Biochemical and Biophysical Research Communications 448 (2014) 308–314

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

# Reciprocal effects between microRNA-140-5p and ADAM10 suppress migration and invasion of human tongue cancer cells

Yang Kai [a], Wang Peng [a], Wu Ling [a], Hao Jiebing [b,*], Bian Zhuan [a,*]

[a] State Key Laboratory Breeding Base of Basic Science of Stomatology and Key Laboratory of Oral Biomedicine Ministry of Education, School and Hospital of Stomatology, Wuhan University, Wuhan 430079, China
[b] The Second Charity Hospital of Henan Province, Jiaozuo 454000, China

ARTICLE INFO

*Article history:*
Received 5 February 2014
Available online 13 February 2014

*Keywords:*
MicroRNA
Tongue squamous cell carcinoma
ADAM10
ERBB4

ABSTRACT

ADAM10, overexpressed in tongue squamous cell carcinoma (TSCC), has been well documented for its role in tumor progression and metastasis. In the present study, we evaluated the inhibition effect of microRNAs (miRNAs) on the TSCC and identified that miR-140-5p could directly targets ADAM10 and inhibits the invasion and migration of TSCC cells. LAMC1, HDAC7 and PAX6, clustered into migration-related genes, were validated to be direct targets of miR-140-5p, while IGF1R and PSEN1 were not responsible to the regulation. Most intriguingly, ERBB4 was upregulated by miR-140-5p even though the interaction between ERBB4 3′UTR and miR-140-5p existed simultaneously. Meanwhile, ADAM10 is involved in the "positive" regulation of ERBB4 and negative regulation of PAX6 by miR-140-5p. Taken together, our results suggest that miR-140-5p play a role in TSCC cell migration and invasion, and two brand new relationships between miRNA and its targets emerged: (1) ADAM10 is not just a direct target of miR-140-5p, the repressed ADAM10 also helps to enhance the effect of miR-140-5p to other target genes: ERBB4 and PAX6; (2) ERBB4 is "positively" regulated by miR-140-5p.



## 1. Introduction

In 2013, cancers of oral cavity & pharynx, mainly characterized by squamous cell carcinoma, account for about 3% of all newly diagnosed cancers in men and tongue is the most invaded site by oral cancer [1]. The poor prognosis of tongue squamous cell carcinoma (TSCC) patients are correlated with the degree of lymph node involvement [2], therefore, it is important to clarify the molecular mechanism underlying tumor cell invasion and metastasis.

A disintegrin and metalloproteinase 10 (ADAM10), with adhesion and protease activity, has been found to be upregulated in many of the studies of human cancers, including TSCC [3–6]. The major function of ADAM10 is to serve as a 'sheddase' that is capable of cleaving the ectodomain of transmembrane protein, which highlights its pivotal roles in a variety of cellular processes including cell proliferation, migration and tumor metastasis via the proteolysis of cytokines, growth factors, receptors and adhesion molecules such as Notch1, ERBB2, N-cadherin and E-cadherin [7].

MicroRNAs (miRNAs) are endogenous, small non-coding RNAs that regulate the translation and degradation of messenger RNAs (mRNAs) at post-transcriptional level [8]. The protein expressions from hundreds of genes were directly suppressed, albeit relatively mild, by a single miRNA [9], underlining the broad and subtle influence of miRNAs on a diversity of signaling pathways. Dysregulated miRNAs are correlated with various cancers and can function as tumor suppressors or oncogenes depending on the function of its targets and the cellular context [10]. Thus far, many miRNAs have been found to participate in the pathways that direct cell proliferation, apoptosis and metastasis of TSCC [11].

To identify tumor-suppressive miRNAs associated with TSCC, we analyzed miRNAs that could directly target ADAM10. In this study, miR-140-5p was identified to be tumor suppressor by directly targeting ADAM10 and repressing TSCC cell migration and invasion. We also screened potential cell migration-related target genes of miR-140-5p and unveiled two brand new relationships between miR-140-5p and its targets.

*Abbreviations:* ADAM10, A disintegrin and metalloproteinase 10; TSCC, tongue squamous cell carcinoma; miRNA, microRNA; UTR, untranslated region; WT, wild type; MUT, mutant; siADAM10, siRNAs against ADAM10; ERBB4, v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 4.

* Corresponding authors. Address: The Second Charity Hospital of Henan Province, 369 Xingguang Road, Jiaozuo 454000, China. (H. Jiebing). Address: State Key Laboratory Breeding Base of Basic Science of Stomatology and Key Laboratory of Oral Biomedicine Ministry of Education, School and Hospital of Stomatology, Wuhan University, 237 Luoyu Road, Wuhan 430079, China. Fax: +86 27 87647443 (B. Zhuan).

*E-mail addresses:* haojiebing@126.com (H. Jiebing), BZ@whuss.com (B. Zhuan).

http://dx.doi.org/10.1016/j.bbrc.2014.02.032
0006-291X/

## 2. Materials and methods

### 2.1. Cell culture

Two human tongue squamous carcinoma cell lines were obtained from American Type Culture Collection (CAL27) and the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Tca8113). They were cultured in Dulbecco's Modified Eagle's Medium (CAL27) or RPMI-1640 medium (Tca8113), both supplemented with 10% fetal bovine serum (FBS, Hyclone).

### 2.2. Cell transfections

CAL27 cells were seeded at $1.2 \times 10^6$ cells/well in 6-well plate and Tca8113 cells were seeded at $0.4 \times 10^6$ cells/well in 12-well plate, allowed to stabilize overnight. Then the cells were transfected with 200 nM miR-140-5p or miR-NC mimics, anti-miR-140-5p or anti-miR-NC, and siRNAs against ADAM10 when the cells reached 70–80% confluence, using Turbofect transfection reagent (Fermentas). All the reagents, including mimics and antibodies, used in this study were summarized in the Supplementary data 1.

### 2.3. Western blotting

48 h after transfection, cells were harvested using RIPA lysis buffer. 1% PMSF was added right before use. Protein concentration was measured using BCA protein assay (Pierce). Protein was loaded onto the 10% SDS–PAGE gel and then transferred to PVDF membrane (Millipore). The blot was blocked with 5% skim milk for 2 h and then probed with primary antibody raised against ADAM10 (1:6000), HDAC7 (1:3000), PAX6 (1:3000), LAMC1 (1:5000), IGF1R (1:3000), PSEN1 (1:3000), ERBB4 (1:3000) and β-actin (1:8000). After overnight incubation at 4 °C, the blot was washed with TBST and incubated in secondary antibody (1:10,000). The signal was developed with ECL (Advansta). ImageJ software (http://rsb.info.nih.gov/ij/) was used for the quantification of protein bands by densitometry analysis.

### 2.4. Luciferase reporter assay

3′UTRs of ADAM10, HDAC7, PAX6, LAMC1 and ERBB4 were PCR amplified from human genomic DNA and then ligated into pMIR-report (Ambion). Then the QuikChange Lightning site-directed mutagenesis kit (Stratagene) was used to induce the miR-140-5p target sequences (complementary to the seed region for miR-140-5p) to mutate from ACCACT to TGGTGA. All constructs were confirmed by sequencing. Primers used in this study were summarized in the Supplementary Table 1. Tca8113 cells were co-transfected with wild-type or mutant 3′UTR luciferase reporter construct, Renilla luciferase construct pRL-TK and either miR-140-5p or miR-NC mimics. 48 h after transfection, luciferase activities were measured using Dual Luciferase Reporter Assay System (Promega) and normalized by dividing firefly luciferase activity with Renilla luciferase activity.

### 2.5. Cell proliferation assay

Transfection of miR-140-5p or miR-NC mimics was performed in 96-well plates in quadruplicate. 48 h after transfection, 10 μl CCK-8 reagent was added to each well and incubate the plate for 2 h. Then the absorbance was measured at 450 nm.

### 2.6. Cell migration and invasion assays

For invasion assay, at 48 h post-transfection, miR-140-5p, miR-NC or mock transfected CAL27 cells were trypsinized, washed, serum-free medium resuspended and added to the upper chamber with Matrigel-coated membrane (24-well insert; BD Biosciences). Complete medium was added to the lower chamber served as chemoattractant. 24 h after incubation, non-invading cells were removed by a cotton swab. Invasive cells were fixed, stained with crystal violet and counted (ten random fields/chamber). Chambers were conducted in duplicate in two independent experiments. The migration assay was the same as the invasion assay except that no matrigel was used in the migration assay.

### 2.7. Statistical analyses

Values were expressed as mean ± standard deviation (SD) from triplicate experiments. Unpaired, two-tailed Student's *t*-test was calculated for statistical analysis using Excel (Microsoft) and GraphPad Prism (Prism). $p < 0.05$ was considered statistically significant.

## 3. Results

### 3.1. ADAM10 is a direct target of miR-140-5p

Emerging evidence shows that, ADAM10 is highly expressed in oral squamous cell carcinoma and TSCC when compared to adjacent normal tissues, which is validated by reverse transcription PCR [6], cDNA microarray [4] and immunohistochemistry [5]. Since miRNA could function as a negative regulator of gene expression posttranscriptionally, we reasoned that, miR-140-5p, the only one miRNA with an 8mer seed-match to ADAM10 3′UTR predicted by TargetScan program (http://www.targetscan.org/) [12], may serve as a tumor suppressor by directly targeting ADAM10. The prediction was also made by miRDB (http://mirdb.org/miRDB/) [13] and miRanda (http://www.microrna.org) [14] programs. The miR-140-5p core target site (position 1072–1079 of human ADAM10 3′UTR corresponding to position 1–8 of miR-140-5p (Fig. 1A)) is extremely conserved among 31 out of 36 eutherian mammals displayed by ensembl genomic alignments (http://www.ensembl.org/).

To assess whether ADAM10 is a functional target of miR-140-5p, we constructed a luciferase reporter with 3′UTR of ADAM10 cloned downstream to a firefly luciferase gene. Subsequently, the reporter and synthetic miR-140-5p or miR-NC mimics were transfected into Tca8113 cells to evaluate the effects of miR-140-5p on the ADAM10 3′UTR based on relative luciferase activity. Compared with miR-NC, miR-140-5p had a potent inhibitory effect on the reporter with wild-type ADAM10 3′UTR, but not on the analogous reporter with mutant ADAM10 3′UTR (Fig. 1B). Consistently, Western blot results further showed that overexpression of miR-140-5p suppressed the endogenous protein level of ADAM10, while knockdown of endogenous miR-140-5p led to upregulation of ADAM10 protein level (Fig. 1C and D). These results indicated that, ADAM10 is a direct target of miR-140-5p in TSCC cells.

### 3.2. Overexpression of miR-140-5p inhibits CAL27 cell invasion and migration, but not proliferation

ADAM10 had a profound impact on tumor growth and metastasis by shedding a list of substrates, including growth factors and adhesion molecules, such as EGF and N-, E-cadherin [7]. This prompted us to investigate the effects of miR-140-5p on the TSCC cell invasion, migration and proliferation.

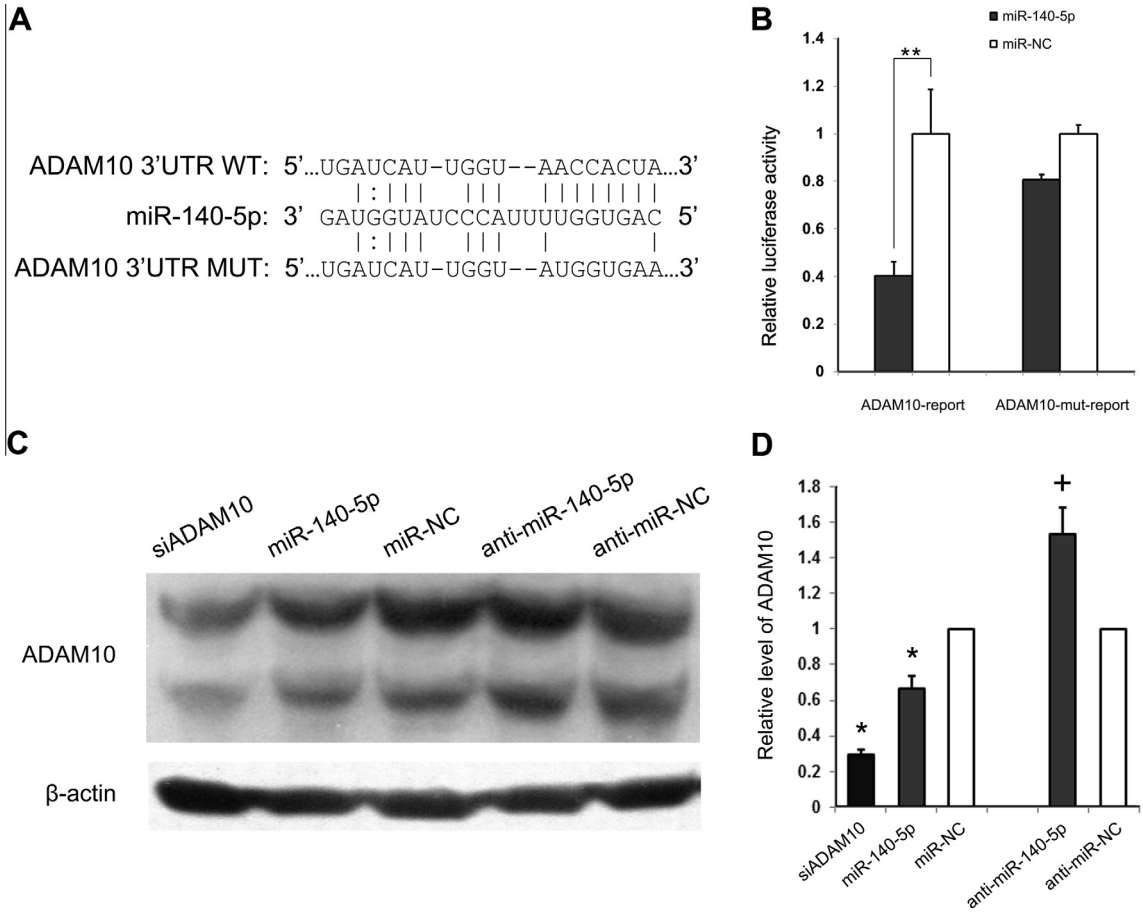


**Fig. 1.** ADAM10 is a direct target of miR-140-5p. (A) Putative interaction between miR-140-5p and its binding sequence in the 3′UTR of ADAM10. Site-directed mutations were generated in the ADAM10 3′UTR sequence in the complementary site for the seed region of miR-140-5p. (B) Analysis of luciferase activity. Tca8113 cells were co-transfected with firefly luciferase reporter containing either wild-type (ADAM10-report) or mutant (ADAM10-mut-report) ADAM10 3′UTR, Renilla luciferase reporter pRL-TK (as internal control), and either miR-140-5p or miR-NC mimics. Relative luciferase activity was measured and normalized by Renilla luciferase activity. The noramlized luciferase activity for the miR-NC transfected cells was set as 1. Data shown are mean ± SD from three independent experiments. $^{**}p < 0.01$; unpaired Student's *t*-test. (C) Western blot results of endogenous ADAM10 protein in CAL27 cells transfected with siRNAs against ADAM10 (siADAM10), miR-140-5p and miRNA-NC mimics, anti-miR-140-5p and anti-miR-NC. siADAM10 was used as positive control. (D) Densitometry analysis. Values are mean ± SD from three independent experiments. $^{*}p < 0.05$ versus miR-NC; $^{+}p < 0.05$ versus anti-miR-NC; unpaired Student's *t*-test.

Transwell invasion assay demonstrated that, increased expression of miR-140-5p suppressed the invasive capacity of CAL27 cells by around 25% to invade through matrigel-coated membranes (Fig. 2A and C). Ectopic expression of miR-140-5p markedly reduced the migration of CAL27 cells by around 40% when compared to the migration of control cells (Fig. 2B and D). Cell proliferation assay indicated that ectopic miR-140-5p had no effect on CAL27 cell proliferation (data not shown).

To comprehensively understand such repressive effect of miR-140-5p on TSCC cell migration and invasion, we performed gene set enrichment analysis using Protein, Signaling, Transcriptional Interactions & Inflammation Networks Gateway (pSTIING) program (http://pstiing.icr.ac.uk/) [15]. 345 target genes of miR-140-5p were uploaded and the top 5 enriched pathways are: regulation of actin cytoskeleton, focal adhesion, calcium signalling, adherens junction and tight junction, thereby partly explained the potent inhibitory effect of miR-140-5p on TSCC cell migration and invasion.

### 3.3. miR-140-5p directly represses cell migration-related genes

To clarify molecular mechanism underlying the repression of TSCC cell migration by miR-140-5p, we submitted all the 345 target genes of miR-140-5p predicted by TargetScan to the programs provided by the Database for Annotation, Visualization and Integrated Discovery (DAVID [16], http://david.abcc.ncifcrf.gov/) to identify genes related to (positive regulation of) cell migration. LAMC1, HDAC7, PAX6, IGF1R, PSEN1 and ERBB4 were chosen for further analysis (Fig. 3A).

CAL27 cells were transfected with either miR-140-5p or miR-NC mimics to assess the inhibitory effect of miR-140-5p on the endogenous protein level of these six genes. Western blot results showed that overexpression of miR-140-5p resulted in around 50% reduction of LAMC1, HDAC7 and PAX6 at protein level, but did not affect the expression of IGF1R and PSEN1 (Fig. 3C and D, ERBB4 was discussed in the next section).

To determine whether such inhibitory effect on the translation is mediated by specific and direct interaction of miR-140-5p with the LAMC1, HDAC7 and PAX6 target site, we constructed luciferase reporter plasmids containing the 3′UTR of these three genes. Dual-luciferase assay showed that the introduction of miR-140-5p significantly reduced the luciferase activity with respect to the miR-NC, whereas such inhibitory effect was absent in the cells transfected with reporter plasmids containing mutant 3′UTR of these three genes (Fig. 3B).

### 3.4. ERBB4 is upregulated by miR-140-5p

Intriguingly, Western blot analysis demonstrated that ectopic miR-140-5p molecules led to upregulation of endogenous ERBB4

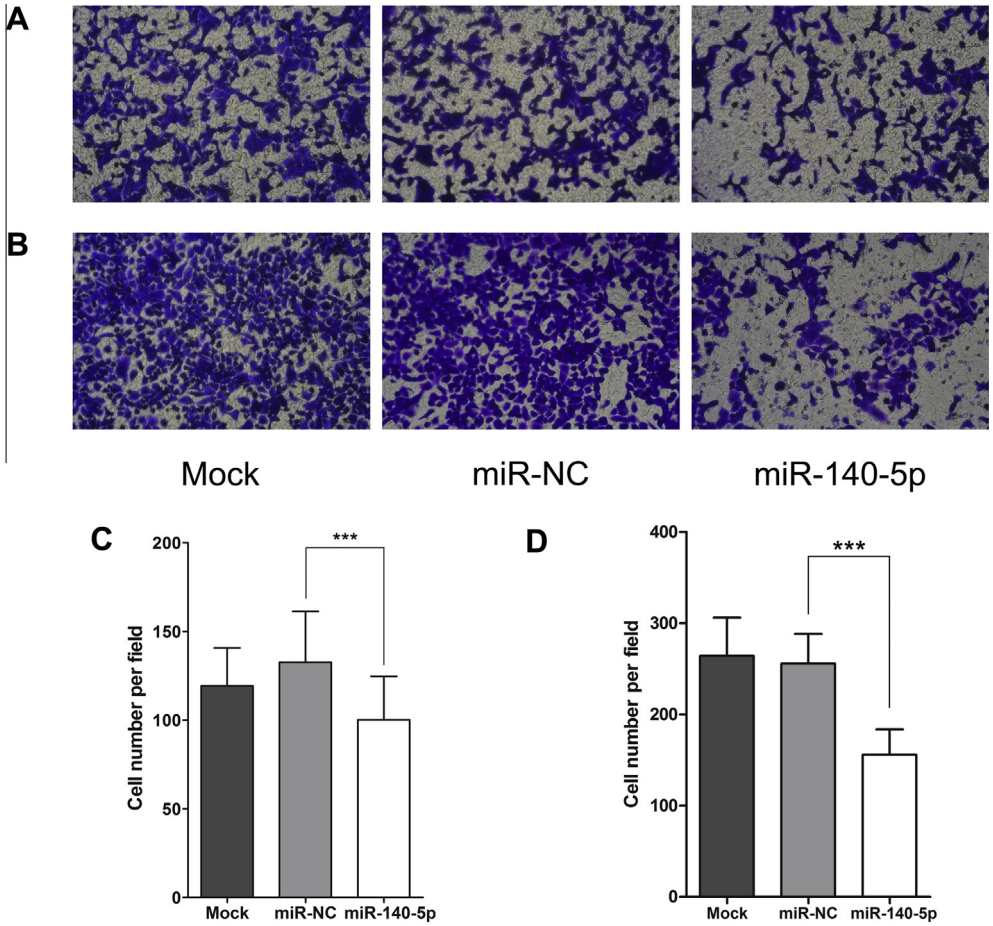


**Fig. 2.** miR-140-5p inhibits CAL27 cell invasion and migration. (A and C) Transwell invasion assay of CAL27 cells transfected with mock, miR-NC, miR-140-5p. (A) Representative images of cell invasion. (C) Quantification of 20 randomly selected fields. (B and D) Transwell migration assay of CAL27 cells transfected with mock, miR-NC, miR-140-5p. (B) Representative images of cell migration. (D) Quantification of 20 randomly selected fields. Data shown are mean ± SD from a single representative experiment. $^{***}p < 0.001$; unpaired Student's *t*-test. Transfection efficiency was monitored by miR-NC with FAM and quantitative real-time PCR (data not shown).

in the pilot experiment, even though the 3′UTR of ERBB4 contain a potential binding site for miR-140-5p (Fig. 4A). Since ADAM10 is a major ectodomain sheddase of ERBB2 [17] and ADAM17 is also essential for the shedding of ERBB4 [18], it's reasonable to deduce that, ADAM10 may also be required, if not essential, for the cleavage of ERBB4. For this reason, the repression of ADAM10 by miR-140-5p may be partly responsible for the upregulation of ERBB4. To verify our hypothesis, siRNAs against ADAM10 were transfected to CAL27 cells along with miR-140-5p and miR-NC mimics, and then Western blot was performed to evaluate the protein expression level of ERBB4. The result of densitometry analysis showed that, the relative protein level of ERBB4 was significantly upregulated by 40% following miR-140-5p transfection, while knocking down ADAM10 by siRNAs only led to 30% upregulation of ERBB4 (Fig. 4C and D).

Compared with miR-140-5p, siRNAs against ADAM10 exert much stronger repression effect on the protein level of ADAM10 (Fig. 1C), which in turn would give rise to higher accumulation of ERBB4 if ADAM10 were a major sheddase for the ERBB4 and main mediator responsible for the upregulation of ERBB4 by miR-140-5p. Above results indicated that ADAM10 may play a minor, not essential, role for the cleavage of ERBB4 and only be partially responsible for the upregulation of ERBB4 by miR-140-5p. There must be some other routes, modulated by miR-140-5p, leading to the upregulation of ERBB4.

Despite all that, we tried to explore the possible interaction between ERBB4 3′UTR and miR-140-5p. A fragment of ERBB4 3′UTR, containing the potential binding site for miR-140-5p, was cloned downstream to a firefly luciferase gene. Co-transfection experiments showed that, overexpression of miR-140-5p markedly repressed the expression of a luciferase gene containing the wild-type 3′UTR of ERBB4, but didn't affect the luciferase gene containing mutant 3′UTR of ERBB4, in which the binding site for the miR-140-5p seed region was mutated (Fig. 4B). Results of luciferase assay argue for a directly targeting ERBB4 by miR-140-5p.

Taken together, these results indicated that ERBB4 was upregulated by miR-140-5p, due in part to the effect of ADAM10, although the interaction between ERBB4 3′UTR and miR-140-5p existed simultaneously.

## 4. Discussion

The role of miR-140-5p in tumorigenesis and progression has not been well documented until very recently. Literatures suggested that miR-140-5p suppressed tumor cell proliferation, migration, and invasion by targeting IGF1R in non-small cell lung cancer (NSCLC) and targeting TGFBR1 and FGF9 in hepatocellular carcinoma. Furthermore, miR-140-5p was significantly downregulated in both carcinomas [19,20]. In the present study, our results suggested that miR-140-5p could directly targets oncogenic ADAM10 and represses TSCC cell migration and invasion, although no significant difference in the expression of miR-140-5p between TSCC tissues and adjacent nontumorous tissues was demonstrated, partly due to insufficient number of tissues (Supplementary Fig. S1).

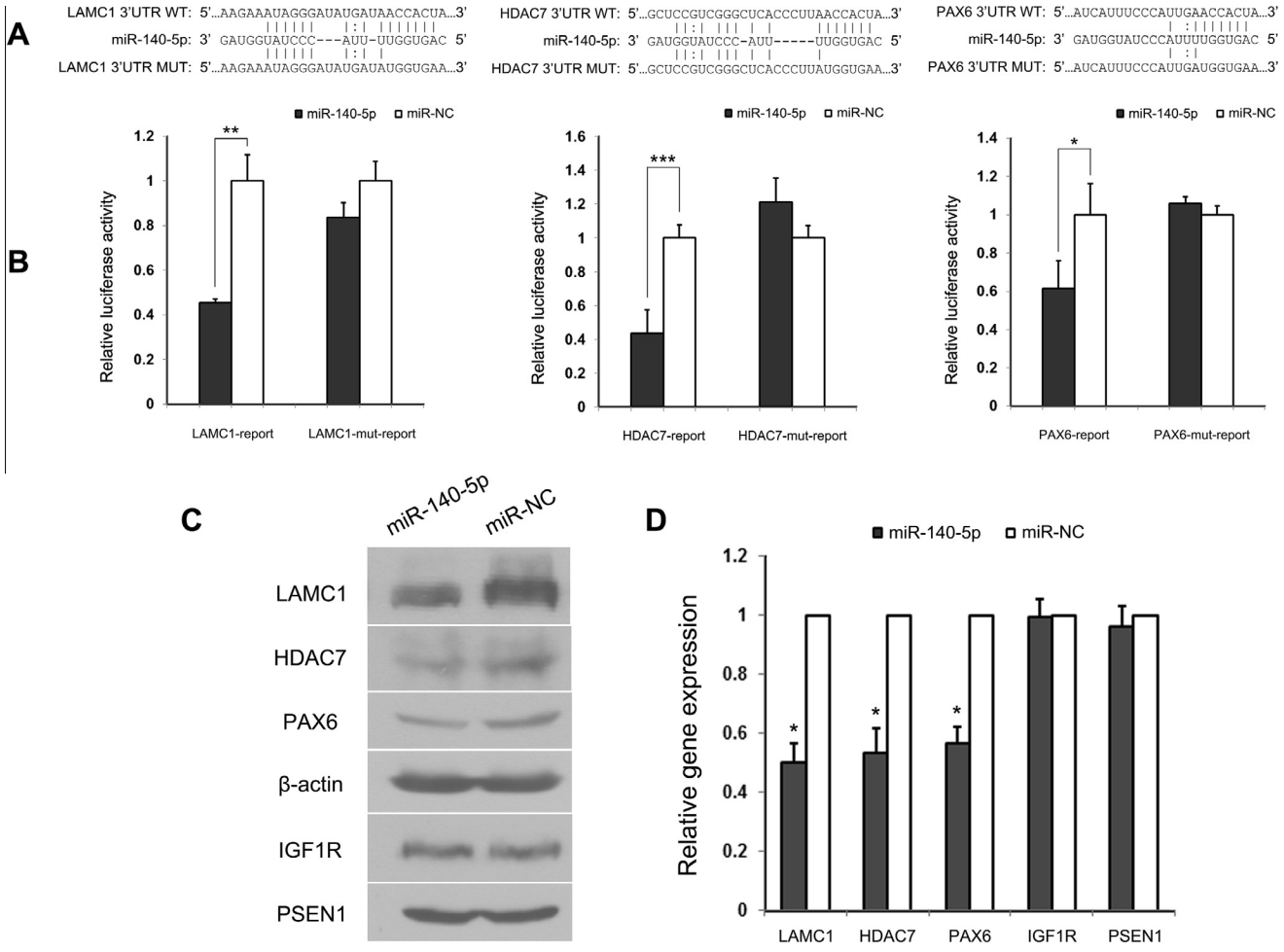


**Fig. 3.** miR-140-5p directly targets cell migration-related genes. (A) Putative interactions between miR-140-5p and its binding sequences in the 3′UTR of LAMC1, HDAC7 and PAX6. Site-directed mutations were generated in the 3′UTR sequences in the complementary sites for the seed region of miR-140-5p. (B) Analysis of luciferase activity. Firefly luciferase reporter containing wild-type (LAMC1/HDAC7/PAX6-report) or mutant (LAMC1/HDAC7/PAX6-mut-report) 3′UTR was used for the luciferase assay. Please refer to Fig. 1B caption for detailed information about experimental procedure. Data shown are mean ± SD from three independent experiments. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; unpaired Student's *t*-test. (C) Western blot results of endogenous LAMC1, HDAC7, PAX6, IGF1R and PSEN1 protein in CAL27 cells transfected with miR-140-5p and miRNA-NC mimics. (D) Densitometry analysis. Values are mean ± SD from three independent experiments. $^{*}p < 0.05$ versus miR-NC; unpaired Student's *t*-test.

In the present study, ADAM10, LAMC1, HDAC7 and PAX6 were confirmed to be specific endogenous targets of miR-140-5p. ADAM10, with adhesion and protease activity, is a key regulator of both cell–cell and cell-extracellular matrix interactions, via shedding cell surface proteins, such as L1 and GPNMB. Then the shed extracellular domain of L1 and GPNMB can promote cell migration by binding to integrin receptors [21,22]. The gamma chain isoform laminin, gamma 1, encoded by LAMC1, can partner with netrin-4 and α6β1 integrin to become a ternary complex and promote migration of neural stem cells [23]. HDAC7 is a key modulator of endothelial cell migration partly by regulating PDGF-B/PDGFR-β and VEGF responsive genes [24,25]. PAX6, essential for the development of CNS and eye, is a delicate regulator of cell migration and correct positioning during development [26,27].

The mysterious upregulation of ERBB4 by miR-140-5p can be inquired from two perspectives. ERBB4, a member of ErbB family of receptor tyrosine kinases, sequentially subjected to ectodomain shedding by ADAM17 and intracellular domain ripping by γ-secretase [18,28]. The released intracellular domain translocates into the nucleus and regulate gene transcription [29]. The upregulation of ERBB4 could come from impaired proteolysis or enhanced transcription or translation, which would be caused by dysregulated genes because of the introduction of miR-140-5p. For example, NCSTN contains a conserved 8mer seed-matched site for miR-140-5p and its encoded protein, nicastrin, is a member of γ-secretase complex, just like presenilin1. If nicastrin is downregulated by miR-140-5p, the ripping of intracellular domain would be affected and the full-length protein of ERBB4 would be accumulated. On the other hand, there are a large number of mRNA binding proteins and they could compete or cooperate with miRNAs to regulate translation. For example, miR-328 can act as a decoy by binding to a repressive RNA binding protein, while miR369-3 can directs the association between AU-rich elements (AREs) and micro-ribonucleoproteins (microRNPs) associated proteins, and thus activate gene translation [30,31]. Likewise, miR-140-5p probably directly or indirectly interacts with regulatory RNA binding proteins and prevents them from blocking translation of ERBB4.

The relationship between miR-140-5p and ADAM10/PAX6/ERBB4 is interesting. Immunofluorescence and Western blot analysis have demonstrated that, inhibition of ADAM10 can also inhibit PAX6 expression using either ADAM10 inhibitor or ADAM10 specific siRNA [32]. In the present study, ADAM10 and PAX6 were identified as direct targets of miR-140-5p. The combined results suggested that, the negative regulation of PAX6 came from two directions, directly targeting by miR-140-5p and indirect repression mediated by ADAM10. Similarly, downregulated ADAM10 was also partly responsible for the "positive" regulation of ERBB4 by miR-140-5p. In summary, ADAM10 is not just a direct target

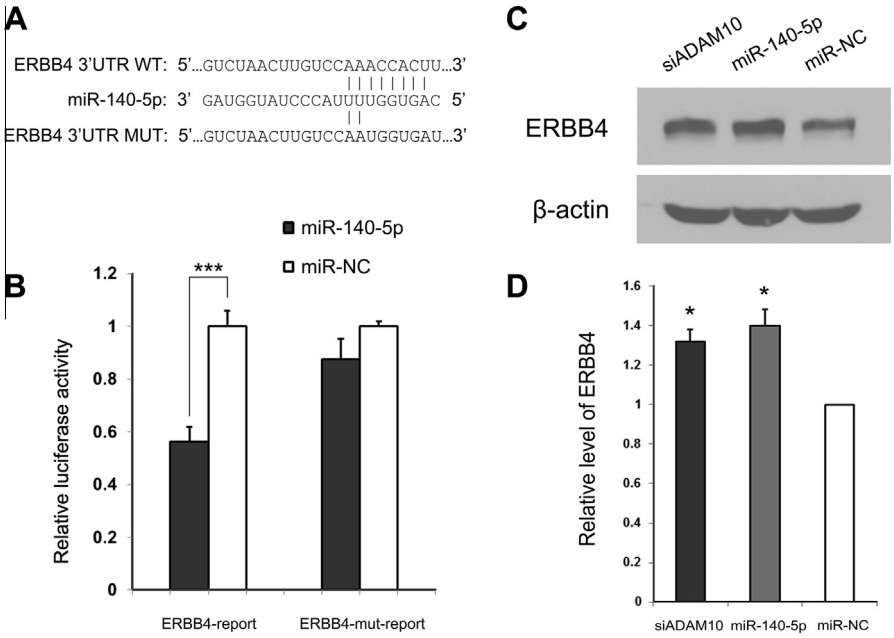


**Fig. 4.** ERBB4 is upregulated by miR-140-5p. (A) Putative interaction between miR-140-5p and its binding sequence in the 3′UTR of ERBB4. Site-directed mutations were generated in the ERBB4 3′UTR sequence in the complementary site for the seed region of miR-140-5p. (B) Analysis of luciferase activity. Firefly luciferase reporter containing wild-type (ERBB4-report) or mutant (ERBB4-mut-report) ERBB4 3′UTR was used for the luciferase assay. Please refer to Fig. 1B caption for detailed information about experimental procedure. Data shown are mean ± SD from three independent experiments. $^{***}p < 0.001$; unpaired Student's *t*-test. (C) Western blot results of endogenous ERBB4 protein in CAL27 cells transfected with siRNAs against ADAM10 (siADAM10), miR-140-5p and miRNA-NC mimics. (D) Densitometry analysis. Values are mean ± SD from three independent experiments. $^{*}p < 0.05$ versus miR-NC; unpaired Student's *t*-test.

of miR-140-5p, the repressed ADAM10 also helps to enhance the effect of miR-140-5p to ERBB4 and PAX6.

Unregulated PSEN1 and IGF1R are really out of expectation, but still can be reconciliated and understandable. In Notch signalling pathway, ADAM10 or ADAM17 cleave the ectodomain of Notch transmembrane protein by shedding, and subsequently, γ-secretase complex (including presenilin1 encoded by PSEN1) cleave the intracellular domain of Notch (NICD) by ripping. Then NICD translocates to the nucleus where it forms a ternary complex to activate target gene expression [33]. PSEN1 and ADAM10 not just cooperate with each other in the same pipeline, they also share the same miRNA:target recognition pattern with miR-140-5p—8mer site. Besides, PSEN1 3′UTR also contain another 7mer-m8 site (Supplementary Fig. S2). It's reasonable to speculate that, just like ADAM10, PSEN1 is also very likely to be a direct target of miR-140-5p, which turned out to be unsubstantiated. Recently, IGF1R has been proved to be a direct target of miR-140-5p in NSCLC [19], while in present study, IGF1R was not responsible to the regulation of miR-140-5p. These seemingly contradictory results can be explained by the emerging paradigm that miRNA can be not only cell-type or tissue-specific "signatures" for certain normal or cancerous tissues, but also functional or inoperative to certain genes depending on the molecular and cellular context. Thus, one must be cautious in extrapolating the result of interaction between miRNA and its target beyond the research field.

In conclusion, miR-140-5p could directly targets ADAM10 in TSCC cells and represses cell migration and invasion, but not proliferation. The repressed ADAM10 also helps to enhance the effect of miR-140-5p to other target genes: ERBB4 and PAX6. The demonstration of miR-140-5p and its target genes would help us to better understand the molecular mechanism underlying TSCC progression.

## Acknowledgments

We thank the patients for participating in the study. This work was supported by Grants 30930099, 81120108010 from the National Natural Science Foundation of China and 2012CB722404 from the Pre-National Basic Research Program of China (973 Plan).

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.02.032.

## References

[1] R. Siegel, D. Naishadham, A. Jemal, Cancer statistics, CA Cancer J. Clin. 63 (2013) (2013) 11–30.
[2] I.K. Kalnins, A.G. Leonard, K. Sako, et al., Correlation between prognosis and degree of lymph node involvement in carcinoma of the oral cavity, Am. J. Surg. 134 (1977) 450–454.
[3] G. Murphy, The ADAMs: signalling scissors in the tumour microenvironment, Nat. Rev. Cancer 8 (2008) 929–941.
[4] S. Zheng-jun, L.I. Jin-rong, Gene expression pattern of oncogene/tumor suppressor gene and apoptosis/stress response-related genes in tongue carcinoma, J. Oral Sci. Res. 25 (2009) 44–47.
[5] Y. Guan, X.-c. Kuang, J.-g. Yao, et al., Expression and significance of ADAM10 in tongue squamous cell carcinomas, J. Oral Sci. Res. 27 (2011) 981–983.
[6] S.-Y. Ko, S.-C. Lin, Y.-K. Wong, et al., Increase of disintergin metalloprotease 10 (ADAM10) expression in oral squamous cell carcinoma, Cancer Lett. 245 (2007) 33–43.
[7] M.L. Moss, A. Stoeck, W. Yan, et al., ADAM10 as a target for anti-cancer therapy, Curr. Pharm. Biotechnol. 9 (2008) 2–8.
[8] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, Cell 116 (2004) 281–297.
[9] D. Baek, J. Villen, C. Shin, et al., The impact of microRNAs on protein output, Nature 455 (2008) 64–71.
[10] A. Esquela-Kerscher, F.J. Slack, Oncomirs – microRNAs with a role in cancer, Nat. Rev. Cancer 6 (2006) 259–269.
[11] M. Gorenchtein, C.F. Poh, R. Saini, et al., MicroRNAs in an oral cancer context – from basic biology to clinical utility, J. Dent. Res. 91 (2012) 440–446.
[12] B.P. Lewis, C.B. Burge, D.P. Bartel, Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets, Cell 120 (2005) 15–20.
[13] X.W. Wang, MiRDB: a microRNA target prediction and functional annotation database with a wiki interface, RNA 14 (2008) 1012–1017.
[14] D. Betel, M. Wilson, A. Gabow, et al., The microRNA.org resource: targets and expression, Nucleic Acids Res. 36 (2008) D149–D153.

[15] A. Ng, B. Bursteinas, Q. Gao, et al., PSTIING: a 'systems' approach towards integrating signalling pathways, interaction and transcriptional regulatory networks in inflammation and cancer, Nucleic Acids Res. 34 (2006) D527–D534.
[16] G. Dennis, B.T. Sherman, D.A. Hosack, et al., DAVID: database for annotation, visualization, and integrated discovery, Genome Biol. 4 (2003).
[17] P.C.C. Liu, X.D. Liu, Y.L. Li, et al., Identification of ADAM10 as a major source of HER2 ectodomain sheddase activity in HER2 overexpressing breast cancer cells, Cancer Biol. Ther. 5 (2006) 657–664.
[18] C. Rio, Tumor necrosis factor-alpha -converting enzyme is required for cleavage of erbB4/HER4, J. Biol. Chem. 275 (2000) 10379–10387.
[19] Y. Yuan, Y. Shen, L. Xue, et al., miR-140 suppresses tumor growth and metastasis of non-small cell lung cancer by targeting insulin-like growth factor 1 receptor, PLoS ONE 8 (2013) e73604.
[20] H. Yang, F. Fang, R. Chang, et al., MicroRNA-140-5p suppresses tumor growth and metastasis by targeting transforming growth factor beta receptor 1 and fibroblast growth factor 9 in hepatocellular carcinoma, Hepatology 58 (2013) 205–217.
[21] M. Yang, Y. Li, K. Chilukuri, et al., L1 stimulation of human glioma cell motility correlates with FAK activation, J. Neurooncol. 105 (2011) 27–44.
[22] A.A. Rose, M.G. Annis, Z. Dong, et al., ADAM10 releases a soluble form of the GPNMB/osteoactivin extracellular domain with angiogenic properties, PLoS ONE 5 (2010) e12093.
[23] F.I. Staquicini, E. Dias-Neto, J. Li, et al., Discovery of a functional protein complex of netrin-4, laminin gamma1 chain, and integrin alpha6beta1 in mouse neural stem cells, Proc. Natl. Acad. Sci. U.S.A. 106 (2009) 2903–2908.
[24] D. Mottet, A. Bellahcene, S. Pirotte, et al., Histone deacetylase 7 silencing alters endothelial cell migration, a key step in angiogenesis, Circ. Res. 101 (2007) 1237–1246.
[25] S. Wang, X. Li, M. Parra, et al., Control of endothelial cell proliferation and migration by VEGF signaling to histone deacetylase 7, Proc. Natl. Acad. Sci. U.S.A. 105 (2008) 7738–7743.
[26] J.M. Collinson, S.A. Chanas, R.E. Hill, et al., Corneal development, limbal stem cell function, and corneal epithelial cell migration in the Pax6(−/−) mouse, Invest. Ophthalmol. Vis. Sci. 45 (2004) 1101–1108.
[27] T. Nomura, N. Osumi, Misrouting of mitral cell progenitors in the Pax6/small eye rat telencephalon, Development 131 (2004) 787–796.
[28] C.Y. Ni, M.P. Murphy, T.E. Golde, et al., Gamma -secretase cleavage and nuclear localization of ErbB-4 receptor tyrosine kinase, Science 294 (2001) 2179–2181.
[29] C.C. Williams, J.G. Allison, G.A. Vidal, et al., The ERBB4/HER4 receptor tyrosine kinase regulates gene expression by functioning as a STAT5A nuclear chaperone, J. Cell Biol. 167 (2004) 469–478.
[30] A.M. Eiring, J.G. Harb, P. Neviani, et al., miR-328 functions as an RNA decoy to modulate hnRNP E2 regulation of mRNA translation in leukemic blasts, Cell 140 (2010) 652–665.
[31] S. Vasudevan, Y. Tong, J.A. Steitz, Switching from repression to activation: microRNAs can up-regulate translation, Science 318 (2007) 1931–1934.
[32] S. Paudel, Y.H. Kim, M.I. Huh, et al., ADAM10 mediates N-cadherin ectodomain shedding during retinal ganglion cell differentiation in primary cultured retinal cells from the developing chick retina, J. Cell. Biochem. 114 (2013) 942–954.
[33] S.J. Bray, Notch signalling: a simple pathway becomes complex, Nat. Rev. Mol. Cell Biol. 7 (2006) 678–689.