTUD1	NM_001145373	OTU deubiquitinase 1	DUBA7, OTDC1	Deubiquitinating enzyme that specifically hydrolyzes 'Lys-63′-linked polyubiquitin to monoubiquitin	
OTX2	NM_001270523	Orthodenticle homeobox 2	CPHD6, MCOPS5	Probably plays a role in the development of the brain and the sense organs	
PCAF	NM_003884	K(lysine) acetyltransferase 2B	CAF, P/CAF, PCAF	Functions as a component of the PCAF complex	
PCDHB8	NM_019120	Protocadherin β 8	PCDH-β8, PCDH3I	Potential calcium-dependent cell-adhesion protein	
PHOX2A	NM_005169	Paired-like homeobox 2a	ARIX, CFEOM2, FEOM2, NCAM2, PMX2A	May be involved in regulating the specificity of expression of the catecholamine biosynthetic genes	
PIM3	NM_001001852	Pim-3 proto-oncogene, serine/threonine kinase	CITF22-49E9.1, pim-3	May be involved in cell cycle progression and antiapoptotic process	
PLA2G4C	NM_001159322	Phospholipase A2, group IVC (cytosolic, calcium-independent)	CPLA2-γ	Has a preference for arachidonic acid at the sn-2 position of phosphatidylcholine as compared with palmitic acid	

(*Continued*)

Table 5 (*Continued*)

Gene symbol	Accession number	Full name	Alias	Function	Cancer gene
PLAG1	NM_001114634	Pleiomorphic adenoma gene 1	PSA, SGPA, ZNF912	Transcription factor whose activation results in upregulation of target genes, such as IGFII, leading to uncontrolled cell proliferation	Yes
PLCL2	NM_001144382	Phospholipase C-like 2	PLCE2	May play a role in the regulation of Ins(1,4,5)P3 around the endoplasmic reticulum	
PLXDC2	NM_001282736	Plexin domain containing 2	UNQ2514/PRO6003, TEM7R	May play a role in tumor angiogenesis	
PNPT1	NM_033109	Polyribonucleotide nucleotidyltransferase 1	COXPD13, DFNB70, OLD35, PNPASE, old-35	Involved in mRNA degradation	
POLR2B	NM_000938	Polymerase (RNA) II (DNA directed) polypeptide B	POL2RB, RPB2, hRPB140, hsRPB2	DNA-dependent RNA polymerase catalyzes the transcription of DNA into RNA using the four ribonucleoside triphosphates as substrates	
PPA1	NM_021129	Pyrophosphatase (inorganic) 1	RP11-367H5.1, HEL-S-66p, IOPPP, PP, PP1, SID6-8061		
PPM1A	NM_021003	Protein phosphatase, Mg^{2+}/Mn^{2+} dependent, 1A	PP2C-ALPHA, PP2CA, PP2Cα	Enzyme with a broad specificity	
PPP2CA	NM_002715	Protein phosphatase 2, catalytic subunit, α isozyme	PP2Ac, PP2CA, PP2Cα, RP-C	PP2A can modulate the activity of phosphorylase B kinase casein kinase 2, mitogen-stimulated S6 kinase, and MAP-2 kinase	
PPP2R5C	NM_001161725	Protein phosphatase 2, regulatory subunit B′, γ	B56G, PR61G	The B regulatory subunit might modulate substrate selectivity and catalytic activity, and also might direct the localization of the catalytic enzyme to a particular subcellular compartment	
PRDX3	NM_006793	Peroxiredoxin 3	AOP-1, AOP1, HBC189, MER5, PRO1748, SP-22, prx-III	Involved in redox regulation of the cell	
PRLR	NM_000949	Prolactin receptor	HPRL, MFAB, hPRLrI	This is a receptor for the anterior pituitary hormone prolactin	
PROSC	NM_007198	Proline synthetase co-transcribed homolog (bacterial)			
PROX1	NM_001270616	Prospero homeobox 1		May play a fundamental role in early development of central nervous system	
PRR4	NM_001098538	Proline rich 4 (lacrimal)	LPRP, PROL4		
PTGS2	NM_000963	Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	COX-2, COX2, GRIPGHS, PGG/HS, PGHS-2, PHS-2, hCox-2	May have a role as a major mediator of inflammation and/or a role for prostanoid signaling in activity-dependent plasticity	
PTPLAD1	NM_016395	Protein tyrosine phosphatase-like A domain containing 1	B-IND1, HACD3, HSPC121	Involved in Rac1-signaling pathways leading to the modulation of gene expression	
PTPRZ1	NM_001206838	Protein tyrosine phosphatase, receptor-type, Z polypeptide 1	HPTPZ, HPTPζ, PTP-ζ, PTP18, PTPRZ, PTPZ, R-PTP-ζ-2, RPTPB, RPTPβ, phosphacan	May be involved in the regulation of specific developmental processes in the central nervous system	
RAB8B	NM_016530	RAB8B, member RAS oncogene family		May be involved in vesicular trafficking and neurotransmitter release	
RASSF6	NM_001270391	Ras association (RalGDS/AF-6) domain family member 6		May act as a Ras effector protein	

Table 5 (*Continued*)

Gene symbol	Accession number	Full name	Alias	Function	Cancer gene
RBM15	NM_001201545	RNA binding motif protein 15	OTT, OTT1, SPEN	May be implicated in HOX gene regulation	
RLF	NM_012421	Rearranged L-myc fusion	RP1-39G22.1, ZN-15L, ZNF292L	May be involved in transcriptional regulation	
ROPN1L	NM_001201466	Rhophilin associated tail protein 1-like	RP11-1C1.7, ASP, RSPH11		
RPS14	NM_001025070	Ribosomal protein S14	PRO2640, EMTB, S14		
RTEL1-TNFRSF6B	NR_037882	RTEL1-TNFRSF6B readthrough (NMD candidate)			
S100A1	NM_006271	S100 calcium binding protein A1	RP1-178F15.1, S100, S100-α, S100A	Weakly binds calcium but binds zinc very tightly-distinct binding sites with different affinities exist for both ions on each monomer	
SCAMP2	NM_005697	Secretory carrier membrane protein 2		Functions in post-Golgi recycling pathways. Acts as a recycling carrier to the cell surface	
SEPT2	NM_001008491	Septin 2	DIFF6, NEDD-5, NEDD5, Pnutl3, hNedd5	Required for normal progress through mitosis. Involved in cytokinesis	
SF3B3	NM_012426	Splicing factor 3b, subunit 3	RSE1, SAP130, SF3b130, STAF13	Subunit of the splicing factor SF3B required for 'A' complex assembly formed by the stable binding of U2 snRNP to the branch point sequence in pre-mRNA	
SH3BGRL	NM_003022	SH3 domain binding glutamate-rich protein like	HEL-S-115, SH3BGR		
SIX6	NM_007374	SIX homeobox 6	MCOPCT2, OPTX2, Six9	May be involved in eye development	
SLC37A3	NM_001287498	Solute carrier family 37, member 3			
SLC7A11	NM_014331	Solute carrier family 7 (anionic amino acid transporter light chain, xc-system), member 11	CCBR1, xCT	Sodium-independent, high-affinity exchange of anionic amino acids with high specificity for anionic form of cystine and glutamate	
SLCO2A1	NM_005630	Solute carrier organic anion transporter family, member 2A1	MATR1, OATP2A1, PGT, PHOAR2, SLC21A2	May mediate the release of newly synthesized prostaglandins from cells, the transepithelial transport of prostaglandins, and the clearance of prostaglandins from the circulation	
SMAD5	NM_001001419	SMAD family member 5	DWFC, JV5-1, MADH5	Transcriptional modulator activated by BMP type I receptor kinase	
SMCHD1	NM_015295	Structural maintenance of chromosomes flexible hinge domain containing 1		Required for maintenance of X inactivation in females and hypermethylation of CpG islands associated with inactive X	
SNAI2	NM_003068	Snail family zinc finger 2	SLUG, SLUGH1, SNAIL2, WS2D	Transcriptional repressor. Involved in the generation and migration of neural crest cells	
SRPK2	NM_001278273	SRSF protein kinase 2	SFRSK2	Phosphorylates RS domain-containing proteins	
TAAR6	NM_175067	Trace amine associated receptor 6	RP11-295F4.3, TA4, TAR4, TAR6, TRAR4, taR-4, taR-6	Orphan receptor. Could be a receptor for trace amines	
TAF15	NM_003487	TAF15 RNA polymerase II, TBP-associated factor	Npl3, RBP56, TAF2N, TAFII68	RNA and ssDNA-binding protein that may play specific roles during transcription initiation at distinct promoters	Yes

(*Continued*)

Table 5 (*Continued*)

Gene symbol	Accession number	Full name	Alias	Function	Cancer gene
TAF2	NM_003184	TAF2 RNA polymerase II, TBP-associated factor	CIF150, MRT40B, TAFII150, TAF2	Transcription factor TFIID is one of the general factors required for accurate and regulated initiation by RNA polymerase II	
TAF6L	NM_006473	TAF6-like RNA PCAF-associated factor	PAF65A	Functions as a component of the PCAF complex	
TBX4	NM_018488	T-box 4	SPS	Involved in the transcriptional regulation of genes required for mesoderm differentiation	
TCF21	NM_003206	Transcription factor 21	POD1, bHLHa23	Involved in epithelial–mesenchymal interactions in kidney and lung morphogenesis that include epithelial differentiation and branching morphogenesis	
THUMPD1	NM_017736	THUMP domain containing 1			
TM9SF3	NM_020123	Transmembrane 9 superfamily member 3	RP11-34E5.1, EP70-P-iso, SMBP		
TMEM14A	NM_014051	Transmembrane protein 14A	PTD011, C6orf73		
TMEM45A	NM_018004	Transmembrane protein 45A	DERP7		
TMPO	NM_001032283	Thymopoietin	CMD1T, LAP2, LEMD4, PRO0868, TP	May help direct the assembly of the nuclear lamina and thereby help maintain the structural organization of the nuclear envelope	
TMPRSS11A	NM_001114387	Transmembrane protease, serine 11A	ECRG1	Probable serine protease, which may play a role in cellular senescence	
TNPO1	NM_002270	Transportin 1	IPO2, KPNB2, MIP, MIP1, TRN	Functions in nuclear protein import as nuclear transport receptor	
TNRC6C	NM_001142640	Trinucleotide repeat containing 6C		Plays a role in RNA-mediated gene silencing by micro-RNAs	
TRUB1	NM_139169	TruB pseudouridine (psi) synthase family member 1	PUS4	May be responsible for synthesis of psi from uracil in transfer RNAs	
TSG101	NM_006292	Tumor susceptibility 101	TSG10, VPS23	Component of the ESCRT-I complex, a regulator of vesicular trafficking process	
TSHR	NM_000369	Thyroid stimulating hormone receptor	CHNG1, LGR3, hTSHR-I	Receptor for thyrothropin. Plays a central role in controlling thyroid cell metabolism	Yes
TUSC1	NM_001004125	Tumor suppressor candidate 1	TSG-9, TSG9		
TWF1	NM_001242397	Twinfilin actin-binding protein 1	A6, PTK9	Actin-binding protein involved in motile and morphological processes	
UGT3A1	NM_001171873	UDP glycosyltransferase 3 family, polypeptide A1		UDP-glucuronosyltransferases catalyze phase II biotransformation reactions	
USP28	NM_001301029	Ubiquitin specific peptidase 28		Deubiquitinase involved in DNA damage response checkpoint and MYC proto-oncogene stability	
VBP1	NM_003372	Von Hippel-Lindau binding protein 1	RP13-228J13.4, PFD3, PFDN3, VBP-1	Binds specifically to c-CPN and transfers target proteins to it	
WDR33	NM_001006622	WD repeat domain 33	NET14, WDC146	Essential for both cleavage and poly-adenylation of pre-mRNA 3′ ends	
WNT16	NM_016087	Wingless-type MMTV integration site family, member 16		Ligand for members of the Frizzled family of seven transmembrane receptors	
WNT2	NM_003391	Wingless-type MMTV integration site family member 2	INT1L1, IRP	Ligand for members of the Frizzled family of seven transmembrane receptors	

Table 5 (*Continued*)

Gene symbol	Accession number	Full name	Alias	Function	Cancer gene
WNT3A	NM_033131	Wingless-type MMTV integration site family, member 3A		Ligand for members of the Frizzled family of seven transmembrane receptors	
YY1	NM_003403	YY1 transcription factor	DELTA, INO80S, NF-E1, UCRBP, YIN-YANG-1	May play an important role in development and differentiation	
ZIC2	NM_007129	Zinc family member 2	HPE5	Involved in cerebellar development	
ZNF12	NM_006956	Zinc finger protein 12	GIOT-3, HZF11, KOX3, ZNF325	May be involved in transcriptional regulation	
ZNF121	NM_001008727	Zinc finger protein 121	D19S204, ZHC32, ZNF20	May be involved in transcriptional regulation	
ZNF132	NM_003433	Zinc finger protein 132	pHZ-12	May be involved in transcriptional regulation	
ZNF180	NM_001278508	Zinc finger protein 180	HHZ168	May be involved in transcriptional regulation	
ZNF238	NM_001278196	Zinc finger and BTB domain containing 18	C2H2-171, MRD22, RP58, TAZ-1, ZNF18	Sequence-specific DNA-binding protein with transcriptional repression activity	
ZNF25	NM_145011	Zinc finger protein 25	KOX19, Zfp9	May be involved in transcriptional regulation	
ZNF30	NM_001099437	Zinc finger protein 30	KOX28	May be involved in transcriptional regulation	
ZNF426	NM_001300883	Zinc finger protein 426		May be involved in transcriptional regulation	
ZNF558	NM_144693	Zinc finger protein 558		May be involved in transcriptional regulation	
ZNF562	NM_001130031	Zinc finger protein 562		May be involved in transcriptional regulation	
ZNF564	NM_144976	Zinc finger protein 564		May be involved in transcriptional regulation	
ZNF594	NM_032530	Zinc finger protein 594	hCG_1775942	May be involved in transcriptional regulation	
ZNF644	NM_016620	Zinc finger protein 644	BM-005, MYP21, NatF, ZEP-2	May be involved in transcriptional regulation	
ZNF652	NM_001145365	Zinc finger protein 652		Functions as a transcriptional repressor	
ZNF700	NM_001271848	Zinc finger protein 700		May be involved in transcriptional regulation	
ZNF703	NM_025069	Zinc finger protein 703	ZEPPO1, ZNF503L, ZPO1	May function as a transcriptional repressor	
ZNF711	NM_021998	Zinc finger protein 711	CMPX1, MRX97, ZNF4, ZNF5, ZNF6, Zfp711, dJ75N13.1	May be involved in transcriptional regulation	
ZNF763	NM_001012753	Zinc finger protein 763	ZNF, ZNF440L	May be involved in transcriptional regulation	
ZNF780A	NM_001010880	Zinc finger protein 780A	ZNF780	May be involved in transcriptional regulation	

Abbreviations: CLL, chronic lymphocytic leukemia; HLA, human leukocyte antigen; HSP, heat shock protein; IGF, insulin-like growth factor; IL, interleukin; mRNA, messenger RNA; NK cells, natural killer cells; ssDNA, single-stranded DNA; UV, ultraviolet.

CCDC6, *CCND1*, *CDX2*, *EP300*, *HOXA11*, *KRAS*, *PLAG1*, *TAF15*, and *TSHR*.

As shown in Table 6, our DAVID analysis showed that there were 16 functional clusters that were identified to be enriched with an enrichment score >1.0 in the target list of hsa-miR-181a-5p, based on TarBase. The functions of these clusters involved negative regulation of macromolecule biosynthetic process, negative regulation of the cellular biosynthetic process, negative regulation of biosynthetic process, negative regulation of apoptosis, negative regulation of programmed cell death, negative regulation of cell death, negative regulation of transcription, negative regulation of nucleobase, nucleoside, nucleotide, and nucleic acid metabolic processes, negative regulation of nitrogen compound metabolic process,

Table 6 The top enriched clusters (enrich score >1) by DAVID for the targets of hsa-miR-181a-5p from TarBase 6.0

Category	Term	Gene count	*P*-value	FDR
Annotation cluster 1	**Enrichment score: 4.3**			
INTERPRO	Zinc finger, C2H2-type	24	2.20E-05	4.60E-03
INTERPRO	Zinc finger, C2H2-like	24	2.90E-05	3.90E-03
SMART	Zinc finger_C2H2	24	2.00E-04	1.90E-02
Annotation cluster 2	**Enrichment score: 3.04**			
GOTERM_BP_FAT	Negative regulation of macromolecule biosynthetic process	17	7.10E-04	9.80E-02
GOTERM_BP_FAT	Negative regulation of cellular biosynthetic process	17	9.30E-04	1.20E-01
GOTERM_BP_FAT	Negative regulation of biosynthetic process	17	1.20E-03	9.50E-02
Annotation cluster 3	**Enrichment score: 2.97**			
GOTERM_BP_FAT	Negative regulation of apoptosis	13	9.70E-04	1.10E-01
GOTERM_BP_FAT	Negative regulation of programmed cell death	13	1.10E-03	1.00E-01
GOTERM_BP_FAT	Negative regulation of cell death	13	1.10E-03	9.70E-02
Annotation cluster 4	**Enrichment score: 2.67**			
GOTERM_BP_FAT	Negative regulation of transcription	15	1.00E-03	1.00E-01
GOTERM_BP_FAT	Negative regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	15	2.90E-03	1.50E-01
GOTERM_BP_FAT	Negative regulation of nitrogen compound metabolic process	15	3.20E-03	1.50E-01
Annotation cluster 5	**Enrichment score: 2.14**			
GOTERM_BP_FAT	Lung development	6	6.20E-03	2.30E-01
GOTERM_BP_FAT	Respiratory tube development	6	7.00E-03	2.40E-01
GOTERM_BP_FAT	Respiratory system development	6	8.90E-03	2.50E-01
Annotation cluster 6	**Enrichment score: 1.75**			
GOTERM_BP_FAT	Positive regulation of protein polymerization	4	3.00E-03	1.50E-01
GOTERM_BP_FAT	Positive regulation of protein complex assembly	4	7.90E-03	2.60E-01
GOTERM_BP_FAT	Regulation of protein polymerization	4	4.60E-02	4.90E-01
GOTERM_BP_FAT	Regulation of protein complex assembly	4	9.00E-02	6.00E-01
Annotation cluster 7	**Enrichment score: 1.59**			
GOTERM_BP_FAT	Regulation of phosphorylation	12	2.20E-02	3.50E-01
GOTERM_BP_FAT	Regulation of phosphate metabolic process	12	2.80E-02	3.90E-01
GOTERM_BP_FAT	Regulation of phosphorus metabolic process	12	2.80E-02	3.90E-01
Annotation cluster 8	**Enrichment score: 1.57**			
INTERPRO	Secreted growth factor Wnt protein, conserved site	3	1.90E-02	7.90E-01
INTERPRO	Secreted growth factor Wnt protein	3	1.90E-02	7.90E-01
INTERPRO	Wnt superfamily	3	1.90E-02	7.90E-01
PIR_SUPERFAMILY	PIRSF001784:int-1 transforming protein	3	2.00E-02	8.60E-01
SMART	Wnt1	3	2.50E-02	5.50E-01
GOTERM_BP_FAT	Wnt receptor signaling pathway, calcium modulating pathway	3	2.50E-02	3.70E-01
KEGG_PATHWAY	Basal cell carcinoma	3	1.40E-01	7.00E-01
Annotation cluster 9	**Enrichment score: 1.56**			
GOTERM_BP_FAT	Positive regulation of microtubule polymerization	3	8.40E-03	2.50E-01
GOTERM_BP_FAT	Regulation of microtubule polymerization	3	9.80E-03	2.60E-01
GOTERM_BP_FAT	Positive regulation of microtubule polymerization or depolymerization	3	9.80E-03	2.60E-01
GOTERM_BP_FAT	Regulation of microtubule polymerization or depolymerization	3	5.10E-02	5.10E-01
GOTERM_BP_FAT	Regulation of microtubule cytoskeleton organization	3	8.70E-02	6.00E-01
GOTERM_BP_FAT	Regulation of microtubule-based process	3	1.10E-01	6.60E-01
Annotation cluster 10	**Enrichment score: 1.49**			
GOTERM_BP_FAT	Positive regulation of cytoskeleton organization	5	1.90E-03	1.20E-01
GOTERM_BP_FAT	Positive regulation of organelle organization	5	1.60E-02	3.30E-01
GOTERM_BP_FAT	Regulation of cytoskeleton organization	5	7.60E-02	5.80E-01
GOTERM_BP_FAT	Regulation of cellular component biogenesis	5	8.60E-02	6.00E-01
GOTERM_BP_FAT	Positive regulation of cellular component organization	5	1.60E-01	7.30E-01
Annotation cluster 11	**Enrichment score: 1.36**			
UP_SEQ_FEATURE	DNA-binding region: Homeobox	7	1.60E-02	4.70E-01
INTERPRO	Homeobox, conserved site	7	4.60E-02	9.10E-01
INTERPRO	Homeobox	7	4.90E-02	9.00E-01
INTERPRO	Homeodomain-related	7	5.10E-02	8.60E-01
SMART	Hox	7	8.50E-02	7.60E-01

Table 6 (*Continued*)

Category	Term	Gene count	*P*-value	FDR
Annotation cluster 12	**Enrichment score: 1.35**			
UP_SEQ_FEATURE	Domain: BTB	6	1.80E-02	4.90E-01
INTERPRO	BTB/POZ-like	6	5.10E-02	8.80E-01
INTERPRO	BTB/POZ fold	6	5.30E-02	8.40E-01
SMART	Btb	6	8.40E-02	8.10E-01
Annotation cluster 13	**Enrichment score: 1.31**			
INTERPRO	Peptidyl-prolyl *cis-trans* isomerase, FKBP-type	3	2.10E-02	7.60E-01
SP_PIR_KEYWORDS	Rotamase	3	5.90E-02	5.00E-01
GOTERM_MF_FAT	Peptidyl-prolyl *cis-trans* isomerase activity	3	6.50E-02	9.50E-01
GOTERM_MF_FAT	*cis-trans* Isomerase activity	3	7.10E-02	9.30E-01
Annotation cluster 14	**Enrichment score: 1.27**			
GOTERM_BP_FAT	Regulation of apoptosis	16	5.10E-02	5.10E-01
GOTERM_BP_FAT	Regulation of programmed cell death	16	5.50E-02	5.20E-01
GOTERM_BP_FAT	Regulation of cell death	16	5.60E-02	5.20E-01
Annotation cluster 15	**Enrichment score: 1.26**			
GOTERM_BP_FAT	Positive regulation of protein kinase activity	7	4.80E-02	5.00E-01
GOTERM_BP_FAT	Positive regulation of kinase activity	7	5.50E-02	5.20E-01
GOTERM_BP_FAT	Positive regulation of transferase activity	7	6.40E-02	5.40E-01
Annotation cluster 16	**Enrichment score: 1.09**			
SP_PIR_KEYWORDS	RNA-mediated gene silencing	3	3.30E-02	4.10E-01
GOTERM_BP_FAT	Gene silencing by RNA	3	5.70E-02	5.20E-01
GOTERM_BP_FAT	Gene silencing	3	1.40E-01	6.90E-01
SP_PIR_KEYWORDS	Translation regulation	3	1.60E-01	7.60E-01

Abbreviations: DAVID, Database for Annotation, Visualization and Integrated Discovery; FDR, false discovery rate.

and lung and respiratory tube development, positive regulation of protein polymerization, positive regulation of protein complex assembly, positive regulation of protein polymerization, and positive regulation of protein complex assembly.

Furthermore, our DAVID analysis revealed that there were nine KEGG pathways significantly enriched in the target list of hsa-miR-181a-5p, based on TarBase (Table 7). These pathways included pathways in cancer pathways (Figure 1), the Wnt signaling pathway (Figure 2), prostate cancer, melanogenesis, cell cycle (Figure 3), hedgehog signaling pathway, p53 signaling pathway (Figure 4), small cell lung cancer, and thyroid cancer.

Validated targets of hsa-miR-181a-5p based on miRTarBase

Based on miRTarBase, 241 targets of hsa-miR-181a-5p have been validated with experimental evidence (Table 8). These included *ACOT12*, *AFTPH*, *AKAP12*, *AMMECR1*, *ANKRD1*, *ANKRD13C*, *AP1M1*, *ARF6*, *ARHGAP12*, *ARL6IP6*, *ATF7IP2*, *ATG10*, *ATM*, *ATP6V0E1*, *ATP8A1*, *BAG2*, *BCL2*, *BCL2L11*, *BDNF*, *BPGM*, *BRCA1*, *BRMS1L*, *BTBD3*, *C1orf109*, *C1QTNF9*, *C8A*, *CCDC6*, *CCNG1*, *CD46*, and *CDKN1B*. Among these validated targets, only 18 are cancer genes (7.47%), including *ATM*, *BCL2*, *CCDC6*, *CDX2*, *FBXO11*, *H3F3B*, *HOOK3*, *HOXA11*, *HRAS*, *KRAS*, *MAP2K1*, *NOTCH1*, *NOTCH2*, *PLAG1*, *PTPN11*, *STAG2*, *TAF15*, and *TSHR* (Table 8). Only half of these cancer genes have been included in TarBase.

As shown in Table 9, our DAVID analysis showed that there were 20 functional clusters that were identified to be enriched with an enrichment score >1.0 in the target list of hsa-miR-181a-5p, based on miRTarBase. The functions of these clusters involved negative regulation of transcription, negative regulation of gene expression, negative regulation of nucleobase, nucleoside, nucleotide, and nucleic acid metabolic processes, negative regulation of nitrogen compound metabolic process, negative regulation of macromolecule biosynthetic process, negative regulation

Table 7 The KEGG pathways by DAVID, for the target list of hsa-miR-181a-5p based on TarBase 6.0

Signaling pathway	Gene count	%	*P*-value	FDR
Pathways in cancer	11	0.5	4.50E-03	1.30E-01
Wnt signaling pathway	8	0.4	1.80E-03	1.50E-01
Prostate cancer	6	0.3	3.80E-03	1.60E-01
Melanogenesis	5	0.2	2.90E-02	4.10E-01
Cell cycle	5	0.2	5.90E-02	5.00E-01
Hedgehog signaling pathway	4	0.2	2.80E-02	4.70E-01
p53 signaling pathway	4	0.2	4.60E-02	4.50E-01
Small cell lung cancer	4	0.2	7.60E-02	5.50E-01
Thyroid cancer	3	0.1	4.50E-02	5.00E-01

Abbreviations: DAVID, Database for Annotation, Visualization and Integrated Discovery; FDR, false discovery rate; KEGG, Kyoto Encyclopedia of Genes and Genomes.

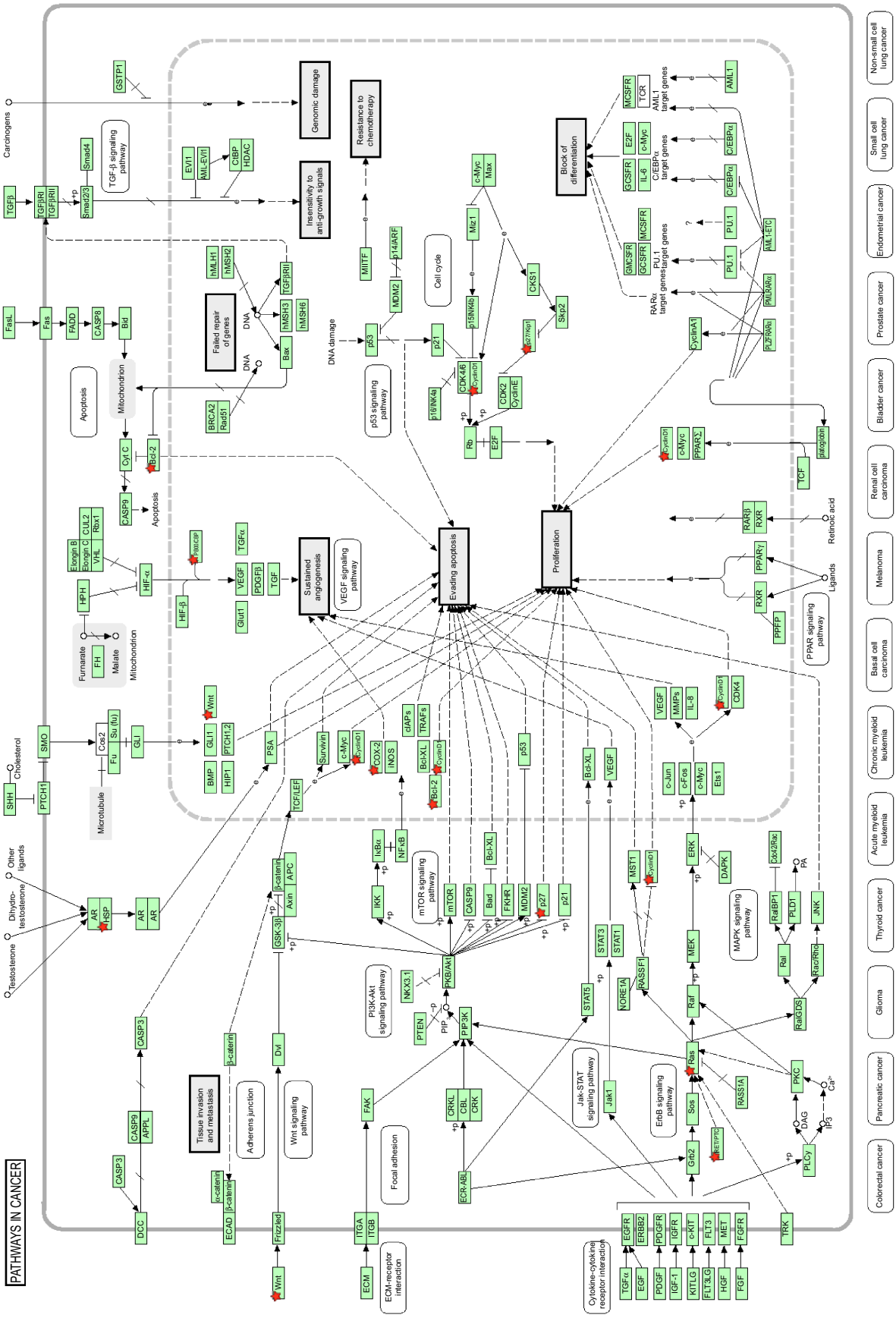

Figure 1 Cancer pathways in the target list of hsa-miR-181a-5p, based on TarBase 6.0.
Notes: Several important oncogenes and tumor suppressors are likely regulated by hsa-miR-181a-5p (marked with a red star), including Wnt, axin, CBP, Bcl-2, p27, cyclin D1, Ras, and HSP. These genes play an important role in the regulation of angiogenesis, cell proliferation, apoptosis, and metastasis.

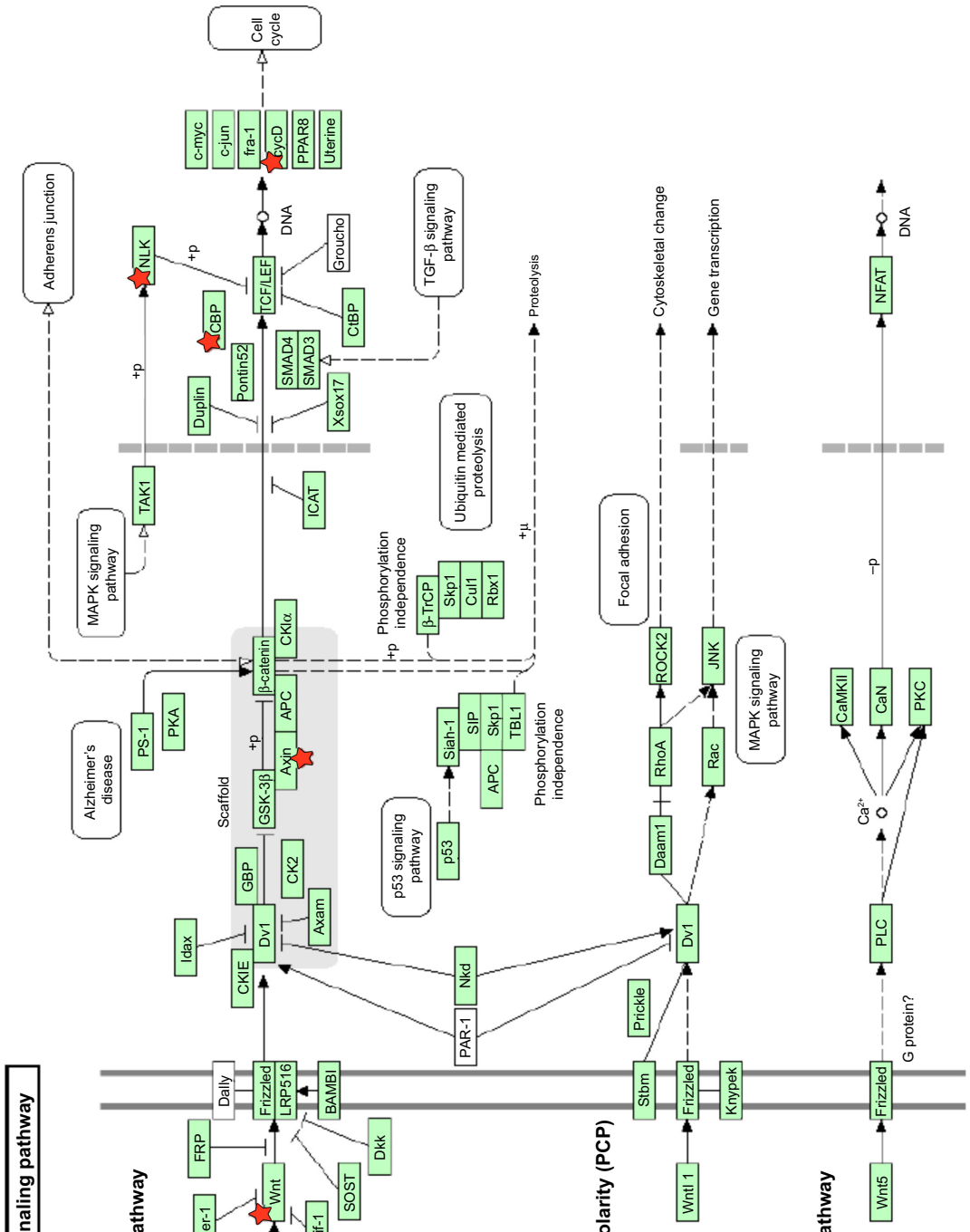

Figure 2 Wnt signaling pathway in the target list of hsa-miR-181a-5p, based on TarBase 6.0.

Notes: hsa-181a-5p is a regulator of Wnt. Three Wnt signaling pathways have been characterized: the canonical Wnt pathway, the noncanonical planar cell polarity pathway, and the noncanonical Wnt/calcium pathway.[103] All three Wnt signaling pathways are activated by the binding of a Wnt-protein ligand to a Frizzled family receptor, which passes the biological signal to the protein Dishevelled inside the cell. The canonical Wnt pathway leads to regulation of gene transcription, the noncanonical planar cell polarity pathway regulates the cytoskeleton that is responsible for the shape of the cell, and the noncanonical Wnt/calcium pathway regulates calcium level inside the cell. Wnt signaling pathways are highly evolutionarily conserved. Wnt signaling has been implicated in the development of breast cancer, EC, and other types of cancer.[103,104] Changes in CTNNB1 expression, which is the gene that encodes β-catenin, can be measured in, not just breast cancer but also, colorectal cancer, melanoma, prostate cancer, lung cancer, EC, and several other cancer types. Increased expression of Wnt ligand-proteins, such as Wnt 1, Wnt2, and Wnt7A, has been observed in the development of glioblastoma, esophageal cancer, EC, and ovarian cancer. There is clinical and experimental evidence that Wnt/β-catenin pathways are deregulated and play an important role in the initiation, development, growth, and metastasis of EC.[105–109] Targets of hsa-miR-181a-5p are marked with a red star.

Abbreviation: EC, endometrial cancer.

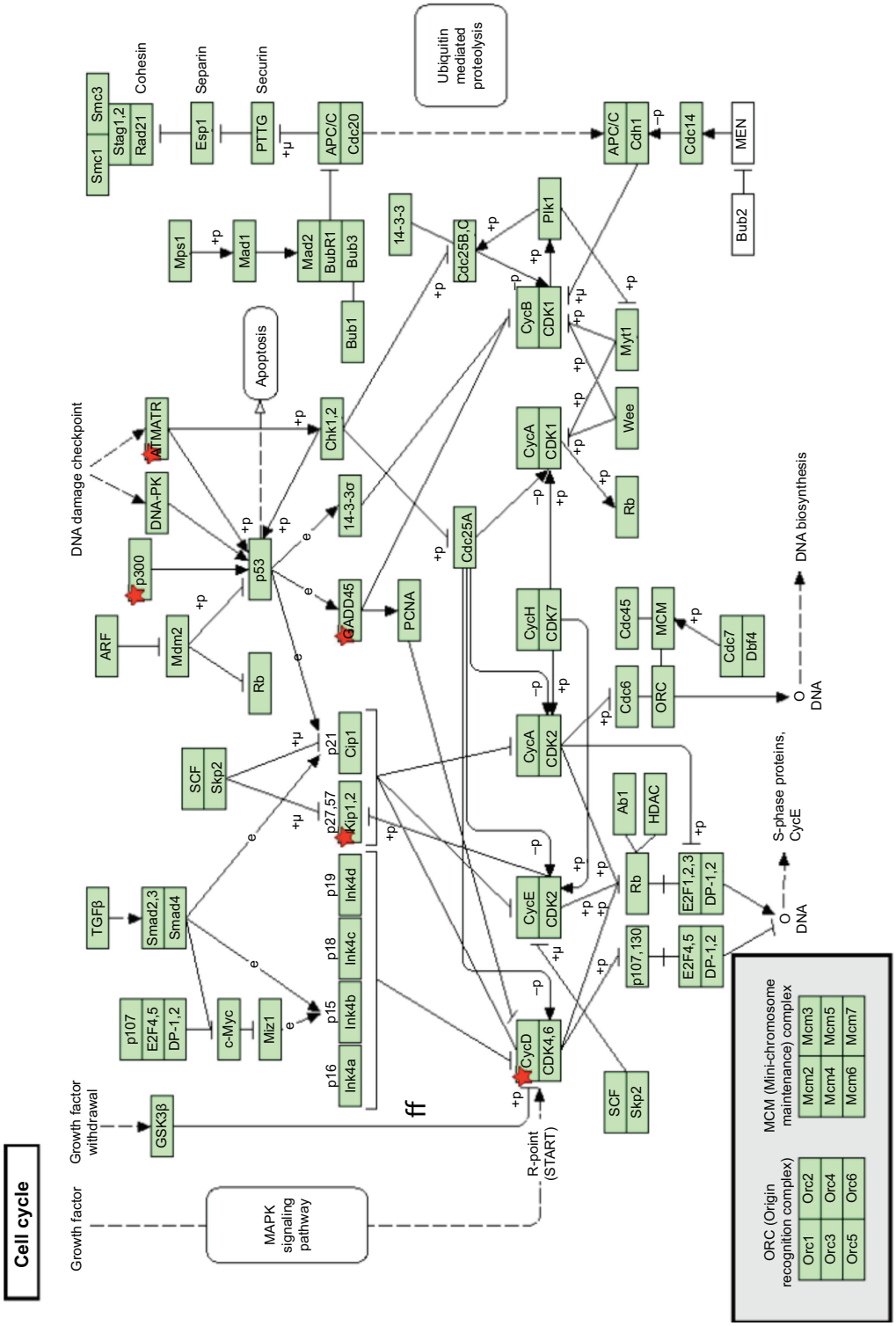

Figure 3 Cell cycle pathway in the target list of hsa-miR-181a-5p, based on TarBase 6.0.

Notes: hsa-miR-181a-5p can regulate a number of important proteins that control the cell cycle. Control of eukaryotic cell growth and division involves molecular circuits known as "checkpoints" that ensure proper timing of cellular events. Passage through a checkpoint from one cell cycle phase to the next needs a coordinated set of proteins that monitor cell growth and DNA integrity. Uncontrolled cell division or propagation of damaged DNA can contribute to genomic instability and tumorigenesis.[110] The G_1/S checkpoint controls progression of cells through the restriction point (R) into the DNA synthesis S phase. During G_1, the tumor suppressor Rb binds and inhibits transcription factor E2F. Phosphorylation of Rb by CDKs in late G_1 induces dissociation of Rb and permits E2F-mediated transcription of S phase-promoting genes. Responding to upstream signals, INK4 and Kip/Cip family inhibitors control CDK activity and prevent entry into S phase.[110] DNA damage activates response pathways through ATM/ATR and Chk1/2 kinases to block CDK activity, leading to cell cycle arrest and DNA repair or cell death. The G_2/M checkpoint prevents cells containing damaged DNA from entering mitosis. Activated CDK1/Cdc2 bound to cyclin B promotes entry into M phase. Wee1 and Myt1 kinases and Cdc25 phosphatase competitively regulate CDK1 activity; Wee1 and Myt1 inhibit CDK1 and prevent entry into M phase, while Cdc25 removes inhibitory phosphates. DNA damage activates multiple kinases that phosphorylate kinases Chk1/2 and tumor suppressor protein p53. Chk1/2 kinases stimulate Wee1 activity and inhibit Cdc25C, preventing entry into M phase. Phosphorylation of p53 promotes dissociation between p53 and MDM2 and allows binding of the transcription factor to DNA. In addition, the spindle checkpoint ensures proper chromatid attachment prior to progression from metaphase to anaphase.[110] The SCF and APC/C protein complexes play prominent roles, with APC-Cdc20 initiating the entry into anaphase by promoting ubiquitin-mediated degradation of multiple substrates, including cyclin B and the regulatory protein securing. Targets of hsa-miR-181a-5p are marked with a red star.

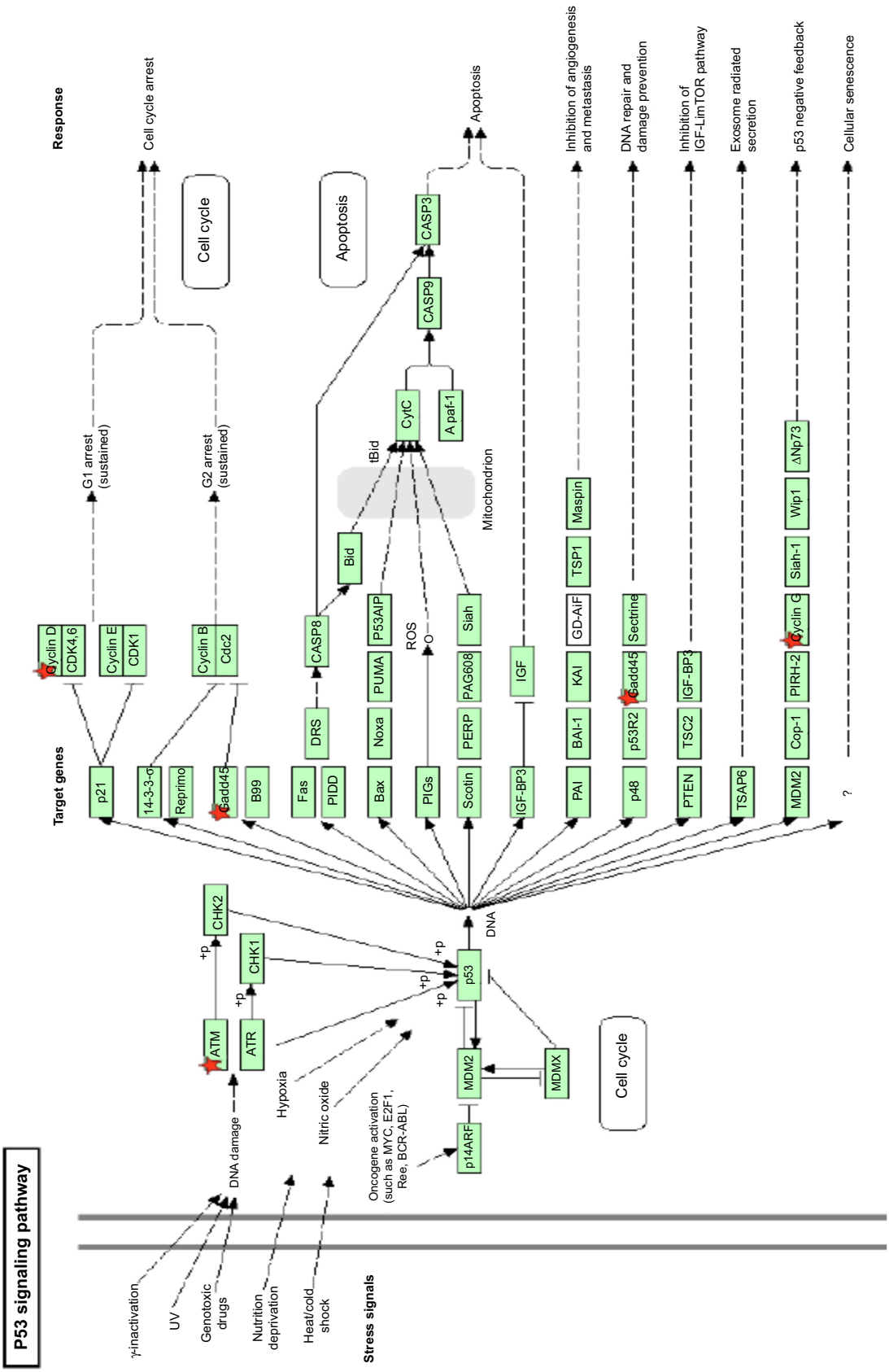

Figure 4 p53 pathway in the target list of hsa-miR-181a-5p, based on TarBase 6.0.

Notes: hsa-miR-181a-5p can regulate the p53 signaling pathway. p53 is situated at the crossroads of a network of signaling pathways that are essential for cell growth regulation and apoptosis induced by genotoxic and nongenotoxic stresses. In normal unstressed cells, the level of p53 protein is downregulated via the binding of proteins such as MDM2, COP1, PIRH2, or JNK, which promote p53 degradation via the ubiquitin/proteasome pathway. As most of these genes are upregulated by p53, this leads to a regulation loop that will keep p53 level very low in normal cells. After exposure to genotoxic or nongenotoxic stresses, p53 protein level is increased via the inhibition of its interaction with MDM2 and the other negative regulators. A series of modulators, such as kinases and acetylases, will activate p53 transcriptional activity. Regardless of the type of stress, the final outcome of p53 activation is either cell cycle arrest and DNA repair or apoptosis. Dysfunctional p53 due to mutations will lead to tumorigenesis. p53 mutations can be found in 50% of human cancers including EC, but their penetrance is highly heterogeneous. Mutations in various pathways upstream of p53 (eg, ATM, p19ARF, or Hcdk2 gene) can be observed in various types of cancer, including EC. Targets of hsa-miR-181a-5p are marked with a red star.

Abbreviation: EC, endometrial cancer.

Table 8 Targets of hsa-miR-181a-5p with experimental evidence based on miRTarBase 4.0

Gene symbol	Accession	Full name	Alias	Function	Cancer gene
ACOT12	NM_130767	Acyl-CoA thioesterase 12	CACH-1, Cach, STARD15, THEAL	Hydrolyzes acetyl-CoA to acetate and CoA	
AFTPH	NM_001002243	Aftiphilin	Nbla10388	May play a role in membrane trafficking	
AKAP12	NM_005100	A kinase anchor protein 12	AKAP250, SSeCKS	Anchoring protein that mediates the subcellular compartmentation of PKA and PKC	
AMMECR1	NM_001025580	Alport syndrome, mental retardation, midface hypoplasia and elliptocytosis chromosomal region gene 1	RP13-360B22.1, AMMERC1		
ANKRD1	NM_014391	Ankyrin repeat domain 1 (cardiac muscle)	ALRP, C-193, CARP, CVARP, MCARP, bA320F15.2	Plays an important role in endothelial cell activation	
ANKRD13C	NM_030816	Ankyrin repeat domain 13C	RP4-677H15.5, dJ677H15.3		
AP1M1	NM_001130524	Adaptor-related protein complex 1, mu 1 subunit	AP47, CLAPM2, CLTNM, MU-1A	Subunit of clathrin-associated adaptor protein complex 1 that plays a role in protein sorting in the trans-Golgi network and endosomes	
ARF6	NM_001663	ADP-ribosylation factor 6		Involved in protein trafficking	
ARHGAP12	NM_001270695	Rho GTPase activating protein 12		GTPase activator for the Rho-type GTPases by converting them to an inactive GDP-bound state	
ARL6IP6	NM_022989	ADP-ribosylation factor-like 6 interacting protein 6	RP23-265N10.1, 2310057C01Rik, 2610529A11Rik, Aip-6	May be involved in protein transport, membrane trafficking, or cell signaling during hematopoietic maturation	
ATF7IP2	NM_001256160	Activating transcription factor 7 interacting protein 2	MCAF2	Recruiter that couples transcriptional factors to general transcription apparatus and thereby modulates transcription regulation and chromatin formation	
ATG10	NM_001131028	Autophagy related 10	PP12616, APG10, APG10L, pp12616	Plays a role in autophagy	
ATM	NM_000051	ATM serine/threonine kinase	AT1, ATA, ATC, ATD, ATDC, ATE, TEL1, TELO1	Serine/threonine protein kinase	Yes
ATP6V0E1	NM_003945	ATPase, H^+ transporting, lysosomal 9 kDa, V0 subunit e1	ATP6H, ATP6V0E, M9.2, Vma21, Vma21p	Vacuolar ATPase is responsible for acidifying a variety of intracellular compartments in eukaryotic cells	
ATP8A1	NM_001105529	ATPase, aminophospholipid transporter (APLT), class I, type 8A, member 1	ATPASEII, ATPIA, ATPP2	May play a role in the transport of aminophospholipids from the outer to the inner leaflet of various membranes and the maintenance of asymmetric distribution of phospholipids	
BAG2	NM_004282	BCL2-associated athanogene 2	RP3-496N17.2, BAG-2, dJ417I1.2	Inhibits the chaperone activity of HSP70/HSC70 by promoting substrate release	
BCL2	NM_000633	B-cell CLL/lymphoma 2	Bcl-2, PPP1R50	Suppresses apoptosis	Yes
BCL2L11	NM_001204106	BCL2-like 11	BAM, BIM, BOD	Induces apoptosis	
BDNF	NM_001143805	Brain-derived neurotrophic factor	ANON2, BULN2	Promotes the survival of neuronal populations	
BPGM	NM_001293085	2,3-bisphosphoglycerate mutase	DPGM	Plays a major role in regulating hemoglobin oxygen affinity	
BRCA1	NM_007294	Breast cancer 1, early onset	BRCAI, BRCC1, BROVCA1, IRIS, PNCA4, PPP1R53, PSCP, RNF53	Plays a central role in DNA repair by facilitating cellular response to DNA repair	

BRMS1L	NM_032352	Breast cancer metastasis-suppressor 1-like	BRMS1	Involved in the HDAC1-dependent transcriptional repression activity	
BTBD3	NM_001282550	BTB (POZ) domain containing 3	RP4-742J24.3, dJ742J24.1	Acts as a key regulator of dendritic field orientation during development of sensory cortex	
C1orf109	NM_017850	Chromosome 1 open reading frame 109			
C1QTNF9	NM_183175	C1q and tumor necrosis factor related protein 9	9130217G22Rik, CTRP9, Ciqtnf9	Activates AMPK, AKT, and p44/42 MAPK signaling pathways	
C8A	NM_000562	Complement component 8, α polypeptide		C8 is a constituent of the membrane attack complex	
CCDC6	NM_005436	Coiled-coil domain containing 6	D10S170, H4, PTC, TPC, TST1	A tumor suppressor	Yes
CCNG1	NM_004060	Cyclin G1	CCNG	May play a role in growth regulation	
CD46	NM_002389	CD46 molecule, complement regulatory protein	AHUS2, MCP, MIC10, TLX, TRA2.10	Acts as a cofactor for complement factor I	
CDKN1B	NM_004064	Cyclin-dependent kinase inhibitor 1B (p27, Kip1)	CDKN4, KIP1, MEN1B, MEN4, P27KIP1	Important regulator of cell cycle progression	
CDX2	NM_001265	Caudal type homeobox 2	CDX-3, CDX3	Involved in the transcriptional regulation of multiple genes expressed in the intestinal epithelium	Yes
CFI	NM_000204	Complement factor I	AHUS3, ARMD13, C3BINA, C3b-INA, FI, IF, KAF	Responsible for cleaving the α-chains of C4b and C3b in the presence of the cofactors C4-binding protein and factor H, respectively	
CHL1	NM_001253387	Cell adhesion molecule L1-like	CALL, L1CAM2	Plays a role in nervous system development and in synaptic plasticity	
CHRFAM7A	NM_139320	CHRNA7 (cholinergic receptor, nicotinic, α 7, exons 5–10) and FAM7A (family with sequence similarity 7A, exons A-E) fusion	CHRNA7, CHRNA7-DR1, D-10	Extracellular ligand-gated ion channel activity	
CLUAP1	NM_015041	Clusterin associated protein 1	CFAP22, FAP22	May play a role in cell proliferation or apoptosis	
COL27A1	NM_032888	Collagen, type XXVII, α 1	RP11-82I1.1	Plays a role during the calcification of cartilage and the transition of cartilage to bone	
COPS2	NM_001143887	COP9 signalosome subunit 2	ALIEN, CSN2, SGN2, TRIP15	Involved in various cellular and developmental processes	
CST5	NM_001900	Cystatin D		Cysteine proteinase inhibitor	
DCST1	NM_001143687	DC-STAMP domain containing 1	RP11-307C12.10-003	Protein and zinc ion binding	
DDIT4	NM_019058	DNA-damage-inducible transcript 4	RP11-442H21.1, Dig2, REDD-1, REDD1	Inhibits cell growth by regulating the frap1 pathway upstream of the tsc1-tsc2 complex and downstream of Akt1	
DDX27	NM_017895	DEAD (Asp-Glu-Ala-Asp) box polypeptide 27	HSPC259, DRS1, Drs1p, PP3241, RHLP, dJ686N3.1	Probable ATP-dependent RNA helicase	
DDX3X	NM_001193416	DEAD (Asp-Glu-Ala-Asp) box helicase 3, X-linked	DBX, DDX14, DDX3, HLP2	ATP-dependent RNA helicase	
DNAJC7	NM_001144766	DnaJ (HSP40) homolog, subfamily C, member 7	DJ11, DJC7, TPR2, TTC2	Acts as co-chaperone regulating the molecular chaperones HSP70 and HSP90 in folding of steroid receptors	
DSCR8	NM_032589	Down syndrome critical region gene 8	C21orf65, CT25.1a, CT25.1b, MMA-1, MMA-1a, MMA-1b, MMA1, MTAG2		
DUSP5	NM_004419	Dual specificity phosphatase 5	DUSP, HVH3	Displays phosphatase activity toward several substrates	

(*Continued*)

Table 8 (*Continued*)

Gene symbol	Accession	Full name	Alias	Function	Cancer gene
DUSP6	NM_001946	Dual specificity phosphatase 6	HH19, MKP3, PYST1	Inactivates MAP kinases	
ENAH	NM_001008493	Enabled homolog	RP11-496N12.7, ENA, MENA, NDPP1	Ena/VASP proteins are actin-associated proteins involved in a range of processes dependent on cytoskeleton remodeling and cell polarity	
EPHA5	NM_001281765	EPH receptor A5	CEK7, EHK-1, EHK1, EK7, HEK7, TYRO4	Receptor for members of the ephrin-A family	
ESR1	NM_000125	Estrogen receptor 1	RP1-130E4.1, ER, ESR, ESRA, ESTRR, Era, NR3A1	Nuclear hormone receptor	
EYA4	NM_001301012	EYA transcriptional coactivator and phosphatase 4	RP11-704J17.4, CMD1J, DFNA10	Tyrosine phosphatase that specifically dephosphorylates 'Tyr-142' of histone H2AX (H2AXY142ph)	
FAM160A2	NM_001098794	Family with sequence similarity 160, member A2	C11orf56		
FAM222B	NM_001077498	Family with sequence similarity 222, member B	C17orf63		
FAM47B	NM_152631	Family with sequence similarity 47, member B	RP13-520K9.1		
FAT1	NM_005245	FAT atypical cadherin 1	CDHF7, CDHR8, FAT, ME5, hFat1	Could function as a cell-adhesion protein	
FBXO11	NM_001190274	F-box protein 11	UG063H01, FBX11, PRMT9, UBR6, VIT1	Substrate recognition component of the SCF E3 ubiquitin-protein ligase complex	Yes
FBXO28	NM_001136115	F-box protein 28	CENP-30, Fbx28	Probably recognizes and binds to some phosphorylated proteins and promotes their ubiquitination and degradation	
FBXO33	NM_203301	F-box protein 33	BMND12, Fbx33, c14_5247	Substrate recognition component of the SCF E3 ubiquitin-protein ligase complex, which mediates the ubiquitination and subsequent proteasomal degradation of target proteins	
FBXO34	NM_017943	F-box protein 34	CGI-301, Fbx34	Substrate recognition component of the SCF E3 ubiquitin-protein ligase complex, which mediates the ubiquitination and subsequent proteasomal degradation of target proteins	
FKBP10	NM_021939	FK506 binding protein 10	PSEC0056, FKBP65, OI11, OI6, PPIASE, hFKBP65	PPIases accelerate the folding of proteins during protein synthesis	
FKBP7	NM_001135212	FK506 binding protein 7	UNQ670/PRO1304, FKBP23, PPIase	PPIases accelerate the folding of proteins during protein synthesis	
FOS	NM_005252	FBJ murine osteosarcoma viral oncogene homolog	AP-1, C-FOS, p55	Nuclear phosphoprotein, which forms a tight but noncovalently linked complex with the JUN/AP-1 transcription factor	
FRA10AC1	NM_145246	Fragile site, folic acid type, rare, fra(10)(q23.3) or fra(10)(q24.2) candidate 1	PRO2972, C10orf4, F26C11.1-like, FRA10A		
FSIP1	NM_152597	Fibrous sheath interacting protein 1	HSD10		
FXYD6	NM_001164831	FXYD domain containing ion transport regulator 6	UNQ521/PRO1056		
GADD45G	NM_006705	Growth arrest and DNA-damage-inducible, γ	RP11-260L6.1, CR6, DDIT2, GADD45 γ, GRP17	Involved in the regulation of growth and apoptosis	

GANAB	NM_001278192	Glucosidase, α; neutral AB	G2AN, GLUII	Cleaves sequentially the 2 innermost α-1,3-linked glucose residues from the Glc(2) Man(9)GlcNAc(2) oligosaccharide precursor of immature glycoproteins	
GATA6	NM_005257	GATA binding protein 6		Regulates terminal differentiation and/or proliferation	
GATAD2B	NM_020699	GATA zinc finger domain containing 2B	RP11-216N14.6, MRD18, P66β, p68	Has transcriptional repressor activity	
GCNT1	NM_001097633	Glucosaminyl (N-acetyl) transferase 1, core 2	RP11-214N16.1, C2GNT, C2GNT-L, C2GNT1, G6NT, NACGT2, NAGCT2	Forms critical branches in O-glycans	
GIGYF1	NM_022574	GRB10 interacting GYF protein 1	PP3360, GYF1, PERQ1	May act cooperatively with GRB10 to regulate tyrosine kinase receptor signaling	
GNAI3	NM_006496	G protein, α inhibiting activity polypeptide 3	RP5-1160K1.2, 87U6, ARCND1	G proteins are involved as modulators or transducers in various transmembrane signaling systems	
GPR137B	NM_003272	G protein-coupled receptor 137B	RP5-985L19.1, TM7SF1		
GPR78	NM_080819	G protein-coupled receptor 78	UNQ5925/PRO19818	Orphan receptor	
GPR83	NM_016540	G protein-coupled receptor 83	GIR, GPR72	Orphan receptor. Could be a neuropeptide y receptor	
GPRIN3	NM_198281	GPRIN family member 3	GRIN3	May be involved in neurite outgrowth	
GSTM2	NM_000848	Glutathione *S*-transferase mu 2 (muscle)	GST4, GSTM-2, GTHMUS, GSTM2	Conjugation of reduced glutathione to a wide number of exogenous and endogenous hydrophobic electrophiles	
H1F0	NM_005318	H1 histone family, member 0	H10, H1FV	Histones H1 are necessary for the condensation of nucleosome chains into higher order structures	
H2AFY	NM_001040158	H2A histone family, member Y	H2A.y, H2A/y, H2AF12M, H2AFJ, MACROH2A1.1, mH2A1, macroH2A1.2	Plays a central role in transcription regulation, DNA repair, DNA replication, and chromosomal stability	
H3F3B	NM_005324	H3 histone, family 3B	H3.3B	Plays a central role in transcription regulation, DNA repair, DNA replication, and chromosomal stability	Yes
HDAC6	NM_006044	Histone deacetylase 6	JM21, CPBHM, HD6, PPP1R90	Responsible for the deacetylation of lysine residues on the N-terminal part of the core histones (H2A, H2B, H3, and H4)	
HERC3	NM_001271602	HECT and RLD domain containing E3 ubiquitin protein ligase 3		E3 ubiquitin-protein ligase	
HEY2	NM_012259	Hes-related family bHLH transcription factor with YRPW motif 2	RP1-293L8.3, CHF1, GRIDLOCK, GRL, HERP1, HESR2, HRT2, bHLHb32	Downstream effector of Notch signaling, which may be required for cardiovascular development	
HIPK2	NM_001113239	Homeodomain interacting protein kinase 2	PRO0593	Protein kinase acting as a corepressor of several transcription factors	
HMGB2	NM_001130688	High mobility group box 2	HMG2	Binds preferentially ssDNA and unwinds double-stranded DNA	
HNRNPAB	NM_004499	Heterogeneous nuclear ribonucleoprotein A/B	ABBP1, HNRPAB	Binds single-stranded RNA	
HOOK3	NM_032410	Hook microtubule-tethering protein 3	HK3	Probable cytoskeletal linker protein, involved in tethering the Golgi complex to the cytoskeleton	Yes

(*Continued*)

Table 8 (*Continued*)

Gene symbol	Accession	Full name	Alias	Function	Cancer gene
HOXA11	NM_005523	Homeobox A11	HOX1, HOX11	Sequence-specific transcription factor	Yes
HRAS	NM_001130442	Harvey rat sarcoma viral oncogene homolog	C-BAS/HAS, C-H-RAS, C-HA-RAS1, CTLO, H-RASIDX, HAMSV1, RASH1, p21ras, HRAS	Ras proteins bind GDP/GTP and possess intrinsic GTPase activity	Yes
HSD17B3	NM_000197	Hydroxysteroid (17-β) dehydrogenase 3	RP11-240L7.3, EDH17B3, SDR12C2	Favors the reduction of androstenedione to testosterone	
HSPA13	NM_006948	Heat shock protein 70 kDa family, member 13	STCH	Has peptide-independent ATPase activity	
HUWE1	NM_031407	HECT, UBA and WWE domain containing 1	RP3-339A18.4, ARF-BP1, HECTH9, HSPC272, Ib772, LASU1, MULE, URE-B1, UREB1	E3 ubiquitin-protein ligase mediating ubiquitination and subsequent proteasomal degradation of target proteins	
IDS	NM_000202	Iduronate 2-sulfatase	MPS2, SIDS	Required for the lysosomal degradation of heparan sulfate and dermatan sulfate	
INCENP	NM_001040694	Inner centromere protein antigens		Component of the chromosomal passenger complex, a complex that acts as a key regulator of mitosis	
IQCG	NM_001134435	IQ motif containing G	CFAP122, DRC9		
KAT2B	NM_003884	K(lysine) acetyltransferase 2B	CAF, P/CAF, PCAF	Functions as a HAT to promote transcriptional activation	
KBTBD3	NM_152433	Kelch repeat and BTB (POZ) domain containing 3	BKLHD3		
KCTD2	NM_015353	Potassium channel tetramerization domain containing 2			
KCTD3	NM_016121	Potassium channel tetramerization domain containing 3	RP11-5F19.1, NY-REN-45		
KIAA0100	NM_014680	KIAA0100	BCOX, BCOX1, CT101		
KIAA0101	NM_001029989	KIAA0101	L5, NS5ATP9, OEATC, OEATC-1, OEATC1, PAF, PAF15, p15(PAF), p15/PAF, p15PAF	May be involved in protection of cells from UV-induced cell death	
KIAA1462	NM_020848	KIAA1462	JCAD		
KLF6	NM_001160124	Kruppel-like factor 6	RP11-184A2.1, BCD1, CBA1, COPEB, CPBP, GBF, PAC1, ST12, ZF9	Plays a role in B-cell growth and development	
KLHL15	NM_030624	Kelch-like family, member 15	HEL-S-305	Probable substrate-specific adapter of an E3 ubiquitin-protein ligase complex, which mediates the ubiquitination and subsequent proteasomal degradation of target proteins	
KLHL42	NM_020782	Kelch-like family, member 42	Ctb9, KLHDC5	Substrate-specific adapter of a BCR (BTB-CUL3-RBX1) E3 ubiquitin-protein ligase complex required for mitotic progression and cytokinesis	
KLRC4	NM_013431	Killer cell lectin-like receptor subfamily C, member 4	NKG2-F, NKG2F	May play a role as a receptor for the recognition of MHC class I HLA-E molecules by NK cells	
KRAS	NM_004985	Kirsten rat sarcoma viral oncogene homolog	C-K-RAS, CFC2, K-RAS2A, K-RAS2B, K-RAS4A, K-RAS4B, KI-RAS1, KRAS2, NS, NS3, RASK2, KRAS	Binds GDP/GTP and possesses intrinsic GTPase activity	Yes

LAMA3	NM_000227	Laminin, α 3	BM600, E170, LAMNA, LOCS, lama3a	Binding to cells via a high-affinity receptor, mediating the attachment, migration, and organization of cells into tissues	
LBR	NM_002296	Lamin B receptor	PRO0650, DHCR14B, LMN2R, PHA, TDRD18	Anchors the lamina and the heterochromatin to the inner nuclear membrane	
LCLAT1	NM_001002257	Lysocardiolipin acyltransferase 1	UNQ1849/PRO3579, 1AGPAT8, AGPAT8, ALCAT1, HSRG1849, LYCAT, UNQ1849	Acyl-CoA: lysocardiolipin acyltransferase	
LFNG	NM_001040167	LFNG *O*-fucosylpeptide 3-β-*N*-acetylglucosaminyltransferase	SCDO3	Glycosyltransferase	
LGALSL	NM_014181	Lectin, galactoside-binding-like	HSPC159, GRP	Does not bind lactose and may not bind carbohydrates	
LPGAT1	NM_014873	Lysophosphatidylglycerol acyltransferase 1	FAM34A, FAM34A1, NET8	Lysophoshatidylglycerol-specific acyltransferase	
LRRC17	NM_001031692	Leucine rich repeat containing 17	UNQ3076/PRO9909, P37NB	Involved in bone homeostasis, acting as a negative regulator of RANKL-induced osteoclast precursor differentiation from bone marrow precursors	
LRRN3	NM_001099658	Leucine rich repeat neuronal 3	Nbla10363, FIGLER5, NLRR-3, NLRR3		
LYSMD3	NM_001286812	LysM, putative peptidoglycan-binding, domain containing 3			
MAP2K1	NM_002755	Mitogen-activated protein kinase kinase 1	CFC3, MAPKK1, MEK1, MKK1, PRKMK1	Catalyzes the concomitant phosphorylation of a threonine and a tyrosine residue in a Thr-Glu-Tyr sequence located in MAP kinases	Yes
MAZ	NM_001042539	MYC-associated zinc finger protein (purine-binding transcription factor)	PUR1, Pur-1, SAF-1, SAF-2, SAF-3, ZF87, ZNF801, Zif87	May function as a transcription factor with dual roles in transcription initiation and termination	
MCL1	NM_001197320	Myeloid cell leukemia 1	BCL2L3, EAT-ES, MCL1L, MCL1S, Mcl-1, TM, bcl2-L-3, mcl1/EAT, MCL1	Involved in the regulation of apoptosis versus cell survival, and in the maintenance of viability but not of proliferation	
METAP1	NM_015143	Methionyl aminopeptidase 1	MAP1A, MetAP1A	Removes the amino terminal methionine from nascent proteins	
MGAT5	NM_002410	Mannosyl (α-1,6-)-glycoprotein β-1,6-*N*-acetyl-glucosaminyltransferase	GNT-V, GNT-VA	Catalyzes the addition of N-acetylglucosamine in β 1–6 linkage to the α-linked mannose of biantennary N-linked oligosaccharides	
MOB1A	NM_018221	MOB kinase activator 1A	C2orf6, MATS1, MOB1, MOBK1B, MOBKL1B, Mob4B	Activator of LATS1/2 in the Hippo signaling pathway	
MOB1B	NM_001244766	MOB kinase activator 1B	MATS2, MOB4A, MOBKL1A	Activator of LATS1/2 in the Hippo signaling pathway	
MOB3B	NM_024761	MOB kinase activator 3B	C9orf35, MOB1D, MOBKL2B	May regulate the activity of kinases	
MRPS14	NM_022100	Mitochondrial ribosomal protein S14	DJ262D12.2, HSMRPS14, MRP-S14, S14mt		
MTMR12	NM_001040446	Myotubularin related protein 12	3-PAP, PIP3AP	Inactive phosphatase that plays a role as an adapter for the phosphatase myotubularin, to regulate myotubularin intracellular location	
MTMR3	NM_021090	Myotubularin related protein 3	hCG_2011013, FYVE-DSP1, ZFYVE10	Phosphatase that acts on lipids with a phosphoinositol head group	

(*Continued*)

Table 8 (*Continued*)

Gene symbol	Accession	Full name	Alias	Function	Cancer gene
ND2		NADH dehydrogenase subunit 2		Core subunit of the mitochondrial membrane respiratory chain NADH dehydrogenase (Complex I)	
NFYB	NM_006166	Nuclear transcription factor Yβ	CBF-A, CBF-B, HAP3, NF-YB	Stimulates the transcription of various genes by recognizing and binding to a CCAAT motif in promoters	
NKX3-2	NM_001189	NK3 homeobox 2	BAPX1, NKX3.2, NKX3B, SMMD	Transcriptional repressor that acts as a negative regulator of chondrocyte maturation	
NLK	NM_016231	Nemo-like kinase		Role in cell fate determination, required for differentiation of bone marrow stromal cells	
NMRK2	NM_001289117	Nicotinamide riboside kinase 2	ITGB1BP3, MIBP, NRK2		
NOL4	NM_001198546	Nucleolar protein 4	HRIHFB2255, CT125, NOLP		
NOTCH1	NM_017617	Notch 1	TAN1, hN1	Functions as a receptor for membrane-bound ligands Jagged1, Jagged2, and Delta1 to regulate cell fate determination	Yes
NOTCH2	NM_001200001	Notch 2	AGS2, HJCYS, hN2	Functions as a receptor for membrane-bound ligands Jagged1, Jagged2, and Delta1 to regulate cell fate determination	Yes
NR6A1	NM_001278546	Nuclear receptor subfamily 6, group A, member 1	CT150, GCNF, GCNF1, NR61, RTR, hGCNF, hRTR	Orphan nuclear receptor. May be involved in the regulation of gene expression in germ cell development during gametogenesis	
NRP1	NM_001024628	Neuropilin 1	RP11-342D11.1, BDCA4, CD304, NP1, NRP, VEGF165R	The membrane-bound isoform 1 is a receptor involved in the development of the cardiovascular system, in angiogenesis, in the formation of certain neuronal circuits, and in organogenesis outside the nervous system	
NUDT12	NM_001300741	Nudix-type motif 12		Hydrolyzes NAD(P)H to NMNH and AMP (2′,5′-ADP), and diadenosine diphosphate to AMP	
NUPL1	NM_001008564	Nucleoporin like 1	RP11-206I15.1, PRO2463	Component of the nuclear pore complex, a complex required for the trafficking across the nuclear membrane	
OCA2	NM_000275	Oculocutaneous albinism II	BEY, BEY1, BEY2, BOCA, D15S12, EYCL, EYCL2, EYCL3, HCL3, P, PED, SHEP1	Could be involved in the transport of tyrosine	
OFCC1	NM_153003	Orofacial cleft 1 candidate 1	MRDS1		
OR11A1	NM_013937	Olfactory receptor, family 11, subfamily A, member 1	DAAP-3411.2, 6M1-18, OR11A2, dJ994E9.6, hs6M1-18	Odorant receptor	
OTUD1	NM_001145373	OTU deubiquitinase 1	DUBA7, OTDC1	Deubiquitinating enzyme that specifically hydrolyzes 'Lys-63'-linked polyubiquitin to monoubiquitin	
OTX2	NM_001270523	Orthodenticle homeobox 2	CPHD6, MCOPS5	Probably plays a role in the development of the brain and the sense organs	
PABPC1	NM_002568	Poly(A) binding protein, cytoplasmic 1	PAB1, PABP, PABP1, PABPC2, PABPL1	Binds the poly(A) tail of mRNA	

PCDHB8	NM_019120	Protocadherin β8	PCDH-β8, PCDH31	Potential calcium-dependent cell-adhesion protein	
PFKFB2	NM_001018053	6-phosphofructo-2-kinase/fructose-2, 6-biphosphatase 2	RP11-164O23.2, PFK-2/FBPase-2	Synthesis and degradation of fructose 2,6-bisphosphate	
PGD	NM_002631	Phosphogluconate dehydrogenase	6PGD	Catalyzes the oxidative decarboxylation of 6-phosphogluconate to ribulose 5-phosphate and CO2, with concomitant reduction of NADP to NADPH	
PHOX2A	NM_005169	Paired-like homeobox 2a	ARIX, CFEOM2, FEOM2, NCAM2, PMX2A	May be involved in regulating the specificity of expression of the catecholamine biosynthetic genes	
PHPT1	NM_001135861	Phosphohistidine phosphatase 1	RP11-216L13.10-005, CGI-202, HEL-S-132P, HSPC141, PHP14	Exhibits phosphohistidine phosphatase activity	
PITPNB	NM_001284277	Phosphatidylinositol transfer protein β	RP3-353E16.2, PI-TP-β, PtdInsTP, VIB1B	Catalyzes the transfer of PtdIns and phosphatidylcholine between membranes	
PLA2G4C	NM_001159322	Phospholipase A2, group IVC	CPLA2-γ	Has a preference for arachidonic acid at the sn-2 position of phosphatidylcholine as compared with palmitic acid	
PLAG1	NM_001114634	Pleiomorphic adenoma gene 1	PSA, SGPA, ZNF912	Transcription factor whose activation results in upregulation of target genes, such as IGFII, leading to uncontrolled cell proliferation	Yes
PLCL2	NM_001144382	Phospholipase C-like 2	PLCE2	May play a role in the regulation of Ins(1,4,5)P3 around the endoplasmic reticulum	
PLXDC2	NM_001282736	Plexin domain containing 2	UNQ2514/PRO6003, TEM7R	May play a role in tumor angiogenesis	
PPP1R9A	NM_001166160	protein phosphatase 1, regulatory subunit 9A	NRB1, NRBI, Neurabin-I	Binds to actin filaments (F-actin) and shows crosslinking activity	
PRAP1	NM_001145201	Proline-rich acidic protein 1	RP11-122K13.6, PRO1195, UPA	May play an important role in maintaining normal growth homeostasis in epithelial cells	
PRDX3	NM_006793	Peroxiredoxin 3	AOP-1, AOP1, HBC189, MER5, PRO1748, SP-22, prx-III	Involved in redox regulation of the cell	
PRLR	NM_000949	Prolactin receptor	HPRL, MAB, hPRLrI	This is a receptor for the anterior pituitary hormone prolactin	
PROSC	NM_007198	Proline synthetase co-transcribed homolog			
PROX1	NM_001270616	Prospero homeobox 1		May play a fundamental role in early development of the central nervous system	
PRR4	NM_001098538	Proline rich 4	LPRP, PROL4		
PRRC2B	NM_013318	Proline-rich coiled-coil 2B	RP11-334J6.1, BAT2L, BAT2L1, KIAA0515, LQFBS-1		
PTGS2	NM_000963	Prostaglandin-endoperoxide synthase 2	COX-2, COX2, GRIPGHS, PGG/HS, PGHS-2, PHS-2, hCox-2	May have a role as a major mediator of inflammation and/or a role for prostanoid signaling in activity-dependent plasticity	
PTPLAD1	NM_016395	Protein tyrosine phosphatase-like A domain containing 1	B-IND1, HACD3, HSPC121	Involved in Rac1-signaling pathways leading to the modulation of gene expression	

(*Continued*)

Table 8 (*Continued*)

Gene symbol	Accession	Full name	Alias	Function	Cancer gene
PTPN11	NM_002834	Protein tyrosine phosphatase, non-receptor type 11	BPTP3, CFC, NS1, PTP-1D, PTP2C, SH-PTP2, SH-PTP3, SHP2	Acts downstream of various receptor and cytoplasmic protein tyrosine kinases to participate in the signal transduction from the cell surface to the nucleus	Yes
PTPN22	NM_001193431	Protein tyrosine phosphatase, non-receptor type 22	LYP, LYP1, LYP2, PEP, PTPN8	Seems to act on Cbl	
PTPRZ1	NM_001206838	Protein tyrosine phosphatase, receptor-type, Z polypeptide 1	HPTPZ, HPTPζ, PTP-ζ, PTP18, PTPRZ, PTPZ, R-PTP-ζ-2, RPTPB, RPTPβ, phosphacan	May be involved in the regulation of specific developmental processes in the central nervous system	
PUM1	NM_001020658	Pumilio RNA-binding family member 1	RP1-65J11.4, HSPUM, PUMH, PUMH1, PUML1	Sequence-specific RNA-binding protein that regulates translation and mRNA stability by binding the 3′-UTR of mRNA targets	
RALA	NM_005402	V-ral simian leukemia viral oncogene homolog A	RAL	Multifunctional GTPase involved in a variety of cellular processes, including gene expression, cell migration, cell proliferation, oncogenic transformation, and membrane trafficking	
RASSF6	NM_001270391	Ras association (RalGDS/AF-6) domain family member 6		May act as a Ras effector protein	
RLF	NM_012421	Rearranged L-myc fusion	RP1-39G22.1, ZN-15L, ZNF292L	May be involved in transcriptional regulation.	
RNF2	NM_007212	Ring finger protein 2	GS1-120K12.1, BAP-1, BAP1, DING, HIPI3, RING1B, RING2	E3 ubiquitin-protein ligase that mediates mono-ubiquitination of "Lys-119" of histone H2A, playing a central role in histone code and gene regulation	
RNF34	NM_001256858	Ring finger protein 34	CARP-1, CARP1, RFI, RIF, RIFF, hRFI	Has E3 ubiquitin-protein ligase activity. Regulates the levels of CASP8 and CASP10 by targeting them for proteasomal degradation	
ROPN1L	NM_001201466	Rhophilin associated tail protein 1-like	RP11-1C1.7, ASP, RSPH11		
RPL14	NM_001034996	Ribosomal protein L14	CAG-ISL-7, CTG-B33, L14, RL14, hRL14		
RPS8	NM_001012	Ribosomal protein S8	RP11-269F19.3, S8		
S100A1	NM_006271	S100 calcium binding protein A1	RP1-178F15.1, S100, S100-α, S100A	Weakly binds calcium but binds zinc very tightly – distinct binding sites with different affinities exist for both ions on each monomer	
SCD	NM_005063	Stearoyl-CoA desaturase	PRO1933, FADS5, MSTP0081, SCDOS, SCD	Terminal component of the liver microsomal stearyl-CoA desaturase system	
SH3BGRL	NM_003022	SH3 domain binding glutamate-rich protein like	HEL-S-115, SH3BGR		
SIK2	NM_015191	Salt-inducible kinase 2	LOH11CR1I, QIK, SNF1LK2	Phosphorylates Ser794 of IRS1 in insulin-stimulated adipocytes	
SIRT1	NM_001142498	Sirtuin 1	RP11-57G10.3, SIR2L1	NAD-dependent deacetylase, which regulates processes such as apoptosis and muscle differentiation by deacetylating key proteins	
SIX6	NM_007374	SIX homeobox 6	MCOPCT2, OPTX2, Six9	May be involved in eye development	

SLC35B4	NM_032826	Solute carrier family 35 (UDP-xylose/UDP-*N*-acetylglucosamine transporter), member B4	PSEC0055, YEA, YEA4	Sugar transporter that specifically mediates the transport of UDP-Xyl and UDP-GlcNAc from cytosol into Golgi	
SLC37A3	NM_001287498	Solute carrier family 37, member 3			
SLC7A11	NM_014331	Solute carrier family 7 (anionic amino acid transporter light chain, xc-system), member 11	CCBR1, xCT	Sodium-independent, high-affinity exchange of anionic amino acids with high specificity for anionic form of cystine and glutamate	
SLCO2A1	NM_005630	Solute carrier organic anion transporter family, member 2A1	MATR1, OATP2A1, PGT, PHOAR2, SLC21A2	May mediate the release of newly synthesized prostaglandins from cells, the transepithelial transport of prostaglandins, and the clearance of prostaglandins from the circulation	
SMCHD1	NM_015295	Structural maintenance of chromosomes flexible hinge domain containing 1		Required for maintenance of X inactivation in females and hypermethylation of CpG islands associated with inactive X	
SMG1	NM_015092	SMG1 phosphatidylinositol 3-kinase-related kinase	61E3.4, ATX, LIP	Serine/threonine protein kinase involved in both mRNA surveillance and genotoxic stress response pathways	
SNAI2	NM_003068	Snail family zinc finger 2	SLUG, SLUGH1, SNAIL2, WS2D	Transcriptional repressor. Involved in the generation and migration of neural crest cells	
SOGA2	NM_015210	Microtubule crosslinking factor 1	CCDC165, KIAA0802, MTCL1		
SPRY2	NM_005842	Sprouty homolog 2	hSPRY2	May function as an antagonist of fibroblast growth factor pathways and may negatively modulate respiratory organogenesis	
SRPK2	NM_001278273	SRSF protein kinase 2	SFRSK2	Phosphorylates RS domain-containing proteins	
SRSF7	NM_001031684	Serine/arginine-rich splicing factor 7	9G8, AAG3, SFRS7	Required for pre-mRNA splicing	
STAG2	NM_001042749	Stromal antigen 2	RP11-517O1.1, SA-2, SA2, SCC3B, bA517O1.1	Component of cohesion complex, a complex required for the cohesion of sister chromatids after DNA replication	Yes
TAAR6	NM_175067	Trace amine associated receptor 6	RP11-295F4.3, TA4, TAR4, TAR6, TRAR4, taR-4, taR-6	Orphan receptor. Could be a receptor for trace amines	
TAB2	NM_001292034	TGF-β activated kinase 1/MAP3K7 binding protein 2	CHTD2, MAP3K7IP2, TAB-2	Adapter linking MAP3K7/TAK1 and TRAF6, and mediator of MAP3K7 activation in the IL1 signaling pathway	
TAF15	NM_003487	TAF15 RNA polymerase II, TBP-associated factor	Npl3, RBP56, TAF2N, TAFII68	RNA and ssDNA-binding protein that may play specific roles during transcription initiation at distinct promoters	Yes
TAF2	NM_003184	TAF2 RNA polymerase II, TBP-associated factor	CIF150, MRT40B, TAFII150, TAF2	Transcription factor TFIID is one of the general factors required for accurate and regulated initiation by RNA polymerase II	
TAF6L	NM_006473	TAF6-like RNA PCAF-associated factor	PAF65A	Functions as a component of the PCAF complex	
TBX4	NM_018488	T-box 4	SPS	Involved in the transcriptional regulation of genes required for mesoderm differentiation	

(*Continued*)

Table 8 (*Continued*)

Gene symbol	Accession	Full name	Alias	Function	Cancer gene
TCF21	NM_003206	Transcription factor 21	POD1, bHLHa23	Involved in epithelial–mesenchymal interactions in kidney and lung morphogenesis that include epithelial differentiation and branching morphogenesis	
TEAD4	NM_003213	TEA domain family, member 4	EFTR-2, RTEF1, TCF13L1, TEF-3, TEF3, TEFR-1, hRTEF-1B	Binds specifically and noncooperatively to the Sph and GT-IIC "enhansons" (5′-GTGGAATGT-3′) and activates transcription	
TGFBR3	NM_001195683	Transforming growth factor β receptor III	BGCAN, β-glycan	Binds to TGF-β	
TGIF2	NM_001199513	TGFB-induced factor homeobox 2		Transcriptional repressor. Probably represses transcription via the recruitment of HDAC proteins	
TIAL1	NM_001033925	TIA1 cytotoxic granule-associated RNA binding protein-like 1	TCBP, TIAR	RNA-binding protein. Possesses nucleolytic activity against cytotoxic lymphocyte target cells May be involved in apoptosis	
TM9SF3	NM_020123	Transmembrane 9 superfamily member 3	RP11-34E5.1, EP70-P-iso, SMBP		
TMED4	NM_182547	Transmembrane emp24 protein transport domain containing 4	ERS25, HNLF	Involved in endoplasmic reticulum stress response. May play a role in the regulation of heat-shock response and apoptosis	
TMEM132B	NM_001286219	Transmembrane protein 132B			
TMEM14A	NM_014051	Transmembrane protein 14A	PTD011, C6orf73		
TMEM192	NM_001100389	Transmembrane protein 192			
TMEM257	NM_004709	Transmembrane protein 257	CXorf1		
TMEM45A	NM_018004	Transmembrane protein 45A	DERP7		
TMEM64	NM_001008495	Transmembrane protein 64			
TMPRSS11A	NM_001114387	Transmembrane protease, serine 11A	ECRG1	Probable serine protease, which may play a role in cellular senescence	
TNIP1	NM_001252385	TNFAIP3 interacting protein 1	ABIN-1, NAF1, VAN, nip40-1	Interacts with zinc finger protein A20/TNFAIP3 and inhibits TNF-induced NF-κB-dependent gene expression by interfering with an RIP- or TRAF2-mediated transactivation signal	
TRIM2	NM_001130067	Tripartite motif containing 2	CMT2R, RNF86	May contribute to the alteration of neural cellular mechanisms	
TSHR	NM_000369	Thyroid stimulating hormone receptor	CHNG1, LGR3, hTSHR-I	Receptor for thyrothropin. Plays a central role in controlling thyroid cell metabolism	Yes
TUSC1	NM_001004125	Tumor suppressor candidate 1	TSG-9, TSG9		
UBA2	NM_005499	Ubiquitin-like modifier activating enzyme 2	HRIHFB2115, ARX, SAE2	The dimeric enzyme acts as a E1 ligase for SUMO1, SUMO2, SUMO3, and probably SUMO4	
UCHL1	NM_004181	Ubiquitin carboxyl-terminal esterase L1	HEL-117, NDGOA, PARK5, PGP 9.5, PGP9.5, PGP95, Uch-L1	Ubiquitin-protein hydrolase involved both in the processing of ubiquitin precursors and of ubiquitinated proteins	

UGT3A1	NM_001171873	UDP glycosyltransferase 3 family, polypeptide A1		UDP-glucuronosyltransferases catalyze phase II biotransformation reactions
USP28	NM_001301029	Ubiquitin specific peptidase 28		Deubiquitinase involved in DNA damage response checkpoint and MYC proto-oncogene stability
WNT16	NM_016087	Wingless-type MMTV integration site family, member 16		Ligand for members of the Frizzled family of seven transmembrane receptors
WNT2	NM_003391	Wingless-type MMTV integration site family, member 2	INT1L1, IRP	Ligand for members of the Frizzled family of seven transmembrane receptors
WNT3A	NM_033131	Wingless-type MMTV integration site family, member 3A		Ligand for members of the Frizzled family of seven transmembrane receptors
XIAP	NM_001167	X-linked inhibitor of apoptosis	RP1-315G1.5, API3, BIRC4, IAP-3, ILP1, MIHA, XLP2, hIAP-3, hIAP3	Apoptotic suppressor
YOD1	NM_001276320	YOD1 deubiquitinase	RP11-164O23.1, DUBA8, OTUD2, PRO0907	May play an important regulatory role at the level of protein turnover, by preventing degradation
ZEB2	NM_001171653	Zinc finger E-box binding homeobox 2	HRIHFB2411, HSPC082, SIP-1, SIP1, SMADIP1, ZFHX1B	Transcriptional inhibitor that binds to DNA sequence 5′-CACCT-3′ in different promoters Represses transcription of E-cadherin
ZFP36L2	NM_006887	Zinc finger protein 36 homolog	BRF2, ERF-2, ERF2, RNF162C, TIS11D	Probable regulatory protein involved in regulating the response to growth factors
ZNF12	NM_006956	Zinc finger protein 12	GIOT-3, HZF11, KOX3, ZNF325	May be involved in transcriptional regulation
ZNF148	NM_021964	Zinc finger protein 148	BERF-1, BFCOL1, HT-β, ZBP-89, ZFP148, pHZ-52	Involved in transcriptional regulation
ZNF25	NM_145011	Zinc finger protein 25	KOX19, Zfp9	May be involved in transcriptional regulation
ZNF35	NM_003420	Zinc finger protein 35	HF.10, HF10, Zfp105	May be involved in transcriptional regulation
ZNF350	NM_021632	Zinc finger protein 350	ZBRK1, ZFQR	Transcriptional repressor
ZNF426	NM_001300883	Zinc finger protein 426		May be involved in transcriptional regulation
ZNF445	NM_181489	Zinc finger protein 445	ZKSCAN15, ZNF168, ZSCAN47	May be involved in transcriptional regulation
ZNF558	NM_144693	Zinc finger protein 558		May be involved in transcriptional regulation
ZNF562	NM_001130031	Zinc finger protein 562		May be involved in transcriptional regulation
ZNF594	NM_032530	Zinc finger protein 594	hCG_1775942	May be involved in transcriptional regulation
ZNF652	NM_001145365	Zinc finger protein 652		Functions as a transcriptional repressor
ZNF763	NM_001012753	Zinc finger protein 763	ZNF, ZNF440L	May be involved in transcriptional regulation

Abbreviations: CLL, chronic lymphocytic leukemia; HLA, human leukocyte antigen; IGF, insulin-like growth factor; IL, interleukin; mRNA, messenger RNA; NK cells, natural killer cells; ssDNA, single-stranded DNA; TGF, transforming growth factor; TNF, tumor necrosis factor; UV, ultraviolet.

Table 9 The top enriched clusters (enrich score >1) by DAVID for the targets of hsa-miR-181a-5p from miRTarBase 4.0

Category	Term	Gene count	*P*-value	FDR
Annotation cluster 1	**Enrichment score: 2.9**			
GOTERM_BP_FAT	Negative regulation of transcription	17	3.10E-04	7.80E-02
GOTERM_BP_FAT	Negative regulation of gene expression	17	8.50E-04	9.90E-02
GOTERM_BP_FAT	Negative regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	17	1.00E-03	1.00E-01
GOTERM_BP_FAT	Negative regulation of nitrogen compound metabolic process	17	1.20E-03	1.00E-01
GOTERM_BP_FAT	Negative regulation of macromolecule biosynthetic process	17	2.00E-03	1.20E-01
GOTERM_BP_FAT	Negative regulation of cellular biosynthetic process	17	2.50E-03	1.20E-01
GOTERM_BP_FAT	Negative regulation of biosynthetic process	17	3.10E-03	1.20E-01
Annotation cluster 2	**Enrichment score: 2.86**			
GOTERM_BP_FAT	Regulation of phosphorylation	16	1.10E-03	1.00E-01
GOTERM_BP_FAT	Regulation of phosphorus metabolic process	16	1.60E-03	1.10E-01
GOTERM_BP_FAT	Regulation of phosphate metabolic process	16	1.60E-03	1.10E-01
Annotation cluster 3	**Enrichment score: 2.66**			
GOTERM_BP_FAT	Lung development	7	1.80E-03	1.10E-01
GOTERM_BP_FAT	Respiratory tube development	7	2.10E-03	1.20E-01
GOTERM_BP_FAT	Respiratory system development	7	2.80E-03	1.20E-01
Annotation cluster 4	**Enrichment score: 2.38**			
GOTERM_BP_FAT	Positive regulation of transcription, DNA-dependent	16	1.30E-03	1.00E-01
GOTERM_BP_FAT	Positive regulation of transcription	16	6.50E-03	1.70E-01
GOTERM_BP_FAT	Positive regulation of gene expression	16	8.50E-03	1.80E-01
Annotation cluster 5	**Enrichment score: 2.37**			
GOTERM_BP_FAT	Mesenchymal cell differentiation	5	4.20E-03	1.50E-01
GOTERM_BP_FAT	Mesenchymal cell development	5	4.20E-03	1.50E-01
GOTERM_BP_FAT	Mesenchyme development	5	4.50E-03	1.50E-01
Annotation cluster 6	**Enrichment score: 2.16**			
GOTERM_BP_FAT	Negative regulation of apoptosis	12	6.40E-03	1.70E-01
GOTERM_BP_FAT	Negative regulation of programmed cell death	12	7.10E-03	1.70E-01
GOTERM_BP_FAT	Negative regulation of cell death	12	7.20E-03	1.70E-01
Annotation cluster 7	**Enrichment score: 2.12**			
BIOCARTA	IGF-1 signaling pathway	4	7.60E-03	3.80E-01
BIOCARTA	IL-6 signaling pathway	4	7.60E-03	3.80E-01
BIOCARTA	Insulin signaling pathway	4	7.60E-03	3.80E-01
Annotation cluster 8	**Enrichment score: 2.03**			
SP_PIR_KEYWORDS	Ubl conjugation pathway	15	2.80E-03	9.30E-02
GOTERM_BP_FAT	Modification-dependent macromolecule catabolic process	15	1.70E-02	2.40E-01
GOTERM_BP_FAT	Modification-dependent protein catabolic process	15	1.70E-02	2.40E-01
Annotation cluster 9	**Enrichment score: 2**			
GOTERM_CC_FAT	Intracellular organelle lumen	33	7.40E-03	3.90E-01
GOTERM_CC_FAT	Organelle lumen	33	1.00E-02	4.20E-01
GOTERM_CC_FAT	Membrane-enclosed lumen	33	1.30E-02	4.50E-01
Annotation cluster 10	**Enrichment score: 1.9**			
GOTERM_BP_FAT	Proteolysis involved in cellular protein catabolic process	16	1.10E-02	2.00E-01
GOTERM_BP_FAT	Cellular protein catabolic process	16	1.20E-02	2.00E-01
GOTERM_BP_FAT	Protein catabolic process	16	1.50E-02	2.20E-01
Annotation cluster 11	**Enrichment score: 1.86**			
INTERPRO	Zinc finger, C2H2-type	19	7.50E-03	6.70E-01
INTERPRO	Zinc finger, C2H2-like	19	8.80E-03	6.30E-01
SMART	Zinc finger_C2H2	19	4.00E-02	9.00E-01
Annotation cluster 12	**Enrichment score: 1.76**			
GOTERM_BP_FAT	Regulation of apoptosis	19	1.60E-02	2.30E-01
GOTERM_BP_FAT	Regulation of programmed cell death	19	1.80E-02	2.40E-01
GOTERM_BP_FAT	Regulation of cell death	19	1.80E-02	2.40E-01
Annotation cluster 13	**Enrichment score: 1.75**			
GOTERM_BP_FAT	Determination of left/right symmetry	4	1.70E-02	2.40E-01
GOTERM_BP_FAT	Determination of symmetry	4	1.80E-02	2.40E-01
GOTERM_BP_FAT	Determination of bilateral symmetry	4	1.80E-02	2.40E-01

Table 9 (*Continued*)

Category	Term	Gene count	*P*-value	FDR
Annotation cluster 14	**Enrichment score: 1.73**			
GOTERM_BP_FAT	Neuron projection morphogenesis	9	6.50E-03	1.70E-01
GOTERM_BP_FAT	Cell projection morphogenesis	9	1.40E-02	2.20E-01
GOTERM_BP_FAT	Cell part morphogenesis	9	1.80E-02	2.40E-01
GOTERM_BP_FAT	Neuron projection development	9	1.80E-02	2.40E-01
GOTERM_BP_FAT	Neuron development	9	7.40E-02	4.90E-01
Annotation cluster 15	**Enrichment score: 1.49**			
INTERPRO	Homeobox, conserved site	8	2.40E-02	7.40E-01
INTERPRO	Homeobox	8	2.50E-02	7.20E-01
SMART	HOX	8	5.50E-02	7.30E-01
Annotation cluster 16	**Enrichment score: 1.38**			
INTERPRO	Wnt superfamily	3	2.20E-02	7.60E-01
INTERPRO	Secreted growth factor Wnt protein	3	2.20E-02	7.60E-01
INTERPRO	Secreted growth factor Wnt protein, conserved site	3	2.20E-02	7.60E-01
PIR_SUPERFAMILY	PIRSF001784: int-1 transforming protein	3	2.60E-02	7.60E-01
GOTERM_BP_FAT	Wnt receptor signaling pathway, calcium modulating pathway	3	3.00E-02	3.10E-01
SMART	WNT1	3	3.10E-02	9.70E-01
KEGG_PATHWAY	Basal cell carcinoma	3	1.90E-01	6.00E-01
KEGG_PATHWAY	Hedgehog signaling pathway	3	2.00E-01	6.00E-01
Annotation cluster 17	**Enrichment score: 1.26**			
GOTERM_BP_FAT	Positive regulation of apoptosis	11	5.30E-02	4.30E-01
GOTERM_BP_FAT	Positive regulation of programmed cell death	11	5.50E-02	4.40E-01
GOTERM_BP_FAT	Positive regulation of cell death	11	5.60E-02	4.40E-01
Annotation cluster 18	**Enrichment Score: 1.22**			
UP_SEQ_FEATURE	Domain: F-box	4	5.30E-02	9.50E-01
INTERPRO	Cyclin-like F-box	4	5.40E-02	8.70E-01
SMART	FBOX	4	7.90E-02	8.00E-01
Annotation cluster 19	**Enrichment score: 1.05**			
BIOCARTA	Cadmium induces DNA synthesis and proliferation in macrophages	3	3.20E-02	7.40E-01
BIOCARTA	IL-3 signaling pathway	3	3.70E-02	6.90E-01
BIOCARTA	NGF pathway	3	5.30E-02	7.40E-01
BIOCARTA	EPO signaling pathway	3	5.90E-02	7.10E-01
BIOCARTA	Inhibition of cellular proliferation by Gleevec	3	6.50E-02	6.90E-01
BIOCARTA	TPO signaling pathway	3	7.10E-02	6.80E-01
BIOCARTA	Signaling pathway from G-protein families	3	7.80E-02	6.70E-01
BIOCARTA	IL-2 signaling pathway	3	7.80E-02	6.70E-01
BIOCARTA	PDGF signaling pathway	3	1.00E-01	7.40E-01
BIOCARTA	BCR signaling pathway	3	1.10E-01	7.40E-01
BIOCARTA	EGF signaling pathway	3	1.10E-01	7.40E-01
BIOCARTA	Fc epsilon receptor I signaling in mast cells	3	1.40E-01	7.70E-01
BIOCARTA	T-cell receptor signaling pathway	3	1.70E-01	7.90E-01
BIOCARTA	MAP kinase signaling pathway	3	5.40E-01	1.00E+00
Annotation cluster 20	**Enrichment score: 1.03**			
UP_SEQ_FEATURE	Short sequence motif: BH3	3	1.70E-02	7.10E-01
INTERPRO	Apoptosis regulator Bcl-2, BH	3	2.90E-02	7.30E-01
SMART	Bcl	3	4.00E-02	7.90E-01
GOTERM_CC_FAT	Mitochondrial outer membrane	3	2.80E-01	9.50E-01
GOTERM_CC_FAT	Organelle outer membrane	3	3.40E-01	9.60E-01
GOTERM_CC_FAT	Outer membrane	3	3.60E-01	9.60E-01

Abbreviations: Bcl, B-cell lymphoma; BCR, B-cell receptor; DAVID, Database for Annotation, Visualization and Integrated Discovery; EGF, epidermal growth factor; EPO, erythropoietin; FDR, false discovery rate; IGF, insulin-like growth factor; IL, interleukin; NGF, nerve growth factor; PDGF, platelet-derived growth factor; TPO, thrombopoietin.

of cellular biosynthetic process, negative regulation of biosynthetic process, regulation of phosphorylation, regulation of phosphate metabolic process, lung development, respiratory tube development, positive regulation of transcription, positive regulation of gene expression, mesenchymal cell differentiation and development, negative regulation of apoptosis and programmed cell death, the insulin-like growth factor (IGF)-1 signaling pathway, interleukin (IL)-6 signaling pathway, insulin signaling pathway, Ubl conjugation pathway, modification-dependent macromolecule catabolic process, and modification-dependent protein catabolic process.

Table 10 The KEGG pathways by DAVID for the target list of hsa-miR-181a-5p based on miRTarBase

Signaling pathway	Gene count	%	*P*-value	FDR
Pathways in cancer	14	6.2	8.70E-04	8.20E-02
MAPK signaling pathway	9	4	4.20E-02	3.70E-01
Melanogenesis	7	3.1	3.20E-03	1.40E-01
Chronic myeloid leukemia	5	2.2	2.40E-02	3.80E-01
Small cell lung cancer	5	2.2	3.50E-02	3.90E-01
Prostate cancer	5	2.2	4.20E-02	4.10E-01
Dorsoventral axis formation	4	1.8	5.60E-03	1.70E-01
Thyroid cancer	4	1.8	8.60E-03	1.90E-01
Notch signaling pathway	4	1.8	3.10E-02	4.10E-01
Long-term depression	4	1.8	8.10E-02	5.60E-01
Renal cell carcinoma	4	1.8	8.40E-02	5.40E-01
B-cell receptor signaling pathway	4	1.8	9.80E-02	5.40E-01
VEGF signaling pathway	4	1.8	9.80E-02	5.40E-01
Prion diseases	3	1.3	9.40E-02	5.50E-01

Abbreviations: DAVID, Database for Annotation, Visualization and Integrated Discovery; FDR, false discovery rate; KEGG, Kyoto Encyclopedia of Genes and Genomes; VEGF, vascular endothelial growth factor.

Furthermore, our DAVID analysis revealed that there were 14 KEGG pathways significantly enriched in the target list of hsa-miR-181a-5p, based on miRTarBase (Table 10). These pathways included pathways in cancer pathways (Figure 5), the MAPK signaling pathway (Figure 6), melanogenesis, chronic myeloid leukemia, small cell lung cancer, prostate cancer, dorsoventral axis formation, thyroid cancer, the Notch signaling pathway (Figure 7), long-term depression, renal cell carcinoma, the B cell receptor signaling pathway, vascular endothelial growth factor (VEGF) signaling pathway (Figure 8), and prion diseases.

Combined validated targets of hsa-miR-181a-5p, based on both TarBase 6.0 and miRTarBase 4.0

When we combined the target lists of hsa-miR-181a-5p with experimental evidence from both TarBase 6.0 and miRTarBase 4.0, there were 313 validated targets for hsa-miR-181a-5p (Table 11). As shown in Table 12, our DAVID analysis showed that there were 26 functional clusters that were identified to be enriched with an enrichment score >1.0 in the combined target list of hsa-miR-181a-5p, based on both TarBase 6.0 and miRTarBase 4.0. The functions of these clusters involved response to hormone stimulus, response to endogenous stimulus, response to organic substance, negative regulation of apoptosis, negative and positive regulation of programmed cell death, negative and positive regulation of cell death, the DNA damage checkpoint, DNA integrity checkpoint, DNA damage response and signal transduction, the cell cycle checkpoint, response to DNA damage stimulus, bladder cancer, endometrial cancer (Figure 9), non-small-cell lung cancer, acute myeloid leukemia, glioma, melanoma, developmental growth, cell fate commitment, tissue morphogenesis, positive regulation of macromolecule biosynthetic process, positive regulation of cellular biosynthetic process, positive regulation of biosynthetic process, regulation of phosphorylation, regulation of phosphate metabolic process, regulation of phosphorus metabolic process, positive regulation of transcription, positive regulation of gene expression, positive regulation of protein kinase activity, positive regulation of kinase activity, positive regulation of transferase activity, regulation of protein kinase activity, regulation of kinase activity, positive regulation of cellular protein metabolic process, positive regulation of protein metabolic process, branching morphogenesis of a tube, positive regulation of cell development, morphogenesis of a branching structure, tube morphogenesis, regulation of cell development, neuron projection morphogenesis, cell projection morphogenesis, neuron projection development, cell part morphogenesis, neuron development, cell morphogenesis, cell projection organization, cellular component morphogenesis, neuron differentiation, IGF-1 signaling pathway, IL6 signaling pathway, insulin signaling pathway, signaling of hepatocyte growth factor receptor, embryonic appendage morphogenesis, embryonic limb morphogenesis, limb morphogenesis, appendage morphogenesis, limb development, appendage development, embryonic morphogenesis, response to ethanol, response to metal ion, response to inorganic substance, response to drug, response to estrogen stimulus, positive regulation of protein modification process, regulation of protein modification process, protein amino acid phosphorylation, phosphorylation, phosphate metabolic process, phosphorus metabolic process, cell aging, negative regulation of neuron apoptosis, aging, actin cytoskeleton organization, actin filament-based process, membrane organization, membrane insoluble fraction, Ras protein signal transduction, long-term depression, the B cell receptor signaling pathway, VEGF signaling pathway, Fc epsilon RI signaling pathway, ErbB signaling pathway, gap junction, gonadotropin-releasing hormone (GnRH) signaling pathway, T cell receptor signaling pathway, insulin signaling pathway, small GTPase-mediated signal transduction, chemokine signaling pathway, regulation of actin cytoskeleton, MAPK signaling pathway, axonogenesis, cell morphogenesis involved in neuron differentiation, cell morphogenesis involved in differentiation, nucleoplasm,

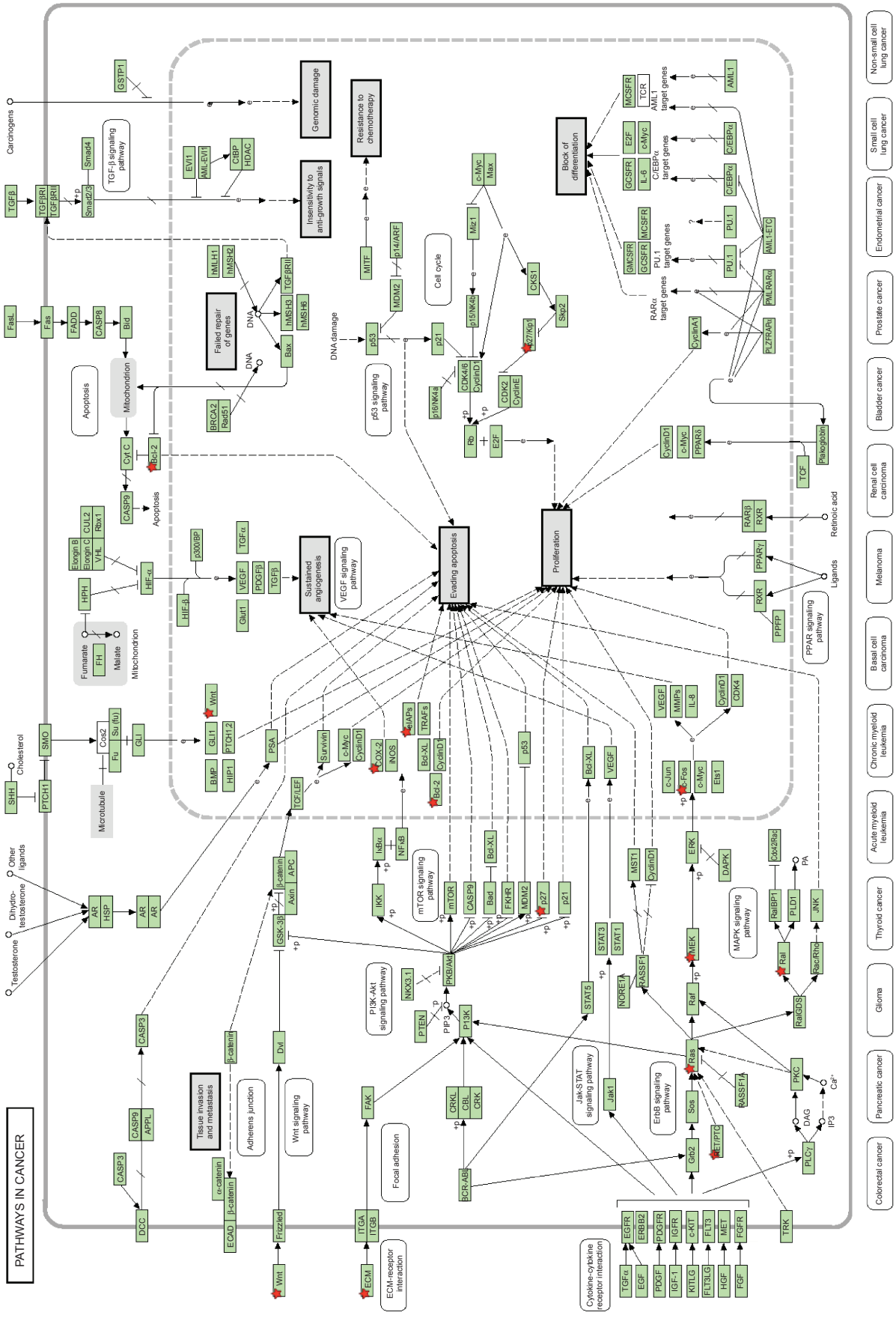

Figure 5 Cancer pathways in the target list of hsa-miR-181a-5p based on miRTarBase 4.0.
Notes: Several important oncogenes and tumor suppressors are likely regulated by hsa-miR-181a-5p (marked with a red star), including ATM, p300, p27, GADD45, and cyclin D1. These genes play an important role in the regulation of angiogenesis, cell proliferation, apoptosis, and metastasis.

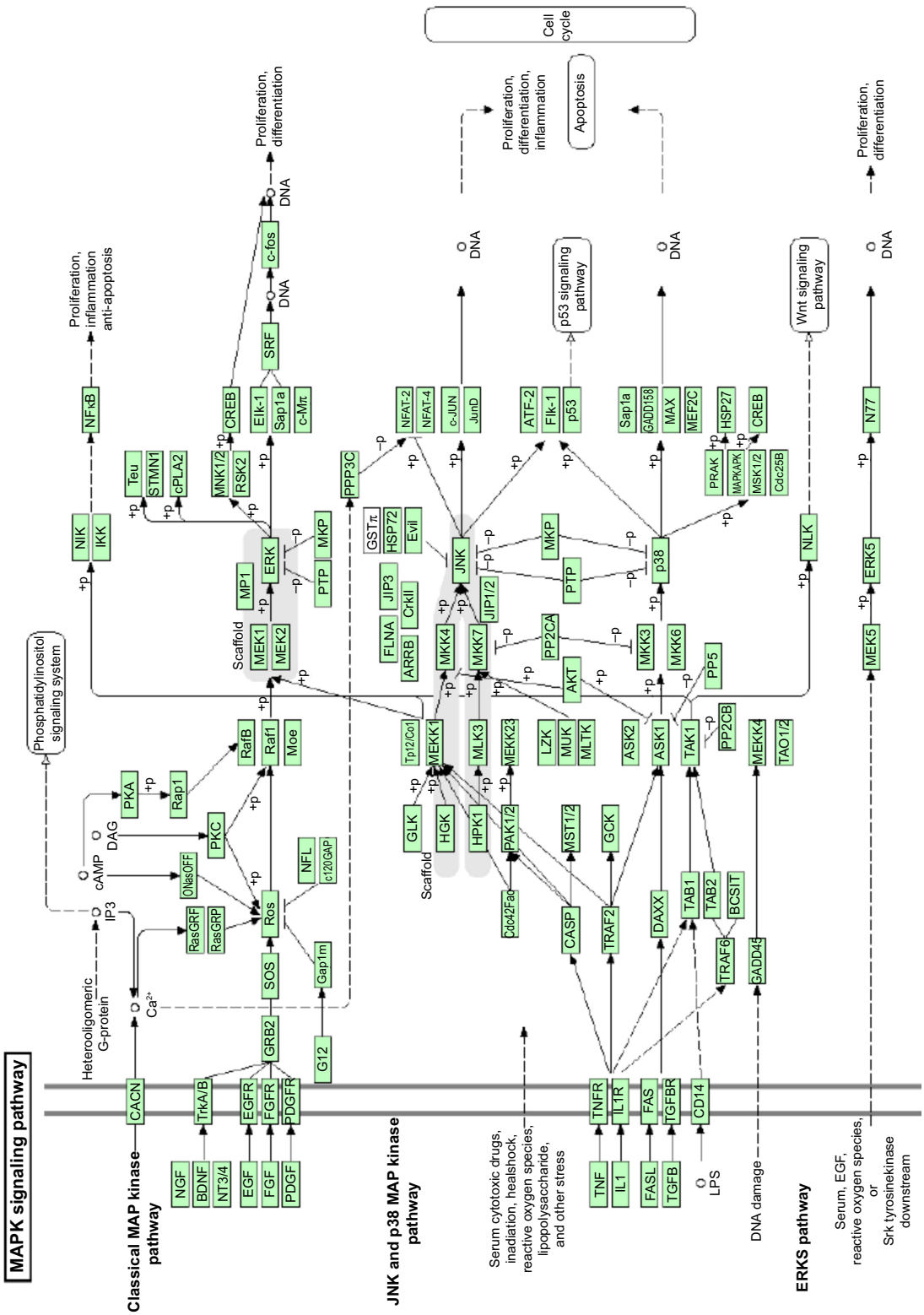

Figure 6 MAPK signaling pathway in the target list of hsa-miR-181a-5p based on miRTarBase 4.0.

Notes: hsa-miR-181a-5p can regulate MAPK signaling pathways. The MAPK/Erk signaling cascade is activated by a wide variety of receptors involved in growth and differentiation, including receptor tyrosine kinases, integrins, and ion channels.[11] The specific components of the cascade vary greatly among different stimuli, but the architecture of the pathway usually includes a set of adaptors (Shc, GRB2, Crk, etc) linking the receptor to a guanine nucleotide exchange factor (SOS, C3G, etc) transducing the signal to small GTP-binding proteins (Ras, Rap1), which in turn activate the core unit of the cascade composed of a MAPKKK (Raf), a MAPKK (MEK1/2), and MAPK (Erk). An activated Erk dimer can regulate targets in the cytosol and also translocates to the nucleus, where it phosphorylates a variety of transcription factors regulating gene expression. p38 MAPKs (α, β, γ, and δ) are members of the MAPK family that are activated by a variety of environmental stresses and inflammatory cytokines. As with other MAPK cascades, the membrane-proximal component is a MAPKKK, typically a MEKK or a mixed lineage kinase (MLK). The MAPKKK phosphorylates and activates MKK3/6, the p38 MAPK kinases.[11] MKK3/6 can also be activated directly by ASK1, which is stimulated by apoptotic stimuli. p38 MAPK is involved in regulation of HSP27, MAPKAPK-2 (MK2), MAPKAPK-3 (MK3), and several transcription factors, including ATF-2, Stat1, the Max/Myc complex, MEF-2, Elk-1, and indirectly, CREB via activation of MSK1.

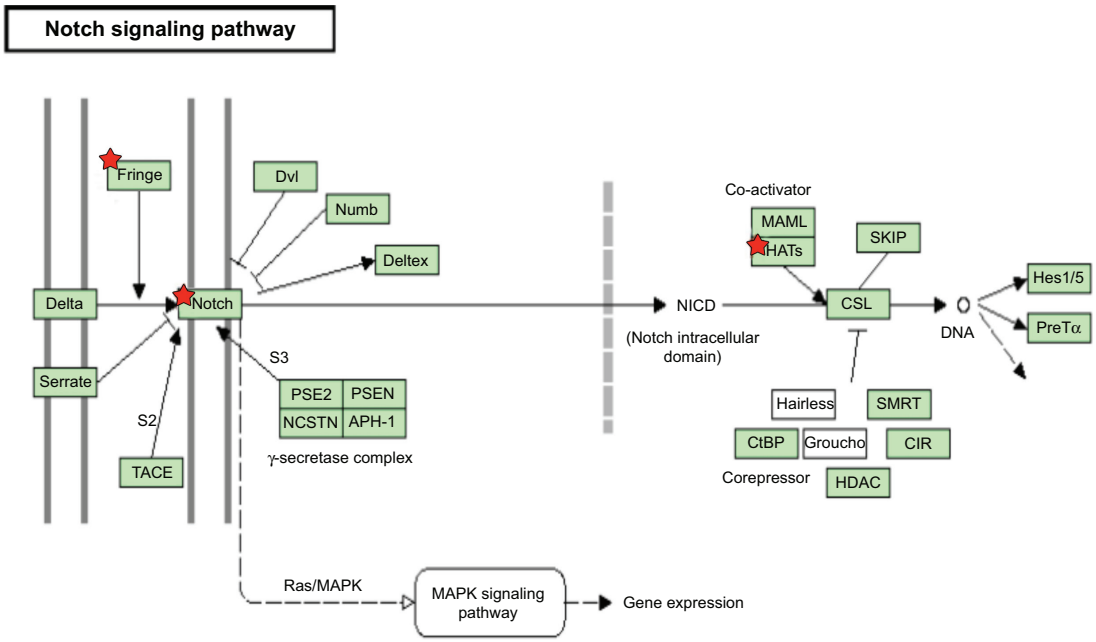

Figure 7 Notch signaling pathway in the target list of hsa-miR-181a-5p based on miRTarBase 4.0.
Notes: hsa-miR-181a-5p can regulate the function of the Notch signaling pathway. Notch signaling is an evolutionarily conserved pathway in multicellular organisms that regulates cell fate determination during development and maintains adult tissue homeostasis. In mammalian signal-sending cells, members of the Delta-like (DLL1, DLL3 & DLL4) and the Jagged (JAG1 & JAG2) families serve as ligands for Notch signaling receptors.[112] Upon ligand binding, the NECD is cleaved away (S2 cleavage) from the TM-NICD domain by TACE (TNF-α ADAM metalloprotease converting enzyme). The NECD remains bound to the ligand, and this complex undergoes endocytosis/recycling within the signal-sending cell in a manner dependent on ubiquitination by Mib. In the signal-receiving cell, γ-secretase (also involved in Alzheimer's disease) releases the NICD from the TM (S3 cleavage), which allows for nuclear translocation where it associates with the CSL (CBF1/Su(H)/Lag-1) transcription factor complex, resulting in subsequent activation of the canonical Notch target genes, including Myc, p21, and the HES-family members. Abnormal expression of Notch and related proteins has been observed in EC, and the Notch signaling pathway may play a role in the development, growth, and metastasis of EC.[113–116] Targets of hsa-miR-181a-5p are marked with a red star.
Abbreviations: EC, endometrial cancer; TNF, tumor necrosis factor.

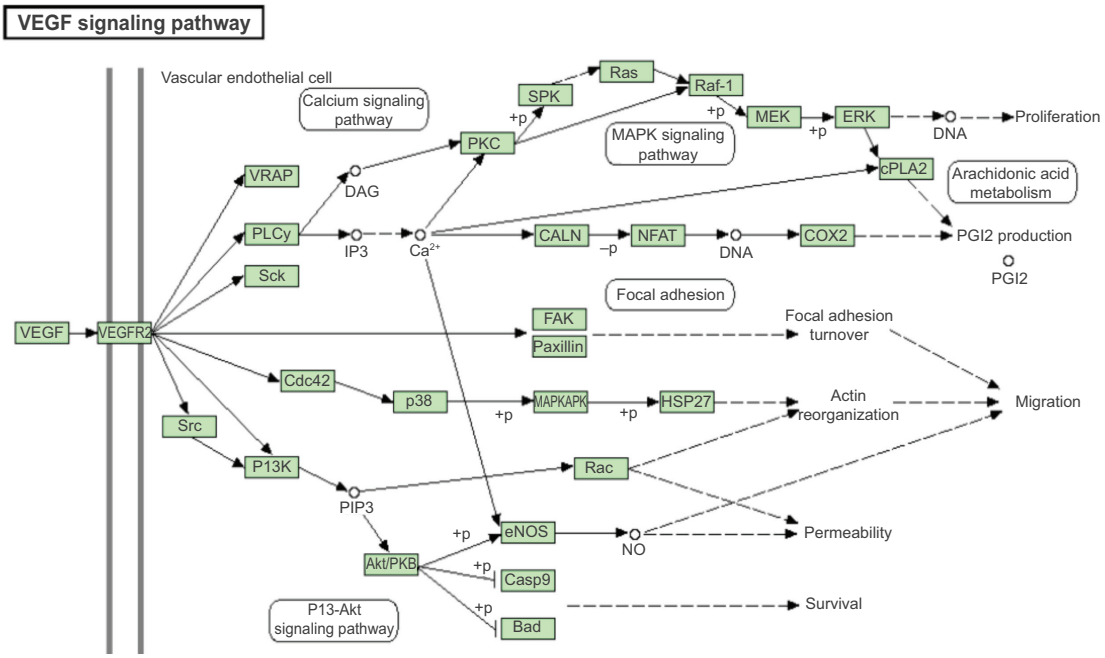

Figure 8 VEGF signaling pathway in the target list of hsa-miR-181a-5p based on miRTarBase 4.0.
Notes: hsa-miR-181a-5p can regulate the VEGF signaling pathway. VEGF is an important signaling protein involved in both vasculogenesis and angiogenesis. All members of the VEGF family stimulate cellular responses by binding to tyrosine kinase receptors (the VEGFRs) on the cell surface, causing them to dimerize and become activated through transphosphorylation. This triggers a signaling cascade that activates several signaling pathways, such as PI3K/Akt, Erk1/2, Smad, and Notch, and results in endothelial cell proliferation and migration. A number of studies have shown that VEGF and its associated proteins are aberrant in EC.[117–122] These proteins represent useful targets in the treatment of EC.
Abbreviations: EC, endometrial cancer; VEGF, vascular endothelial growth factor.

Table 11 Combined targets of hsa-miR-181a-5p with experimental evidence based on both TarBase and miRTarBase 4.0

Gene symbol	Full name	Alias	Function
ACOT12	Acyl-CoA thioesterase 12	CACH-1, Cach, STARD15, THEAL	Hydrolyzes acetyl-CoA to acetate and CoA
AFTPH	Aftiphilin	Nbla10388	May play a role in membrane trafficking
AKAP12	A kinase anchor protein 12	AKAP250, SSeCKS	Anchoring protein that mediates the subcellular compartmentation of PKA and PKC
ALG10B	α-1,2-Glucosyltransferase	ALG10, KCR1	Transfers glucose from dolichyl phosphate glucose onto the lipid-linked oligosaccharide Glc(2)Man(9)GlcNAc(2)-PP-Dol
AMMECR1	Alport syndrome, mental retardation, midface hypoplasia and elliptocytosis chromosomal region gene 1	RP13-360B22.1, AMMERC1	
ANKRD1	Ankyrin repeat domain 1 (cardiac muscle)	ALRP, C-193, CARP, CVARP, MCARP, bA320F15.2	Plays an important role in endothelial cell activation
ANKRD13C	Ankyrin repeat domain 13C	RP4-677H15.5, dJ677H15.3	
AP1M1	Adaptor-related protein complex 1, mu 1 subunit	AP47, CLAPM2, CLTNM, MU-1A	Subunit of clathrin-associated adaptor protein complex 1 that plays a role in protein sorting in the trans-Golgi network and endosomes
ARF6	ADP-ribosylation factor 6		Involved in protein trafficking
ARHGAP11A	Rho GTPase activating protein 11A	RP11-1000B6.5, GAP (1–12)	GTPase activator activity
ARHGAP12	Rho GTPase activating protein 12		GTPase activator for the Rho-type GTPases, by converting them to an inactive GDP-bound state
ARL6IP1	ADP-ribosylation factor-like 6 interacting protein 1	AIP1, ARL6IP, ARMER, SPG61	May be involved in protein transport, membrane trafficking, or cell signaling during hematopoietic maturation
ARL6IP6	ADP-ribosylation factor-like 6 interacting protein 6	RP23-265N10.1, 2310057C01Rik, 2610529A11Rik, Aip-6	May be involved in protein transport, membrane trafficking, or cell signaling during hematopoietic maturation
ATF7IP2	Activating transcription factor 7 interacting protein 2	MCAF2	Recruiter that couples transcriptional factors to general transcription apparatus and thereby modulates transcription regulation and chromatin formation
ATG10	Autophagy related 10	PP12616, APG10, APG10L, pp12616	Plays a role in autophagy
ATM	ATM serine/threonine kinase	AT1, ATA, ATC, ATD, ATDC, ATE, TEL1, TELO1	Serine/threonine protein kinase
ATP6V0E1	ATPase, H^+ transporting, lysosomal 9 kDa, V0 subunit e1	ATP6H, ATP6V0E, M9.2, Vma21, Vma21p	Vacuolar ATPase is responsible for acidifying a variety of intracellular compartments in eukaryotic cells
ATP8A1	ATPase, aminophospholipid transporter (APLT), class I, type 8A, member 1	ATPASEII, ATPIA, ATPP2	May play a role in the transport of aminophospholipids from the outer to the inner leaflet of various membranes and the maintenance of asymmetric distribution of phospholipids
BAG2	BCL2-associated athanogene 2	RP3-496N17.2, BAG-2, dJ417I1.2	Inhibits the chaperone activity of HSP70/HSC70 by promoting substrate release
BCL2	B-cell CLL/lymphoma 2	Bcl-2, PPP1R50	Suppresses apoptosis
BCL2L11	BCL2-like 11	BAM, BIM, BOD	Induces apoptosis
BDNF	Brain-derived neurotrophic factor	ANON2, BULN2	Promotes the survival of neuronal populations
BPGM	2,3-bisphosphoglycerate mutase	DPGM	Plays a major role in regulating hemoglobin oxygen affinity
BRCA1	Breast cancer 1, early onset	BRCAI, BRCC1, BROVCA1, IRIS, PNCA4, PPP1R53, PSCP, RNF53	Plays a central role in DNA repair by facilitating cellular response to DNA repair
BRIX1	Biogenesis of ribosomes, homolog (*S. cerevisiae*)	BRIX, BXDC2	Required for biogenesis of the 60S ribosomal subunit

BRMS1L	Breast cancer metastasis-suppressor 1-like	BRMS1	Involved in the HDAC1-dependent transcriptional repression activity
BTBD3	BTB (POZ) domain containing 3	RP4-742J24.3, dJ742J24.1	Acts as a key regulator of dendritic field orientation during the development of sensory cortex
C1orf109	Chromosome 1 open reading frame 109		
C1orf43	Chromosome 1 open reading frame 43	HSPC012, NICE-3, NS5ATP4, S863-3	
C1QTNF9	C1q and tumor necrosis factor related protein 9	9130217G22Rik, CTRP9, Ciqtnf9	Activates AMPK, AKT, and p44/42 MAPK signaling pathways
C8A	Complement component 8, α polypeptide		C8 is a constituent of the membrane attack complex
CBX3	Chromobox homolog 3	HECH, HP1-GAMMA, HP1Hs-γ	Involved in transcriptional silencing in heterochromatin-like complexes
CCDC6	Coiled-coil domain containing 6	D10S170, H4, PTC, TPC, TST1	Functions as a tumor suppressor
CCDC82	Coiled-coil domain containing 82	HT025, HSPC048	
CCND1	Cyclin D1	BCL1, D11S287E, PRAD1, U21B31	Essential for the control of the cell cycle at the G1/S (start) transition
CCNG1	Cyclin G1	CCNG	May play a role in growth regulation
CD46	CD46 molecule, complement regulatory protein	AHUS2, MCP, MIC10, TLX, TRA2.10	Acts as a cofactor for complement factor I
CDKN1B	Cyclin-dependent kinase inhibitor 1B (p27, Kip1)	CDKN4, KIP1, MEN1B, MEN4, P27KIP1	Important regulator of cell cycle progression
CDX2	Caudal type homeobox 2	CDX-3, CDX3	Involved in the transcriptional regulation of multiple genes expressed in the intestinal epithelium
CEP97	Centrosomal protein 97 kDa	2810403B08Rik, LRRIQ2	Collaborates with cep110, being involved in the suppression of a cilia assembly program
CFI	Complement factor I	AHUS3, ARMD13, C3BINA, C3b-INA, FI, IF, KAF	Responsible for cleaving the α-chains of C4b and C3b, in the presence of the cofactors C4-binding protein and factor H, respectively
CHD1	Chromodomain helicase DNA binding protein 1		Sequence-selective DNA-binding protein
CHL1	Cell adhesion molecule L1-like	CALL, L1CAM2	Plays a role in nervous system development and in synaptic plasticity
CHRFAM7A	CHRNA7 (cholinergic receptor, nicotinic, α 7, exons 5–10) and FAM7A (family with sequence similarity 7A, exons A-E) fusion	CHRNA7, CHRNA7-DR1, D-10	Extracellular ligand-gated ion channel activity
CLUAP1	Clusterin associated protein 1	CFAP22, FAP22	May play a role in cell proliferation or apoptosis
COL27A1	Collagen, type XXVII, α 1	RP11-82I1.1	Plays a role during the calcification of cartilage and the transition of cartilage to bone
COPS2	COP9 signalosome subunit 2	ALIEN, CSN2, SGN2, TRIP15	Involved in various cellular and developmental processes
CST5	Cystatin D		Cysteine proteinase inhibitor
CXorf1	Transmembrane protein 257	CXorf1	
D3R	Dopamine receptor D3	DRD3; D3DR; ETM1; FET1	Inhibits adenylyl cyclase through inhibitory G-proteins, plays a role in cognitive and emotional functions
DCP2	Decapping mRNA 2	NUDT20	Necessary for the degradation of mRNAs
DCST1	DC-STAMP domain containing 1	RP11-307C12.10-003	Protein and zinc ion binding
DDIT4	DNA-damage-inducible transcript 4	RP11-442H21.1, Dig2, REDD-1, REDD1	Inhibits cell growth by regulating the frap1 pathway upstream of the tsc1-tsc2 complex and downstream of Akt1
DDX27	DEAD (Asp-Glu-Ala-Asp) box polypeptide 27	HSPC259, DRS1, Drs1p, PP3241, RHLP, dJ686N3.1	Probable ATP-dependent RNA helicase

(*Continued*)

Table 11 (*Continued*)

Gene symbol	Full name	Alias	Function
DDX3X	DEAD (Asp-Glu-Ala-Asp) box helicase 3, X-linked	DBX, DDX14, DDX3, HLP2	ATP-dependent RNA helicase
DNAJC7	DnaJ (HSP40) homolog, subfamily C, member 7	DJ11, DJC7, TPR2, TTC2	Acts as co-chaperone regulating the molecular chaperones HSP70 and HSP90 in folding of steroid receptors
DSCR8	Down syndrome critical region gene 8	C21orf65, CT25.1a, CT25.1b, MMA-1, MMA-1a, MMA-1b, MMA1, MTAG2	
DUSP5	Dual specificity phosphatase 5	DUSP, HVH3	Displays phosphatase activity toward several substrates
DUSP6	Dual specificity phosphatase 6	HH19, MKP3, PYST1	Inactivates MAP kinases
EIF1	Eukaryotic translation initiation factor 1	A121, EIF-1A, ISO1, SUI1, EIF1	Necessary for scanning and involved in initiation site selection
EIF2C1	Argonaute RISC catalytic component 1	RP4-789D17.1, EIF2C, AGO1, GERP95, Q99	Required for RNA-mediated gene silencing
EIF2C3	Argonaute RISC catalytic component 3	AGO3	Required for RNA-mediated gene silencing
ELAVL1	ELAV like RNA binding protein 1	ELAV1, HUR, Hua, MelG	Binds avidly to the AU-rich element in FOS and IL3 mRNAs
ENAH	Enabled homolog	RP11-496N12.7, ENA, MENA, NDPP1	Ena/VASP proteins are actin-associated proteins involved in a range of processes dependent on cytoskeleton remodeling and cell polarity
EP300	E1A binding protein p300	RP1-85F18.1, KAT3B, RSTS2, p300	Functions as HAT and regulates transcription via chromatin remodeling
EPHA5	EPH receptor A5	CEK7, EHK-1, EHK1, EK7, HEK7, TYRO4	Receptor for members of the ephrin-A family
ESR1	Estrogen receptor 1	RP1-130E4.1, ER, ESR, ESRA, ESTRR, Era, NR3A1	Nuclear hormone receptor
EYA4	EYA transcriptional coactivator and phosphatase 4	RP11-704J17.4, CMD1J, DFNA10	Tyrosine phosphatase that specifically dephosphorylates "Tyr-142" of histone H2AX (H2AXY142ph)
FAM160A2	Family with sequence similarity 160, member A2	C11orf56	
FAM222B	Family with sequence similarity 222, member B	C17orf63	
FAM47B	Family with sequence similarity 47, member B	RP13-520K9.1	
FAT1	FAT atypical cadherin 1	CDHF7, CDHR8, FAT, ME5, hFat1	Could function as a cell-adhesion protein
FBXO11	F-box protein 11	UG063H01, FBX11, PRMT9, UBR6, VIT1	Substrate recognition component of the SCF E3 ubiquitin-protein ligase complex
FBXO28	F-box protein 28	CENP-30, Fbx28	Probably recognizes and binds to some phosphorylated proteins and promotes their ubiquitination and degradation
FBXO33	F-box protein 33	BMND12, Fbx33, c14_5247	Substrate recognition component of the SCF E3 ubiquitin-protein ligase complex, which mediates the ubiquitination and subsequent proteasomal degradation of target proteins
FBXO34	F-box protein 34	CGI-301, Fbx34	Substrate-recognition component of the SCF E3 ubiquitin ligase complex
FKBP10	FK506 binding protein 10	PSEC0056, FKBP65, OI11, OI6, PPIASE, hFKBP65	PPIases accelerate the folding of proteins during protein synthesis
FKBP4	FK506 binding protein 4	FKBP51, FKBP52, FKBP59, HBI, Hsp56, PPIase, p52	May play a role in the intracellular trafficking of heterooligomeric forms of steroid hormone receptors
FKBP7	FK506 binding protein 7	UNQ670/PRO1304, FKBP23, PPIase	PPIases accelerate the folding of proteins during protein synthesis
FOS	FBJ murine osteosarcoma viral oncogene homolog	AP-1, C-FOS, p55	Nuclear phosphoprotein, which forms a tight but noncovalently linked complex with the JUN/AP-1 transcription factor
FRA10AC1	Fragile site, folic acid type, rare, fra(10)(q23.3) or fra(10)(q24.2) candidate 1	PRO2972, C10orf4, F26C11.1-like, FRA10A	
FSIP1	Fibrous sheath interacting protein 1	HSD10	

FXYD6	FXYD domain containing ion transport regulator 6	UNQ521/PRO1056	
GADD45G	Growth arrest and DNA-damage-inducible, γ	RP11-260L6.1, CR6, DDIT2, GADD45γ, GRP17	Involved in the regulation of growth and apoptosis
GANAB	Glucosidase, α; neutral AB	G2AN, GLUII	Cleaves sequentially the 2 innermost α-1,3-linked glucose residues from the Glc(2)Man(9)GlcNAc(2) oligosaccharide precursor of immature glycoproteins
GATA6	GATA binding protein 6		Regulates terminal differentiation and/or proliferation
GATAD2B	GATA zinc finger domain containing 2B	RP11-216N14.6, MRD18, P66β, p68	Has transcriptional repressor activity
GCNT1	Glucosaminyl (*N*-acetyl) transferase 1, core 2	RP11-214N16.1, C2GNT, C2GNT-L, C2GNT1, G6NT, NACGT2, NAGCT2	Forms critical branches in O-glycans
GIGYF1	GRB10 interacting GYF protein 1	PP3360, GYF1, PERQ1	May act cooperatively with GRB10 to regulate tyrosine kinase receptor signaling
GNA13	G protein, α 13	G13	Modulators or transducers in various transmembrane signaling systems
GNAI3	G protein, α inhibiting activity polypeptide 3	RP5-1160K1.2, 87U6, ARCND1	G proteins are involved as modulators or transducers in various transmembrane signaling systems
GNB1	G protein, β polypeptide 1	RP1-283E3.7	A modulator or transducer in various transmembrane signaling systems
GPR137B	G protein-coupled receptor 137B	RP5-985L19.1, TM7SF1	
GPR78	G protein-coupled receptor 78	UNQ5925/PRO19818	Orphan receptor
GPR83	G protein-coupled receptor 83	GIR, GPR72	Orphan receptor. Could be a neuropeptide y receptor
GPRIN3	GPRIN family member 3	GRIN3	May be involved in neurite outgrowth
GSTM2	Glutathione *S*-transferase mu 2 (muscle)	GST4, GSTM-2, GTHMUS, GSTM2	Conjugation of reduced glutathione to a wide number of exogenous and endogenous hydrophobic electrophiles
H1F0	H1 histone family, member 0	H10, H1FV	Histones H1 are necessary for the condensation of nucleosome chains into higher-order structures
H2AFY	H2A histone family, member Y	H2A.y, H2A/y, H2AF12M, H2AFJ, MACROH2A1.1, mH2A1, macroH2A1.2	Plays a central role in transcription regulation, DNA repair, DNA replication, and chromosomal stability
H3F3B	H3 histone, family 3B	H3.3B	Plays a central role in transcription regulation, DNA repair, DNA replication, and chromosomal stability
HDAC6	Histone deacetylase 6	JM21, CPBHM, HD6, PPP1R90	Responsible for the deacetylation of lysine residues on the N-terminal part of the core histones (H2A, H2B, H3 and H4)
HERC3	HECT and RLD domain containing E3 ubiquitin protein ligase 3		E3 ubiquitin-protein ligase
HEY2	Hes-related family bHLH transcription factor with YRPW motif 2	RP1-293L8.3, CHF1, GRIDLOCK, GRL, HERP1, HESR2, HRT2, bHLHb32	Downstream effector of Notch signaling, which may be required for cardiovascular development
HIPK2	Homeodomain interacting protein kinase 2	PRO0593	Protein kinase acting as a corepressor of several transcription factors
HMGB2	High mobility group box 2	HMG2	Binds preferentially ssDNA and unwinds double stranded DNA
HNRNPAB	Heterogeneous nuclear ribonucleoprotein A/B	ABBP1, HNRPAB	Binds single-stranded RNA
HNRPDL	Heterogeneous nuclear ribonucleoprotein D-like	HNRNP, HNRPDL, JKTBP, JKTBP2, laAUF1	Acts as a transcriptional regulator
HOOK3	Hook microtubule-tethering protein 3	HK3	Probable cytoskeletal linker protein, involved in tethering the Golgi complex to the cytoskeleton
HOXA10	Homeobox A10	HOX1, HOX1.8, HOX1H, PL	Sequence-specific transcription factor

(*Continued*)

Table 11 (*Continued*)

Gene symbol	Full name	Alias	Function
HOXA11	Homeobox A11	HOX1, HOX11	Sequence-specific transcription factor
HRAS	Harvey rat sarcoma viral oncogene homolog	C-BAS/HAS, C-H-RAS, C-HA-RAS1, CTLO, H-RASIDX, HAMSV1, RASH1, p21ras, HRAS	Ras proteins bind GDP/GTP and possess intrinsic GTPase activity
HSD17B3	Hydroxysteroid (17-β) dehydrogenase 3	RP11-240L7.3, EDH17B3, SDR12C2	Favors the reduction of androstenedione to testosterone
HSP90B1	Heat-shock protein 90 kDa β (Grp94), member 1	ECGP, GP96, GRP94, HEL-S-125m, HEL35, TRA1	Molecular chaperone that functions in the processing and transport of secreted proteins
HSPA13	Heat-shock protein 70 kDa family, member 13	STCH	Has peptide-independent ATPase activity
HSPA1B	Heat-shock 70 kDa protein 1B	DAAP-21F2.7, HSP70-1B, HSP70-2	Stabilizes preexistent proteins against aggregation and mediates the folding of newly translated polypeptides in the cytosol as well as within organelles
HUWE1	HECT, UBA and WWE domain containing 1	RP3-339A18.4, ARF-BP1, HECTH9, HSPC272, Ib772, LASU1, MULE, URE-B1, UREB1	E3 ubiquitin-protein ligase mediating ubiquitination and subsequent proteasomal degradation of target proteins.
ICMT	Isoprenylcysteine carboxyl methyltransferase	RP1-120G22.4, HSTE14, MST098, MSTP098, PCCMT, PCMT, PPMT	Catalyzes the posttranslational methylation of isoprenylated C-terminal cysteine residues
IDS	Iduronate 2-sulfatase	MPS2, SIDS	Required for the lysosomal degradation of heparan sulfate and dermatan sulfate
INCENP	Inner centromere protein antigens		Component of the chromosomal passenger complex, a complex that acts as a key regulator of mitosis
IQCG	IQ motif containing G	CFAP122, DRC9	
KAT2B	K(lysine) acetyltransferase 2B	CAF, P/CAF, PCAF	Functions as a HAT to promote transcriptional activation
KBTBD3	Kelch repeat and BTB (POZ) domain containing 3	BKLHD3	
KBTBD7	Kelch repeat and BTB (POZ) domain containing 7		
KCTD2	Potassium channel tetramerization domain containing 2		
KCTD3	Potassium channel tetramerization domain containing 3	RP11-5F19.1, NY-REN-45	
KIAA0100	KIAA0100	BCOX, BCOX1, CT101	
KIAA0101	KIAA0101	L5, NS5ATP9, OEATC, OEATC-1, OEATC1, PAF, PAF15, p15(PAF), p15/PAF, p15PAF	May be involved in protection of cells from UV-induced cell death
KIAA1462	KIAA1462	JCAD	
KIAA2026	KIAA2026		
KLF6	Kruppel-like factor 6	RP11-184A2.1, BCD1, CBA1, COPEB, CPBP, GBF, PAC1, ST12, ZF9	Plays a role in B-cell growth and development
KLHL15	Kelch-like family member 15	HEL-S-305	Probable substrate-specific adapter of an E3 ubiquitin-protein ligase complex, which mediates the ubiquitination and subsequent proteasomal degradation of target proteins
KLHL42	Kelch-like family member 42	Ctb9, KLHDC5	Substrate-specific adapter of a BCR (BTB-CUL3-RBX1) E3 ubiquitin-protein ligase complex required for mitotic progression and cytokinesis
KLRC4	Killer cell lectin-like receptor subfamily C, member 4	NKG2-F, NKG2F	May play a role as a receptor for the recognition of MHC class I HLA-E molecules by NK cells

KRAS	Kirsten rat sarcoma viral oncogene homolog	C-K-RAS, CFC2, K-RAS2A, K-RAS2B, K-RAS4A, K-RAS4B, KI-RAS1, KRAS2, NS, NS3, RASK2, KRAS	Binds GDP/GTP and possesses intrinsic GTPase activity
LAMA3	Laminin, α3	BM600, E170, LAMNA, LOCS, lama3a	Binding to cells via a high-affinity receptor, mediating the attachment, migration, and organization of cells into tissues
LBR	Lamin B receptor	PRO0650, DHCR14B, LMN2R, PHA, TDRD18	Anchors the lamina and the heterochromatin to the inner nuclear membrane.
LCLAT1	Lysocardiolipin acyltransferase 1	UNQ1849/PRO3579, 1AGPAT8, AGPAT8, ALCAT1, HSRG1849, LYCAT, UNQ1849	Acyl-CoA: lysocardiolipin acyltransferase
LFNG	LFNG O-fucosylpeptide 3-β-*N*-acetylglucosaminyltransferase	SCDO3	Glycosyltransferase
LGALSL	Lectin, galactoside-binding-like	HSPC159, GRP	Does not bind lactose and may not bind carbohydrates
LPGAT1	Lysophosphatidylglycerol acyltransferase 1	FAM34A, FAM34A1, NET8	Lysophoshatidylglycerol-specific acyltransferase
LRRC17	Leucine rich repeat containing 17	UNQ3076/PRO9909, P37NB	Involved in bone homeostasis, acting as a negative regulator of RANKL-induced osteoclast precursor differentiation from bone marrow precursors
LRRN3	Leucine rich repeat neuronal 3	Nbla10363, FIGLER5, NLRR-3, NLRR3	
LYSMD3	LysM, putative peptidoglycan-binding, domain containing 3		
MAP1B	Microtubule-associated protein 1B	FUTSCH, MAP5, PPP1R102	May play a role in the cytoskeletal changes that accompany neurite extension
MAP2K1	Mitogen-activated protein kinase kinase 1	CFC3, MAPKK1, MEK1, MKK1, PRKMK1	Catalyzes the concomitant phosphorylation of a threonine and a tyrosine residue in a Thr-Glu-Tyr sequence located in MAP kinases
MAZ	MYC-associated zinc finger protein (purine-binding transcription factor)	PUR1, Pur-1, SAF-1, SAF-2, SAF-3, ZF87, ZNF801, Zif87	May function as a transcription factor, with dual roles in transcription initiation and termination
MCL1	Myeloid cell leukemia 1	BCL2L3, EAT-ES, MCL1L, MCL1S, Mcl-1, TM, bcl2-L-3, mcl1/EAT, MCL1	Involved in the regulation of apoptosis versus cell survival, and in the maintenance of viability but not of proliferation
METAP1	Methionyl aminopeptidase 1	MAP1A, MetAP1A	Removes the amino-terminal methionine from nascent proteins
MFAP3	Microfibrillar-associated protein 3		Component of the elastin-associated microfibrils
MGAT5	Mannosyl (α-1,6-)-glycoprotein β1,6-*N*-acetyl-glucosaminyltransferase	GNT-V, GNT-VA	Catalyzes the addition of N-acetylglucosamine in β1-6 linkage to the α-linked mannose of biantennary N-linked oligosaccharides
MIF	Macrophage migration inhibitory factor (glycosylation-inhibiting factor)	GIF, GLIF, MMIF	The expression of MIF at sites of inflammation suggests a role for the mediator in regulating the function of macrophage in host defense. Also acts as a phenylpyruvate tautomerase
MOB1A	MOB kinase activator 1A	C2orf6, MATS1, MOB1, MOBK1B, MOBKL1B, Mob4B	Activator of LATS1/2 in the Hippo signaling pathway
MOB1B	MOB kinase activator 1B	MATS2, MOB4A, MOBKL1A	Activator of LATS1/2 in the Hippo signaling pathway
MOB3B	MOB kinase activator 3B	C9orf35, MOB1D, MOBKL2B	May regulate the activity of kinases
MRPS14	Mitochondrial ribosomal protein S14	DJ262D12.2, HSMRPS14, MRP-S14, S14mt	
MTMR12	Myotubularin related protein 12	3-PAP, PIP3AP	Inactive phosphatase that plays a role as an adapter for the phosphatase myotubularin to regulate myotubularin intracellular location
MTMR3	Myotubularin related protein 3	hCG_2011013, FYVE-DSP1, ZFYVE10	Phosphatase that acts on lipids with a phosphoinositol head group
MTRR	5-methyltetrahydrofolate-homocysteine methyltransferase reductase	MSR, cblE	Involved in the reductive regeneration of cob(I)alamin cofactor required for the maintenance of methionine synthase in a functional state

(*Continued*)

Table 11 (*Continued*)

Gene symbol	Full name	Alias	Function
MYO9A	Myosin IXA		Myosins are actin-based motor molecules with ATPase activity Unconventional myosins serve in intracellular movements
NCAPG	Non-SMC condensin I complex, subunit G	CAPG, CHCG, NY-MEL-3, YCG1	Regulatory subunit of the condensin complex, a complex required for conversion of interphase chromatin into mitotic-like condense chromosomes
ND2	NADH dehydrogenase subunit 2		Core subunit of the mitochondrial membrane respiratory chain NADH dehydrogenase (Complex I)
NFYB	Nuclear transcription factor Y β	CBF-A, CBF-B, HAP3, NF-YB	Stimulates the transcription of various genes by recognizing and binding to a CCAAT motif in promoters
NKX3-2	NK3 homeobox 2	BAPX1, NKX3.2, NKX3B, SMMD	Transcriptional repressor that acts as a negative regulator of chondrocyte maturation
NLK	Nemo-like kinase		Role in cell fate determination, required for differentiation of bone marrow stromal cells
NMRK2	Nicotinamide riboside kinase 2	ITGB1BP3, MIBP, NRK2	
NOL4	Nucleolar protein 4	HRIHFB2255, CT125, NOLP	
NOTCH1	Notch 1	TAN1, hN1	Functions as a receptor for membrane-bound ligands Jagged1, Jagged2 and Delta1 to regulate cell fate determination
NOTCH2	Notch 2	AGS2, HJCYS, hN2	Functions as a receptor for membrane-bound ligands Jagged1, Jagged2, and Delta1 to regulate cell fate determination
NR6A1	Nuclear receptor subfamily 6, group A, member 1	CT150, GCNF, GCNF1, NR61, RTR, hGCNF, hRTR	Orphan nuclear receptor. May be involved in the regulation of gene expression in germ cell development during gametogenesis
NRP1	Neuropilin 1	RP11-342D11.1, BDCA4, CD304, NP1, NRP, VEGF165R	The membrane-bound isoform 1 is a receptor involved in the development of the cardiovascular system, in angiogenesis, in the formation of certain neuronal circuits, and in organogenesis outside the nervous system
NUDT12	Nudix-type motif 12		Hydrolyzes NAD(P)H to NMNH and AMP (2′,5′-ADP), and diadenosine diphosphate to AMP
NUPL1	Nucleoporin like 1	RP11-206I15.1, PRO2463	Component of the nuclear pore complex, a complex required for the trafficking across the nuclear membrane
OAZ1	Ornithine decarboxylase antizyme 1	AZ1, OAZ	Binds to and destabilizes ornithine decarboxylase, which is then degraded. Also inhibits cellular uptake of polyamines by inactivating the polyamine uptake transporter
OCA2	Oculocutaneous albinism II	BEY, BEY1, BEY2, BOCA, D15S12, EYCL, EYCL2, EYCL3, HCL3, P, PED, SHEP1	Could be involved in the transport of tyrosine
OFCC1	Orofacial cleft 1 candidate 1	MRDS1	
OR11A1	Olfactory receptor, family 11, subfamily A, member 1	DAAP-3411.2, 6M1-18, OR11A2, dJ994E9.6, hs6M1-18	Odorant receptor
OTUD1	OTU deubiquitinase 1	DUBA7, OTDC1	Deubiquitinating enzyme that specifically hydrolyzes "Lys-63"-linked polyubiquitin to monoubiquitin
OTX2	Orthodenticle homeobox 2	CPHD6, MCOPS5	Probably plays a role in the development of the brain and the sense organs
PABPC1	Poly(A) binding protein, cytoplasmic 1	PAB1, PABP, PABP1, PABPC2, PABPL1	Binds the poly(A) tail of mRNA

PCAF	K(lysine) acetyltransferase 2B	CAF, P/CAF, PCAF	Functions as a component of the PCAF complex
PCDHB8	Protocadherin β 8	PCDH-β8, PCDH31	Potential calcium-dependent cell-adhesion protein
PFKFB2	6-Phosphofructo-2-kinase/fructose-2, 6-biphosphatase 2	RP11-164O23.2, PFK-2/FBPase-2	Synthesis and degradation of fructose 2,6-bisphosphate
PGD	Phosphogluconate dehydrogenase	6PGD	Catalyzes the oxidative decarboxylation of 6-phosphogluconate to ribulose 5-phosphate and CO_2, with concomitant reduction of NADP to NADPH
PHOX2A	Paired-like homeobox 2a	ARIX, CFEOM2, FEOM2, NCAM2, PMX2A	May be involved in regulating the specificity of expression of the catecholamine biosynthetic genes
PHPT1	Phosphohistidine phosphatase 1	RP11-216L13.10-005, CGI-202, HEL-S-132P, HSPC141, PHP14	Exhibits phosphohistidine phosphatase activity
PIM3	Pim-3 proto-oncogene, serine/threonine kinase	CITF22-49E9.1, pim-3	May be involved in cell cycle progression and antiapoptotic process
PITPNB	Phosphatidylinositol transfer protein β	RP3-353E16.2, PI-TP-β, PtdInsTP, VIB1B	Catalyzes the transfer of PtdIns and phosphatidylcholine between membranes
PLA2G4C	Phospholipase A2, group IVC	CPLA2-γ	Has a preference for arachidonic acid at the sn-2 position of phosphatidylcholine as compared with palmitic acid
PLAG1	Pleiomorphic adenoma gene 1	PSA, SGPA, ZNF912	Transcription factor whose activation results in upregulation of target genes, such as IGFII, leading to uncontrolled cell proliferation
PLCL2	Phospholipase C-like 2	PLCE2	May play an role in the regulation of Ins(1,4,5)P3 around the endoplasmic reticulum
PLXDC2	Plexin domain containing 2	UNQ2514/PRO6003, TEM7R	May play a role in tumor angiogenesis
PNPT1	Polyribonucleotide nucleotidyltransferase 1	COXPD13, DFNB70, OLD35, PNPASE, old-35	Involved in mRNA degradation
POLR2B	Polymerase (RNA) II (DNA directed) polypeptide B	POL2RB, RPB2, hRPB140, hsRPB2	DNA-dependent RNA polymerase catalyzes the transcription of DNA into RNA, using the four ribonucleoside triphosphates as substrates
PPA1	Pyrophosphatase (inorganic) 1	RP11-367H5.1, HEL-S-66p, IOPPP, PP, PP1, SID6-8061	
PPM1A	Protein phosphatase, Mg^{2+}/Mn^{2+} dependent, 1A	PP2C-ALPHA, PP2CA, PP2Cα	Enzyme with a broad specificity
PPP1R9A	Protein phosphatase 1, regulatory subunit 9A	NRB1, NRBI, Neurabin-I	Binds to actin filaments (F-actin) and shows crosslinking activity
PPP2CA	Protein phosphatase 2, catalytic subunit, α isozyme	PP2Ac, PP2CA, PP2Cα, RP-C	PP2A can modulate the activity of phosphorylase B kinase casein kinase 2, mitogen-stimulated S6 kinase, and MAP-2 kinase
PPP2R5C	Protein phosphatase 2, regulatory subunit B', γ	B56G, PR61G	The B regulatory subunit might modulate substrate selectivity and catalytic activity, and also might direct the localization of the catalytic enzyme to a particular subcellular compartment
PRAP1	Proline-rich acidic protein 1	RP11-122K13.6, PRO1195, UPA	May play an important role in maintaining normal growth homeostasis in epithelial cells
PRDX3	Peroxiredoxin 3	AOP-1, AOP1, HBC189, MER5, PRO1748, SP-22, prx-III	Involved in redox regulation of the cell
PRLR	Prolactin receptor	HPRL, MFAB, hPRLrI	This is a receptor for the anterior pituitary hormone prolactin
PROSC	Proline synthetase co-transcribed homolog		
PROX1	Prospero homeobox 1		May play a fundamental role in early development of the central nervous system
PRR4	Proline rich 4	LPRP, PROL4	
PRRC2B	Proline-rich coiled-coil 2B	RP11-334J6.1, BAT2L, BAT2L1, KIAA0515, LQFBS-1	
PTGS2	Prostaglandin-endoperoxide synthase 2	COX-2, COX2, GRIPGHS, PGG/HS, PGHS-2, PHS-2, hCox-2	May have a role as a major mediator of inflammation and/or a role for prostanoid signaling in activity-dependent plasticity

(*Continued*)

Table 11 (*Continued*)

Gene symbol	Full name	Alias	Function
PTPLAD1	Protein tyrosine phosphatase-like A domain containing 1	B-IND1, HACD3, HSPC121	Involved in Rac1-signaling pathways leading to the modulation of gene expression
PTPN11	Protein tyrosine phosphatase, non-receptor type 11	BPTP3, CFC, NS1, PTP-1D, PTP2C, SH-PTP2, SH-PTP3, SHP2	Acts downstream of various receptor and cytoplasmic protein tyrosine kinases to participate in the signal transduction from the cell surface to the nucleus
PTPN22	Protein tyrosine phosphatase, non-receptor type 22	LYP, LYP1, LYP2, PEP, PTPN8	Seems to act on casitas B-lineage lymphoma (Cbl)
PTPRZ1	Protein tyrosine phosphatase, receptor-type, Z polypeptide 1	HPTPZ, HPTPζ, PTP-ζ, PTP18, PTPRZ, PTPZ, R-PTP-ζ-2, RPTPB, RPTPβ, phosphacan	May be involved in the regulation of specific developmental processes in the central nervous system
PUM1	Pumilio RNA-binding family member 1	RP1-65J11.4, HSPUM, PUMH, PUMH1, PUML1	Sequence-specific RNA-binding protein that regulates translation and mRNA stability by binding the 3′-UTR of mRNA targets
RAB8B	RAB8B, member RAS oncogene family		May be involved in vesicular trafficking and neurotransmitter release
RALA	V-ral simian leukemia viral oncogene homolog A	RAL	Multifunctional GTPase involved in a variety of cellular processes, including gene expression, cell migration, cell proliferation, oncogenic transformation, and membrane trafficking
RASSF6	Ras association (RalGDS/AF-6) domain family member 6		May act as a Ras effector protein
RBM15	RNA binding motif protein 15	OTT, OTT1, SPEN	May be implicated in HOX gene regulation
RLF	Rearranged L-myc fusion	RP1-39G22.1, ZN-15L, ZNF292L	May be involved in transcriptional regulation
RNF2	Ring finger protein 2	GS1-120K12.1, BAP-1, BAP1, DING, HIPI3, RING1B, RING2	E3 ubiquitin-protein ligase that mediates monoubiquitination of "Lys-119" of histone H2A, playing a central role in histone code and gene regulation
RNF34	Ring finger protein 34	CARP-1, CARP1, RFI, RIF, RIFF, hRFI	Has E3 ubiquitin-protein ligase activity. Regulates the levels of CASP8 and CASP10 by targeting them for proteasomal degradation
ROPN1L	Rhophilin associated tail protein 1-like	RP11-1C1.7, ASP, RSPH11	
RPL14	Ribosomal protein L14	CAG-ISL-7, CTG-B33, L14, RL14, hRL14	
RPS14	Ribosomal protein S14	PRO2640, EMTB, S14	
RPS8	Ribosomal protein S8	RP11-269F19.3, S8	
RTEL1-TNFRSF6B	RTEL1-TNFRSF6B readthrough (NMD candidate)		
S100A1	S100 calcium binding protein A1	RP1-178F15.1, S100, S100-α, S100A	Weakly binds calcium but binds zinc very tightly – distinct binding sites with different affinities exist for both ions on each monomer
SCAMP2	Secretory carrier membrane protein 2		Functions in post-Golgi recycling pathways. Acts as a recycling carrier to the cell surface
SCD	Stearoyl-CoA desaturase	PRO1933, FADS5, MSTP0081, SCDOS, SCD	Terminal component of the liver microsomal stearyl-CoA desaturase system
SEPT2	Septin 2	DIFF6, NEDD-5, NEDD5, Pnutl3, hNedd5	Required for normal progress through mitosis. Involved in cytokinesis
SF3B3	Splicing factor 3b, subunit 3	RSE1, SAP130, SF3b130, STAF13	Subunit of the splicing factor SF3B required for "A" complex assembly formed by the stable binding of U2 snRNP to the branch point sequence in pre-mRNA
SH3BGRL	SH3 domain binding glutamate-rich protein like	HEL-S-115, SH3BGR	

SIK2	Salt-inducible kinase 2	LOH11CR1I, QIK, SNF1LK2	Phosphorylates "Ser-794" of IRS1 in insulin-stimulated adipocytes
SIRT1	Sirtuin 1	RP11-57G10.3, SIR2L1	NAD-dependent deacetylase, which regulates processes such as apoptosis and muscle differentiation by deacetylating key proteins
SIX6	SIX homeobox 6	MCOPCT2, OPTX2, Six9	May be involved in eye development
SLC35B4	Solute carrier family 35 (UDP-xylose/UDP-*N*-acetylglucosamine transporter), member B4	PSEC0055, YEA, YEA4	Sugar transporter that specifically mediates the transport of UDP-Xyl and UDP-GlcNAc from cytosol into Golgi
SLC37A3	Solute carrier family 37, member 3		
SLC7A11	Solute carrier family 7 (anionic amino acid transporter light chain, xc- system), member 11	CCBR1, xCT	Sodium-independent, high-affinity exchange of anionic amino acids with high specificity for anionic form of cystine and glutamate
SLCO2A1	Solute carrier organic anion transporter family, member 2A1	MATR1, OATP2A1, PGT, PHOAR2, SLC21A2	May mediate the release of newly synthesized prostaglandins from cells, the transepithelial transport of prostaglandins, and the clearance of prostaglandins from the circulation
SMAD5	SMAD family member 5	DWFC, JV5-1, MADH5	Transcriptional modulator activated by BMP type I receptor kinase
SMCHD1	Structural maintenance of chromosomes flexible hinge domain containing 1		Required for maintenance of X inactivation in females and hypermethylation of CpG islands associated with inactive X
SMG1	SMG1 phosphatidylinositol 3-kinase-related kinase	61E3.4, ATX, LIP	Serine/threonine protein kinase involved in both mRNA surveillance and genotoxic stress response pathways
SNAI2	Snail family zinc finger 2	SLUG, SLUGH1, SNAIL2, WS2D	Transcriptional repressor. Involved in the generation and migration of neural crest cells
SOGA2	Microtubule crosslinking factor 1	CCDC165, KIAA0802, MTCL1	
SPRY2	Sprouty homolog 2	hSPRY2	May function as an antagonist of FGF pathways and may negatively modulate respiratory organogenesis
SRPK2	SRSF protein kinase 2	SFRSK2	Phosphorylates RS domain-containing proteins
SRSF7	Serine/arginine-rich splicing factor 7	9G8, AAG3, SFRS7	Required for pre-mRNA splicing
STAG2	Stromal antigen 2	RP11-517O1.1, SA-2, SA2, SCC3B, bA517O1.1	Component of cohesin complex, a complex required for the cohesion of sister chromatids after DNA replication
TAAR6	Trace amine associated receptor 6	RP11-295F4.3, TA4, TAR4, TAR6, TRAR4, taR-4, taR-6	Orphan receptor. Could be a receptor for trace amines
TAB2	TGF-β activated kinase 1/MAP3K7 binding protein 2	CHTD2, MAP3K7IP2, TAB-2	Adapter linking MAP3K7/TAK1 and TRAF6 and mediator of MAP3K7 activation in the IL1 signaling pathway
TAF15	TAF15 RNA polymerase II, TBP-associated factor	Npl3, RBP56, TAF2N, TAFII68	RNA and ssDNA-binding protein that may play specific roles during transcription initiation at distinct promoters
TAF2	TAF2 RNA polymerase II, TBP-associated factor	CIF150, MRT40B, TAFII150, TAF2	Transcription factor TFIID is one of the general factors required for accurate and regulated initiation by RNA polymerase II
TAF6L	TAF6-like RNA polymerase II, PCAF-associated factor	PAF65A	Functions as a component of the PCAF complex
TBX4	T-box 4	SPS	Involved in the transcriptional regulation of genes required for mesoderm differentiation
TCF21	Transcription factor 21	POD1, bHLHa23	Involved in epithelial–mesenchymal interactions in kidney and lung morphogenesis that include epithelial differentiation and branching morphogenesis
TEAD4	TEA domain family member 4	EFTR-2, RTEF1, TCF13L1, TEF-3, TEF3, TEFR-1, hRTEF-1B	Binds specifically and noncooperatively to the Sph and GT-IIC "enhansons" (5′-GTGGAATGT-3′) and activates transcription
TGFBR3	Transforming growth factor β receptor III	BGCAN, β-glycan	Binds to TGF-β

(*Continued*)

Table 11 (*Continued*)

Gene symbol	Full name	Alias	Function
TGIF2	TGFβ-induced factor homeobox 2		Transcriptional repressor. Probably represses transcription via the recruitment of histone deacetylase proteins
THUMPD1	THUMP domain containing 1		
TIAL1	TIA1 cytotoxic granule-associated RNA binding protein-like 1	TCBP, TIAR	RNA-binding protein. Possesses nucleolytic activity against cytotoxic lymphocyte target cells. May be involved in apoptosis
TM9SF3	Transmembrane 9 superfamily member 3	RP11-34E5.1, EP70-P-iso, SMBP	
TMED4	Transmembrane emp24 protein transport domain containing 4	ERS25, HNLF	Involved in endoplasmic reticulum stress response. May play a role in the regulation of heat-shock response and apoptosis
TMEM132B	Transmembrane protein 132B		
TMEM14A	Transmembrane protein 14A	PTD011, C6orf73	
TMEM192	Transmembrane protein 192		
TMEM257	Transmembrane protein 257	CXorf1	
TMEM45A	Transmembrane protein 45A	DERP7	
TMEM64	Transmembrane protein 64		
TMPO	Thymopoietin	CMD1T, LAP2, LEMD4, PRO0868, TP	May help direct the assembly of the nuclear lamina and thereby help maintain the structural organization of the nuclear envelope
TMPRSS11A	Transmembrane protease, serine 11A	ECRG1	Probable serine protease, which may play a role in cellular senescence
TNIP1	TNFAIP3 interacting protein 1	ABIN-1, NAF1, VAN, nip40-1	Interacts with zinc finger protein A20/TNFAIP3 and inhibits TNF-induced NF-κB-dependent gene expression by interfering with an RIP- or TRAF2-mediated transactivation signal
TNPO1	Transportin 1	IPO2, KPNB2, MIP, MIP1, TRN	Functions in nuclear protein import as nuclear transport receptor
TNRC6C	Trinucleotide repeat containing 6C		Plays a role in RNA-mediated gene silencing by miRNAs
TRIM2	Tripartite motif containing 2	CMT2R, RNF86	May contribute to the alteration of neural cellular mechanisms
TRUB1	TruB psi synthase family member 1	PUS4	May be responsible for synthesis of psi from uracil in transfer RNAs
TSG101	Tumor susceptibility 101	TSG10, VPS23	Component of the ESCRT-I complex, a regulator of vesicular trafficking process
TSHR	Thyroid stimulating hormone receptor	CHNG1, LGR3, hTSHR-I	Receptor for thyrothropin. Plays a central role in controlling thyroid cell metabolism
TUSC1	Tumor suppressor candidate 1	TSG-9, TSG9	
TWF1	Twinfilin actin-binding protein 1	A6, PTK9	Actin-binding protein involved in motile and morphological processes
UBA2	Ubiquitin-like modifier activating enzyme 2	HRIHFB2115, ARX, SAE2	The dimeric enzyme acts as an E1 ligase for SUMO1, SUMO2, SUMO3, and probably SUMO4
UCHL1	Ubiquitin carboxyl-terminal esterase L1	HEL-117, NDGOA, PARK5, PGP 9.5, PGP9.5, PGP95, Uch-L1	Ubiquitin-protein hydrolase involved both in the processing of ubiquitin precursors and of ubiquitinated proteins
UGT3A1	UDP glycosyltransferase 3 family, polypeptide A1		UDP-glucuronosyltransferases catalyze phase II biotransformation reactions
USP28	Ubiquitin specific peptidase 28		Deubiquitinase involved in DNA damage response checkpoint and MYC proto-oncogene stability
VBP1	Von Hippel-Lindau binding protein 1	RP13-228J13.4, PFD3, PFDN3, VBP-1	Binds specifically to c-CPN and transfers target proteins to it

WDR33	WD repeat domain 33	NET14, WDC146	Essential for both cleavage and polyadenylation of pre-mRNA 3′ ends
WNT16	Wingless-type MMTV integration site family, member 16		Ligand for members of the Frizzled family of seven transmembrane receptors
WNT2	Wingless-type MMTV integration site family, member 2	INT1L1, IRP	Ligand for members of the Frizzled family of seven transmembrane receptors
WNT3A	Wingless-type MMTV integration site family member 3A		Ligand for members of the Frizzled family of seven transmembrane receptors
XIAP	X-linked inhibitor of apoptosis	RP1-315G1.5, API3, BIRC4, IAP-3, ILP1, MIHA, XLP2, hIAP-3, hIAP3	Apoptotic suppressor
YOD1	YOD1 deubiquitinase	RP11-164O23.1, DUBA8, OTUD2, PRO0907	May play an important regulatory role at the level of protein turnover by preventing degradation
YY1	YY1 transcription factor	DELTA, INO80S, NF-E1, UCRBP, YIN-YANG-1	May play an important role in development and differentiation
ZEB2	Zinc finger E-box binding homeobox 2	HRIHFB2411, HSPC082, SIP-1, SIP1, SMADIP1, ZFHX1B	Transcriptional inhibitor that binds to DNA sequence 5′-CACCT-3′ in different promoters. Represses transcription of E-cadherin
ZFP36L2	ZFP36 ring finger protein-like 2	BRF2, ERF-2, ERF2, RNF162C, TIS11D	Probable regulatory protein involved in regulating the response to growth factors
ZIC2	Zinc family member 2	HPE5	Involved in cerebellar development
ZNF12	Zinc finger protein 12	GIOT-3, HZF11, KOX3, ZNF325	May be involved in transcriptional regulation
ZNF121	Zinc finger protein 121	D19S204, ZHC32, ZNF20	May be involved in transcriptional regulation
ZNF132	Zinc finger protein 132	pHZ-12	May be involved in transcriptional regulation
ZNF148	Zinc finger protein 148	BERF-1, BFCOL1, HT-β, ZBP-89, ZFP148, pHZ-52	Involved in transcriptional regulation
ZNF180	Zinc finger protein 180	HHZ168	May be involved in transcriptional regulation
ZNF238	Zinc finger and BTB domain containing 18	C2H2-171, MRD22, RP58, TAZ-1, ZNF18	Sequence-specific DNA-binding protein with transcriptional repression activity
ZNF25	Zinc finger protein 25	KOX19, Zfp9	May be involved in transcriptional regulation
ZNF30	Zinc finger protein 30	KOX28	May be involved in transcriptional regulation
ZNF35	Zinc finger protein 35	HF.10, HF10, Zfp105	May be involved in transcriptional regulation
ZNF350	Zinc finger protein 350	ZBRK1, ZFQR	Transcriptional repressor
ZNF426	Zinc finger protein 426		May be involved in transcriptional regulation
ZNF445	Zinc finger protein 445	ZKSCAN15, ZNF168, ZSCAN47	May be involved in transcriptional regulation
ZNF558	Zinc finger protein 558		May be involved in transcriptional regulation
ZNF562	Zinc finger protein 562		May be involved in transcriptional regulation
ZNF594	Zinc finger protein 594	hCG_1775942	May be involved in transcriptional regulation
ZNF644	Zinc finger protein 644	BM-005, MYP21, NatF, ZEP-2	May be involved in transcriptional regulation
ZNF652	Zinc finger protein 652		Functions as a transcriptional repressor
ZNF700	Zinc finger protein 700		May be involved in transcriptional regulation
ZNF703	Zinc finger protein 703	ZEPPO1, ZNF503L, ZPO1	May function as a transcriptional repressor
ZNF711	Zinc finger protein 711	CMPX1, MRX97, ZNF4, ZNF5, ZNF6, Zfp711, dJ75N13.1	May be involved in transcriptional regulation
ZNF763	Zinc finger protein 763	ZNF, ZNF440L	May be involved in transcriptional regulation
ZNF780A	Zinc finger protein 780A	ZNF780	May be involved in transcriptional regulation

Abbreviations: FGF, fibroblast growth factor; HLA, human leukocyte antigen; IL, interleukin; miRNA, microRNA; mRNA, messenger RNA; NK cells, natural killer cells; ssDNA, single-stranded DNA; TGF, Transforming growth factor; TNF, tumor necrosis factor; UV, ultraviolet; VEGF, vascular endothelial growth factor.

Table 12 The top enriched clusters (enrich score >1) by DAVID for the combined targets of hsa-miR-181a-5p from both TarBase 6.0 and miRTarBase 4.0

Category	Term	Gene count	*P*-value	FDR
Annotation cluster 1	**Enrichment score: 4.49**			
GOTERM_BP_FAT	Response to hormone stimulus	7	7.70E-06	1.10E-03
GOTERM_BP_FAT	Response to endogenous stimulus	7	1.40E-05	1.50E-03
GOTERM_BP_FAT	Response to organic substance	7	3.30E-04	8.80E-03
Annotation cluster 2	**Enrichment score: 3.97**			
GOTERM_BP_FAT	Negative regulation of apoptosis	6	1.00E-04	5.60E-03
GOTERM_BP_FAT	Negative regulation of programmed cell death	6	1.10E-04	5.00E-03
GOTERM_BP_FAT	Negative regulation of cell death	6	1.10E-04	4.80E-03
Annotation cluster 3	**Enrichment score: 3.57**			
GOTERM_BP_FAT	DNA damage checkpoint	4	3.90E-05	3.10E-03
GOTERM_BP_FAT	DNA integrity checkpoint	4	5.00E-05	3.60E-03
GOTERM_BP_FAT	DNA damage response, signal transduction	4	1.80E-04	6.50E-03
GOTERM_BP_FAT	Cell cycle checkpoint	4	2.60E-04	7.20E-03
GOTERM_BP_FAT	Response to DNA damage stimulus	4	1.50E-02	1.00E-01
Annotation cluster 4	**Enrichment score: 3.38**			
KEGG_PATHWAY	Bladder cancer	4	1.80E-04	1.50E-03
KEGG_PATHWAY	Endometrial cancer	4	3.40E-04	2.30E-03
KEGG_PATHWAY	Non-small-cell lung cancer	4	3.80E-04	2.30E-03
KEGG_PATHWAY	Acute myeloid leukemia	4	4.70E-04	2.60E-03
KEGG_PATHWAY	Glioma	4	6.00E-04	3.00E-03
KEGG_PATHWAY	Melanoma	4	8.50E-04	3.60E-03
Annotation cluster 5	**Enrichment score: 3.2**			
GOTERM_BP_FAT	Regulation of apoptosis	7	6.00E-04	1.40E-02
GOTERM_BP_FAT	Regulation of programmed cell death	7	6.30E-04	1.40E-02
GOTERM_BP_FAT	Regulation of cell death	7	6.50E-04	1.40E-02
Annotation cluster 6	**Enrichment score: 3.13**			
GOTERM_BP_FAT	Developmental growth	4	2.30E-04	7.20E-03
GOTERM_BP_FAT	Cell fate commitment	4	9.10E-04	1.90E-02
GOTERM_BP_FAT	Tissue morphogenesis	4	1.90E-03	3.30E-02
Annotation cluster 7	**Enrichment score: 2.74**			
GOTERM_BP_FAT	Apoptosis	6	1.20E-03	2.20E-02
GOTERM_BP_FAT	Programmed cell death	6	1.30E-03	2.30E-02
GOTERM_BP_FAT	Cell death	6	2.60E-03	3.90E-02
GOTERM_BP_FAT	Death	6	2.70E-03	3.80E-02
Annotation cluster 8	**Enrichment score: 2.7**			
GOTERM_BP_FAT	Positive regulation of macromolecule biosynthetic process	6	1.70E-03	3.00E-02
GOTERM_BP_FAT	Positive regulation of cellular biosynthetic process	6	2.10E-03	3.50E-02
GOTERM_BP_FAT	Positive regulation of biosynthetic process	6	2.20E-03	3.70E-02
Annotation cluster 9	**Enrichment score: 2.56**			
GOTERM_BP_FAT	Positive regulation of apoptosis	5	2.70E-03	3.90E-02
GOTERM_BP_FAT	Positive regulation of programmed cell death	5	2.70E-03	3.80E-02
GOTERM_BP_FAT	Positive regulation of cell death	5	2.80E-03	3.80E-02
Annotation cluster 10	**Enrichment score: 2.41**			
GOTERM_BP_FAT	Regulation of phosphorylation	5	3.60E-03	4.40E-02
GOTERM_BP_FAT	Regulation of phosphate metabolic process	5	4.10E-03	4.50E-02
GOTERM_BP_FAT	Regulation of phosphorus metabolic process	5	4.10E-03	4.50E-02
Annotation cluster 11	**Enrichment score: 2.22**			
GOTERM_BP_FAT	Positive regulation of transcription, DNA-dependent	5	3.90E-03	4.40E-02
GOTERM_BP_FAT	Positive regulation of transcription	5	7.00E-03	6.70E-02
GOTERM_BP_FAT	Positive regulation of gene expression	5	7.80E-03	7.20E-02
Annotation cluster 12	**Enrichment score: 2.15**			
GOTERM_BP_FAT	Positive regulation of protein kinase activity	4	3.50E-03	4.40E-02
GOTERM_BP_FAT	Positive regulation of kinase activity	4	3.90E-03	4.40E-02
GOTERM_BP_FAT	Positive regulation of transferase activity	4	4.30E-03	4.60E-02
GOTERM_BP_FAT	Regulation of protein kinase activity	4	1.20E-02	9.20E-02
GOTERM_BP_FAT	Regulation of kinase activity	4	1.30E-02	9.80E-02
GOTERM_BP_FAT	Regulation of transferase activity	4	1.40E-02	1.00E-01

Table 12 (*Continued*)

Category	Term	Gene count	*P*-value	FDR
Annotation cluster 13	**Enrichment score: 2.1**			
GOTERM_BP_FAT	Positive regulation of cellular protein metabolic process	4	4.00E-03	4.40E-02
GOTERM_BP_FAT	Positive regulation of protein metabolic process	4	4.50E-03	4.70E-02
GOTERM_BP_FAT	Regulation of cellular protein metabolic process	4	2.70E-02	1.80E-01
Annotation cluster 14	**Enrichment score: 2.1**			
GOTERM_BP_FAT	Branching morphogenesis of a tube	3	3.70E-03	4.30E-02
GOTERM_BP_FAT	Positive regulation of cell development	3	4.10E-03	4.50E-02
GOTERM_BP_FAT	Morphogenesis of a branching structure	3	4.80E-03	4.90E-02
GOTERM_BP_FAT	Tube morphogenesis	3	1.30E-02	1.00E-01
GOTERM_BP_FAT	Regulation of cell development	3	3.30E-02	2.00E-01
Annotation cluster 15	**Enrichment score: 2.06**			
GOTERM_BP_FAT	Neuron projection morphogenesis	4	3.10E-03	4.00E-02
GOTERM_BP_FAT	Cell projection morphogenesis	4	4.60E-03	4.80E-02
GOTERM_BP_FAT	Neuron projection development	4	5.20E-03	5.10E-02
GOTERM_BP_FAT	Cell part morphogenesis	4	5.20E-03	5.10E-02
GOTERM_BP_FAT	Neuron development	4	1.10E-02	9.00E-02
GOTERM_BP_FAT	Cell morphogenesis	4	1.30E-02	9.80E-02
GOTERM_BP_FAT	Cell projection organization	4	1.40E-02	1.00E-01
GOTERM_BP_FAT	Cellular component morphogenesis	4	1.70E-02	1.20E-01
GOTERM_BP_FAT	Neuron differentiation	4	2.20E-02	1.50E-01
Annotation cluster 16	**Enrichment score: 2.03**			
BIOCARTA	IGF-1 signaling pathway	3	7.00E-03	3.00E-01
BIOCARTA	IL-6 signaling pathway	3	7.00E-03	3.00E-01
BIOCARTA	Insulin signaling pathway	3	7.00E-03	3.00E-01
BIOCARTA	Signaling of hepatocyte growth factor receptor	3	2.20E-02	3.10E-01
Annotation cluster 17	**Enrichment score: 1.97**			
GOTERM_BP_FAT	Embryonic appendage morphogenesis	3	6.50E-03	6.30E-02
GOTERM_BP_FAT	Embryonic limb morphogenesis	3	6.50E-03	6.30E-02
GOTERM_BP_FAT	Limb morphogenesis	3	8.40E-03	7.50E-02
GOTERM_BP_FAT	Appendage morphogenesis	3	8.40E-03	7.50E-02
GOTERM_BP_FAT	Limb development	3	9.00E-03	7.70E-02
GOTERM_BP_FAT	Appendage development	3	9.00E-03	7.70E-02
GOTERM_BP_FAT	Embryonic morphogenesis	3	6.80E-02	3.40E-01
Annotation cluster 18	**Enrichment score: 1.81**			
GOTERM_BP_FAT	Response to ethanol	3	3.60E-03	4.30E-02
GOTERM_BP_FAT	Response to metal ion	3	1.40E-02	1.00E-01
GOTERM_BP_FAT	Response to inorganic substance	3	3.30E-02	2.00E-01
GOTERM_BP_FAT	Response to drug	3	3.60E-02	2.10E-01
Annotation cluster 19	**Enrichment score: 1.59**			
GOTERM_BP_FAT	Response to estrogen stimulus	3	9.40E-03	7.90E-02
GOTERM_BP_FAT	Positive regulation of protein modification process	3	2.80E-02	1.80E-01
GOTERM_BP_FAT	Regulation of protein modification process	3	6.40E-02	3.20E-01
Annotation cluster 20	**Enrichment score: 1.57**			
GOTERM_BP_FAT	Protein amino acid phosphorylation	5	1.30E-02	9.70E-02
GOTERM_BP_FAT	Phosphorylation	5	2.30E-02	1.50E-01
GOTERM_BP_FAT	Phosphate metabolic process	5	4.30E-02	2.40E-01
GOTERM_BP_FAT	Phosphorus metabolic process	5	4.30E-02	2.40E-01
Annotation cluster 21	**Enrichment score: 1.55**			
GOTERM_BP_FAT	Cell aging	3	9.60E-04	1.90E-02
GOTERM_BP_FAT	Negative regulation of neuron apoptosis	3	2.30E-03	3.70E-02
GOTERM_BP_FAT	Aging	3	1.00E-02	8.50E-02
GOTERM_BP_FAT	Actin cytoskeleton organization	3	3.90E-02	2.20E-01
GOTERM_BP_FAT	Actin filament-based process	3	4.40E-02	2.40E-01
GOTERM_BP_FAT	Membrane organization	3	9.90E-02	4.40E-01
GOTERM_CC_FAT	Membrane fraction	3	3.20E-01	9.40E-01
GOTERM_CC_FAT	Insoluble fraction	3	3.30E-01	9.30E-01
Annotation cluster 22	**Enrichment score: 1.47**			
GOTERM_BP_FAT	Ras protein signal transduction	3	9.40E-03	7.90E-02
KEGG_PATHWAY	Long-term depression	3	1.50E-02	4.40E-02

(*Continued*)

Table 12 (*Continued*)

Category	Term	Gene count	*P*-value	FDR
KEGG_PATHWAY	B cell receptor signaling pathway	3	1.70E-02	4.50E-02
KEGG_PATHWAY	VEGF signaling pathway	3	1.70E-02	4.50E-02
KEGG_PATHWAY	Fc epsilon RI signaling pathway	3	1.90E-02	4.60E-02
KEGG_PATHWAY	ErbB signaling pathway	3	2.30E-02	5.40E-02
KEGG_PATHWAY	Gap junction	3	2.40E-02	5.50E-02
KEGG_PATHWAY	GnRH signaling pathway	3	2.90E-02	6.30E-02
KEGG_PATHWAY	T-cell receptor signaling pathway	3	3.40E-02	7.20E-02
KEGG_PATHWAY	Insulin signaling pathway	3	5.20E-02	1.00E-01
GOTERM_BP_FAT	Small GTPase-mediated signal transduction	3	6.70E-02	3.40E-01
KEGG_PATHWAY	Chemokine signaling pathway	3	9.20E-02	1.70E-01
KEGG_PATHWAY	Regulation of actin cytoskeleton	3	1.20E-01	2.10E-01
KEGG_PATHWAY	MAPK signaling pathway	3	1.70E-01	2.80E-01
Annotation cluster 23	**Enrichment score: 1.45**			
GOTERM_BP_FAT	Axonogenesis	3	3.00E-02	1.80E-01
GOTERM_BP_FAT	Cell morphogenesis involved in neuron differentiation	3	3.40E-02	2.00E-01
GOTERM_BP_FAT	Cell morphogenesis involved in differentiation	3	4.50E-02	2.50E-01
Annotation cluster 24	**Enrichment score: 1.31**			
GOTERM_CC_FAT	Nucleoplasm	6	6.20E-03	4.90E-01
GOTERM_CC_FAT	Nuclear lumen	6	4.50E-02	5.70E-01
GOTERM_CC_FAT	Intracellular organelle lumen	6	9.40E-02	7.40E-01
GOTERM_CC_FAT	Organelle lumen	6	1.00E-01	7.20E-01
GOTERM_CC_FAT	Membrane-enclosed lumen	6	1.10E-01	7.10E-01
Annotation cluster 25	**Enrichment score: 1.3**			
GOTERM_BP_FAT	Hemopoiesis	3	4.30E-02	2.40E-01
GOTERM_BP_FAT	Hemopoietic or lymphoid organ development	3	5.10E-02	2.70E-01
GOTERM_BP_FAT	Immune system development	3	5.60E-02	2.90E-01
Annotation cluster 26	**Enrichment score: 1.06**			
SP_PIR_KEYWORDS	Transcription regulation	6	6.30E-02	3.80E-01
SP_PIR_KEYWORDS	Transcription	6	6.80E-02	3.90E-01
GOTERM_BP_FAT	Transcription	6	1.60E-01	6.10E-01

Abbreviations: DAVID, Database for Annotation, Visualization and Integrated Discovery; FDR, false discovery rate; GnRH, gonadotropin releasing hormone; IGF, insulin-like growth factor; IL, interleukin; VEGF, vascular endothelial growth factor.

nuclear lumen, intracellular organelle lumen, organelle lumen, membrane-enclosed lumen, hemopoiesis, hemopoietic or lymphoid organ development, immune system development, and transcription regulation (Table 12).

Furthermore, our DAVID analysis revealed that there were 33 KEGG pathways significantly enriched in the target list of hsa-miR-181a-5p, based on both TarBase and miRTarBase (Table 13). These pathways included pathways in cancer, prostate cancer, thyroid cancer, renal cell carcinoma, chronic myeloid leukemia, neurotrophin signaling pathway, dorsoventral axis formation, bladder cancer, endometrial cancer, non-small-cell lung cancer, acute myeloid leukemia, glioma, long-term potentiation, melanoma, colorectal cancer, melanogenesis, cell cycle, natural killer cell–mediated cytotoxicity, focal adhesion, notch signaling pathway, long-term depression, pancreatic cancer, B cell receptor signaling pathway, VEGF signaling pathway, Fc epsilon RI signaling pathway, ErbB signaling pathway, gap junction, GnRH signaling pathway, T cell receptor signaling pathway, insulin signaling pathway, Jak-STAT signaling pathway, chemokine signaling pathway, and prion diseases.

Among the 313 validated targets of hsa-miR-181a-5p, 22 were cancer genes (Table 14). These included *ATM*, *BCL2*, *BRCA1*, *CCDC6*, *CCND1*, *CDX2*, *EP300*, *FBXO11*, *H3F3B*, *HOOK3*, *HOXA11*, *HRAS*, *KRAS*, *MAP2K1*, *NOTCH1*, *NOTCH2*, *PLAG1*, *PTPN11*, *RBM15*, *STAG2*, *TAF15*, and *TSHR*. The KEGG pathway analysis also indicate that the targets regulated by hsa-miR-181a that are cancer genes are all involved in the tumorigenesis of bladder cancer, endometrial cancer, non-small-cell lung cancer, acute myeloid leukemia, glioma, melanoma, and colorectal cancer.

Now when we looked at the prediction accuracy of all the nine algorithms we used, we found that all the predicting programs behaved poorly. The reasons for this may include: a) the predicting criteria were not set up properly; b) the matching criteria for hsa-miR-181a with the complementary sites of the target mRNAs may have been too restrictive or too loose; c) the value for the threshold was not properly set prior to prediction;

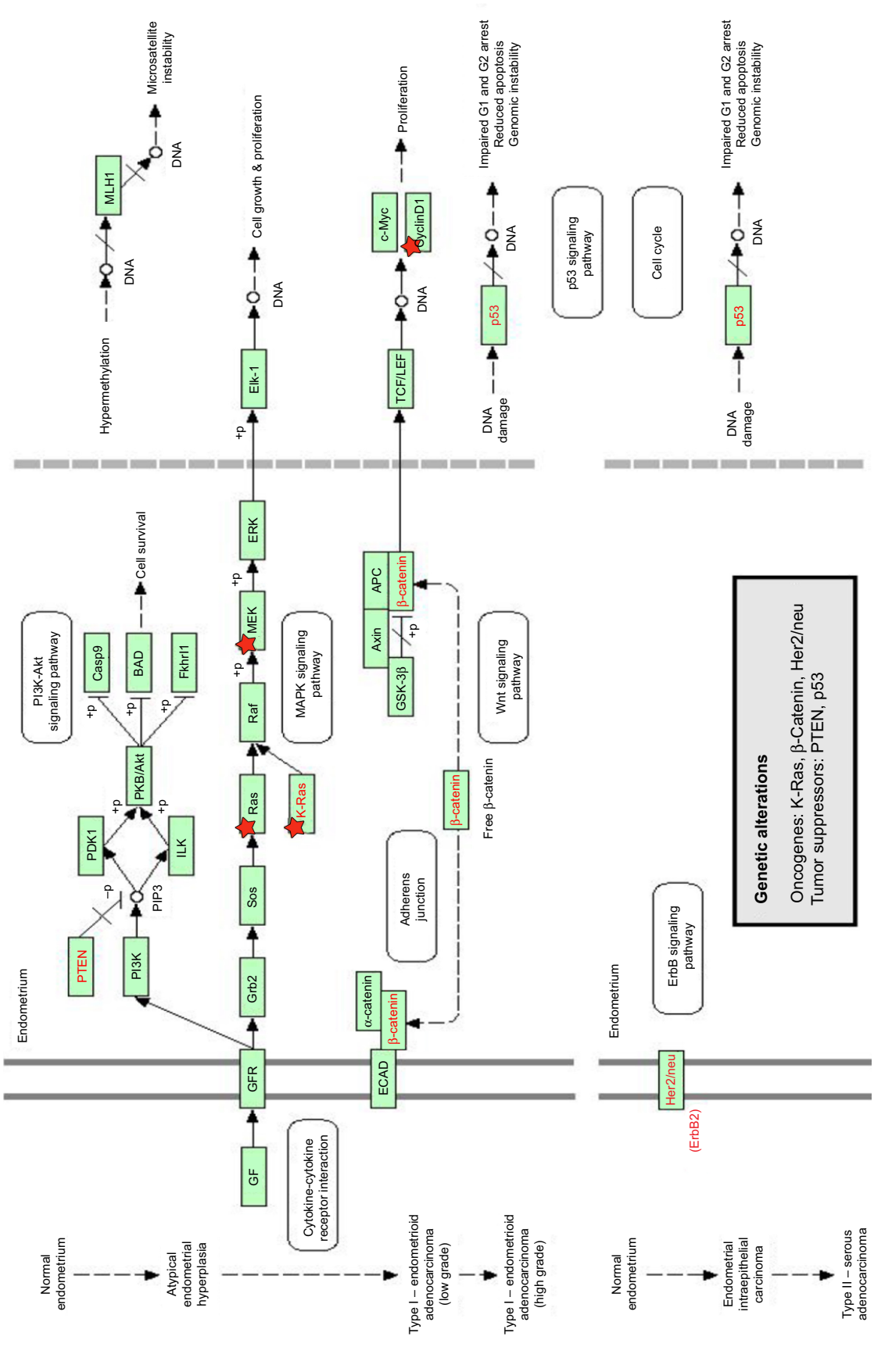

Figure 9 Endometrial carcinoma pathways in the combined target list of hsa-miR-181a-5p based on both TarBase and miRTarBase 4.0.

Notes: EC has two types. Type I EC, or estrogen-dependent endometrioid EC, represents the most common subtype. It is an estrogen-associated lesion often seen in conjunction with endometrial hyperplasia. The histological subtypes that correspond to endometrioid adenocarcinoma and its variants, as well as mucinous adenocarcinoma, are allocated to this group. Type II EC, or nonendometrioid EC, tends to affect older, postmenopausal women and is a non-estrogen-associated lesion. These cancers are not preceded by endometrial hyperplasia, though they can occasionally arise in endometrial polyps or from precancerous lesions, endometrial intraepithelial carcinoma, or in the vicinity of atrophic endometrium. The clinicopathological differences between the two types are paralleled by specific genetic alterations, with type I EC showing microsatellite instability and mutations in PTEN, PIK3CA, KRAS, and CTNNB1 (β-catenin), and type II exhibiting p53 mutations and chromosomal instability.[17] hsa-miR-181a-5p has been found to regulate these genes and eventually promote EC initiation, development, growth, and metastasis.

Abbreviation: EC, endometrial cancer.

Table 13 KEGG pathways for the combined targets of hsa-miR-181a-5p based on TarBase 6.0 and miRTarBase 4.0

Signaling pathway	Gene count	%	*P*-value	FDR
Pathways in cancer	7	31.8	1.30E-04	1.30E-03
Prostate cancer	6	27.3	2.60E-06	7.80E-05
Thyroid cancer	5	22.7	8.20E-07	4.90E-05
Renal cell carcinoma	5	22.7	3.00E-05	5.90E-04
Chronic myeloid leukemia	5	22.7	3.90E-05	4.70E-04
Neurotrophin signaling pathway	5	22.7	2.80E-04	2.10E-03
Dorsoventral axis formation	4	18.2	3.70E-05	5.50E-04
Bladder cancer	4	18.2	1.80E-04	1.50E-03
Endometrial cancer	4	18.2	3.40E-04	2.30E-03
Non-small-cell lung cancer	4	18.2	3.80E-04	2.30E-03
Acute myeloid leukemia	4	18.2	4.70E-04	2.60E-03
Glioma	4	18.2	6.00E-04	3.00E-03
Long-term potentiation	4	18.2	7.50E-04	3.50E-03
Melanoma	4	18.2	8.50E-04	3.60E-03
Colorectal cancer	4	18.2	1.40E-03	5.50E-03
Melanogenesis	4	18.2	2.20E-03	8.30E-03
Cell cycle	4	18.2	4.30E-03	1.50E-02
Natural killer cell–mediated cytotoxicity	4	18.2	5.20E-03	1.70E-02
Focal adhesion	4	18.2	1.60E-02	4.50E-02
Notch signaling pathway	3	13.6	7.10E-03	2.20E-02
Long-term depression	3	13.6	1.50E-02	4.40E-02
Pancreatic cancer	3	13.6	1.60E-02	4.30E-02
B-cell receptor signaling pathway	3	13.6	1.70E-02	4.50E-02
VEGF signaling pathway	3	13.6	1.70E-02	4.50E-02
Fc epsilon RI signaling pathway	3	13.6	1.90E-02	4.60E-02
ErbB signaling pathway	3	13.6	2.30E-02	5.40E-02
Gap junction	3	13.6	2.40E-02	5.50E-02
GnRH signaling pathway	3	13.6	2.90E-02	6.30E-02
T cell receptor signaling pathway	3	13.6	3.40E-02	7.20E-02
Insulin signaling pathway	3	13.6	5.20E-02	1.00E-01
Jak-STAT signaling pathway	3	13.6	6.60E-02	1.30E-01
Chemokine signaling pathway	3	13.6	9.20E-02	1.70E-01
Prion diseases	2	9.1	9.20E-02	1.70E-01

Abbreviations: FDR, false discovery rate; GnRH, gonadotropin releasing hormone; KEGG, Kyoto Encyclopedia of Genes and Genomes; VEGF, vascular endothelial growth factor.

and d) the calculation of the *P*-values may have been too simple or too complicated. The poor predictive ability for all these algorithms also emphasizes the importance of experimental validation of the targets of any specific miRNAs.

Clinical validation of the role of hsa-miR-181a in EC tumorigenesis

Next, we aimed to validate the function of hsa-miR-181a in the pathogenesis of EC by measuring and comparing the expression levels of hsa-miR-181a in normal, benign, and malignant endometrial tissues. The association of disease progression of EC with the expression profile of hsa-miR-181a was also determined. There are two types of EC with distinct histological characteristics.[15,51] Herein, a total of 78 tissue samples were classified by immunohistochemical staining. There were 47, 18, and 13 samples that were categorized as EC, endometrial hyperplasia, or normal endometrium, respectively. For the EC group, there were 37 samples, and 10 samples that belonged to type I and type II EC, respectively. The type I EC was ER- and PR-positive (Figure 10), and the type II EC was ER- and PR-negative (Figure 11).

To examine the potential role of hsa-miR-181a in the development and progression of EC, the expression profile of hsa-miR-181a was tested in all collected clinical samples, using RT-PCR (Figure S1). In comparison with normal endometrium, the expression level of hsa-miR-181a was increased 8.5-, 31.2-, and 4.1-fold in type I EC, type II EC, and endometrial hyperplasia, respectively ($P<0.05$, by one-way ANOVA) (Tables 15 and 16). The expression level of hsa-miR-181a in type II EC was higher (3.7-fold) than that in type I EC ($P<0.05$). In addition, hsa-miR-181a had a higher expression level in EC than that in endometrial hyperplasia.

Since we have observed the differential expression profile of hsa-miR-181a in all examined clinical samples, we further

Table 14 Combined targets of hsa-miR-181a-5p based on TarBase 6.0 and miRTarBase 4.0, that are cancer genes

Gene symbol	Name	Tumor types (somatic)	Tumor types (germline)
ATM	Ataxia telangiectasia mutated	T-PLL	Leukemia; lymphoma; medulloblastoma; glioma
BCL2	B-cell CLL/lymphoma 2	NHL; CLL	
BRCA1	Familial breast/ovarian cancer gene 1	Ovarian	Breast; ovarian
CCDC6	Coiled-coil domain containing 6	NSCLC	
CCND1	Cyclin D1	CLL; B-ALL; breast	
CDX2	Caudal type homeobox transcription factor 2	AML	
EP300	300 kDa E1A-binding protein gene	Colorectal; breast; pancreatic; AML; ALL; DLBCL	
FBXO11	F-box protein 11	DLBCL	
H3F3B	H3 histone; family 3B (H3.3B)	Chondroblastoma	
HOOK3	Hook homolog 3	Papillary thyroid	
HOXA11	Homeobox A11	CML	
HRAS	v-Ha-ras Harvey rat sarcoma viral oncogene homolog	Infrequent sarcomas; rare other tumor types	Rhabdomyosarcoma; ganglioneuroblastoma; bladder
KRAS	v-Ki-ras2 Kirsten rat sarcoma 2 viral oncogene homolog	Pancreatic; colorectal; lung; thyroid; AML; other tumor types	
MAP2K1	Mitogen-activated protein kinase kinase 1	NSCLC; melanoma; colorectal	
NOTCH1	Notch homolog 1; translocation-associated (Drosophila) (TAN1)	T-ALL	
NOTCH2	Notch homolog 2	Marginal zone lymphoma; DLBCL	
PLAG1	Pleiomorphic adenoma gene 1	Salivary adenoma	
PTPN11	Protein tyrosine phosphatase; nonreceptor type 11	JMML; AML; MDS	
RBM15	RNA binding motif protein 15	Acute megakaryocytic leukemia	
STAG2	Stromal antigen 2	Bladder carcinoma; glioblastoma; melanoma; Ewing's sarcoma; myeloid neoplasms	
TAF15	TAF15 RNA polymerase II; TBP-associated factor; 68 kDa	Extraskeletal myxoid chondrosarcoma; ALL	
TSHR	Thyroid stimulating hormone receptor	Toxic thyroid adenoma	Thyroid adenoma

Abbreviations: ALL, acute lymphocytic leukemia; AML, adult acute myeloid leukemia; B-ALL, B-cell ALL; CLL, chronic lymphocytic leukemia; CML, chronic myelogenous leukemia; DLBCL, diffuse large B-cell lymphoma; JMML, juvenile myelomonocytic leukemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin lymphoma; NSCLC, non-small-cell lung cancer; T-ALL, T-cell ALL; T-PLL, T-cell subtype of prolymphocytic leukemia.

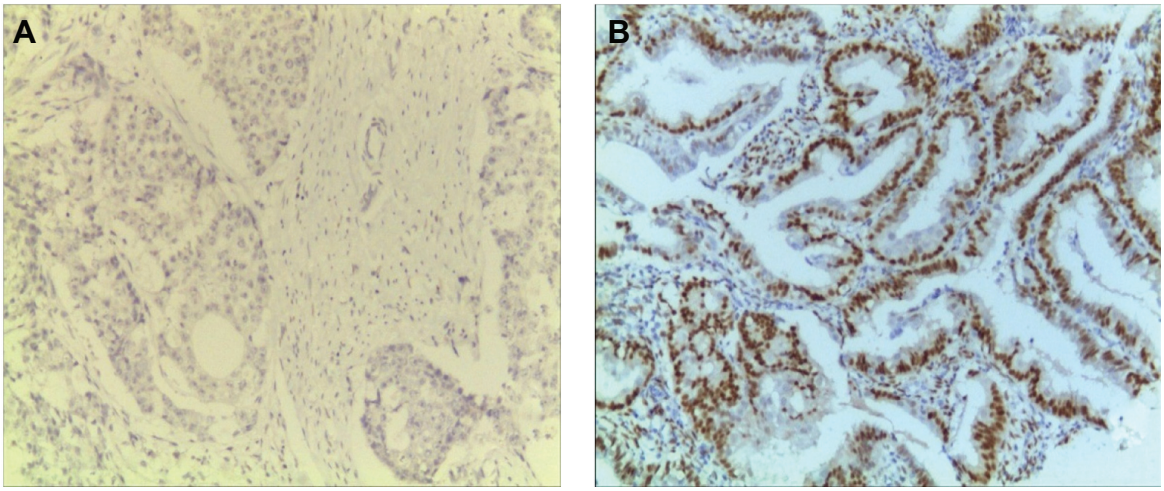

Figure 10 The expression of ER and PR in type I EC.
Notes: The expression of ER and PR was evaluated by immunohistochemistry. Dewaxed and dehydrated sections were washed with PBS and then, incubated with 3% peroxyl in methanol to terminate the activity of endogenous peroxidases. The sections were washed with PBS and immersed into boiled citrate-buffered solution for 10 minutes. The sections were blocked with 5% bovine serum albumin in PBS for 20 minutes at room temperature. Following that, the sections were probed with primary antibody against ER or PR, followed by biotinylated second anti-rabbit antibody. (**A**) Positive expression of ER; and (**B**) positive expression of PR.
Abbreviations: EC, endometrial cancer; ER, estrogen receptor; PBS, phosphate-buffered saline; PR, progesterone receptor.

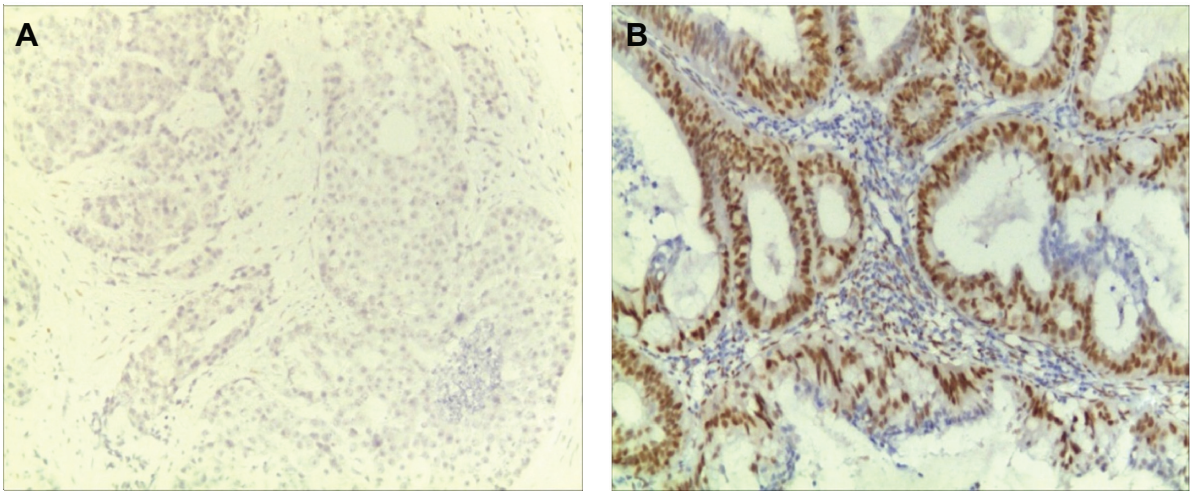

Figure 1 The expression of ER and PR in type II EC.
Notes: The expression of ER and PR was evaluated by immunohistochemistry. Dewaxed and dehydrated sections were washed with PBS and then, incubated with 3% peroxyl in methanol to terminate the activity of endogenous peroxidases. The sections were washed with PBS and immersed into boiled citrate-buffered solution for 10 minutes. The sections were blocked with 5% bovine serum albumin in PBS for 20 minutes at room temperature. Following that, the sections were probed with primary antibody against ER or PR, followed by biotinylated second anti-rabbit antibody. (**A**) Negative expression of ER; and (**B**) negative expression of PR.
Abbreviations: EC, endometrial cancer; ER, estrogen receptor; PBS, phosphate-buffered saline; PR, progesterone receptor.

assessed the association between the expression of hsa-miR-181a and the development of EC with regard to histological type, stage, grade, migration, and invasion. As shown in Table 17, compared with the stage I and II EC, there was a remarkable increase in the expression level of hsa-miR-181a in stage III and VI EC (P=0.01, by one-way ANOVA). The expression level of hsa-miR-181a was increased in EC with lymph migration and myometrial invasion. In addition, compared with the grade 1 EC, there was an increase in the expression level of hsa-miR-181a in grade 2 and grade 3 EC (P>0.05; Table 17). Taken together, these results indicate that there is an association between the expression level of hsa-miR-181a and the clinical development and progression of EC.

Discussion

miRNAs play critical roles in regulating proliferation, differentiation, apoptosis, development, metabolism, and immunity.[2] miRNAs may act as oncogenes or tumor suppressors, and they could play a potential role as diagnostic and prognostic biomarkers of cancers.[7,52] Specific miRNAs are expressed in various tissues, and changes in regulation of gene expression are thought to cause carcinogenesis. Thus, tissue-specific miRNAs may be used as effective biomarkers for cancer diagnosis, treatment, and prognosis.[8] Hsa-miR-181a has been proposed to play a role in the pathogenesis, development, progression, metastasis, prognosis, and therapeutic response to chemo- and radiotherapy in EC,[4,53] ovarian cancer,[54] glioma,[30,55] liver cancer,[56] colorectal cancer,[57–59] gastric cancer,[60,61] lung cancer,[62] breast cancer,[63–67] cervical carcinoma,[68,69] pancreatic cancer,[46] osteosarcoma,[70] oral squamous cell carcinoma,[71,72] B-cell lymphoma,[73] thyroid cancer,[74] salivary adenoid cystic carcinoma,[75] and acute

Table 15 Expression level of hsa-miR-181a in normal endometrium, endometrial hyperplasia, and EC

Tissue	N	ΔCt	$2^{-\Delta\Delta Ct}$
Endometrial cancer	47	−3.356±2.401	10.240
Endometrial hyperplasia	18	−1.893±2.568	4.073
Normal endometrium	13	0.133±2.527	1.000

Note: ΔCt, normalized threshold cycle; $2^{-\Delta\Delta Ct}$, a method used to calculate relative changes in the gene expression determined from real-time quantitative polymerase chain reaction experiments and it means the fold change.
Abbreviations: ΔCt, normalized threshold cycle; EC, endometrial cancer.

Table 16 Differential expression level of hsa-miR181a in endometrium tissues

Tissue	$2^{-\Delta\Delta Ct}$	*P*
Endometrial hyperplasia vs normal endometrial tissue	4.073	0.027
Type II endometrial cancer vs type I endometrial cancer	3.668	0.032
Type II endometrial cancer vs endometrial hyperplasia	7.669	0.003
Type II endometrial cancer vs normal endometrial tissue	31.233	0.000
Type I endometrial cancer vs endometrial hyperplasia	2.091	0.127
Type I endometrial cancer vs normal endometrial tissue	8.515	0.000

Note: $2^{-\Delta\Delta Ct}$ a method used to calculate relative changes in the gene expression determined from real-time quantitative polymerase chain reaction experiments and it means the fold change.

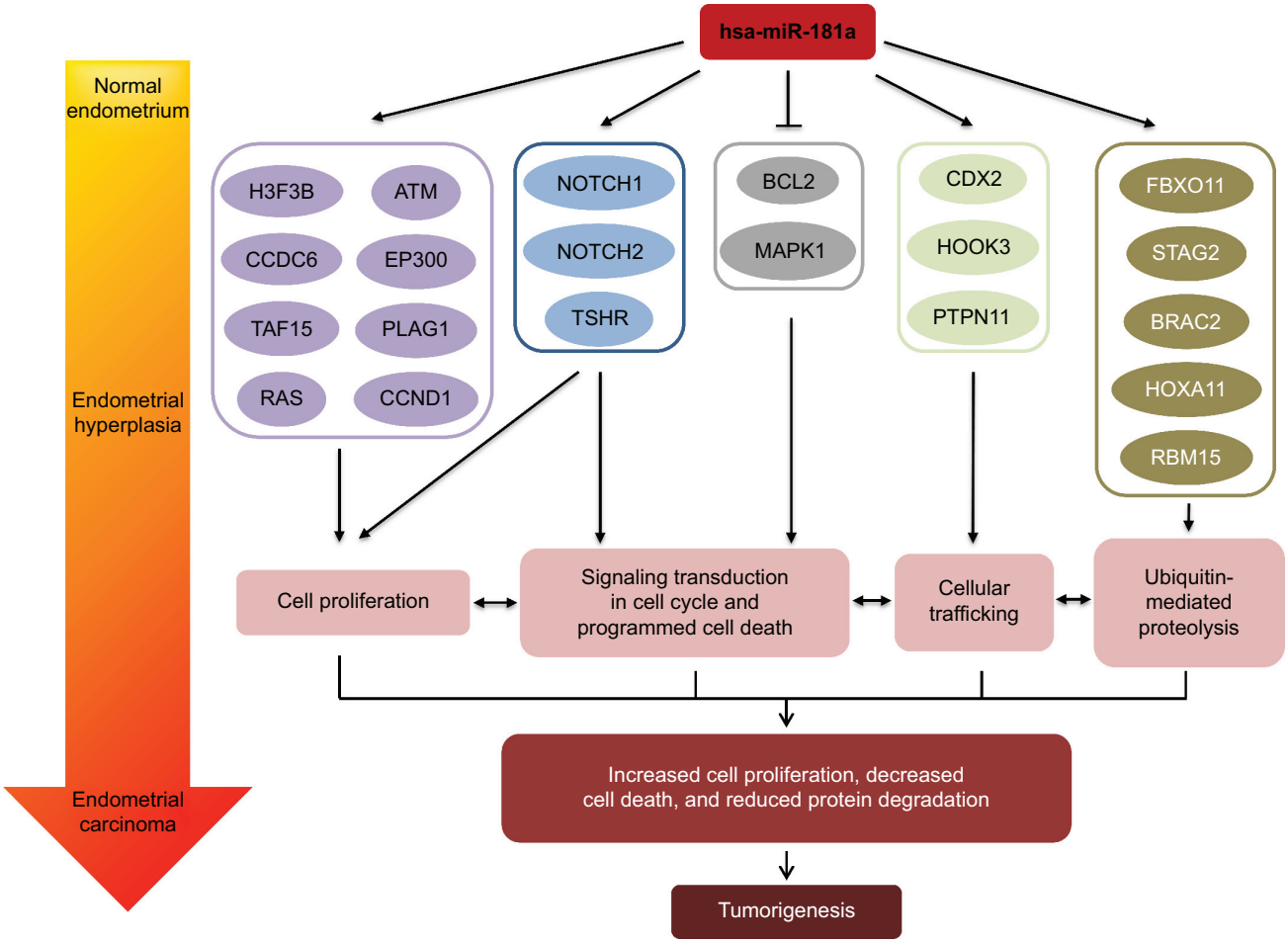

Figure 12 Proposed mechanisms for how hsa-miR-181a regulates cancer genes and may serve as an oncogene for the development of EC.

Notes: hsa-miR-181a may play a role in the tumorigenesis of EC via multiple pathways that interplay with many important oncogenes and tumor suppressors. hsa-miR-181a may interact with H3F3B, ATM, CCDC6, TAF15, RAS, and PLAG1 to promote cell proliferation. It may suppress cell apoptosis via interaction with NOTCH1, NOTCH2, MAPK1, and BCL2. It may affect ubiquitin-mediated proteolysis via regulation of FBXO11, STAG2, BRCA2, HOXA11, and RBM15. All these actions may transform the normal endometrial cells into tumor cells.

Abbreviation: EC, endometrial cancer; ATM, ATM serine/threonine kinase; BCL2, B-cell lymphoma 2; CCDC6, coiled-coil domain containing 6; PLAG1, pleiomorphic adenoma gene 1; MAPK1, mitogen activated kinase-like protein 1; RAS, rat sarcoma viral oncogene homolog; BRCA2, breast cancer 2, early onset; STAG2, stromal antigen 2; HOXA11, homeobox A11; RBM15, RNA binding motif protein 15; NOTCH1, notch 1; H3F3B, H3 histone, family 3B; FBXO11, F-box protein 11; TAF15, TAF15 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 68 kDa; HOOK3, hook microtubule-tethering protein 3; PTPN11, protein tyrosine phosphatase, non-receptor type 11; TSHR, thyroid stimulating hormone receptor; CDX2, caudal type homeobox 2; EP300, E1A binding protein p300.

Table 17 Association between hsa-miR-181a expression and pathological characteristics of EC

Pathological characteristics	N	ΔCt	*F*	*P*
FIGO stage				
I+II	38	−2.929±2.306	7.120	0.011
III+VI	9	−5.160±2.002		
Tumor grade				
G1	32	−3.152±2.528	0.723	0.400
G2 + G3	15	−3.792±2.118		
Lymph migration				
No	42	−3.183±2.370	2.095	0.155
Yes	5	−4.808±2.401		
Myometrial invasion				
≤1/2	39	−3.086±2.406	3.033	0.088
>1/2	8	−4.674±2.015		

Abbreviations: ΔCt, normalized threshold cycle; EC, endometrial cancer; FIGO, International Federation of Gynecology and Obstetrics.

and chronic leukemias.[76–81] Ciafrè et al,[82] firstly reported that the expression of hsa-miR-181a was significantly downregulated in primary glioblastomas and human glioblastoma cell lines compared with normal brain tissue. As in glioblastoma, significant downregulation of hsa-miR-181a was also observed in squamous lung cell carcinoma,[83] oral squamous cell carcinoma,[71] luminal A-like breast cancer,[67] and non-small-cell lung cancer.[84] However, hsa-miR-181a was significantly overexpressed in MCF-7 breast cancer cells,[85] colorectal cancer,[28] and hepatocellular carcinoma cells.[56,86,87] Hsa-miR-181a was upregulated in acute myeloid leukemia,[76] especially in the M1 and M2 subtypes, and in myelodysplastic syndromes[88] but downregulated in multiple myeloma[89] and chronic lymphocyte leukemia.[79,90] hsa-miR-181a can serve as an oncogene[46,54,56–58,60,61,65,66,68–70,87,91]

or tumor suppressor,[55,67,71,75,78,79,92] implicating its multifaceted and complex roles in the regulation of its target genes and signaling pathways associated with cancer initiation, growth, development, progression, and metastasis.

In the present study, our bioinformatic study predicted that hsa-miR-181a could regulate a large number of targets, including proteins that participate in regulation of cell proliferation, cell cycle, apoptosis, autophagy, metabolism, signaling transduction, and transport. A further search in TarBase and miRTarBase identified 313 targets of hsa-miR-181a-5p, and 22 of these genes are cancer genes that play critical roles in the tumorigenesis of various cancers.

During the prediction process, we employed ten different predicting programs that are based on different matching criteria and calculating algorithms. All the algorithms displayed disappointing predictive accuracy and ability when compared with the validated targets of hsa-miR-181a. It appears that there is a need to refine or combine these algorithms to improve their predictive accuracy and ability. Indeed, most of these prediction algorithms, including RNAhybrid, miRanda, TargetScan, DIANA microT, and PicTar exhaustively analyze all the possible miRNA: mRNA pairs, searching for structural evidence that could suggest the existence of an interaction. Although these approaches are significantly cheaper than those based on experimental validation, results of these methods are in many cases uncorrelated to each other, and their degree of overlap is low as shown in this study. The weakness of these algorithms depends on many factors, especially on the impossibility of incorporating in a single model all the possible interplaying variants/factors that can affect miRNA targeting and the prediction outcomes, especially in mammals. Different results can also depend on the approach used and on the rules considered for the miRNA targeting, as well as on the type of resource of sequences they use as a reference dataset.[93] Shirdel et al[94] found that the precision and recall values computed against validated interactions of a specific algorithm were generally poor, but a combination of these algorithms can improve the prediction precision. Recently, some machine learning approaches have been incorporated, to learn to combine the outputs of distinct prediction algorithms and improve their accuracy.[95–97] Zhang and Verbeek[97] proposed the application of a supervised learning algorithm, ie, a Bayesian network learner, to distinct sets of features considered in three prediction algorithms, including RNAhybrid, miRanda, and TargetScan. Pio et al[96] proposed a semisupervised ensemble learning approach using miRTarBase as the set of labeled (positive) interactions and microRNA Data Integration Portal (mirDIP) as the set of unlabeled interactions, and the predictive accuracy was improved.

We next compared the expression levels of hsa-miR-181a in normal endometrium, endometrial hyperplasia, and type I and type II EC. We found that the expression level of hsa-miR-181a was significantly higher in EC than that in normal endometrium and that advanced EC exhibited a higher expression level of hsa-miR-181a. These observations demonstrate that there was an association between the expression level of hsa-miR-181a and the progression of EC and that hsa-miR-181a might serve as an oncogene in the development and progression of EC.

Many miRNAs are aberrantly expressed in cancer, resulting in functional alterations in cell differentiation, proliferation, migration, invasion, programmed cell death, and survival.[5,6,8,53] A number of oncogenes and tumor-suppressor genes could be potentially regulated by miRNAs. miRNAs are presumed to be a class of genes involved in human tumorigenesis, and miRNA-mediated gene regulation is an important cellular biologic process in cancer development.[5,6,8,53] For example, let-7 acts as tumor suppressor gene, which was found to be downregulated in lung tumors and associated with a poor postoperative prognosis.[98] It has been showed that the RAS oncogene was regulated by let-7 and that a decreased expression level of let-7 in lung cancer resulted in an increase in the expression level of the RAS oncogene.[99]

Many studies have showed that upregulation of hsa-miR-181a promotes carcinogenesis, cancer cell growth, and metastasis in a variety of cancers, via regulation of a number of molecular targets and signaling pathways related to cell proliferation, invasion, migration, survival, and cell death.[46,54,56–58,60,61,65,66,68–70,87,91] Zou et al[87] observed an increase in the expression level of hsa-miR-181a, which may contribute to the development and progression of hepatocellular carcinoma via targeting of E2F transcription factor 5, p130-binding (E2F5). hsa-miR-181a was also upregulated in hepatocellular cancer stem cells.[86,100] Silencing hsa-miR-181 led to a decreased motility and invasion of hepatocellular cancer stem cells, via targeting of the putative tumor suppressor Ras association domain family 1 isoform A (RASSF1), metalloproteinase inhibitor 3 (ie, TIMP3), and nemo-like kinase (NLK).[100] hsa-miR-181 could directly target hepatic transcriptional regulators of differentiation, including caudal type homeobox 2 (CDX2), GATA binding protein 6 (GATA6), and NLK, an inhibitor of Wnt/β-catenin signaling.[86] hsa-miR-181a promoted tumor growth and liver metastasis in colorectal cancer patients by targeting the tumor suppressor WNT inhibitory factor 1 (WIF1).[28] hsa-miR-181a was most elevated in these colorectal cancer patients with liver metastases and could serve as an independent prognostic factor of poor overall survival.[28] hsa-miR-181a

showed a potent tumor-promoting effect through inhibition of the expression of WIF1 and promotion of epithelial–mesenchymal transition.[28] Moreover, upregulation of hsa-miR-181a plays a potential role in the development of gastric cancer by targeting the tumor suppressor ATM serine/threonine kinase (ATM). Consequently, it leads to promotion of gastric cancer cell proliferation and inhibition of apoptosis. Wei et al[101] showed that the PTEN/Akt signaling pathway was involved in the regulatory effect of hsa-miR-181a in the development of colon cancer, by promoting cell growth. hsa-miR-181a also played an important role in ovarian cancer progression, by promoting epithelial–mesenchymal transition.[54] These data indicate that hsa-miR-181a may function as oncogenic miRNA in cancer development and progression. In agreement with previous studies, our findings showed that there was a significant increase in the expression level of hsa-miR-181a in EC compared with that in normal endometrium. Moreover, our results showed that advanced EC had a significant higher expression level of hsa-miR-181a than that in early stage of EC, suggesting that hsa-miR-181a may have a critical role in tumor metastasis of advanced EC.

On the other hand, hsa-miR-181 may function as a tumor suppressor. In glioma, hsa-miR-181a was shown to be downregulated.[30] Both hsa-miR-181a and hsa-miR-181b triggered growth inhibition, induced apoptosis, and inhibited invasion in glioma cells. Transiently overexpressed hsa-miR-181a significantly sensitized malignant glioma U87MG cells to radiation with downregulated BCL2.[55] In chronic lymphocytic leukemia, hsa-miR-181a together with hsa-miR-15a, hsa-miR-16-1, hsa-miR-29b, and hsa-miR-181b were all downregulated.[90] These miRNAs may play a role in the pathogenesis of chronic lymphocytic leukemia and serve as new biomarkers for the prediction of prognosis in chronic lymphocytic leukemia. hsa-miR-181a expression level was found to be significantly lower in poor prognosis patients, and a low expression of hsa-miR-181a and hsa-miR-181b was associated with shorter overall survival and treatment-free survival in patients with chronic lymphocytic leukemia.[79] Furthermore, hsa-miR-181a inhibited the migration, invasion, and proliferation of salivary adenoid cystic carcinoma cells, and suppressed tumor growth and lung metastasis in nude mice, via targeting of MAP2K1, MAPK1, and SNAI2.[75]

Based on our DAVID and KEGG pathway analysis, PI3K/Akt, MAPK, and Wnt signaling pathways played important roles in the development of type I EC. CCND1, HRAS, and KRAS are all key components in these pathways, which are all validated targets of hsa-miR-181a.

The expression level of hsa-miR-181a has been proposed as a potential biomarker for assessing prognosis and therapeutic response in cancer. Ouyang et al[102] suggested that hsa-miR-181a may be a potential biomarker for predicting chemoresistance in the treatment of triple negative breast cancer. It also has been showed that hsa-miRNA-181a enhanced the chemoresistance of human cervical squamous cell carcinoma to cisplatin by targeting protein kinase Cδ[69] and that hsa-miR-181a may serve an oncologic miRNA biomarker for luminal A-like breast cancer.[67] Interestingly, Pichler et al[59] showed a reverse correlation between hsa-miR-181a expression level and survival rate in patients with colorectal cancer. In our study, we observed a significant difference in the expression level of hsa-miR-181a among normal endometrium, endometrial hyperplasia, and EC, and a higher expression level of hsa-miR-181a in advanced EC. Our findings suggest that the expression level of hsa-miR-181a might serve as a useful biomarker for the prediction of prognosis of EC in clinic.

In summary, our bioinformatics studies have showed that hsa-miR-181a might regulate a large number of target genes that are important in the regulation of critical cell processes. To date, 313 targets of hsa-miR-181a have been validated, and 22 of these targets are cancer genes. Many of these genes are involved in tumorigenesis of various cancers, including EC. Our data demonstrate that hsa-miR-181a is upregulated in EC, with a possible role in the development and progression of EC (Figure 12). It might serve as a new biomarker for prognosis prediction in EC in clinical practice and has important implication in the treatment of EC. More mechanistic and functional studies are needed to validate the role of hsa-miR-181a in the pathogenesis of EC and to establish the association between the expression level of hsa-miR-181a and the clinical phenotypes, including disease status and therapeutic response of EC to chemo- and radiotherapy.

Acknowledgment

This work was supported by Guangdong Natural Science Foundation (grant number S2012010010024), Guangzhou, People's Republic of China. Dr Zhi-Wei Zhou is a postdoctoral fellow supported by the College of Pharmacy, University of South Florida, Tampa, FL, USA.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Croce CM, Calin GA. miRNAs, cancer, and stem cell division. *Cell*. 2005; 122(1):6–7.
2. Yates LA, Norbury CJ, Gilbert RJ. The long and short of microRNA. *Cell*. 2013;153(3):516–519.
3. Lu J, Getz G, Miska EA, et al. MicroRNA expression profiles classify human cancers. *Nature*. 2005;435(7043):834–838.

4. Banno K, Yanokura M, Kisu I, Yamagami W, Susumu N, Aoki D. MicroRNAs in endometrial cancer. *Int J Clin Oncol*. 2013;18(2):186–192.
5. Lujambio A, Lowe SW. The microcosmos of cancer. *Nature*. 2012; 482(7385):347–355.
6. Esquela-Kerscher A, Slack FJ. Oncomirs – microRNAs with a role in cancer. *Nat Rev Cancer*. 2006;6(4):259–269.
7. Ryan BM, Robles AI, Harris CC. Genetic variation in microRNA networks: the implications for cancer research. *Nat Rev Cancer*. 2010; 10(6):389–402.
8. Nana-Sinkam SP, Croce CM. Clinical applications for microRNAs in cancer. *Clin Pharmacol Ther*. 2013;93(1):98–104.
9. Ritchie W, Rasko JE. Refining microRNA target predictions: sorting the wheat from the chaff. *Biochem Biophys Res Commun*. 2014; 445(4):780–784.
10. Peterson SM, Thompson JA, Ufkin ML, Sathyanarayana P, Liaw L, Congdon CB. Common features of microRNA target prediction tools. *Front Genet*. 2014;5:23.
11. Ferlay J, Soerjomataram I, Ervik M, et al. *GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11* [Internet]. Lyon: International Agency for Research on Cancer; 2013. Available from: http://globocan.iarc.fr. Accessed November 10, 2014.
12. Howlader N, Noone AM, Krapcho M, et al. *SEER Cancer Statistics Review, 1975–2011*. Bethesda, MD: National Cancer Institute; 2014.
13. Balch C, Matei DE, Huang TH, Nephew KP. Role of epigenomics in ovarian and endometrial cancers. *Epigenomics*. 2010;2(3): 419–447.
14. Amant F, Moerman P, Neven P, Timmerman D, Van Limbergen E, Vergote I. Endometrial cancer. *Lancet*. 2005;366(9484):491–505.
15. Colombo N, Preti E, Landoni F, et al; ESMO Guidelines Working Group. Endometrial cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2013;24 Suppl 6: vi33–vi38.
16. Hecht JL, Mutter GL. Molecular and pathologic aspects of endometrial carcinogenesis. *J Clin Oncol*. 2006;24(29):4783–4791.
17. Yeramian A, Moreno-Bueno G, Dolcet X, et al. Endometrial carcinoma: molecular alterations involved in tumor development and progression. *Oncogene*. 2013;32(4):403–413.
18. National Cancer Institute. Endometrial Cancer. 2014. Available from http://www.cancer.gov/cancertopics/types/endometrial. Accessed December 15, 2014.
19. Dong P, Kaneuchi M, Konno Y, Watari H, Sudo S, Sakuragi N. Emerging therapeutic biomarkers in endometrial cancer. *Biomed Res Int*. 2013; 2013:130362.
20. Ow TJ, Sandulache VC, Skinner HD, Myers JN. Integration of cancer genomics with treatment selection: from the genome to predictive biomarkers. *Cancer*. 2013;119(22):3914–3928.
21. Mitra S, Das S, Chakrabarti J. Systems biology of cancer biomarker detection. *Cancer Biomark*. 2013;13(4):201–213.
22. Widschwendter M, Jones A, Teschendorff AE. Epigenetics makes its mark on women-specific cancers – an opportunity to redefine oncological approaches? *Gynecol Oncol*. 2013;128(1):134–143.
23. Schwarzenbach H, Hoon DS, Pantel K. Cell-free nucleic acids as biomarkers in cancer patients. *Nat Rev Cancer*. 2011;11(6):426–437.
24. Kasinski AL, Slack FJ. Epigenetics and genetics. MicroRNAs en route to the clinic: progress in validating and targeting microRNAs for cancer therapy. *Nat Rev Cancer*. 2011;11(12):849–864.
25. Chen CZ, Li L, Lodish HF, Bartel DP. MicroRNAs modulate hematopoietic lineage differentiation. *Science*. 2004;303(5654):83–86.
26. Lin Y, Nie Y, Zhao J, et al. Genetic polymorphism at miR-181a binding site contributes to gastric cancer susceptibility. *Carcinogenesis*. 2012; 33(12):2377–2383.
27. Havelange V, Stauffer N, Heaphy CC, et al. Functional implications of microRNAs in acute myeloid leukemia by integrating microRNA and messenger RNA expression profiling. *Cancer*. 2011;117(20):4696–4706.
28. Ji D, Chen Z, Li M, et al. MicroRNA-181a promotes tumor growth and liver metastasis in colorectal cancer by targeting the tumor suppressor WIF-1. *Mol Cancer*. 2014;13:86.
29. Fei J, Li Y, Zhu X, Luo X. miR-181a post-transcriptionally downregulates oncogenic RalA and contributes to growth inhibition and apoptosis in chronic myelogenous leukemia (CML). *PLoS One*. 2012;7(3):e32834.
30. Shi L, Cheng Z, Zhang J, et al. hsa-mir-181a and hsa-mir-181b function as tumor suppressors in human glioma cells. *Brain Res*. 2008;1236: 185–193.
31. Panda H, Chuang TD, Luo X, Chegini N. Endometrial miR-181a and miR-98 expression is altered during transition from normal into cancerous state and target *PGR*, *PGRMC1*, *CYP19A1*, *DDX3X*, and *TIMP3*. *J Clin Endocrinol Metab*. 2012;97(7):E1316–E1326.
32. Kozomara A, Griffiths-Jones S. miRBase: annotating high confidence microRNAs using deep sequencing data. *Nucleic Acids Res*. 2014;42(Database issue):D68–D73.
33. Kozomara A, Griffiths-Jones S. miRBase: integrating microRNA annotation and deep-sequencing data. *Nucleic Acids Res*. 2011;39(Database issue):D152–D157.
34. Griffiths-Jones S, Grocock RJ, van Dongen S, Bateman A, Enright AJ. miRBase: microRNA sequences, targets and gene nomenclature. *Nucleic Acids Res*. 2006;34(Database issue):D140–D144.
35. Betel D, Koppal A, Agius P, Sander C, Leslie C. Comprehensive modeling of microRNA targets predicts functional non-conserved and non-canonical sites. *Genome Biol*. 2010;11(8):R90.
36. Maragkakis M, Alexiou P, Papadopoulos GL, et al. Accurate microRNA target prediction correlates with protein repression levels. *BMC Bioinformatics*. 2009;10:295.
37. Paraskevopoulou MD, Georgakilas G, Kostoulas N, et al. DIANA-microT web server v5.0: service integration into miRNA functional analysis workflows. *Nucleic Acids Res*. 2013;41(Web Server issue):W169–W173.
38. Wang X. miRDB: a microRNA target prediction and functional annotation database with a wiki interface. *RNA*. 2008;14(6):1012–1017.
39. Miranda KC, Huynh T, Tay Y, et al. A pattern-based method for the identification of MicroRNA binding sites and their corresponding heteroduplexes. *Cell*. 2006;126(6):1203–1217.
40. Bandyopadhyay S, Mitra R. TargetMiner: microRNA target prediction with systematic identification of tissue-specific negative examples. *Bioinformatics*. 2009;25(20):2625–2631.
41. 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(1):15–20.
42. Garcia DM, Baek D, Shin C, Bell GW, Grimson A, Bartel DP. Weak seed-pairing stability and high target-site abundance decrease the proficiency of lsy-6 and other microRNAs. *Nat Struct Mol Biol*. 2011;18(10): 1139–1146.
43. Krek A, Grün D, Poy MN, et al. Combinatorial microRNA target predictions. *Nat Genet*. 2005;37(5):495–500.
44. Dweep H, Sticht C, Pandey P, Gretz N. miRWALK – database: prediction of possible miRNA binding sites by "walking" the genes of three genomes. *J Biomed Inform*. 2011;44(5):839–847.
45. Vergoulis T, Vlachos IS, Alexiou P, et al. TarBase 6.0: capturing the exponential growth of miRNA targets with experimental support. *Nucleic Acids Res*. 2012;40(Database issue):D222–D229.
46. Hsu SD, Tseng YT, Shrestha S, et al. miRTarBase update 2014: an information resource for experimentally validated miRNA-target interactions. *Nucleic Acids Res*. 2014;42(Database issue):D78–D85.
47. Futreal PA, Coin L, Marshall M, et al. A census of human cancer genes. *Nat Rev Cancer*. 2004;4(3):177–183.
48. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr, Kinzler KW. Cancer genome landscapes. *Science*. 2013;339(6127): 1546–1558.
49. Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc*. 2009;4(1):44–57.
50. Huang da W, Sherman BT, Tan Q, et al. DAVID bioinformatics resources: expanded annotation database and novel algorithms to better extract biology from large gene lists. *Nucleic Acids Res*. 2007;35(Web Server issue):W169–W175.

51. Pecorelli S. Revised FIGO staging for carcinoma of the vulva, cervix, and endometrium. *Int J Gynaecol Obstet*. 2009;105(2):103–104.
52. Di Leva G, Garofalo M, Croce CM. MicroRNAs in cancer. *Annu Rev Pathol*. 2014;9:287–314.
53. Gilabert-Estelles J, Braza-Boils A, Ramon LA, et al. Role of microRNAs in gynecological pathology. *Curr Med Chem*. 2012;19(15): 2406–2413.
54. Parikh A, Lee C, Peronne J, et al. microRNA-181a has a critical role in ovarian cancer progression through the regulation of the epithelial-mesenchymal transition. *Nat Commun*. 2014;5:2977.
55. Chen G, Zhu W, Shi D, et al. MicroRNA-181a sensitizes human malignant glioma U87MG cells to radiation by targeting Bcl-2. *Oncol Rep*. 2010;23(4): 997–1003.
56. Brockhausen J, Tay SS, Grzelak CA, et al. miR-181a mediates TGF-β-induced hepatocyte EMT and is dysregulated in cirrhosis and hepatocellular cancer. *Liver Int*. 2015;35(1):240–253.
57. Nishimura J, Handa R, Yamamoto H, et al. microRNA-181a is associated with poor prognosis of colorectal cancer. *Oncol Rep*. 2012; 28(6):2221–2226.
58. Zou C, Chen J, Chen K, et al. Functional analysis of miR-181a and Fas involved in hepatitis B virus-related hepatocellular carcinoma pathogenesis. *Exp Cell Res*. 2015 [Epub ahead of print].
59. Pichler M, Winter E, Ress AL, et al. miR-181a is associated with poor clinical outcome in patients with colorectal cancer treated with EGFR inhibitor. *J Clin Pathol*. 2014;67(3):198–203.
60. Zhang X, Nie Y, Du Y, Cao J, Shen B, Li Y. MicroRNA-181a promotes gastric cancer by negatively regulating tumor suppressor KLF6. *Tumour Biol*. 2012;33(5):1589–1597.
61. Zhang X, Nie Y, Li X et al., MicroRNA-181a functions as an oncomir in gastric cancer by targeting the tumour suppressor gene *ATM*. *Pathol Oncol Res*. 2014;20(2):381–389.
62. Galluzzi L, Morselli E, Vitale I, et al. miR-181a and miR-630 regulate cisplatin-induced cancer cell death. *Cancer Res*. 2010;70(5): 1793–1803.
63. Farazi TA, Horlings HM, Ten Hoeve JJ, et al. MicroRNA sequence and expression analysis in breast tumors by deep sequencing. *Cancer Res*. 2011;71(13):4443–4453.
64. Guo LJ, Zhang QY. Decreased serum miR-181a is a potential new tool for breast cancer screening. *Int J Mol Med*. 2012;30(3):680–686.
65. Taylor MA, Sossey-Alaoui K, Thompson CL, Danielpour D, Schiemann WP. TGF-β upregulates miR-181a expression to promote breast cancer metastasis. *J Clin Invest*. 2013;123(1):150–163.
66. Bisso A, Faleschini M, Zampa F, et al. Oncogenic miR-181a/b affect the DNA damage response in aggressive breast cancer. *Cell Cycle*. 2013; 12(11):1679–1687.
67. McDermott AM, Miller N, Wall D, et al. Identification and validation of oncologic miRNA biomarkers for luminal A-like breast cancer. *PLoS One*. 2014;9(1):e87032.
68. Ke G, Liang L, Yang JM, et al. MiR-181a confers resistance of cervical cancer to radiation therapy through targeting the pro-apoptotic *PRKCD* gene. *Oncogene*. 2013;32(25):3019–3027.
69. Chen Y, Ke G, Han D, Liang S, Yang G, Wu X. MicroRNA-181a enhances the chemoresistance of human cervical squamous cell carcinoma to cisplatin by targeting *PRKCD*. *Exp Cell Res*. 2014;320(1):12–20.
70. Jianwei Z, Fan L, Xiancheng L, Enzhong B, Shuai L, Can L. MicroRNA 181a improves proliferation and invasion, suppresses apoptosis of osteosarcoma cell. *Tumour Biol*. 2013;34(6):3331–3337.
71. Shin KH, Bae SD, Hong HS, Kim RH, Kang MK, Park NH. miR-181a shows tumor suppressive effect against oral squamous cell carcinoma cells by downregulating K-ras. *Biochem Biophys Res Commun*. 2011;404(4):896–902.
72. Liu M, Wang J, Huang H, Hou J, Zhang B, Wang A. miR-181a-Twist1 pathway in the chemoresistance of tongue squamous cell carcinoma. *Biochem Biophys Res Commun*. 2013;441(2):364–370.
73. Alencar AJ, Malumbres R, Kozloski GA, et al. MicroRNAs are independent predictors of outcome in diffuse large B-cell lymphoma patients treated with R-CHOP. *Clin Cancer Res*. 2011;17(12):4125–4135.
74. Keutgen XM, Filicori F, Crowley MJ, et al. A panel of four miRNAs accurately differentiates malignant from benign indeterminate thyroid lesions on fine needle aspiration. *Clin Cancer Res*. 2012;18(7): 2032–2038.
75. He Q, Zhou X, Li S, et al. MicroRNA-181a suppresses salivary adenoid cystic carcinoma metastasis by targeting MAPK-Snai2 pathway. *Biochim Biophys Acta*. 2013;1830(11):5258–5266.
76. Debernardi S, Skoulakis S, Molloy G, Chaplin T, Dixon-McIver A, Young BD. MicroRNA miR-181a correlates with morphological subclass of acute myeloid leukaemia and the expression of its target genes in global genome-wide analysis. *Leukemia*. 2007;21(5):912–916.
77. Cuesta R, Martínez-Sánchez A, Gebauer F. miR-181a regulates cap-dependent translation of p27(kip1) mRNA in myeloid cells. *Mol Cell Biol*. 2009;29(10):2841–2851.
78. Bai H, Cao Z, Deng C, Zhou L, Wang C. miR-181a sensitizes resistant leukaemia HL-60/Ara-C cells to Ara-C by inducing apoptosis. *J Cancer Res Clin Oncol*. 2012;138(4):595–602.
79. Zhu DX, Zhu W, Fang C, et al. miR-181a/b significantly enhances drug sensitivity in chronic lymphocytic leukemia cells via targeting multiple anti-apoptosis genes. *Carcinogenesis*. 2012;33(7):1294–1301.
80. Dahlhaus M, Schult C, Lange S, Freund M, Junghanss C. MicroRNA 181a influences the expression of *HMGB1* and *CD4* in acute leukemias. *Anticancer Res*. 2013;33(2):445–452.
81. Lin S, Pan L, Guo S, et al. Prognostic role of microRNA-181a/b in hematological malignancies: a meta-analysis. *PLoS One*. 2013;8(3): e59532.
82. Ciafrè SA, Galardi S, Mangiola A, et al. Extensive modulation of a set of microRNAs in primary glioblastoma. *Biochem Biophys Res Commun*. 2005;334(4):1351–1358.
83. Gao W, Shen H, Liu L, Xu J, Xu J, Shu Y. MiR-21 overexpression in human primary squamous cell lung carcinoma is associated with poor patient prognosis. *J Cancer Res Clin Oncol*. 2011;137(4):557–566.
84. Gao W, Yu Y, Cao H, et al. Deregulated expression of miR-21, miR-143 and miR-181a in non small cell lung cancer is related to clinicopathologic characteristics or patient prognosis. *Biomed Pharmacother*. 2010;64(6):399–408.
85. Miller TE, Ghoshal K, Ramaswamy B, et al. MicroRNA-221/222 confers tamoxifen resistance in breast cancer by targeting p27Kip1. *J Biol Chem*. 2008;283(44):29897–29903.
86. Ji J, Yamashita T, Budhu A, et al. Identification of microRNA-181 by genome-wide screening as a critical player in EpCAM-positive hepatic cancer stem cells. *Hepatology*. 2009;50(2):472–480.
87. Zou C, Li Y, Cao Y, et al. Up-regulated MicroRNA-181a induces carcinogenesis in hepatitis B virus-related hepatocellular carcinoma by targeting E2F5. *BMC Cancer*. 2014;14:97.
88. Pons A, Nomdedeu B, Navarro A, et al. Hematopoiesis-related microRNA expression in myelodysplastic syndromes. *Leuk Lymphoma*. 2009;50(11):1854–1859.
89. Pichiorri F, Suh SS, Ladetto M, et al. MicroRNAs regulate critical genes associated with multiple myeloma pathogenesis. *Proc Natl Acad Sci U S A*. 2008;105(35):12885–12890.
90. Zhu DX, Miao KR, Fang C, et al. Aberrant microRNA expression in Chinese patients with chronic lymphocytic leukemia. *Leuk Res*. 2011; 35(6):730–734.
91. Albulescu R. TGF-β upregulates miR-181a expression to promote breast cancer metastasis. *Biomark Med*. 2013;7(2):204.
92. Jiao X, Zhao L, Ma M, et al. MiR-181a enhances drug sensitivity in mitoxantone-resistant breast cancer cells by targeting breast cancer resistance protein (BCRP/ABCG2). *Breast Cancer Res Treat*. 2013; 139(3):717–730.
93. Ritchie W, Flamant S, Rasko JE. Predicting microRNA targets and functions: traps for the unwary. *Nat Methods*. 2009;6(6):397–398.
94. Shirdel EA, Xie W, Mak TW, Jurisica I. NAViGaTing the micronome – using multiple microRNA prediction databases to identify signalling pathway-associated microRNAs. *PLoS One*. 2011;6(2):e17429.
95. Godsey B. Discovery of miR-mRNA interactions via simultaneous Bayesian inference of gene networks and clusters using sequence-based predictions and expression data. *J Integr Bioinform*. 2013;10(1):227.

96. Pio G, Malerba D, D'Elia D, Ceci M. Integrating microRNA target predictions for the discovery of gene regulatory networks: a semi-supervised ensemble learning approach. *BMC Bioinformatics*. 2014;15 Suppl 1:S4.
97. Zhang Y, Verbeek FJ. Comparison and integration of target prediction algorithms for microRNA studies. *J Integr Bioinform*. 2010;7(3).
98. Takamizawa J, Konishi H, Yanagisawa K, et al. Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. *Cancer Res*. 2004;64(11):3753–3756.
99. Johnson SM, Grosshans H, Shingara J, et al. RAS is regulated by the let-7 microRNA family. *Cell*. 2005;120(5):635–647.
100. Meng F, Glaser SS, Francis H, et al. Functional analysis of microRNAs in human hepatocellular cancer stem cells. *J Cell Mol Med*. 2012; 16(1):160–173.
101. Wei Z, Cui L, Mei Z, Liu M, Zhang D. miR-181a mediates metabolic shift in colon cancer cells via the PTEN/AKT pathway. *FEBS Lett*. 2014; 588(9):1773–1779.
102. Ouyang M, Li Y, Ye S, et al. MicroRNA profiling implies new markers of chemoresistance of triple-negative breast cancer. *PLoS One*. 2014; 9(5):e96228.
103. Clevers H, Nusse R. Wnt/β-catenin signaling and disease. *Cell*. 2012; 149(6):1192–1205.
104. MacDonald BT, Tamai K, He X. Wnt/β-catenin signaling: components, mechanisms, and diseases. *Dev Cell*. 2009;17(1):9–26.
105. Pijnenborg JM, Kisters N, van Engeland M, et al. APC, β-catenin, and E-cadherin and the development of recurrent endometrial carcinoma. *Int J Gynecol Cancer*. 2004;14(5):947–956.
106. van der Zee M, Jia Y, Wang Y, et al. Alterations in Wnt-β-catenin and Pten signalling play distinct roles in endometrial cancer initiation and progression. *J Pathol*. 2013;230(1):48–58.
107. Liu Y, Meng F, Xu Y, et al. Overexpression of Wnt7a is associated with tumor progression and unfavorable prognosis in endometrial cancer. *Int J Gynecol Cancer*. 2013;23(2):304–311.
108. Zhao Y, Yang Y, Trovik J, et al. A novel wnt regulatory axis in endometrioid endometrial cancer. *Cancer Res*. 2014;74(18):5103–5117.
109. Liu Y, Patel L, Mills GB, et al. Clinical significance of *CTNNB1* mutation and Wnt pathway activation in endometrioid endometrial carcinoma. *J Natl Cancer Inst*. 2014;106(9):dju245.
110. Malumbres M, Barbacid M. Cell cycle, CDKs and cancer: a changing paradigm. *Nat Rev Cancer*. 2009;9(3):153–166.
111. Plotnikov A, Zehorai E, Procaccia S, Seger R. The MAPK cascades: signaling components, nuclear roles and mechanisms of nuclear translocation. *Biochim Biophys Acta*. 2011;1813(9):1619–1633.
112. Ranganathan P, Weaver KL, Capobianco AJ. Notch signalling in solid tumours: a little bit of everything but not all the time. *Nat Rev Cancer*. 2011;11(5):338–351.
113. Suzuki T, Aoki D, Susumu N, Udagawa Y, Nozawa S. Imbalanced expression of TAN-1 and human Notch4 in endometrial cancers. *Int J Oncol*. 2000;17(6):1131–1139.
114. Cobellis L, Caprio F, Trabucco E, et al. The pattern of expression of Notch protein members in normal and pathological endometrium. *J Anat*. 2008;213(4):464–472.
115. Mitsuhashi Y, Horiuchi A, Miyamoto T, Kashima H, Suzuki A, Shiozawa T. Prognostic significance of Notch signalling molecules and their involvement in the invasiveness of endometrial carcinoma cells. *Histopathology*. 2012;60(5):826–837.
116. Jonusiene V, Sasnauskiene A, Lachej N, et al. Down-regulated expression of Notch signaling molecules in human endometrial cancer. *Med Oncol*. 2013;30(1):438.
117. Nickkho-Amiry M, McVey R, Holland C. Peroxisome proliferator-activated receptors modulate proliferation and angiogenesis in human endometrial carcinoma. *Mol Cancer Res*. 2012;10(3):441–453.
118. Pengchong H, Tao H. Expression of IGF-1R, VEGF-C and D2-40 and their correlation with lymph node metastasis in endometrial adenocarcinoma. *Eur J Gynaecol Oncol*. 2011;32(6):660–664.
119. Dobrzycka B, Mackowiak-Matejczyk B, Kinalski M, Terlikowski SJ. Pretreatment serum levels of bFGF and VEGF and its clinical significance in endometrial carcinoma. *Gynecol Oncol*. 2013;128(3):454–460.
120. Saarelainen SK, Staff S, Peltonen N, et al. Endoglin, VEGF, and its receptors in predicting metastases in endometrial carcinoma. *Tumour Biol*. 2014;35(5):4651–4657.
121. Wang J, Taylor A, Showeil R, et al. Expression profiling and significance of VEGF-A, VEGFR2, VEGFR3 and related proteins in endometrial carcinoma. *Cytokine*. 2014;68(2):94–100.
122. Saharinen P, Eklund L, Pulkki K, Bono P, Alitalo K. VEGF and angiopoietin signaling in tumor angiogenesis and metastasis. *Trends Mol Med*. 2011;17(7):347–362.

Supplementary materials

Table S1 A full list of cancer genes, based on Futreal et al[1]. Reprinted by permission from Macmillan Publishers Ltd: *Nature Reviews Cancer*. Futreal PA, Coin L, Marshall M, et al. A census of human cancer genes. *Nat Rev Cancer*. 2004;4(3):177–183. Copyright © 2004.[1]

Table S2 Predicted targets of hsa-miR-181a-5p, by DIANA microT v5.0

Table S3 Predicted targets of hsa-miR-181a-5p, by miRanda-mirSVR

Table S4 Predicted targets of hsa-miR-181a-5p, by miRanda-mirSVR, that are cancer genes

Table S5 Predicted targets of hsa-miR-181a-5p, by miRDB

Table S6 Predicted targets of hsa-miR-181a-5p, by RNA22 v2

Table S7 Predicted targets of hsa-miR-181a-5p, by RNA22 v2, that are cancer genes

Table S8 Predicted targets of hsa-miR-181a-5p, by TargetMiner

Table S9 Predicted targets of hsa-miR-181a-5p, by TargetScan 6.2

Table S10 Predicted targets of hsa-miR-181a-5p, by PicTar

Table S11 Predicted targets of hsa-miR-181a-5p, by MicroCosm Targets v5

Table S12 Predicted targets of hsa-miR-181a-3p, by DIANA microT v5.0

Table S13 Predicted targets of hsa-miR-181a-3p, by miRanda-mirSVR

Table S14 Predicted targets of hsa-miR-181a-3p, by miRanda-mirSVR, that are cancer genes

Table S15 Predicted targets of hsa-miR-181a-3p, by miRDB

Table S16 Predicted targets of hsa-miR-181a-3p, by RNA22 v2

Table S17 Predicted targets of hsa-miR-181a-3p, by TargetMiner

Table S18 Predicted targets of hsa-miR-181a-5p, by MicroCosm Targets v5

Table S19 Predicted targets of hsa-miR-181a, by miRWALK

Table S20 Predicted genes of hsa-miR-181a, by miRWALK

Table S21 A summarized table that includes all the targets predicted to be regulated by hsa-miR-181a by the eight algorithms

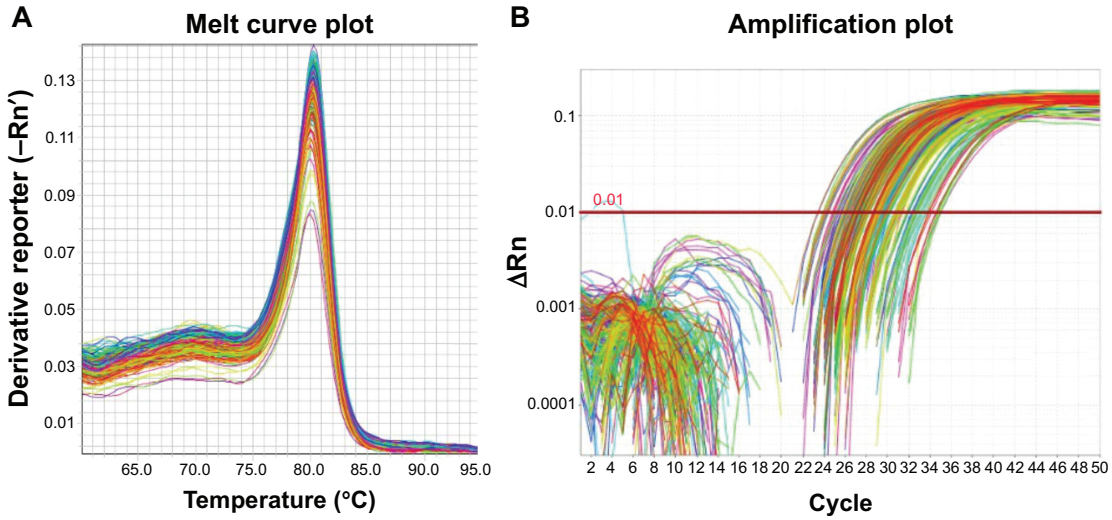

Figure S1 Analysis of hsa-miR-181a expression in a human endometrial specimen by real-time PCR.
Notes: (**A**) Melting curve showing the single melt peak for hsa-miR-181a and U6, respectively; and (**B**) the amplification plot of the target gene.
Abbreviation: PCR, polymerase chain reaction.

Reference

1. Futreal PA, Coin L, Marshall M, et al. A census of human cancer genes. *Nat Rev Cancer*. 2004;4(3):177–183.