Overexpression of microRNA-34a Attenuates Proliferation and Induces Apoptosis in Pituitary Adenoma Cells via SOX7

Zijiang Yang,1,4,5 Ting Zhang,2,5 Qiping Wang,3 and Heng Gao3

Pituitary adenomas constitute one of the most common intracranial tumors and are typically benign. However, the role of the tumor suppressor microRNA-34a (miR-34a), which is implicated in other cancers, in pituitary adenoma pathogenesis remains largely unknown. miR-34a expression was compared between GH4C1 cancer cells and normal cells derived from the pituitary gland of Rattus norvegicus, and the effects of miR-34a on GH4C1 cell proliferation and apoptosis were examined. miR-34a target genes were identified and analyzed computationally. The mRNA levels of the miR-34a target genes were measured using qRT-PCR, and the protein levels of the differentially expressed targets were assessed by western blotting. miR-34a expression was significantly lower in GH4C1 cells, whereas miR-34a overexpression significantly inhibited GH4C1 cell proliferation and promoted cell apoptosis through SRY-box 7 (SOX7). Our data facilitate the development of a better understanding of the pathogenesis and treatment of pituitary adenomas by elucidating the crucial role of miR-34a in the development of pituitary adenomas.

INTRODUCTION

Pituitary adenomas constitute the most common intracranial tumors after gliomas and meningiomas, accounting for approximately 22.5% of cases.1 Most pituitary adenomas are benign and are rarely malignant, with only 0.1%–0.2% of cases progressing to pituitary carcinoma.2 Conversely, the associated dysregulation of hormone secretion and occupation of intracranial space may cause serious morbidity, which is often compounded by typically late diagnosis and lack of effective treatments for recurrent cases. The etiology of pituitary adenoma remains unclear, although surveys in recent decades have identified possible diagnostic and therapeutic biomarkers. However, these surveys were limited by small sample size, lack of normal human pituitary tissues as controls, and the absence of rigorous high-throughput studies. Hence, elucidation of the molecular pathogenesis of pituitary adenomas and identification of reliable diagnostic and therapeutic biomarkers remain critical.

Pituitary adenomas are classified based on residual hormone secretion. In China,4 42.70% of cases show no hormone secretion, whereas 24.50%, 10.20%, 9.00%, and 2.10% show prolactin, growth hormone, follicle-stimulating hormone, and adrenocorticotropic hormone secretion, respectively. All other cases are characterized by the secretion of multiple hormones, including luteinizing hormone and thyroid-stimulating hormone. The molecular mechanisms underlying different types of adenomas may vary; therefore, for example, the GH4C1 rat pituitary cell line is suggested as a model of growth hormone-secreting adenomas. Furthermore, depending on whether the tumor diameter is more or less than 10 mm, 45.5% and 54.5% of cases are classified as macro- and microadenomas, respectively,5 of which the latter are more likely to be aggressive.

Although genes such as vascular endothelial growth factor precursor4 and cyclin D1 have been demonstrated both in vitro and in vivo to regulate proliferation, invasion, and apoptosis in pituitary adenomas and GH4C1 cells, several other genes may also be involved. In general, the development of many cancers is driven by specific genes. For example, MYC is a well-established oncogene that promotes cell growth, proliferation, and malignant transformation.6 BCL-2 and BAX8 are also associated with cancer development. Similarly, high-mobility group box 1 protein (HMGB1) and interleukin (IL)-16 have been shown to play dual roles in cancer development.9 SOX7, a member of the F group of SOX proteins that contains a high-mobility group DNA-binding domain, was also recently demonstrated to inhibit tumorigenesis.10

MicroRNAs are short noncoding RNAs of 18–25 nt that bind to the 3′ UTR of target mRNAs to suppress translation or promote posttranscriptional mRNA cleavage, depending on the degree of sequence complementarity.11 Conversely, microRNAs may activate translation by binding to the 5′ UTR of the target gene or to the protein itself.12 Finally, microRNAs may also directly bind or modulate methylation

Received 12 September 2017; accepted 5 July 2018; https://doi.org/10.1016/j.omto.2018.07.001.

These authors contributed equally to this work.

Correspondence: Heng Gao, PhD, Jiangyin People’s Hospital Affiliated to Nantong University, Shoushanlu No. 163, Jiangyin, Wuxi 214400, China.

E-mail: drgaoheng1971@126.com
at the promoter of target genes. In the past decade, an increasing number of microRNAs have been demonstrated to regulate the development and progression of pituitary adenomas, including let-7, miR-15a, miR-16-1, miR-26b, miR-122, miR-128, and miR-493. – 17 miR-34a is a well-established tumor suppressor that is widely implicated in many tumors and inhibits cancer cell proliferation, invasion, and metastasis by targeting platelet-derived growth factor receptor beta, mesenchymal-epithelial transition (MET) proto-oncogene, and transforming growth factor beta receptor 2. However, its role in pituitary adenomas remains unknown. The aim of the present study was, therefore, to investigate the role of this microRNA (miRNA) in pituitary adenomas, using GH4C1 cells as a model system. Moreover, the relationship between miR-34a and Sox7, Bcl2, Bax, Myc, Hmgb1, and Il16 was additionally investigated, as this is yet undefined in pituitary adenomas.

RESULTS

miR-34a Is Downregulated in Pituitary Adenoma Cells

miR-34a expression was more than 3- to 4-fold lower in GH4C1 cells than in normal rat pituitary tissue (p < 0.05; Figure 1A). To determine the specific functions of miR-34a in pituitary adenomas, we transfected GH4C1 cells with a miR-34a mimic to obtain a cell line with 5-fold higher miR-34a levels than cells transfected with the negative control (p < 0.05; Figure 1B).

miR-34a Overexpression Inhibits Proliferation

To determine whether miR-34a exerted anti-proliferative effects, cell proliferation was measured after 1–6 days in mimic-, negative control oligonucleotide-transfected, and GH4C1 cells. Microscopy showed that high miR-34a levels significantly suppressed cell proliferation (Figures 2A and 2D). Moreover, a colony-formation assay revealed that clonogenic survival was obviously decreased following increase in miR-34a levels (Figures 2B and 2C; p < 0.05).

miR-34a Overexpression Induces Apoptosis

Apoptosis was measured by flow cytometry in cells transfected with the miR-34a mimic or the negative or blank GH4C1 cell controls. In the resulting plots (Figures 2E and 2F), cells clustered in the lower right quadrant are in early apoptosis. In contrast, late apoptotic and necrotic cells are located in the upper right quadrant, and cells in the last stage of apoptosis cluster are located in the upper left quadrant. The proportions of cells in early, late, and final stages of apoptosis were 1.7%, 1.1%, and 1.9%, respectively, in GH4C1 cells transfected with the negative control; the percentages were 0.7%, 1.5%, and 2.3%, respectively, in blank control cells and 17.1%, 3.1%, 1.7%.
and 2.0%, respectively, in blank control cells transfected with miR-34a (Figure 2E). The proportion of cells in early apoptosis and in late apoptosis in the miR-34a mimic group was significantly higher than that in the negative control group and in the blank control (Figure 2F; p < 0.05). These results indicate that miR-34a elicits apoptosis in GH4C1 cells.

miR-34a Target Genes and Functional Enrichment

miR-34a target genes in GH4C1 cells were predicted with high stringency based on four miRNA databases and two sets of gene array data. Briefly, 3,409 candidate target genes were collected from the miRanda, TargetScan, miRDB, and DIANA databases (the targets from the four databases are summarized in Table S1) and filtered in Excel to identify genes repeated at least twice in different databases. For 413 genes, candidates that appeared twice in the same database were also retained, of which, 332 were present in at least two databases (Figure 3A; summarized in Table S2). In contrast, the gene arrays GEO: GSE2175 and GSE4237 in gnen cloud of biotechnology information (GCBI) indicated that 4,656 mRNAs were significantly dysregulated by at least 2-fold (p < 0.05) in pituitary adenomas (summarized in Table S3). A Venn diagram (Figure 3B) for dysregulated mRNAs from the array data and target genes in miRNA databases identified 108 candidates for further analysis (summarized in Table S4).

Enrichment analyses with ONTO-TOOLS and GOEAST GO indicated that 54 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and 446 GO processes were enriched in the 108 candidates. Most of the top 15 KEGG pathways are implicated in cancer development (summarized in Table S5). In contrast, transcriptional regulation was significantly enriched in the top five enriched GO terms “molecular function,” “biological process,” and “cellular component” (summarized in Table S5). Based on protein-protein interactions (Figure 3C) and sequence complementarity measured in RNA22 (Figure 3D), Myc, Bcl2, Sp100, Sox7, Hmgb1, Mtor, Map3k9, Foxp4, Il16, and Bax were selected for further analysis by qRT-PCR. MYC, BAX, BCL-2, HMGB1, SOX7, and IL-16 were also selected for validation by western blot.

Validation of miR-34a Target Genes

Western blotting demonstrated that GH4C1 cell transfection with the miR-34a mimic significantly suppressed MYC, BCL-2, HMGB1, and SOX7 expression, even as BAX and IL-16 accumulated (Figure 4A). Furthermore, experimental validation of the miR-34a targets, BCL-2, MYC, and SOX7, using a luciferase reporter system was performed (Figure 4B). Luciferase reporter assays showed that miR-34a reduced the luciferase activity of the Sox7 3’ UTR but not that of Bcl2 and Myc. However, mutated Sox7 alleviated the inhibitory effect of miR-34a on the luciferase activity (Figures 4C and 4D). These results suggested that miR-34a directly regulates Sox7 mRNA expression.

SOX7 Downregulation Suppresses Proliferation and Induces Apoptosis

To determine whether the regulation of SOX7 expression by miR-34a plays an important role in cell proliferation and apoptosis, three SOX7 small interfering RNAs (siRNAs) were constructed. Small interfering (Si)-SOX7 #2 and #3 sequence significantly dysregulated by at least 2-fold (p < 0.05) in pituitary adenomas (summarized in Table S3). A Venn diagram (Figure 3B) for dysregulated mRNAs from the array data and target genes in miRNA databases identified 108 candidates for further analysis (summarized in Table S4).
Cell Counting Kit-8 (CCK-8) at 1–5 days and microscopic observation at 4 days after SOX7 knockdown (Figures 5C and 5D). Knockdown of SOX7 yielded much higher apoptosis rates than that observed in the control group. The proportions of cells in the early, late, and final stages of apoptosis were 1.2%, 0.9%, and 2.3%, respectively, in GH4C1 cells transfected with the negative control; however, the percentages were 16.4%, 6.6%, and 4.5%, respectively, in siRNA-SOX7 #2 cells; and 23.5%, 7.3%, and 2.2%, respectively, in siRNA-SOX7 #3 cells (Figure 5E). The proportion of cells in early and late apoptosis in siRNA-SOX7 #2 and in siRNA-SOX7 #3 was significantly higher than that in the negative control group (Figure 5F; p < 0.05). These results indicated that SOX7 inhibits apoptosis in GH4C1 cells.

DISCUSSION

miR-34a suppresses many forms of cancers, and several studies have shown that it is downregulated in cancers such as glioma,20 breast cancer,21 and bladder carcinoma.22 In particular, miR-34a elicits a range of biological effects by directly or indirectly regulating the expression of genes crucial for differentiation (sirtuin 1; SIRT1),23 proliferation and apoptosis (TGFB2),19 cell-cycle progression (CD44),22 invasion and metastasis (tumor protein D52),21 protein phosphorylation (VEGF),24 angiogenesis (CD44),24 epithelial-mesenchymal transition (sterile alpha motif domain-containing 3),26 autophagy (HMGB1),27 and chemosensitivity (AXL receptor tyrosine kinase).28 Notably, this miRNA is encoded in chromosome 1p36, which is strongly regulated by p53.29 Yamakuchi et al.30 described a positive-feedback loop, in which p53 induces miR-34a, which, in turn, suppresses SIRT1 to stimulate p53 activity. However, whether the p53-miR-34a positive-feedback loop actually exists in pituitary adenomas requires further investigation.

Herein, we report that Sox7, Myc, Bcl2, Bax, Hmgb1, and Il16 were predicted to contain potential miR-34a binding sites. In addition to the Myc oncogene,31 the Hmgb1 transcription factor, and the pleiotropic immune response factor IL-16,32,34 dysregulation of anti-apoptotic proteins such as BCL-2 and pro-apoptotic proteins such as Bax35 is also associated with cancer development. Paradoxically, both BCL-2 and BAX are simultaneously expressed in some cancers.5 In turn, SOX7 inhibits tumorigenesis.11 We subjectively chose three of the target genes for luciferase reporter validation experiments, which demonstrated miR-34a directly binding on the Sox7 3′ UTR. Our findings demonstrate, for the first time, that miR-34a suppresses pituitary adenomas by directly downregulating Sox7. We observed that, similar to pituitary adenomas, miR-34a is less abundantly expressed in GH4C1 cells than in normal rat pituitary tissue and that miR-34a overexpression inhibits cell proliferation. miR-34a also stimulates apoptosis, which corroborates previous observations in non-small-cell lung cancer.19

SOX7 constitutes a key molecule associated with tumorigenesis,11 although the underlying mechanisms are unclear. For example, SOX7 was demonstrated to suppress lung21 and breast cancer.22 Recently, Cuvertino et al.33 showed that, in contrast to the conventional view, SOX7 is an oncogene that promotes the proliferation of acute lymphoblastic cells. Katoh34 reported that SOX7 expression was upregulated in pancreatic cancer cell lines and in some gastric and esophageal cancer cell lines. In addition, a study demonstrated that overexpression of Sox7 induced embryonal carcinoma cell differentiation by regulating Gata-4 and Gata-6 expression.35 SOX7 promotes glioma cell growth by increasing vascular endothelial growth factor receptor 2 (VEGFR2)-mediated vascular abnormality, with high SOX7 expression levels correlating with poor survival, early recurrence, and impaired vascular function.36 However, Yang et al.37 reported that SOX7 overexpression induces IL-33 expression to promote metastasis of tumor-associated macrophages. These results suggest that SOX7 does not necessarily play the same role in all cancers. Indeed, the role of SOX7 in pituitary adenomas is unknown, although we found that miR-34a overexpression directly suppressed Sox7 expression at both the mRNA and protein levels, which, in turn, restrained GH4C1 cell proliferation and induced apoptosis. In summary, we report that miR-34a was suppressed in GH4C1 cells. Moreover, we demonstrated that miR-34a inhibits proliferation and induces apoptosis in GH4C1 cells by directly regulating SOX7 levels. SOX7 plays an oncogene role in pituitary adenomas. Nonetheless, collectively, the data suggest that miR-34a plays a crucial role in regulating the expression of SOX7 in GH4C1 cells.

![Figure 4. Sox7 Dual-Luciferase Reporter](image-url)
role in the development of pituitary adenomas and that targeting of miR-34a activity may provide new opportunities for treatment.

MATERIALS AND METHODS

Tissue Collection and Cell Culture

Owing to the lack of normal human pituitary tissue for use as negative control, the pituitary glands were collected from 10-week-old healthy female *Rattus norvegicus* after euthanization with 5% chloral hydrate on ice and washed in PBS (pH 7.2). Procedures involving animals and their care were conducted in conformity with NIH guidelines (NIH Pub. No. 85-23, revised 1996) and were approved by the Animal Care and Use Committee of the Medical School of Nantong University.

The rat pituitary adenoma cell line GH4C1 was purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA) and cultured in Ham’s F10 medium (12390-035, Gibco, Life Technologies, Gaithersburg, MD, USA) supplemented with 10% fetal bovine serum (10099-141, Gibco, Life Technologies) at 37°C in an incubator with 5% CO₂ and 95% air.

**RNA Isolation and Real-Time qRT-PCR**

Total RNA was extracted from tissues and cells using TRIzol (15596026, Life Technologies), following the manufacturer’s instructions. miR-34a was reverse-transcribed and quantified with stem-loop primers, using U6 as an internal control. Target gene expression was quantified using qRT-PCR on a CFX96 real-time system (785BR10081, Bio-Rad, Hercules, CA, USA) using the SYBR Green PCR master mix (RR820A, TaKaRa, Shiga, Japan) and normalized to Gapdh expression. Fold change was calculated according to the 2⁻ⁿDΔCt method. qRT-PCR primers are listed in Table 1, and miR-34a mimic and negative control oligos are shown in Table 2.

**Transfection**

GH4C1 cells (2.0 x 10⁵) were plated in a 6-well dish and allowed to adhere overnight. Cells were transfected with a 5- or 10-nM equimolar mix of miR-34a mimic (GenePharma, Shanghai, China) or negative control oligos (GenePharma) (2 oligos per category) using Lipofectamine 2000 (Invitrogen) and opti-modified Eagle medium (MEM) (Invitrogen, Carlsbad, CA, USA), according to the manufacturer’s instructions. Transfection efficiency was assessed by fluorescence microscopy and confirmed by qRT-PCR 48 hr after transfection. The same methods were used to knock down SOX7. Cells (2.0 x 10⁵) were plated into a 6-well dish and allowed to adhere overnight. Cells were then transfected with 3 siRNAs designed against SOX7 combined in an equimolar ratio (20 or 40 nM; SOX7-Rat-1, SOX7-Rat-2, and SOX7-Rat-3, Invitrogen-Thermo Fisher Scientific, Waltham, MA, USA) or a negative control siRNA (Invitrogen-Thermo Fisher Scientific). Transfection efficiency was monitored via fluorescence microscopy and confirmed using qRT-PCR 48 hr after transfection. siRNA transfections were performed in GH4C1 cell lines and utilized in proliferation and apoptosis assays. siRNA-SOX7 and negative control sequence are listed in Table 3.

**Western Blot**

Western blotting was used to assess MYC, BAX, BCL-2, HMGB1, SOX7, and IL-16 levels in GH4C1 cells 48 hr after transfection with miRNA oligos. Briefly, equal amounts of protein (40 μg) were separated by 12% SDS-PAGE, transferred to polyvinylidene fluoride membranes (Millipore, Bedford, MA, USA), and then blocked in 5% milk in Tris-buffered saline (TBS)-Tween 20 (0.5%). Proteins were then probed with rabbit antibodies against human MYC, BCL2, SOX7, HMGB1, BAX, AND IL-16 (Cell Signaling Technology, Danvers, MA, USA) and corresponding peroxidase-conjugated secondary antibodies (Cell Signaling Technology) and visualized using

---

**Figure 5. Effect of Sox7 on Cell Proliferation and Apoptosis**

Effects of SOX7 expression on proliferation and apoptosis in GH4C1 cells. (A and B) Knockdown of SOX7 with different SOX7 siRNA sequences showed that si-SOX7 #2 and #3 significantly inhibited SOX7 expression at the mRNA and protein levels in GH4C1 cells. Beta-actin and GAPDH were used as controls. (C and D) Proliferation was assessed using CCK-8 at 1–5 days after knockdown with siRNAs, negative control, and blank GH4C1 cell controls. Effects of siRNAs on proliferation at 4 days after SOX7 knockdown are indicated. Original magnification: 40×. (E) Apoptosis of GH4C1 cells transfected with SOX7 siRNAs and negative control was tested by annexin V-FITC and propidium iodide staining and flow cytometry. (F) Statistical analysis of the apoptosis index after transfection with SOX7 siRNAs or negative control. E, early; L, late. OD, optical density. *p < 0.05; **p < 0.01.
enhanced chemiluminescence (ECL) solution (Santa Cruz Biotechnology, Dallas, TX, USA). For Cell Signaling Technology c-MYC (#13987), BAX (#2772), HMBG1 (#6893), and GAPDH (#2118), and for Abcam BCL-2 (ab196495), SOX7 (ab89954), and IL-16 (ab13987), BAX (#2772), HMGB1 (#6893), and GAPDH (#2118), the dilution ratio was 1:1,000.

**Table 1. Primers for qRT-PCR**

| Target | Primers (5′-3′) |
|--------|----------------|
| GAPDH | B661284 (Sangon, Shanghai, China) |
| mir-34a RT | GTGATTTGAATGTTCTTACTCCCATC |
| mir-34a-F | TGGCCATCTGGAGATGACAAACC |
| mir-34a-R | ATCCAGTGACCCTCCGAGG |
| U6 RT | AAGCGCTTCAGAAATTTGCGTG |
| U6-F | GCTGGCTTCGGCAGCACA |
| U6-R | GAGGTATTCGCACCAGAGGA |
| MYC-F | CTCACGACAACATACCGACC |
| MYC-R | GCTGGTGCATTTTCGGTTGT |
| Map3k9-F | AAACGAGGAGTGGCTCTTCC |
| Map3k9-R | GAGGACATCAGCCAGGACCAT |
| Bcl-2-F | AAGCAGAGGAGAAATCAAACA |
| Bcl-2-R | GCGGCGGTGGCAGTGTCTTAGC |
| BAX-F | CCTGGTGCAAGTCTCGT |
| BAX-R | GCTGGTGCATTTTCGGTTGT |
| nTOR-R | TTTGTCCTTGATGTTAGGGT |
| nTOR-F | GGATATGGTGTGCTTGGT |
| Foxp4-R | CGATAAGGGAGGAGCATG |
| Foxp4-F | GCAGAGAGAGACACAGCAG |
| IL-16-R | CAGACGAGCTGCGATAG |
| IL-16-F | CTTGCTACAACACCCAGGAT |
| HMGB1-R | GCACAGCGTGTTGCTTCCCAT |
| HMGB1-F | CCGCCTTTCTGTGTTTCTG |
| SOX7-R | GCCAAGGGCCAAGAACCA |
| SOX7-F | GCCACATATCCCTACCCG |
| SP100-R | GTTGCGAGGTTGCTCTG |
| SP100-F | GACCCAAAGCAAGCAGT |

**Table 2. miR-34a Mimic and Negative Control Oligos**

| Oligo | Sequence |
|-------|----------|
| mir-34a (RAT) | 5′-UUGGCAGUGU/CUUAUCUGGUUGU-3′ |
| Negative control | 5′-UUUCCCGAAGGUCGACGGT-3′ |

**Cell Proliferation and Colony-Formation Assay**

GH4C1 cells were seeded in 96-well microplates at 5,000 cells per well and allowed to adhere and grow for 2, 4, 6, 8, and 10 days after transfection. Proliferation was measured at each time point using the CCK-8 (Dojindo, Kumamoto, Japan), following the manufacturer’s instructions. Each assay was repeated six times, and the results were averaged. To assess colony formation, cells were seeded in 6-well plates at 2,000 cells per well and transfected with the miR-34a mimic and the negative control. After 1 week, the colonies were fixed with 4% paraformaldehyde and stained with crystal violet (Sigma, St. Louis, MO, USA). Data were collected from three replicates.

**Cell Apoptosis**

Apoptosis was measured by annexin V-FITC (fluorescein isothiocyanate) and propidium iodide staining (BD PharMingen, San Jose, CA, USA), according to the manufacturer’s instructions. Briefly, GH4C1 cells were collected 48 hr after transfection and resuspended in 100 μL of 1× binding buffer supplemented with 5 μL annexin V-FITC, followed by incubation at room temperature for 15 min in the dark and mixing with 5 μL propidium iodide 5 min before analysis. Samples were then diluted with 400 μL binding buffer and analyzed by flow cytometry (BD LSRFortessa, Bedford, MA, USA).

**miRNA Target Prediction**

Putative miR-34a targets were identified using miRanda, TargetScan (http://www.targetscan.org/vert_71/), miRDB (http://mirdb.org/miRDB/index.html), and DIANA (http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=microT_CDS/index). RNA22 v2.0 (https://cm.jefferson.edu/rna22/) was then used to test sequence complementarity between miR34a and candidate target genes. Consensus targets were defined as genes appearing at least twice in different databases, with p < 0.1 for sequence complementarity against miR-34a.

**GCB1** (https://www.gcbi.com.cn/gclib/html/index) was also mined for genes significantly dysregulated in human pituitary adenomas. Using “neoplasms, pituitary,” “neoplasms, pituitary,” “pituitary neoplasms,” “pituitary tumors,” “pituitary tumor,” “tumor, pituitary,” “tumors, pituitary,” “pituitary adenoma,” “adenoma, pituitary,” “adenomas, pituitary,” or “pituitary adenomas” as simple search terms.

Array data were analyzed to compare mRNA expression between pituitary adenomas and normal pituitary tissue, between invasive and non-invasive pituitary adenomas, and between recurrent and nonrecurrent pituitary adenomas. Array data from gene mutants, stem cells, and transgenic systems were excluded. Finally, the array datasets GEO: GSE2175 and GSE4237 were selected for further analysis.

**Enrichment Analysis**

Enrichment for KEGG pathways and Gene Ontology (GO) terms was assessed using ONTO-TOOLS and GOEAST (http://omicslab.genetics.ac.cn/GOEAST/php/batch_genes.php), respectively. Protein-protein interactions among miR-34a target genes were reconstructed in String (https://string-db.org/cgi/input.pl). Targets were selected for validation by qRT-PCR and western blot.

**Dual-Luciferase Reporter Assay**

For dual-luciferase assays, the Sox7 3′ UTR containing predicted miR-34a seed-matching sites (wild-type; WT) and corresponding
Experiments were performed in triplicate with three independent using a dual luciferase assay kit (Promega, Madison, WI, USA). using Lipofectamine 2000 according to the manufacturer amounts of mir-34a and miR-NC (GenePharma, Shanghai, China), fected with either WT or mutant luciferase reporter plasmids con- sequencing. GH4C1 cells were seeded in a 24-well plate and cotrans- mediated by the pMIR-REPORTER vector (Ambion; Austin, TX, USA). WT and mutant constructs were con- primed by the SOX7-Rat-86, SOX7-Rat-288, and SOX7-Rat-1052. mutant sites (mut) were amplified by PCR from cDNA of GH4C1 and inserted into the pMIR-REPORTER vector (Ambion; Austin, TX, USA). WT and mutant constructs were confirmed by sequencing. GH4C1 cells were seeded in a 24-well plate and cotrans- fected with either WT or mutant luciferase reporter plasmids contain- ing the Sox7-3’ UTR, pGL4.74 vector (Ambion), and equal amounts of mir-34a and miR-NC (GenePharma, Shanghai, China), using Lipofectamine 2000 according to the manufacturer’s instruc- tions. Luciferase activities were measured 24 hr after transfection using a dual luciferase assay kit (Promega, Madison, WI, USA). Experiments were performed in triplicate with three independent replicates.

Statistical Analysis
All experiments were replicated three times. Results are reported as the means ± SEM and were compared using the Student’s t test in GraphPad PRISM software 5.0 (La Jolla, CA, USA). Differences were considered statistically significant at p < 0.05.

SUPPLEMENTAL INFORMATION
Supplemental Information includes five tables and can be found with this article online at https://doi.org/10.1016/j.omto.2018.07.001.

AUTHOR CONTRIBUTIONS
Z.Y. and T.Z. designed and performed the experiments and revised the manuscript. Z.Y. statistically analyzed the data and drafted the manuscript. Q.W. participated in sample collection and reviewed the experimental results. H.G. participated in the design of this study and helped to draft and revise the manuscript. All authors read and approved the final manuscript.

ACKNOWLEDGMENTS
I would like to express my gratitude to all those who have helped me during the research working. I gratefully acknowledge the help of my mentor, Heng Gao. I do appreciate his patience, encouragement, and professional instructions. The Jiangsu Provincial Natural Science Foundation of China (grant no. BK2011188) supported our work.

REFERENCES
1. Ezzat, S., Asa, S.L., Couldwell, W.T., Barr, C.E., Dodge, W.E., Vance, M.L., and McCutcheon, I.E. (2004). The prevalence of pituitary adenomas: a systematic review. Cancer 101, 613–619.
2. Fichard, C., Gerber, S., Laloï, M., Kujas, M., ClerenCeau, S., Ponvert, D., Bruckert, E., and Turpin, G. (2002). Pituitary carcinoma: report of an exceptional case and review of the literature. J. Endocrinol. Invest. 25, 65–72.
3. Zhu, X., Wang, Y., Zhao, X., Jiang, C., Zhang, Q., Jiang, W., Wang, Y., Chen, H., Shou, X., Zhao, Y., et al. (2015). Incidence of pituitary apoplexy and its risk factors in Chinese people: a database study of patients with pituitary adenoma. PLoS ONE 10, e0139088.
4. Cristina, C., Luque, G.M., Demarchi, G., Lopez Vicchi, F., Zubeldia-Brenner, L., Perez Millan, M.L., Perrone, S., Ormsiek, A.M., Lacau-Mengido, I.M., Berner, S.L., and Becu-Villalobos, D. (2014). Angiogenesis in pituitary adenomas: human studies and new mutat mouse models. Int. J. Endocrinol. 2014, 308497.
5. Hewedi, I.H., Osman, W.M., and El Mahdy, M.M. (2011). Differential expression of cyclin D1 in human pituitary tumors: relation to MIB-1 and p27/Kip1 labeling indices. J. Egypt. Nail. Canc. Inst. 23, 171–179.
6. Stine, Z.E., Walton, Z.E., Altman, B.J., Hsieh, A.L., and Dang, C.V. (2015). MYC, metastasis, and cancer. Cancer Discov. 5, 1024–1039.
7. Yang, E., and Korsmeyer, S.J. (1996). Molecular thanatopsis: a discourse on the BCL2 family and cell death. Blood 88, 386–401.
8. Soni, Y., Piaàkko, P., and Lehto, V.P. (1998). Histopathological evaluation of apoptosis in cancer. Am. J. Pathol. 153, 1041–1053.
9. Cebrian, M.J., Bauden, M., Andersson, R., Holdenrieder, S., and Ansari, D. (2016). Paradoxical role of HMGR1 in pancreatic cancer: tumor suppressor or tumor pro- meor? Anticancer Res. 36, 4381–4389.
10. Richardson, J., Tuzova, M., Cruikshank, W., and Center, D. (2014). Regulation of cellular processes by interleukin-16 in homeostasis and cancer. J. Cell. Physiol. 229, 139–147.
11. Stovall, D.B., Cao, P., and Sai, G. (2014). SOX7: from a developmental regulator to an emerging tumor suppressor. Histol. Histopathol. 29, 439–445.
12. Ambros, V. (2004). The functions of animal microRNAs. Nature 431, 350–355.
13. Garzon, R., Marcucci, G., and Croce, C.M. (2010). Targeting microRNAs in cancer: rationale, strategies and challenges. Nat. Rev. Drug Discov. 9, 775–789.
14. Palumbo, T., Fauz, F.R., Azevedo, M., Xekouki, P., Iliopoulos, D., and Stratakis, C.A. (2013). Functional screen analysis reveals miR-26b and miR-128 as central regulators of pituitary adenomas. Mod. Pathol. 26, 1651–1659.
15. Bottoni, A., Piccin, D., Tagliati, F., Luchin, A., Zatelli, M.C., and deghi Uberti, E.C. (2005). miR-15a and miR-16-1 down-regulation in pituitary adenomas. J. Cell. Physiol. 204, 280–285.
16. Qian, Z.R., Asa, S.L., Siomi, H., Siomi, M.C., Yoshimoto, K., Yamada, S., Wang, E.L., Rahman, M.M., Inoue, H., Itakura, M., et al. (2009). Overexpression of HMGAI2 re- lates to reduction of the let-7 and its relationship to clinicopathological features in pitu- itary adenomas. Mod. Pathol. 22, 431–441.
17. Stillling, G., Sun, Z., Zhang, S., Jin, L., Righi, A., Kovacs, G., Korbonits, M., Scheithauer, B.W., Kovacs, K., and Lloyd, R.V. (2010). MicroRNA expression in ACTH-producing pituitary tumors: up-regulation of microRNA-122 and -493 in pitui- tary carcinomas. Endocrine 38, 67–75.
18. Peng, Y., Guo, J.J., Liu, Y.M., and Wu, X.L. (2014). MicroRNA-34A inhibits the growth, invasion and metastasis of gastric cancer by targeting PDGFR and MET expression. Biosci. Rep. 34, e00112.
19. Ma, Z.J., Hou, P.P., Li, Y.L., Wang, D.T., Yuan, T.W., Wei, J.L., Zhao, B.T., Lou, J.T., Zhao, X.T., Jin, Y., and Jin, Y.X. (2015). MicroRNA-34A inhibits the proliferation and promotes the apoptosis of non-small cell lung cancer H1299 cell line by targeting TGFBR2. Tumour Biol. 36, 2481–2490.
20. Li, S.Z., Hu, Y.Y., Zhao, J., Zhao, Y.B., Sun, J.D., Yang, Y.F., Ji, C.C., Liu, Z.B., Cao, W.D., Qu, Y., et al. (2014). MicroRNA-34A induces apoptosis in the human glioma cell line, A172, through enhanced ROS production and NOX2 expression. Biochem. Biophys. Res. Commun. 444, 6–12.
21. Li, G., Yao, L., Zhang, J., Li, X., Dang, S., Zeng, K., Zhou, Y., and Gao, F. (2016). Tumor-suppressive microRNA-34a inhibits breast cancer cell migration and invasion via targeting oncogenic TPD52. Tumour Biol. 37, 7481–7491.
22. Yu, G., Xu, K., Xu, S., Zhang, X., Huang, Q., and Lang, B. (2015). MicroRNA-34a regulates cell cycle by targeting CD44 in human bladder carcinoma cells. Nan Fang Yi Ke Da Xue Xue Bao 35, 935–940.
23. Yu, X., Zhang, L., Wen, G., Zhao, H., Luong, L.A., Chen, Q., Huang, Y., Zhu, J., Ye, S., Xu, Q., et al. (2015). Upregulated siruin 1 by miRNA-34a is required for smooth muscle cell differentiation from pluripotent stem cells. Cell Death Differ. 22, 1170–1180.
24. Zhang, D., Zhou, J., and Dong, M. (2014). Dysregulation of microRNA-34a expression in colorectal cancer inhibits the phosphorylation of FAK via VEGF. Dig. Dis. Sci. 59, 958–967.
25. Yu, G., Yao, W., Xiao, W., Li, H., Xu, H., and Lang, B. (2014). MicroRNA-34a functions as an anti-metastatic microRNA and suppresses angiogenesis in bladder cancer by directly targeting CD44. J. Exp. Clin. Cancer Res. 33, 779.
26. Qiao, P., Li, G., Bi, W., Yang, L., Yao, L., and Wu, D. (2015). microRNA-34a inhibits epithelial mesenchymal transition in human cholangiocarcinoma by targeting Smad4 through transforming growth factor-beta/Smad pathway. BMC Cancer 15, 469.
27. Liu, K., Huang, J., Xie, M., Yu, Y., Zhu, S., Kang, R., Cao, L., Tang, D., and Duan, X. (2014). MIR34A regulates autophagy and apoptosis by targeting HMGB1 in the retinoblastoma cell. Autophagy 10, 442–452.
28. Li, X.Y., Wen, J.Y., Jia, C.C., Wang, T.T., Li, X., Dong, M., Lin, Q.U., Chen, Z.H., Ma, X.K., Wei, L., et al. (2015). MicroRNA-34a-5p enhances sensitivity to chemotherapy by targeting AXL in hepatocellular carcinoma MHCC-97L cells. Oncol. Lett. 10, 2691–2698.
29. Li, X.J., Ren, Z.J., and Tang, J.H. (2014). MicroRNA-34a: a potential therapeutic target in human cancer. Cell Death Dis. 5, e1327.
30. Yamakuchi, M., and Lowenstein, C.J. (2009). MiR-34: SIRT1 and p53: the feedback loop. Cell Cycle 8, 712–715.
31. Li, B., Ge, Z., Song, S., Zhang, S., Yan, H., Huang, B., and Zhang, Y. (2012). Decreased expression of SOX7 is correlated with poor prognosis in lung adenocarcinoma patients. Pathol. Oncol. Res. 18, 1039–1045.
32. Stovall, D.B., Wan, M., Miller, L.D., Cao, P., Maglic, D., Zhang, Q., Stampfer, M.R., Liu, W., Xu, J., and Sui, G. (2013). The regulation of SOX7 and its tumor suppressive role in breast cancer. Am. J. Pathol. 183, 1645–1653.
33. Cuvertino, S., Filiciotto, G., Masurekar, A., Saha, V., Lacaud, G., and Kouskoff, V. (2016). SOX7 promotes the maintenance and proliferation of B cell precursor acute lymphoblastic cells. Oncotarget 8, 64974–64983.
34. Katoh, M. (2002). Expression of human SOX7 in normal tissues and tumors. Int. J. Mol. Med. 9, 363–368.
35. Futaki, S., Hayashi, Y., Emoto, T., Weber, C.N., and Sekiguchi, K. (2004). Sox7 plays crucial roles in parietal endoderm differentiation in F9 embryonal carcinoma cells through regulating Gata-4 and Gata-6 expression. Mol. Cell. Biol. 24, 10492–10503.
36. Kim, I.K., Kim, K., Lee, E., Oh, D.S., Park, C.S., Park, S., Yang, J.M., Kim, J.H., Kim, H.S., Shima, D.T., et al. (2018). Sox7 promotes high-grade glioma by increasing VEGFR2-mediated vascular abnormality. J. Exp. Med. 215, 963–983.
37. Yang, Y., Andersson, P., Hosaka, K., Zhang, Y., Cao, R., Iwamoto, H., Yang, X., Nakamura, M., Wang, J., Zhuang, R., et al. (2016). The PDGF-BB-SOX7 axis modulated IL-33 in pericytes and stromal cells promotes metastasis through tumour-associated macrophages. Nat. Commun. 7, 11385.