# The tumor-suppressive *microRNA-23b/27b* cluster regulates the *MET* oncogene in oral squamous cell carcinoma

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**Abstract.** Our recent studies of microRNA (miRNA) expression signatures in human cancers revealed that two clustered miRNAs, *microRNA-23b* (*miR-23b*) and *microRNA-27b* (*miR-27b*), were significantly reduced in cancer tissues. Few reports have provided functional analyses of these clustered miRNAs in oral squamous cell carcinoma (OSCC). The aim of this study was to investigate the functional significance of *miR-23b* and *miR-27b* in OSCC and to identify novel *miR-23b/27b*-mediated cancer pathways and target genes involved in OSCC oncogenesis and metastasis. Expression levels of *miR-23b* and *miR-27b* were significantly reduced in OSCC specimens. Restoration of *miR-23b* or *miR-27b* in cancer cells revealed that both miRNAs significantly inhibited cancer cell migration and invasion. Our *in silico* analyses and luciferase reporter assays showed that the receptor tyrosine kinase *MET*, was directly regulated by these miRNAs. Moreover, downregulating the *MET* gene by use of siRNA significantly inhibited cell migration and invasion by OSCC cells. The identification of novel molecular pathways regulated by *miR-23b* and *miR-27b* may lead to a better understanding of the oncogenesis and metastasis of this disease.

## Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer in the world, and it consists of a heterogeneous group of malignancies arising from the oral cavity, paranasal sinus, pharynx, larynx and salivary glands (1). Most of oral squamous cell carcinoma (OSCC) occurs from oral cavity (accounts for >95%) and is the most common type of HNSCC (2). Despite recent advances in various treatment modalities, including surgery, radiotherapy, chemotherapy and molecularly targeted therapy, the survival rate of patients with OSCC has not markedly improved (5-year survival is <50%) due to the high rate of locoregional recurrence and distinct metastasis (3). We suggest that it would be possible to significantly improve diagnosis, therapy, and prevention of OSCC through a better understanding of the molecular oncogenic processes and metastatic pathways underlying the disease. We further suggest that this could be achieved through the use of current genome-based approaches.

The discovery of microRNA (miRNA) in the human genome provided new directions in cancer study. miRNAs are endogenous small non-coding RNAs (19-22 bases long) that regulate protein-coding/non protein-coding gene expression by repressing translation or degradation of RNA transcripts in a sequence-specific manner (4). A growing body of studies have shown that miRNAs are aberrantly expressed in many human cancers. Thus, they act pivotal roles in the initiation, progression and metastasis of such cancers (5). Moreover, normal RNA networks can be disrupted by the aberrant expression of tumor-suppressive or oncogenic miRNAs in cancer cells. Therefore, identifying aberrantly expressed miRNAs is an important first step toward understanding miRNA-mediated RNA networks.

Based on this proposal, we have constructed miRNA expression signatures through genetic analysis of hypopharyngeal-SCC, maxillary sinus-SCC and OSCC clinical specimens (6-9). Using these miRNA expression signatures, we have identified molecular pathways in HNSCC that are mediated by aberrantly expressed miRNAs. For example, downregulation of tumor-suppressive *miR-375* inhibited cancer cell apoptosis through dysregulation of *AEG-1/MTDH* in HNSCC cells (10). Moreover, downregulation of *miR-874* is a frequent event in HNSCC and *miR-874* acted as a tumor suppressor that directly targets *HDAC1* (11). More recently, we found that *miR-26a* and

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*miR-26b* function as tumor suppressors through regulating of *TMEM184B* based on the OSCC signature (9).

Our miRNA expression signatures of human cancers, including OSCC, revealed that clustered miRNAs, *miR-23b* and *miR-27b* were frequently downregulated in several types of cancer tissues (9,12-14). Several studies showed that these miRNAs act as tumor suppressive miRNAs through their targeting of oncogenic genes (15-17). Up to now, few reports have provided functional analyses of these clustered miRNAs in OSCC. The aims of the study were to investigate the functional roles of *miR-23b* and *miR-27b* in OSCC and to identify novel *miR-23b/27b*-mediated cancer pathways and target genes involved in OSCC oncogenesis and metastasis. We expect that this analysis will provide novel insights into the pivotal molecular mechanisms of OSCC oncogenesis and metastasis. This new knowledge will facilitate the development of therapeutic strategies for the treatment of the disease.

## Materials and methods

*Clinical specimens in patients with OSCC and cell lines*. A total of 37 pairs of cancer tissues and corresponding normal epithelial tissues were obtained from patients with OSCC at Chiba University Hospital from 2008 to 2013. The patients were classified according to the 2002 Union for International Cancer Control (UICC) staging criteria before treatment. Prior written informed consent and approval were obtained from all patients. The patients' backgrounds and clinicopathological characteristics are shown in Table I. The following human OSCC cell lines were used: SAS (derived from a primary tongue SCC) and HSC3 (derived from a lymph node metastasis of tongue SCC).

*RNA isolation*. Tissues were immersed in RNAlater (Ambion, Austin, TX, USA), and stored at 4˚C until RNA was extracted. Total RNA was isolated using TRIzol reagent according to the manufacturer's instructions.

*Quantitative of miRNAs and messenger RNA by real-time RT-PCR*. The procedure for PCR quantification was described previously (6-11). The expression levels of *miR-23b* (assay ID: 000400) and *miR-27b* (assay ID: 000409) were analyzed by TaqMan quantitative real-time PCR and normalized to *RNU48* (assay ID: 001006). TaqMan probes and primers for *MET* (P/N: Hs01565584_m1), *GUSB* (P/N: Hs 00939627_ml) and *GAPDH* (P/N: Hs02758991_g1) as an internal control were obtained from Applied Biosystems.

*Function assays by miRNA and small-interfering RNA transfection*. The following miRNAs mimics were used in this study: mirVana miRNA mimic for *hsa-miR-23b* (product ID: PM10711) and *hsa-miR-27b* (product ID: PM10750). The transfection procedures and transfection efficiencies of miRNA for SAS and HSC3 cells were reported in previous studies (6-9,11,15,18). To investigate the functional significance of *miR-23b, miR-27b* and *si-MET*, we performed cell proliferation, migration and invasion assays using OSCC cell lines. The experimental procedures were described in previous studies (8,9,15,18).

*Identification of target genes regulated by miR-23b, miR-27b by using genome-wide gene expression and in silico analysis*. The miRNA public database (TargetScan) was used for *in silico* identification of candidate target genes that contained *miR-23b* and *miR-27b* binding sites in their 3'-untranslated region. These genes were then categorized into KEGG pathways using the GeneCodis program (http://genecodis.dacya.ucm.es). To identify upregulated genes in OSCC, we analyzed a publicly available gene expression data set in GEO (accession no. GSE6631).

*Western blotting*. Cells were harvested 72 h after transfection and lysates were prepared. From each lysate, an aliquot containing 20 $\mu$g of protein was separated on Mini-PROTEAN TGX Gels (Bio-Rad, Hercules, CA, USA) and transferred to PVDF membranes. Immunoblotting was performed with rabbit anti-MET antibodies (1:1,000); mouse anti-GAPDH antibodies (1:4,000) were used as an internal loading control. The experimental procedures were performed as described in our previous studies (6-9,11,15,18).

*Immunohistochemistry*. Two OSCC clinical specimens were immunostained following the manufacturer's protocol with the Ultra-Vision detection system (Thermo Scientific, Fremont, CA, USA). Primary rabbit polyclonal antibodies against MET were diluted 1:300. The slides were treated with biotinylated goat antibodies.

*Plasmid construction and dual-luciferase reporter assays*. The partial wild-type sequence of the *MET* 3'-untranslated region or those with mutated *miR-23b/miR-27b* target sites were inserted between the *Xho*I-*Pme*I restriction sites in the 3'-UTR of the *hRluc* gene in the psiDHECK-2 vector (C8021; Promega, Madison, WI, USA). The procedure for the dual-luciferase reporter assay was described previously (6-9,11,15,18).

*Statistical analysis*. The relationships between two groups and numerical values obtained by real-time RT-qPCR were analyzed using Mann-Whitney U tests. Spearman's rank test was used to evaluate the correlation between the expression levels of *miR-23b, miR-27b* and *MET* mRNA. The relationships among more than three variables and numerical values were analyzed using the Mann-Whitney U test after Bonferroni adjustment. All analyses were performed using Expert Stat View (version 5, SAS Institute Inc., Cary, NC, USA).

## Results

*Expression levels of miR-23b and miR-27b in OSCC tissues and cell lines*. We evaluated the expression levels of the clustered miRNAs in 37 OSCC clinical specimens and two cell lines. The expression levels of *miR-23b* and *miR-27b* were significantly lower in tumor tissues and cell lines than in corresponding normal tissues (Fig. 1A and B). Spearman's rank test showed a positive correlation between the expression levels of *miR-23b* and *miR-27b* (Fig. 1C).

*Gain-of-function assay of miR-23b and miR-27b in OSCC cell lines: effects on cell proliferation, migration and invasion*.

Table I. Clinical features of 37 OSCC patients.

| No. | Age | Sex | Location | T | N | M | Stage | Differentiation |
|---|---|---|---|---|---|---|---|---|
| 1 | 66 | M | Tongue | 2 | 0 | 0 | II | Moderate |
| 2 | 65 | M | Oral floor | 4a | 1 | 0 | IVA | Moderate |
| 3 | 67 | M | Tongue | 4a | 2c | 0 | IVA | Moderate |
| 4 | 36 | F | Tongue | 3 | 1 | 0 | III | Moderate |
| 5 | 73 | M | Tongue | 3 | 2b | 0 | IVA | Poor |
| 6 | 63 | F | Oral floor | 2 | 2b | 0 | IVA | Basaloid SCC |
| 7 | 77 | M | Gum | 2 | 0 | 0 | II | Moderate |
| 8 | 68 | M | Tongue | 2 | 0 | 0 | II | Well |
| 9 | 76 | F | Tongue | 1 | 0 | 0 | I | Well |
| 10 | 69 | M | Tongue | 1 | 0 | 0 | I | Well |
| 11 | 73 | F | Tongue | 1 | 0 | 0 | I | Well |
| 12 | 64 | M | Tongue | 1 | 0 | 0 | I | Well |
| 13 | 64 | M | Tongue | 1 | 0 | 0 | I | Well |
| 14 | 82 | M | Oral floor | 1 | 0 | 0 | I | Well |
| 15 | 67 | M | Oral floor | 4a | 2b | 0 | IVA | Well |
| 16 | 67 | M | Tongue | 3 | 0 | 0 | III | Moderate |
| 17 | 64 | M | Tongue | 3 | 2b | 0 | IVA | Moderate |
| 18 | 59 | M | Tongue | 1 | 2a | 0 | IVA | Moderate |
| 19 | 47 | M | Oral floor | 1 | 0 | 0 | I | Moderate |
| 20 | 67 | M | Tongue | 2 | 0 | 0 | II | Poor-moderate |
| 21 | 70 | M | Tongue | 1 | 0 | 0 | I | Well |
| 22 | 38 | M | Tongue | 1 | 0 | 0 | I | Well |
| 23 | 70 | M | Tongue, oral floor | 2 | 0 | 0 | II | Well |
| 24 | 51 | M | Tongue | 1 | 0 | 0 | I | Well |
| 25 | 81 | M | Tongue | is | 0 | 0 | 0 | Extremely well |
| 26 | 34 | F | Tongue | 1 | 0 | 0 | I | Poor |
| 27 | 42 | M | Gum | 4a | 0 | 0 | IVA | Moderate |
| 28 | 70 | M | Tongue | 1 | 0 | 0 | I | Moderate |
| 29 | 71 | M | Tongue | 1 | 0 | 0 | I | Well |
| 30 | 60 | F | Tongue | 2 | I | 0 | III | Well |
| 31 | 77 | M | Tongue | 2 | 2b | 0 | IVA | Poorly |
| 32 | 64 | F | Oral floor | 4a | 2c | 0 | IVA | Moderate |
| 33 | 68 | M | Tongue | 1 | 0 | 0 | I | Well |
| 34 | 39 | M | Tongue | 4a | 0 | 0 | IVA | Well |
| 35 | 29 | F | Tongue | 1 | 0 | 0 | I | Poorly |
| 36 | 71 | M | Buccal mucosa | 2 | 1 | 0 | III | Poorly |
| 37 | 39 | M | Tongue | 4a | 0 | 0 | IVA | Moderate |

The functional significance of *miR-23b* and *miR-27b* were investigated using miRNA transfection of OSCC cell lines. XTT assays demonstrated that SAS cell proliferation was significantly inhibited in *miR-23b*- and *miR-27b*-transfectants compared with the mock or miR-control transfected SAS cells. On the other hand, proliferation was inhibited only in *miR-27b* transfectant in HSC3 (Fig. 1D). Migration and invasion assays demonstrated that cell migration and invasion activity were significantly inhibited in *miR-23b* and *miR-27b* transfectants compared with the mock or miR-control transfectants in OSCC cell lines (Fig. 1E and F).

*Selection of genes targeted by miR-23b and miR-27b in OSCC.* To identify genes targeted by *miR-23b* and *miR-27b*, we use *in silico* analyses and genome-wide expression analyses. Our strategy for identification of target genes is shown in Fig. 2.

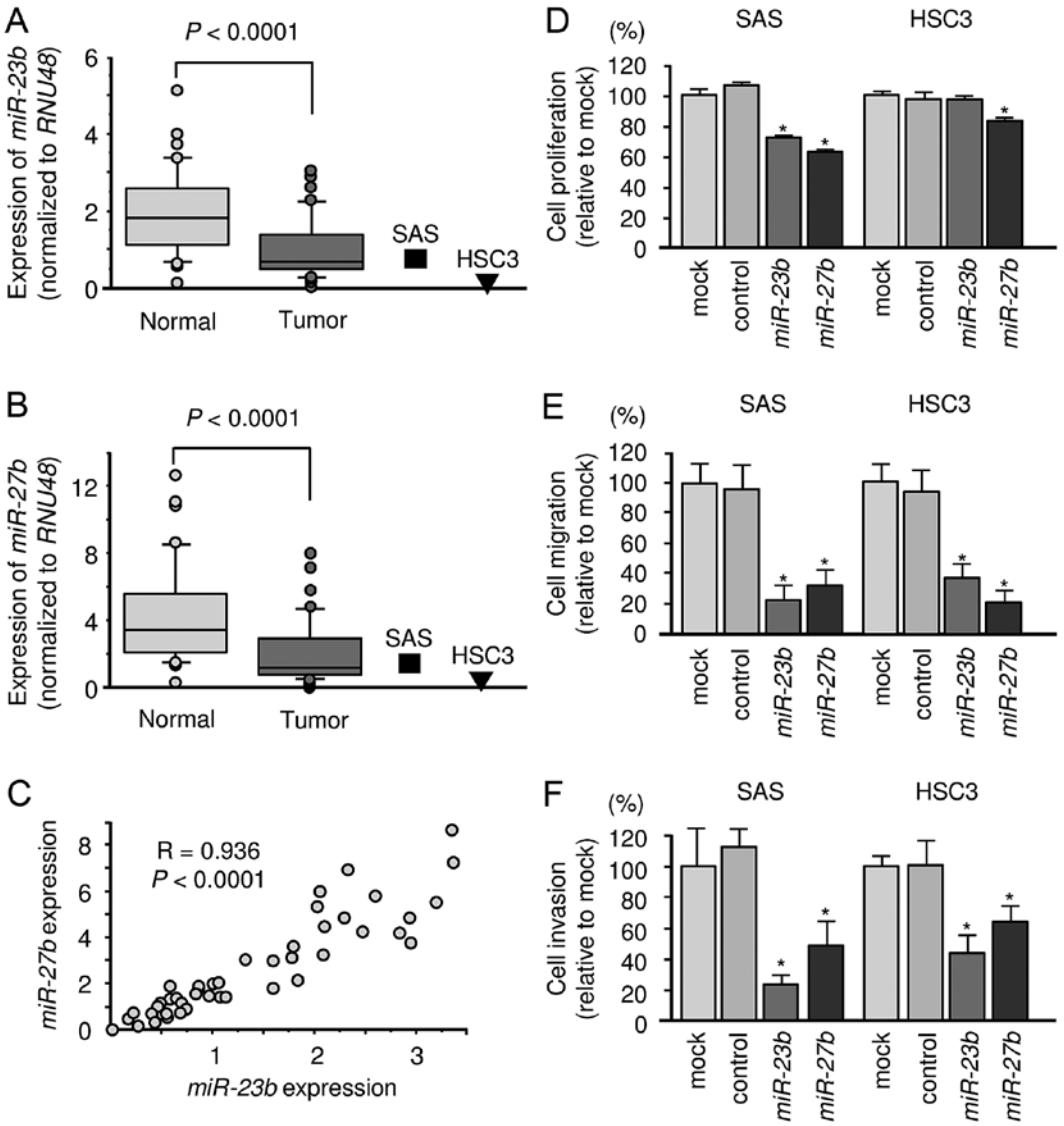


Figure 1. Expression levels of *miR-23b* and *miR-27b* and functional significance of *miR-23b* and *miR-27b* in OSCC cell lines. (A and B) Expression levels of *miR-23b* and *miR-27b* in OSCC clinical specimens and cell lines. *RNU48* was used for internal control. (C) Correlations between the expression levels of *miR-23b* and *miR-27b* in OSCC clinical specimens. (D) Cell proliferation was determined with the XTT assay 72 h after transfection with *miR-23b* or *miR-27b*. (E) Cell migration was assessed with the migration assay 48 h after transfection with *miR-23b* or *miR-27b*. (F) Cell invasion was characterized with an invasion assay 48 h after transfection with *miR-23b* or *miR-27b*. *P<0.0001.

First, we screened putative candidate target genes using the TargetScan database and identified 229 potential targets. These genes were classified into KEGG pathways using GeneCodis analysis and four pathways and 18 genes were identified as significantly enriched pathways (Table IIA) and genes (Table IIB-E). The gene set was then analyzed with a publicly available gene expression data set in GEO (accession no. GSE6631). In this group of genes, we focused on the hepatocyte growth factor receptor (*MET*) because it was the most significantly upregulated in HNSCC (Fig. 3).

*Expression of MET in OSCC clinical specimens and cell lines*. We investigated the expression levels of *MET* in 37 OSCC clinical specimens and cell lines. First, qRT-PCR revealed that *MET* was significantly upregulated in cancer tissues and cell lines compared with normal tissues (Fig. 4A). Spearman's rank test showed negative correlations between the expression levels of miR-23b/miR-27b and MET (Fig. 4B and C). Next, immunohistochemistry revealed that MET was strongly expressed in cancer tissues, while low expression was observed in normal tissues (Fig. 4D and E).

*Direct regulation of MET gene by miR-23b and miR-27b in OSCC cells*. We investigated the expression levels of *MET* in OSCC cell lines. We performed quantitative real-time RT-PCR and western blotting in OSCC cell lines to investigate whether restoration of *miR-23b* or *miR-27b* altered *MET* gene and protein expression. mRNA expression levels of *MET* were significantly repressed in *miR-23b* and *miR-27b* transfectants compared with mock or miR-control transfectant in OSCC cell lines (Fig. 5A). Protein expression levels of MET were repressed in *miR-23b* and *miR-27b* transfectants compared

Table II. The KEGG pathways.

A, Significantly enriched KEGG pathway regulated by *miR-23b/27b* cluster

| No. of genes | Annotations | P-value |
|---|---|---|
| 10 | (KEGG) 05200: Pathways in cancer | 0.0082 |
| 7 | (KEGG) 04810: Regulation of actin cytoskeleton | 0.0210 |
| 8 | (KEGG) 04010: MAPK signaling pathway | 0.0235 |
| 4 | (KEGG) 05218: Melanoma | 0.0372 |

B, Pathway in cancer

| Gene symbol | Gene name | HNSCC log2 ratio |
|---|---|---|
| *LAMC2* | Laminin, γ2 | 2.33 |
| *FGF1* | Fibroblast growth factor 1 (acidic) | 2.32 |
| *PTCH1* | Patched 1 | 2.24 |
| *FZD7* | Frizzled family receptor 7 | 2.18 |
| *PAX8* | Paired box 8 | 1.43 |
| *FGF12* | Fibroblast growth factor 12 | 1.39 |
| *RUNX1* | Runt-related transcription factor 1 | 1.27 |
| *MET* | Met proto-oncogene (hepatocyte growth factor receptor) | 1.26 |
| *MAPK10* | Mitogen-activated protein kinase 10 | 1.22 |
| *EGFR* | Epidermal growth factor receptor | 1.15 |

C, Regulation of actin cytoskeleton

| Gene symbol | Gene name | HNSCC log2 ratio |
|---|---|---|
| *FGF1* | Fibroblast growth factor 1 (acidic) | 2.32 |
| *FGF12* | Fibroblast growth factor 12 | 1.39 |
| *ARHGEF7* | Rho guanine nucleotide exchange factor (GEF) 7 | 1.34 |
| *SSH1* | Slingshot homolog 1 (*Drosophila*) | 1.20 |
| GNA13 | Guanine nucleotide binding protein (G protein), α13 | 1.18 |
| *EGFR* | Epidermal growth factor receptor | 1.15 |
| *ENAH* | Enabled homolog (*Drosophila*) | 1.09 |

D, MAPK signaling pathway

| Gene symbol | Gene name | HNSCC log2 ratio |
|---|---|---|
| *NTRK2* | Neurotrophic tyrosine kinase, receptor, type 2 | 3.33 |
| *CACNA1B* | Calcium channel, voltage-dependent, N type, α1B subunit | 2.86 |
| *FGF1* | Fibroblast growth factor 1 (acidic) | 2.32 |
| *FGF12* | Fibroblast growth factor 12 | 1.39 |
| *MAP4K3* | Mitogen-activated protein kinase kinase kinase kinase 3 | 1.24 |
| *MAPK10* | Mitogen-activated protein kinase 10 | 1.22 |
| *PRKX* | Protein kinase, X-linked | 1.16 |
| *EGFR* | Epidermal growth factor receptor | 1.15 |

E, Melanoma

| Gene symbol | Gene name | H average |
|---|---|---|
| FGF1 | Fibroblast growth factor 1 (acidic) | 2.32 |
| FGF12 | Fibroblast growth factor 12 | 1.39 |
| MET | Met proto-oncogene (hepatocyte growth factor receptor) | 1.26 |
| EGFR | Epidermal growth factor receptor | 1.15 |

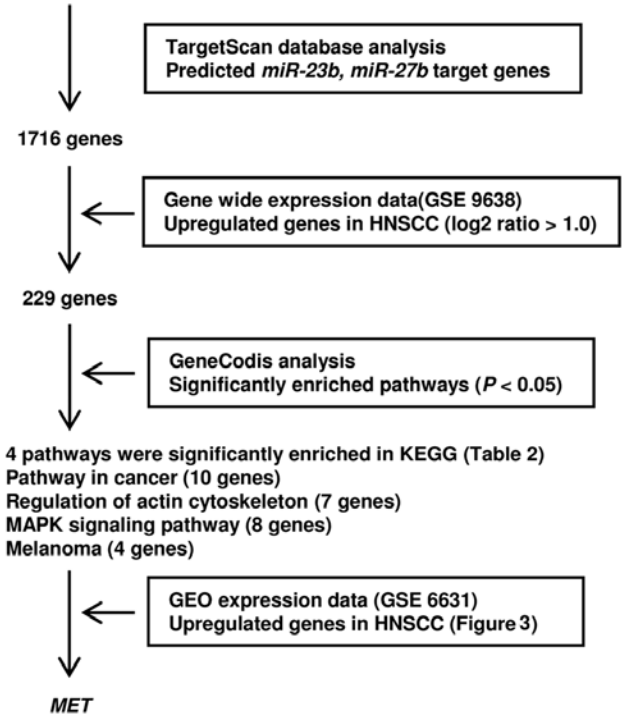


Figure 2. Selection for target genes regulated by *miR-23b/27b* cluster. A total of 1,716 genes were identified as putative target genes containing binding sites for *miR-23b* and *miR-27b*. Among these, 229 genes were upregulated in HNSCC (GSE9638). These genes were classified into KEGG pathways, and four pathways and 18 genes were identified as enriched pathways and genes.

with mock or miR-control in SAS. Although restoration of *miR-27b* significantly suppressed MET protein expression, no significant downregulation of MET was observed in *miR-23b*

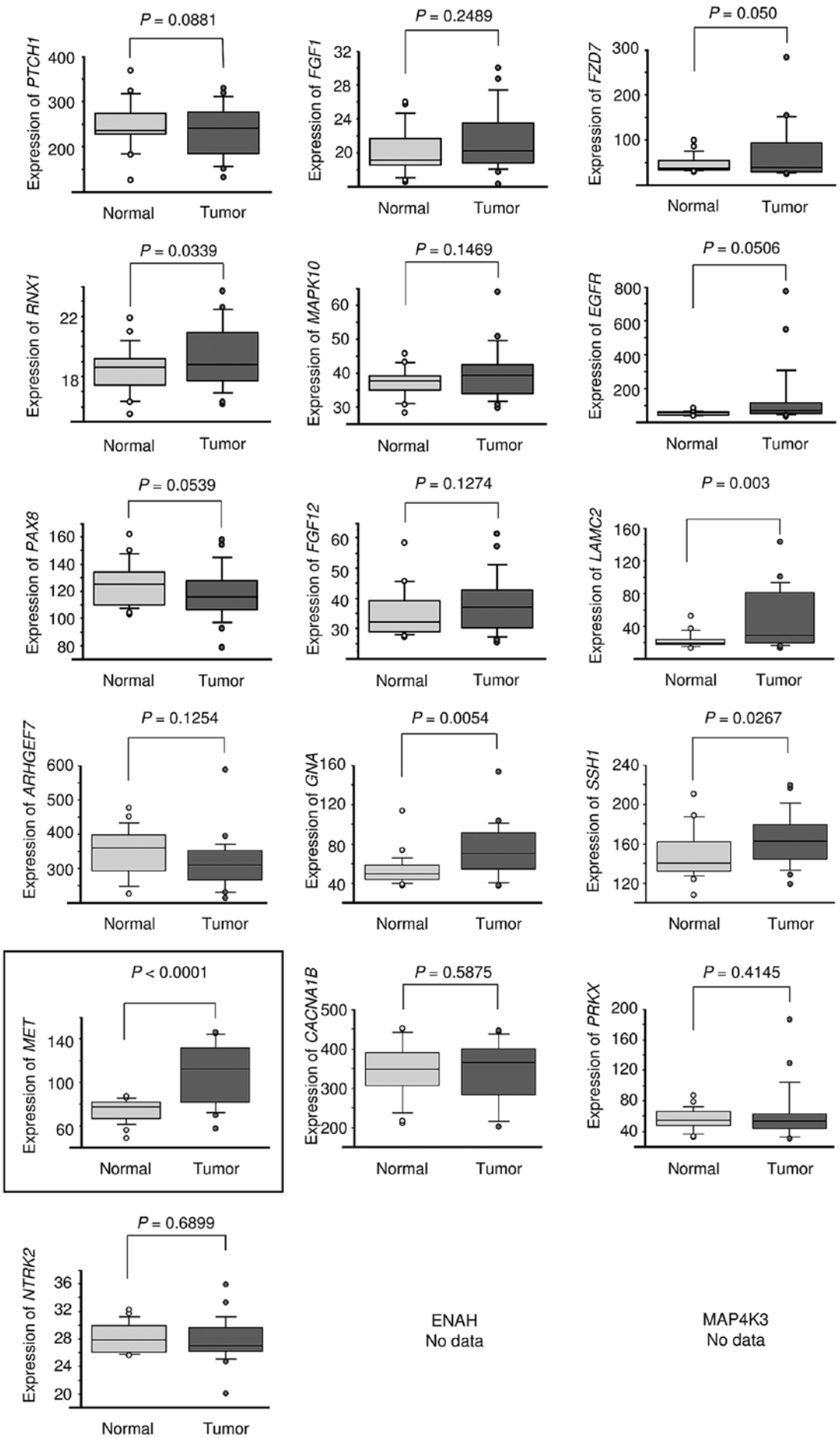


Figure 3. *In silico* analysis of HNSCC clinical specimens. The expression levels of putative target genes in HNSCC clinical specimens were investigated by GEO expression database (accession no. GSE6631).

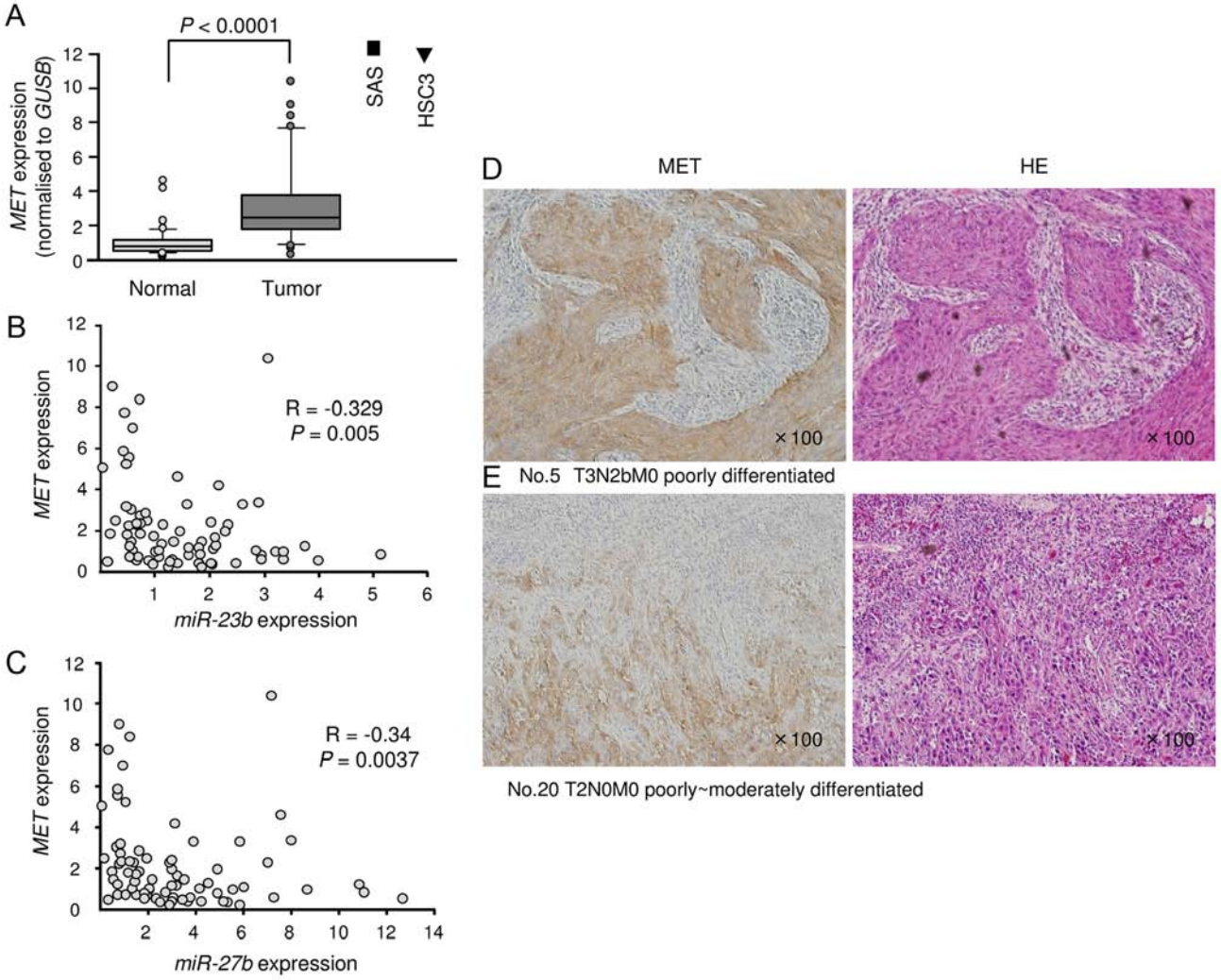


Figure 4. Expression levels of *MET* in OSCC. (A) The mRNA expression levels of *MET* in OSCC clinical specimens and cell lines was measured. *GUSB* was used for the internal control. (B and C) Correlation between *MET* expression and *miR-23b* (B) or *miR-27b* (C). (D and E) Immunohistochemical staining for detection of MET and H&E staining in two patients with OSCC.

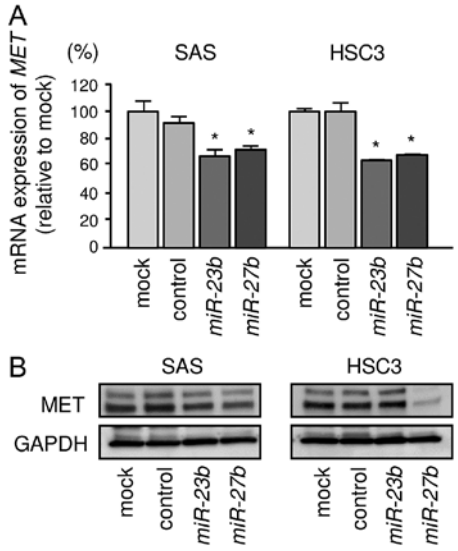


Figure 5. MET expression was directly regulated by *miR-23b*/*miR-27b* in OSCC cell lines. (A) *MET* mRNA expression 72 h after transfection with 10 nM *miR-23b* or *miR-27b*. *GAPDH* was used for the internal control. (B) MET protein expression 72 h after transfection with 10 nM *miR-23b* or *miR-27b*. GAPDH was used for loading control. *P<0.001.

transfectant in HSC3 (Fig. 5B). Next, we performed luciferase reporter assays in OSCC cell lines to determine whether *MET* mRNA contained target sites for *miR-23b* and *miR-27b*. We used vectors encoding either a partial wild-type sequence or a sequence in which the miRNA binding site had been mutated from the 3'-UTR of *MET* mRNA. Our data showed that the luminescence intensity was significantly reduced by co-transfection with *miR-23b*/*miR-27b* and the vector carrying the wild-type 3'-UTR of *MET* mRNA (Fig. 6A and B).

*Effect of silencing MET gene on cell proliferation, migration, and invasion in OSCC cells*. To investigate the functional role of MET in OSCC, we performed loss-of-function studies using *si-MET* transfectants. First, we checked the knockdown efficiency of *si-MET* transfection. Western blotting and qRT-PCR revealed that the si-RNA effectively reduced the expression levels of MET in OSCC cell lines (Fig. 7A and B). Cell proliferation assays showed that SAS cell viability was significantly inhibited in si-RNA transfectants compared with mock or si-control. On the other hand, proliferation was not inhibited in HSC3 cells (Fig. 7C). Migration and invasion

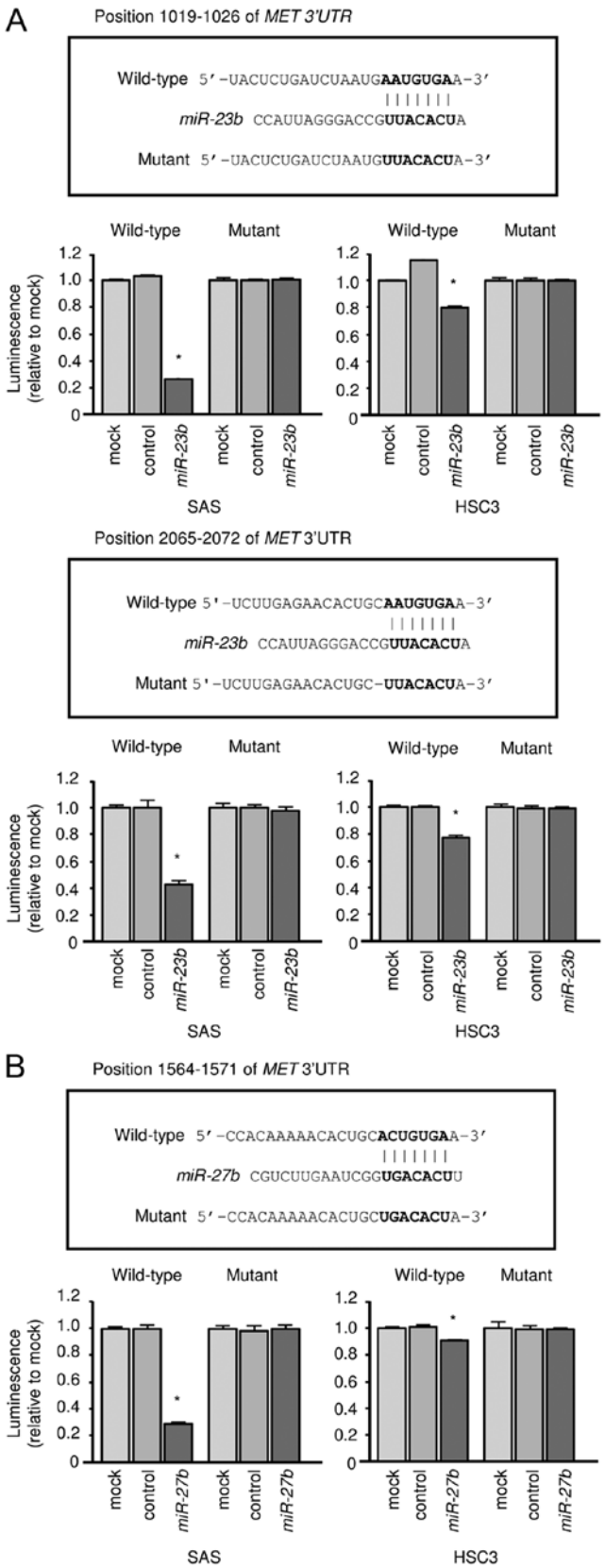


Figure 6. Luciferase reporter assays using OSCC cell lines. Luciferase reporter assays using the vectors encoding putative *miR-23b* (A) or *miR-27b* (B) target sites of the *MET* 3'-UTR for both wild-type and mutant co-transfectants. *Renilla* luciferase values were normalized to firefly luciferase values. *P<0.001.

assays showed that cell migration activity was significantly inhibited in OSCC cells (Fig. 7D and E).

## Discussion

A significant amount of evidence suggests that aberrantly expressed miRNAs are closely involved in human oncogenesis, metastasis and drug resistance (19). The cause of the poor prognosis of OSCC is distant metastasis of the cancer cells. Thus, identification of tumor-suppressive miRNAs that regulate novel metastatic pathways and metastasis-promoting genes may improve our understanding of OSCC progression and metastasis. We have sequentially identified tumor-suppressive miRNA-mediated novel cancer pathways in HNSCC and OSCC (18,20-24). We hypothesize that identification of novel metastatic pathways and targets regulated by tumor-suppressive miRNAs could lead to a better understanding of OSCC and the development of new therapeutic strategies to treat this disease.

Here, we focused on two clustered miRNAs, *miR-23b* and *miR-27b*, based on miRNA expression signatures. Thus, we investigated the functional significance of these miRNAs in OSCC cells. We found that *miR-23b* and *miR-27b* were downregulated in cancer specimens and that restoration of *miR-23b* and *miR-27b* significantly inhibited cancer cell migration and invasion. These results strongly suggested that these miRNAs functioned as tumor suppressors in OSCC cells. Our previous studies of prostate cancer, renal cell carcinoma and bladder cancer showed that *miR-23b* and *miR-27b* act as tumor suppressors regulating several oncogenic genes (15-17). In renal cell carcinoma, significantly poor prognosis was observed in patients with lower expression levels of the *miR-23b/miR-27b* cluster, suggesting that low expression of these miRNAs increased the risk of disease progression and predicted poor survival (18).

Other research groups have shown tumor-suppressive roles of *miR-23b* and *miR-27b* in several cancers (25-28). For example, *miR-23b* directly controls the proto-oncogenes *SRC* and *AKT*, and overexpression of *miR-23b* suppresses cell viabilities, cell cycle arrest, and apoptosis (29). Another report has shown that *miR-23b* and *miR-27b* are downregulated in metastatic and castration-resistant prostate cancer (CRPC) tumors and that ectopic expression of these miRNAs suppresses cell invasion and migration in CRPC cell lines (30). Contrary to our data showing tumor suppressive roles of *miR-23b* and *miR-27b* in human cancers, expression levels of these miRNAs were significantly upregulated in human breast cancer, and *miR-23b* and *miR-27b* knockdown substantially represses breast cancer cell growth. These results indicate that these miRNAs function as oncogenes in this cellular context (31). Elucidation of the molecular mechanisms controlling expression of the *miR-23b/27b* cluster is an important theme in this field.

Identification of miRNA-regulated pathways and targets is important to elucidate the molecular functions of tumor-suppressive *miR-23b* and *miR-27b* in OSCC cells. Towards that end, we combined expression data from OSCC clinical specimens and *in silico* miRNA database analysis. In this screening, several putative pathways and targets were annotated to be subject to *miR-23b* and *miR-27b* in OSCC cells. Among them, we focused on the *MET* oncogene because overexpression of *MET* was indicated by gene expression data and it is well known that MET activates signaling that contributes to cancer

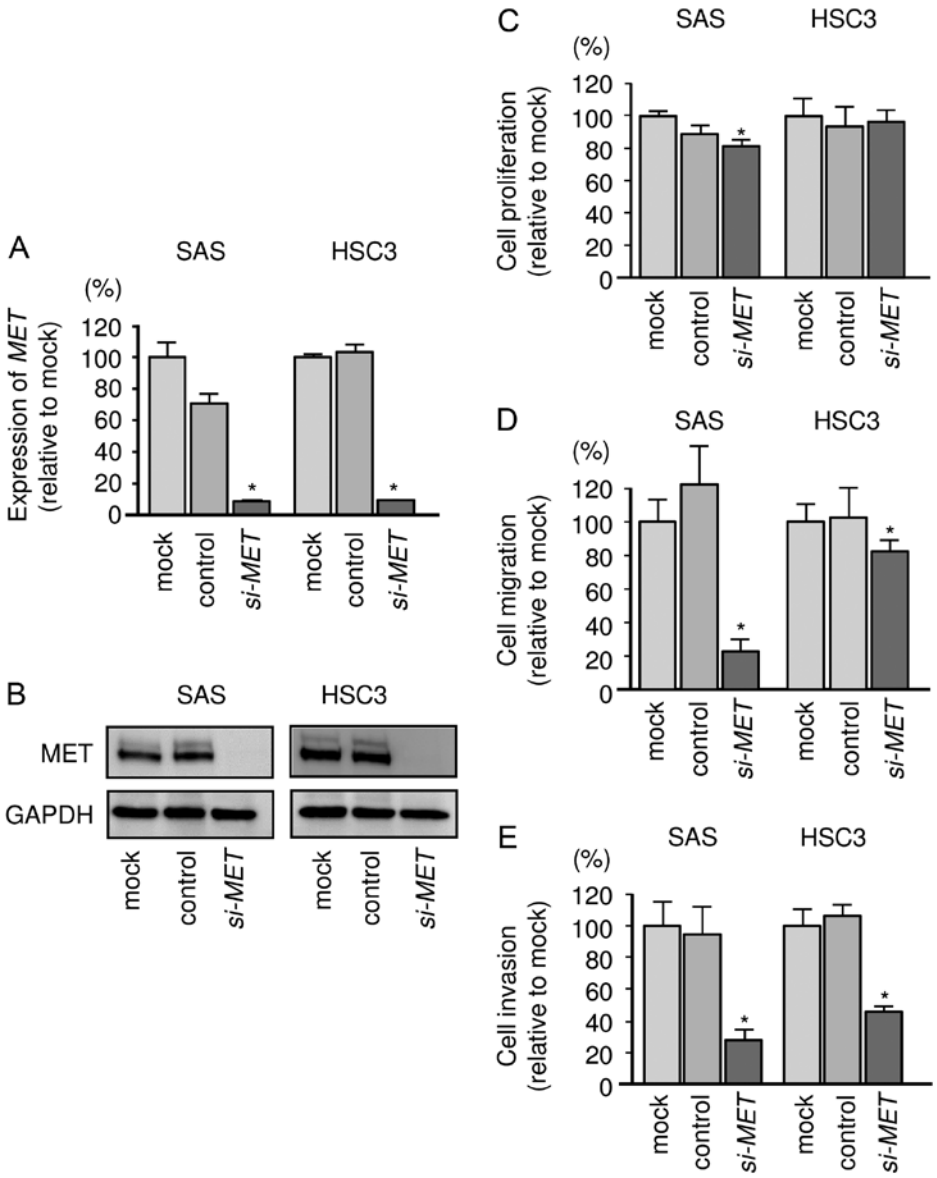


Figure 7. Effect of *MET* silencing by si-*MET* transfection of OSCC cell lines. (A) *MET* mRNA expression levels 72 h after transfection with 10 nM *si-MET*. (B) MET protein expression levels 72 h after transfection with 10 nM *si-MET*. (C-E) Effect of silencing of MET in OSCC cell lines. (C) Cell proliferation was determined with the XTT assay 72 h after transfection with 10 nM *si-MET*. (D) Cell migration was assessed with the migration assay 48 h after transfection with 10 nM *si-MET*. (E) Cell invasion was determined with the invasion assay 48 h after transfection with 10 nM *si-MET*. $^{*}$P<0.05.

cell proliferation, metastasis and drug resistance (32). One study reported that overexpression of *MET* was observed in 90% of HNSCC cell lines and 84% of HNSCC patient samples (33). Moreover, HGF overexpression has also been described in ~60% of HNSCC, and co-expression of MET/HGF has been correlated with more aggressive disease behavior (33). Thus, the control of HGF/MET oncogenic signaling is the pivotal treatment target of the disease.

Cetuximab, a monoclonal antibody directed against the EGFR, is now available for targeted molecular therapy in HNSCC, including OSCC (34). Cetuximab is currently approved in combination with radiation therapy as a first-line treatment in combination with platinum and 5-fluorouracil in recurrent or metastatic disease (35,36). However, the curative effects of these treatments are limited, and it is difficult to recover completely from this disease. Many studies have suggested different mechanisms that may be contributing to targeted EGFR resistance (37). A recent study showed that cetuximab-induced MET activation enhanced to cetuximab-resistance in colon cancer cells (38). Aberrant MET expression and hepatocyte growth factor (HGF) signaling might be contributing as salvage pathways for EGFR blockade-resistant cancer cells. Therefore, dual blocking therapeutic strategies of EGFR and MET oncogenic signaling are indispensable for HNSCC and OSCC treatment.

In conclusion, *miR-23b* and *miR-27b* were frequently reduced in OSCC clinical specimens and appeared to act as tumor suppressors through targeting of the *MET* oncogene in this disease. Elucidation of novel target genes and pathways regulating by tumor-suppressive *miR-23b/27b* cluster may provide new information of OSCC and the development of new treatment strategies of this disease.

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