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FoxM1 promotes epithelial–mesenchymal transition, invasion, and migration of tongue squamous cell carcinoma cells through a c-Met/AKT-dependent positive feedback loop

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Forkhead box protein M1 (FoxM1) has been associated with cancer progression and metastasis. However, the function of FoxM1 in tongue squamous cell carcinoma (TSCC) remains largely unknown. The purpose of this study was to determine the role of FoxM1 in regulation of epithelial–mesenchymal transition (EMT) and migration of TSCC cells. We found that FoxM1 induced EMT and increased invasion/migration capacity in SCC9 and SCC25 cells. FoxM1 stimulation increased c-Met, pAKT, and vimentin levels but decreased E-cadherin level. Chromatin immunoprecipitation assay established that FoxM1 is bound to the promoter of c-Met to activate its transcription. In turn, c-Met promoted the expression of FoxM1 and pAKT. Blocking AKT signaling attenuated the invasion and migration of SCC9 and SCC25 cells stimulated by FoxM1 or c-Met. These results indicate that a positive feedback loop controls the EMT and migration of TSCC cells induced by FoxM1 and c-Met through AKT. Furthermore, the expression levels of FoxM1, pAKT, and c-Met were found to significantly increase in TSCC tissues compared with normal tissues, and these three biomarkers were concomitantly expressed in TSCC tissues. Clinical association analyses indicated that the expression of FoxM1, c-Met, and pAKT was associated with clinicopathological characteristics of patients with TSCC including tumor stage, tumor size, and lymph node metastasis. Taken together, our findings suggest that FoxM1 promotes the EMT, invasion and migration of TSCC cells, and cross-talks with c-Met/AKT signaling to form a positive feedback loop to promote TSCC development. ***Anti-Cancer Drugs*** **29:216–226 Copyright © 2018 The Author(s). Published by Wolters Kluwer Health, Inc.**

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Introduction

Cancer is a severe disease leading to millions of death worldwide [1]. Oral cavity cancer is a malignant tumor with high incidence, which is known for its poor prognosis and high mortality rates because of features such as high invasiveness and metastasis [2,3]. The oral tongue is the most common primary site of oral cavity cancer, and squamous cell carcinoma represents the majority of the pathologic types. Although tongue squamous cell carcinoma (TSCC) diagnosed early has favorable prognosis, the 5-year survival rate for advanced-stage disease is only 40–60% [4]. Despite significant advances in surgery and chemotherapy over the past few decades, therapeutic failure and disease progression are still quite frequent [4]. Therefore, there is an urgent requirement for new biomarkers for TSCC to develop better early diagnostic and therapeutic strategies.

Forkhead box protein M1 (FoxM1) is a typical transcription factor that belongs to the Forkhead Box family, which is evolutionarily conserved and is defined by having a common DNA-binding domain called Forkhead or winged-helix domain [5]. FoxM1 mainly exerts its function in tumorigenesis through transcriptional regulation of its target genes to initiate various cellular responses, including cell growth, proliferation, differentiation, longevity, and transformation [6–8]. Previous studies have shown that FoxM1 is frequently overexpressed in many human cancers, and its expression is associated with poor cancer outcomes [8–15]. Furthermore, it has been reported that FoxM1 could promote tumor

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progression through regulating epithelial–mesenchymal transition (EMT) in a variety of cancers [16–20]. EMT is a process in which epithelial cells acquire a mesenchymal phenotype, and it plays a crucial role in tumor [21]. However, the specific role and molecular basis of FoxM1 in TSCC metastasis are still elusive.

EMT can induced by many growth factors, such as hepatocyte growth factor, which can activate c-Met signaling pathway [22]. The c-Met signaling pathway has been implicated in the development and progression of various cancers including oral cavity cancer, but the mechanisms regulating its expression are not fully understood [23–27]. AKT is regarded as a downstream factor of c-Met pathway [22,28], and increasing evidence indicates AKT and c-Met are activated in cancers [28,29]. The c-Met amplification results in continuous activation of the phosphoinositide 3-kinase (PI3K)/AKT signaling pathway, which is strongly activated and plays a very important role in cancer development [23,30]. Moreover, the function of FoxM1 was reported to be mediated by PI3K/AKT signaling pathway [13,31–33]. These findings suggest that c-Met/AKT/FoxM1 signaling pathway may play an important role in the development of TSCC.

In the present study, we investigate the role of FoxM1 in regulating EMT and metastasis in TSCC cells and define a novel regulatory pathway directly linking the c-Met/AKT signaling axis to FoxM1. We found that FoxM1 is bound directly to the promoter regions of c-Met and regulates expression of c-Met at the transcriptional level. We also found that FoxM1 was regulated by c-Met through PI3K/AKT signaling pathway and that the feedback regulation loop between FoxM1 and c-Met/AKT signaling pathway could play a pivotal role in regulating EMT and metastasis in TSCC cells. Furthermore, immunohistochemical analysis showed that expression levels of FoxM1, c-Met, and pAKT were significantly higher in TSCC tissues than in normal tongue tissues, and these three biomarkers were concomitantly overexpressed in TSCC tissues. The expression of FoxM1, c-Met, and pAKT is associated with tumor stage, tumor size, and lymph node metastasis. Thus, our findings elucidated a novel FoxM1/c-Met/AKT regulatory feedback loop and identified that FoxM1 might be a potential therapeutic target for treatment of patients with TSCC.

Methods

Clinical specimens

This study was approved by the Ethics Committee of First Affiliated Hospital of Fourth Military Medical University. (KY20163010-1). A total of 58 pairs of TSCC specimens and adjacent noncancerous specimens were collected from patients who underwent resection at Stomatological Hospital Affiliated with Fourth Military Medical University, after a written informed consent from the patients. A part of each tissue was immediately snap-frozen in liquid nitrogen, whereas the other part was fixed in formalin for histological examination.

Cell culture and reagents

Human TSCC SCC9 and SCC25 cells were obtained from American Type Culture Collection (Manassas, Virginia, USA). Cells were maintained in a 1 : 1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium (Invitrogen, Carlsbad, California, USA) supplemented with 10% fetal bovine serum (Gibco BRL, Gaithersburg, Maryland, USA), 400 ng/ml hydrocortisone (Sigma-Aldrich, St Louis, Missouri, USA), and penicillin (100 U/ml)/streptomycin (100 μg/ml) (Invitrogen). The cells were maintained at 37°C in 5% CO_2 humidified air. The specific PI3K/AKT inhibitor LY294002 was purchased from Cell Signaling Technology (Beverly, Massachusetts, USA) and was used at a final concentration of 10 μmol/l.

Plasmids, shRNA, and transfection

The pcDNA3.1-FoxM1 (FoxM1) and pcDNA3.1-c-Met (c-Met) plasmids were constructed in our laboratory. The shRNA for FoxM1 (shFoxM1), shRNA for c-Met (shc-Met), and negative control shRNA (shNC) were obtained from Santa Cruz Biotechnology (Dallas, Texas, USA). Transfections were performed with Lipofectamine 2000 reagent (Invitrogen) using 1–2 mg of expression vector/ml serum-free medium as described by the manufacturer.

Quantitative real-time PCR

Total RNA isolation from cell lines and tissues was performed using Trizol (Invitrogen). A reverse transcription reaction was performed using a reverse transcription kit (Applied Biosystems, Foster City, California, USA). Quantitative real-time PCR (qRT-PCR) was performed on an ABI 7500 real-time system (Applied Biosystems) according to the manufacturer's protocol. Data were analyzed according to the comparative C_t method [34]. The β-actin was used as an internal control for each specific gene. Three independent experiments were performed to analyze the relative gene expression. Primer sequences were listed in Supplementary Table S1 (Supplemental digital content 1, *http://links.lww.com/ACD/A243*).

Western blot analysis

Cells were lysed in lysis buffer containing protease inhibitor cocktail. Protein concentration was determined using a BCA protein assay kit (Pierce Biotechnology, Rockford, Illinois, USA). Total protein was separated on 10% SDS-PAGE gels and transferred to PVDF membrane (Millipore, Bedford, Massachusetts, USA). The membrane was blocked with 5% nonfat dry milk in TBS and then probed with the antibody against FoxM1 (Santa Cruz Biotechnology), pAKT (Abcam, Cambridge, Massachusetts, USA), AKT (Abcam), c-Met (Abcam), pc-Met (Abcam), and β-actin (Abcam). After washing, horseradish peroxidase-conjugated anti-rabbit IgG (Santa Cruz Biotechnology) was used as a secondary antibody and

incubated for 1 h at room temperature. The immunoreactive proteins were then detected using the ECL system (Pierce Biotechnology, Illinois, USA). Quantification of band intensity was performed using Image J software (National Institutes of Health, Bethesda, Maryland, USA).

Cell migration and invasion assays

A transwell system that incorporated a polycarbonate filter membrane with a pore size of 8 μm (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA) was used to assess cell migration and invasion. The cells were grown to log phase and treated with 10 μmol/l LY294002 for 12 h. For cell invasion assays, cells were seeded on the upper chamber at a density of 3.0×10^5 cells/well in serum-free medium. Medium containing 10% fetal bovine serum medium was applied to the lower chamber as chemoattractant. After 48 h of incubation at 37°C, noninvasive cells remaining on the upper surface of the membrane were removed by wiping with cotton-tipped swabs. Cells that invaded through the matrix gel and were adherent to the lower surface of the filter were fixed with methanol, stained with 0.5% crystal violet, photographed, and counted. Cell migration assay was performed according to the aforementioned protocol, except that the cells were added into the inserts with 24 h incubation without matrix gel precoated. Each test group was assayed in triplicate.

Chromatin immunoprecipitation assay

Chromatin immunoprecipitation (ChIP) assays were performed in TSCC cells following the protocol provided by the manufacturer (Millipore). In summary, after cross-linking with formaldehyde at 1% final concentration for 10 min at 37°C, the reaction was quenched by addition of glycine to a final concentration of 0.125 M. The cells were lysed in SDS buffer, and the pellet was resuspended in nuclei lysis buffer and sonicated. Immunoprecipitation was carried out with FoxM1 antibody (Santa Cruz Biotechnology). The PCR primer sequences for DNA fragments as parts of the targeted promoters are provided in Supplementary Table S2 (Supplemental digital content 1, *http://links.lww.com/ACD/A243*).

Luciferase reporter assay

The luciferase assays were performed using a luciferase assay kit (Promega, Madison, Wisconsin, USA) according to the manufacturer's protocol. Cells were plated in 24-well plates and transiently transfected with pGL3-c-Met vector and Renillar luciferase reporter with FoxM1 shRNA, pcDNA3.1-FoxM1, or control vector using Lipofectamine 2000 (Invitrogen). Relative firefly luciferase activity was measured using a dual luciferase assay system (Promega) 24 h after transfection.

Immunohistochemistry

The immunostaining technique was conducted as described previously [14]. The intensity of staining was scored as 0 (negative), 1 (weak), 2 (medium), or 3 (strong), whereas the extent of staining was scored as 0 (0% of cells stained), 1 (1–25%), 2 (26–50%), 3 (51–75%), or 4 (76–100%). The scores of each tumor sample were multiplied to give a final score of 0–12.

Statistical analysis

All data were expressed as mean ± SD and then processed using GraphPad Prism, v5.0 software (Graphpad Software Inc., San Diego, California, USA). A Student's *t*-test was performed to compare the differences between treated groups relative to their paired controls. Analyses for an association between immunohistochemical expression and clinicopathologic variables were done by χ^2-test or Fisher's exact test. Pearson correlation coefficient was used to measure the strength of the association between FoxM1, c-Met, and pAKT expression levels. Values of P less than 0.05 were considered significant.

Results

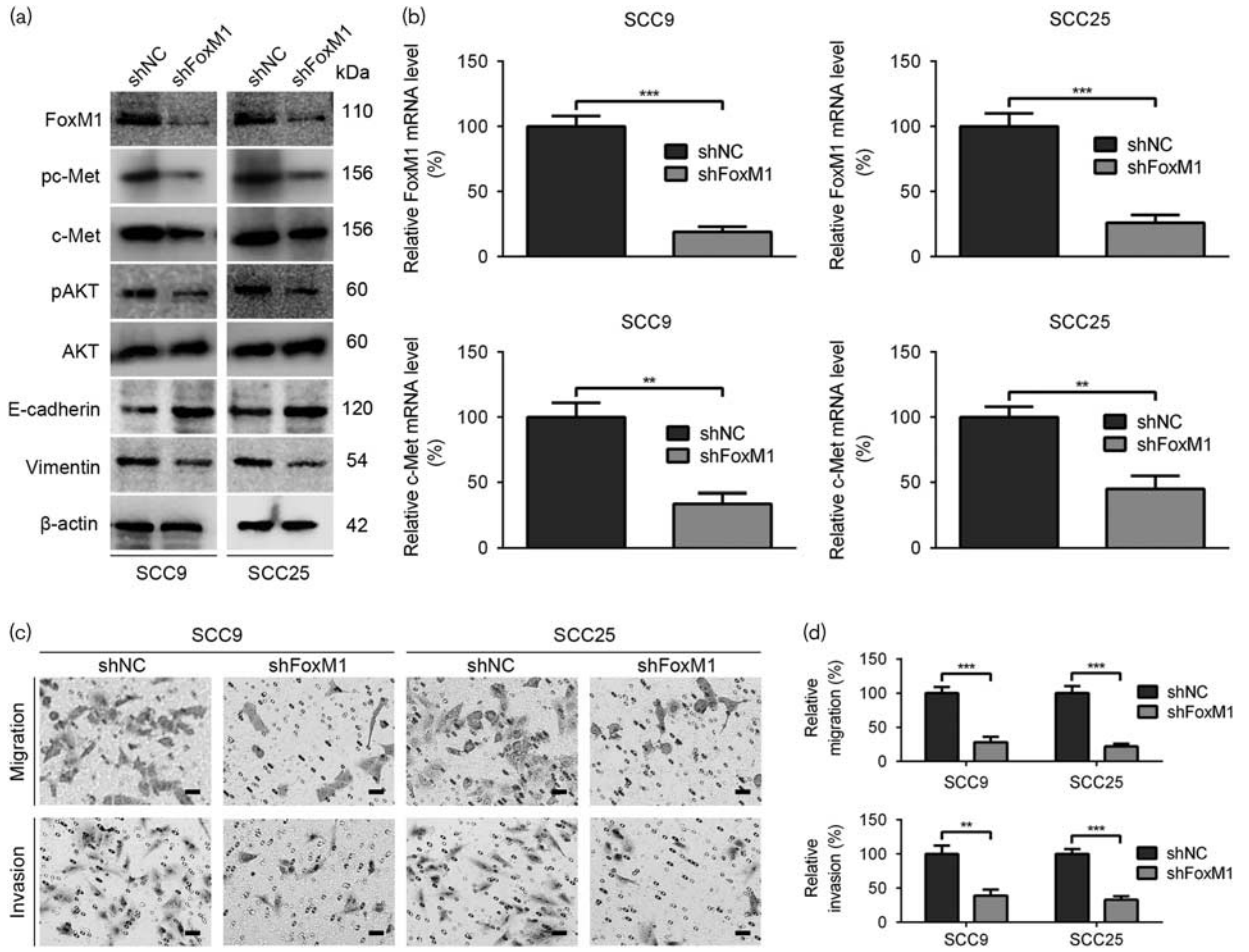
FoxM1 promotes the invasion and migration of tongue squamous cell carcinoma cells through c-Met/AKT signaling

To investigate the functional roles of FoxM1 in an in-vitro setting, SCC9 and SCC25 cells were correspondingly selected to perform in-vitro loss-of-function and gain-of-function experiments. As shown in Fig. 1a, western blot assays showed that the protein levels of pc-Met, c-Met, pAKT, and vimentin were significantly decreased, but E-cadherin expression was increased by FoxM1 knockdown in SCC9 and SCC25 cells. In line with our aforementioned results, qRT-PCR analyses showed that c-Met mRNA levels were significantly decreased by FoxM1 knockdown in SCC9 and SCC25 cells (Fig. 1b). To confirm the effects of FoxM1 silencing on cell invasion and migration, FoxM1 was down-regulated in SCC9 and SCC25 cells using shRNA against FoxM1 transcripts. As shown in Fig. 1c and d, SCC9 and SCC25 cells that were transfected with FoxM1 shRNA exhibited a significant decrease in cellular migration and invasion as compared with control cells. Furthermore, FoxM1 overexpression significantly enhanced the expressions of pc-Met, c-Met, pAKT, and vimentin and inhibited the expressions of E-cadherin in SCC9 and SCC25 cells, but this effect was reversed by LY294002 treatment (Fig. 2a and b). As shown in Fig. 2c and d, SCC9 and SCC25 cells that were transfected with FoxM1-expressing plasmid exhibited a significant increase in cellular migration and invasion as compared with control cells, but this effect was reversed by LY294002 treatment. Collectively, these results indicate that FoxM1 promotes the invasion and migration of TSCC cells through c-Met/AKT signaling.

c-Met promotes the invasion and migration of tongue squamous cell carcinoma cells through AKT/FoxM1 signaling

To investigate the functional roles of c-Met in an in-vitro setting, SCC9 and SCC25 cells were correspondingly

Fig. 1



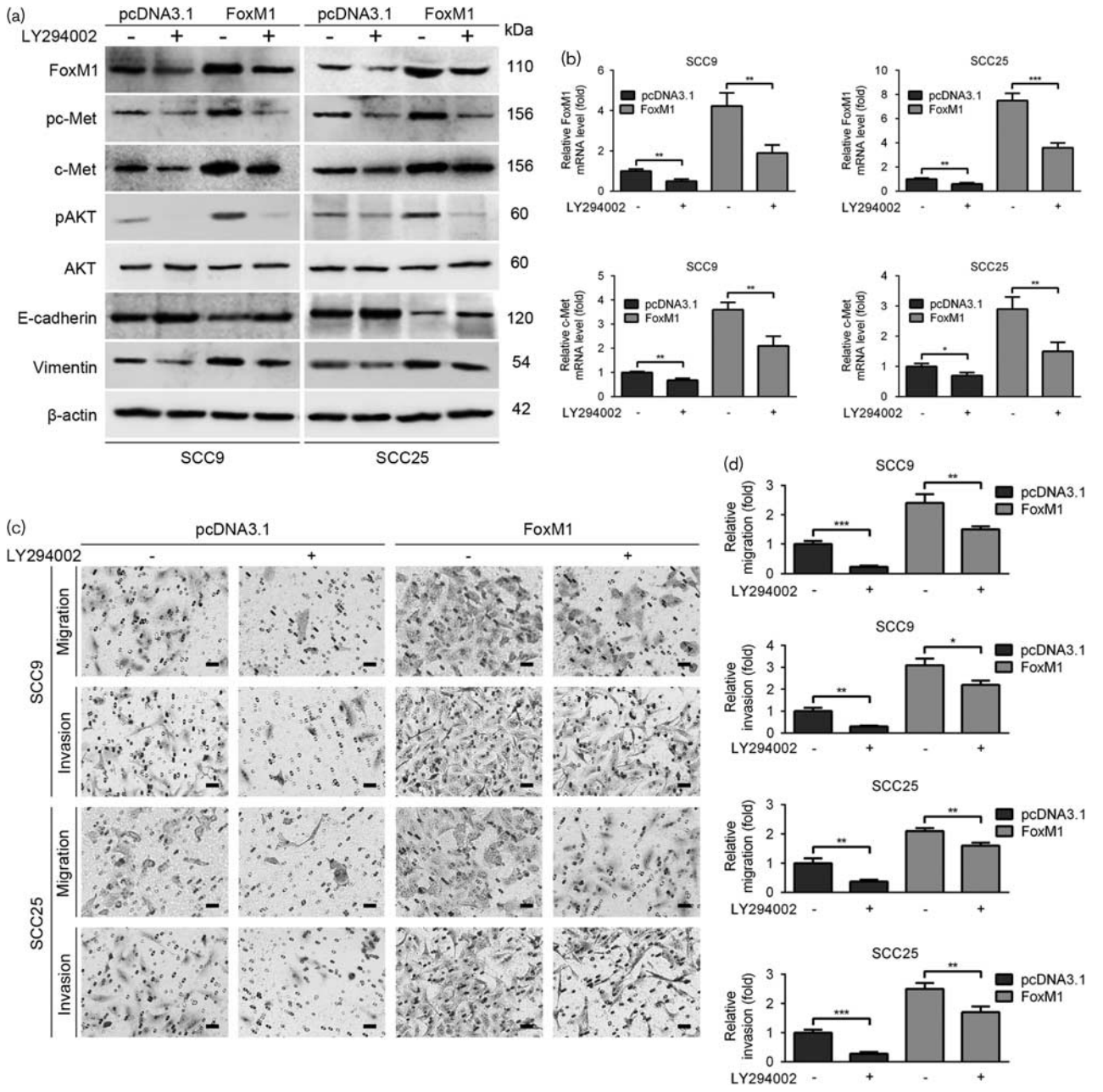
The effects of FoxM1 knockdown on the expression of pc-Met, c-Met, pAKT, AKT, E-cadherin, and vimentin and the abilities of migration and invasion of tongue squamous cell carcinoma cells. (a) SCC9 and SCC25 cells were transfected with FoxM1 shRNA or shNC, and the protein levels of FoxM1, pc-Met, c-Met, pAKT and AKT, E-cadherin, and vimentin were analyzed by western blot analysis. (b) The mRNA levels of FoxM1 and c-Met were analyzed by quantitative real-time PCR analysis. (c, d) The effects of FoxM1 knockdown on the abilities of migration and invasion of SCC9 and SCC25 cells were measured by transwell assay (**$P < 0.01$, ***$P < 0.001$).

selected to perform in-vitro loss-of-function and gain-of-function experiments. As shown in Fig. 3a, western blot assays showed that the protein levels of FoxM1, pc-Met, Pakt, and vimentin were significantly decreased, but E-cadherin expression was increased by c-Met knockdown in SCC9 and SCC25 cells. In line with our aforementioned results, qRT-PCR analyses showed that FoxM1 mRNA levels were significantly decreased by c-Met knockdown in SCC9 and SCC25 cells (Fig. 3b). To confirm the effects of c-Met silencing on cell invasion and migration, c-Met was downregulated in SCC9 and SCC25 cells using shRNA against c-Met transcripts. As shown in Fig. 3c and d, SCC9 and SCC25 cells that were transfected with c-Met shRNA exhibited a significant decrease in cellular migration and invasion as compared with control cells. In contrast, the protein levels of FoxM1, pc-Met, pAKT, and vimentin were significantly increased, but E-cadherin expression was decreased by c-Met overexpression in SCC9 and SCC25 cells. Furthermore, c-Met overexpression significantly enhanced the expressions of FoxM1, pc-Met, pAKT, and vimentin and inhibited the expressions of E-cadherin in SCC9 and SCC25 cells, but this effect was reversed by LY294002 treatment (Fig. 4a and b). As shown in Fig. 4c and d, SCC9 and SCC25 cells that were transfected with c-Met-expressing plasmid exhibited a significant increase in cellular migration and invasion as compared with control cells, but this effect was reversed by LY294002 treatment. These data combined with that FoxM1 promotes the invasion and migration through c-Met/AKT signaling demonstrate that there exists a positive feedback regulation between FoxM1 and the c-Met/AKT signaling pathway in TSCC cells.

FoxM1 is a transcriptional activator of c-Met

To dissect the molecular mechanism of the effects of FoxM1 on c-Met expression, we analyzed the sequences of c-Met

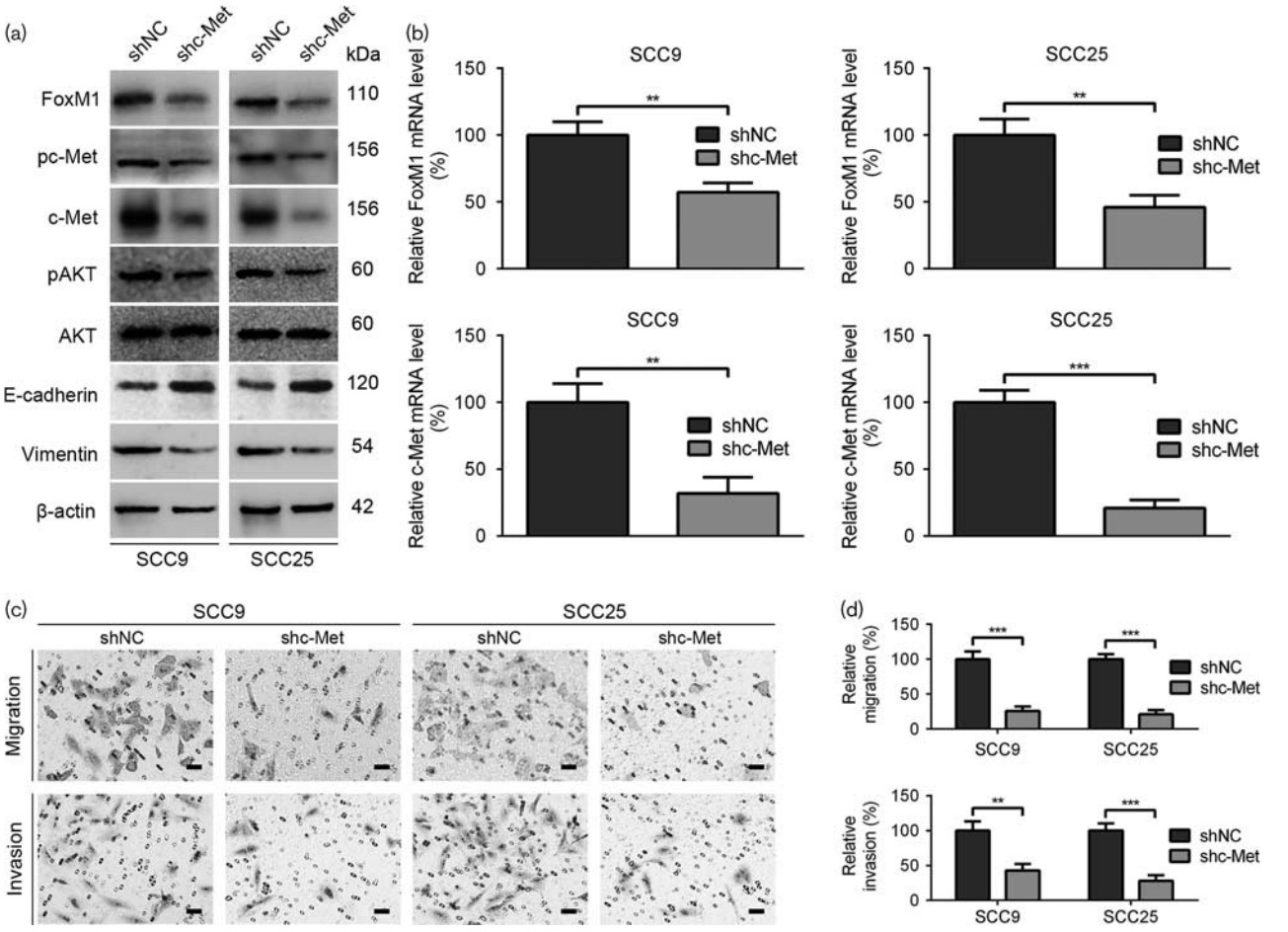
Fig. 2



The effects of FoxM1 overexpression and LY294002 on the expression of pc-Met, c-Met, pAKT, AKT, E-cadherin, and vimentin and the abilities of migration and invasion of tongue squamous cell carcinoma cells. (a) SCC9-FoxM1 and SCC25-FoxM1 cells were treated with LY294002 for 12 h, and the protein levels of FoxM1, pc-Met, c-Met, pAKT and AKT, E-cadherin, and vimentin were analyzed by western blot analysis. (b) The mRNA levels of FoxM1 and c-Met were analyzed by quantitative real-time PCR analysis. (c, d) The effects of FoxM1 overexpression and LY294002 on the abilities of migration and invasion of SCC9 and SCC25 cells were measured by transwell assay (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

promoter for the potential FoxM1-binding elements. Intriguingly, we identified a putative FoxM1-binding element in the c-Met promoter region (Fig. 5a). To explore whether FoxM1 directly regulates c-Met, we first performed ChIP assays in SCC9 and SCC25 cells. The results suggested that c-Met chromatins were specifically immunoprecipitated with antibody against FoxM1, compared with the IgG control (Fig. 5b). Moreover, a series of reporter gene constructs based on the potential binding sites were generated (Fig. 5a). These reporter constructs were cotransfected into SCC9 and SCC25 cells with FoxM1 shRNA, pcDNA3.1-FoxM1, or control vector. As shown in Fig. 5c, knockdown of FoxM1

Fig. 3



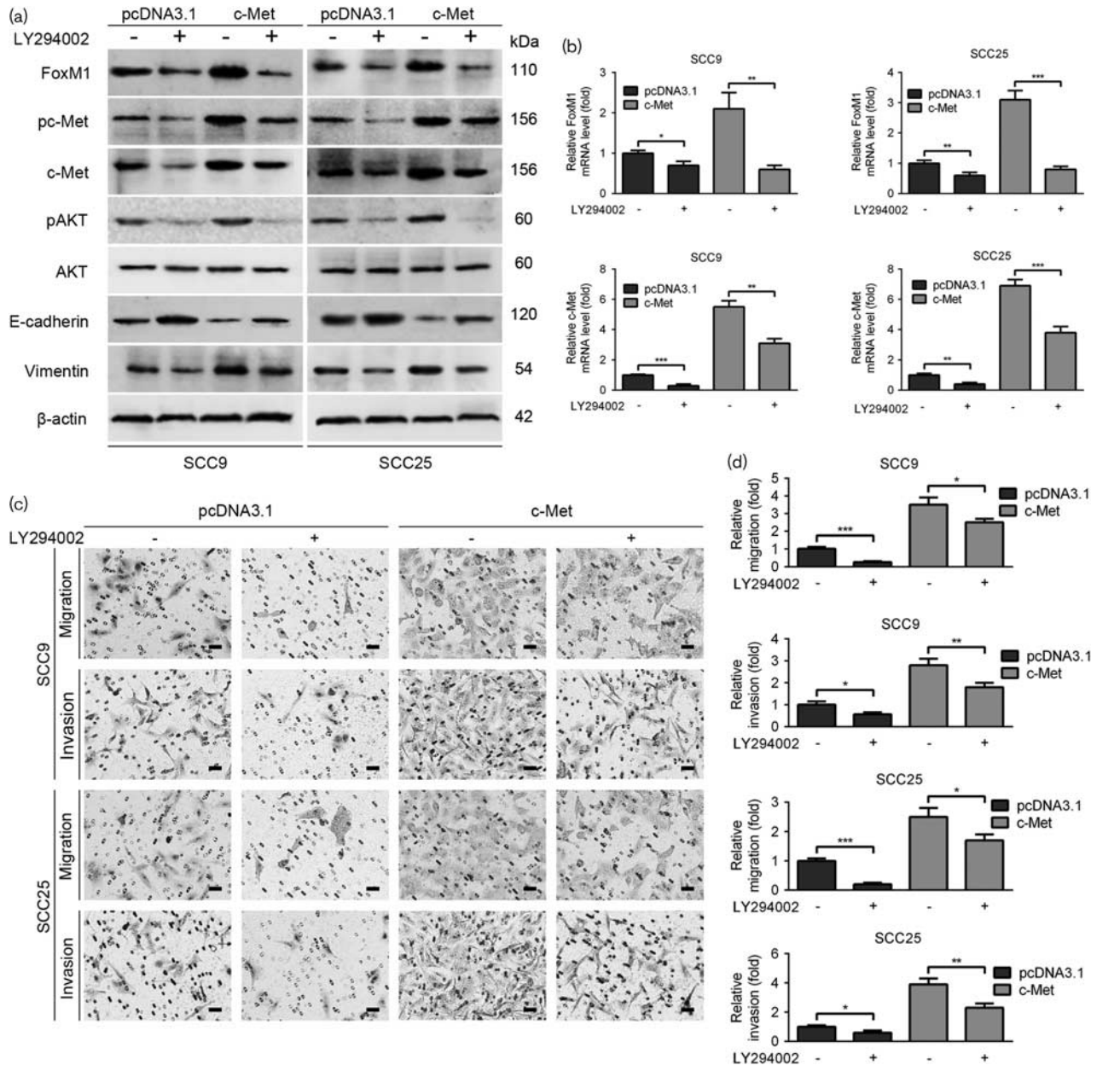
The effects of c-Met knockdown on the expression of FoxM1, pc-Met, pAKT, AKT, E-cadherin, and vimentin and the abilities of migration and invasion of tongue squamous cell carcinoma cells. (a) SCC9 and SCC25 cells were transfected with c-Met shRNA or shNC, and the protein levels of FoxM1, pc-Met, c-Met, pAKT and AKT, E-cadherin, and vimentin were analyzed by western blot analysis. (b) The mRNA levels of FoxM1 and c-Met were analyzed by quantitative real-time PCR analysis. (c, d) The effects of c-Met knockdown on the abilities of migration and invasion of SCC9 and SCC25 cells were measured by transwell assay (**$P < 0.01$, ***$P < 0.001$).

significantly decreased the c-Met promoter activity in the P2605 construct, and altered expression of FoxM1 did not change the promoter activity in the P2118 construct, which did not contain the potential FoxM1-binding site. We mutated the putative binding sites within the luciferase reporter constructs (Fig. 5a). As shown in Fig. 5d, knockdown of FoxM1 significantly reduced the activity of the wild-type pLuc-c-Met construct in SCC9 and SCC25 cells, and altered expression of FoxM1 did not change the activity of the MT (mutant) pLuc-c-Met construct. In addition, FoxM1 overexpression markedly increased the c-Met promoter activity in the P2605 construct, and altered expression of FoxM1 did not change the promoter activity in the P2118 construct (Fig. 5e). Collectively, these results support that FoxM1 is an authentic and direct transcriptional activator for c-Met.

Immunohistochemical detection of the expression of FoxM1, c-Met, and pAKT in tongue squamous cell carcinoma specimens

To explore the role of FoxM1, c-Met, and pAKT for TSCC tumorigenesis, we characterized their expression status by immunohistochemical staining in 58 pairs of human TSCC specimens and adjacent noncancerous specimens. As shown in Fig. 6a, the expression levels of FoxM1, c-Met, and pAKT were confirmed to be higher in human TSCC specimens than in adjacent noncancerous specimens. Moreover, Spearman's rank correlation analysis showed significant positive correlations between FoxM1 and c-Met protein levels, FoxM1 and pAKT protein levels, and c-Met and pAKT protein levels (Fig. 6b). We next sought to determine whether the expression levels of FoxM1, c-Met, and pAKT were associated with the pathological progression of TSCC.

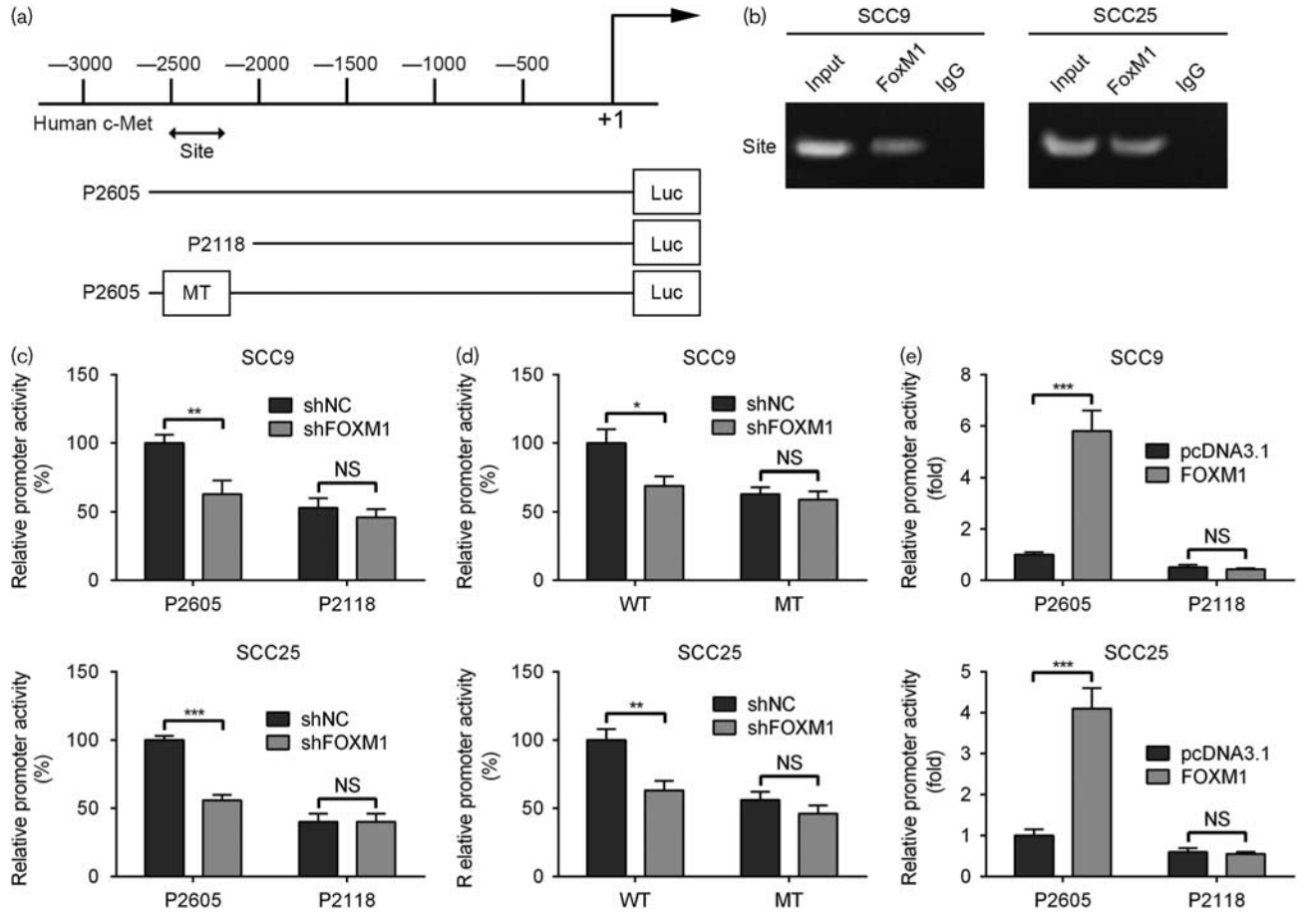
Fig. 4



The effects of c-Met overexpression and LY294002 on the expression of FoxM1, pc-Met, pAKT, AKT, E-cadherin, and vimentin and the abilities of migration and invasion of tongue squamous cell carcinoma cells. (a) SCC9-c-Met and SCC25-c-Met cells were treated with LY294002 for 12 h, and the protein levels of FoxM1, pc-Met, c-Met, pAKT and AKT, E-cadherin, and vimentin were analyzed by western blot analysis. (b) The mRNA levels of FoxM1 and c-Met were analyzed by quantitative real-time PCR analysis. (c, d) The effects of c-Met overexpression and LY294002 on the abilities of migration and invasion of SCC9 and SCC25 cells were measured by transwell assay (*$P < 0.05$,**$P < 0.01$, ***$P < 0.001$).

As shown in Fig. 7, the expression levels of FoxM1, c-Met, and pAKT were significantly increased in TSCC samples from stage III–IV patients, than the levels in TSCC samples from stage I–II patients, respectively. The expression levels of FoxM1, c-Met, and pAKT were significantly increased in TSCC samples from stage T3–T4 patients than the levels in TSCC samples from stage T1–T2 patients (Fig. 7). Furthermore, we observed that the expression levels of FoxM1, c-Met ,and pAKT in TSCC specimens with lymph node metastasis were significantly higher than those in specimens without lymph node metastasis (Fig. 7). Taken together, these results revealed that the expression levels of FoxM1, c-Met, and pAKT were upregulated in TSCC and were correlated with cancer progression and malignancy.

Fig. 5



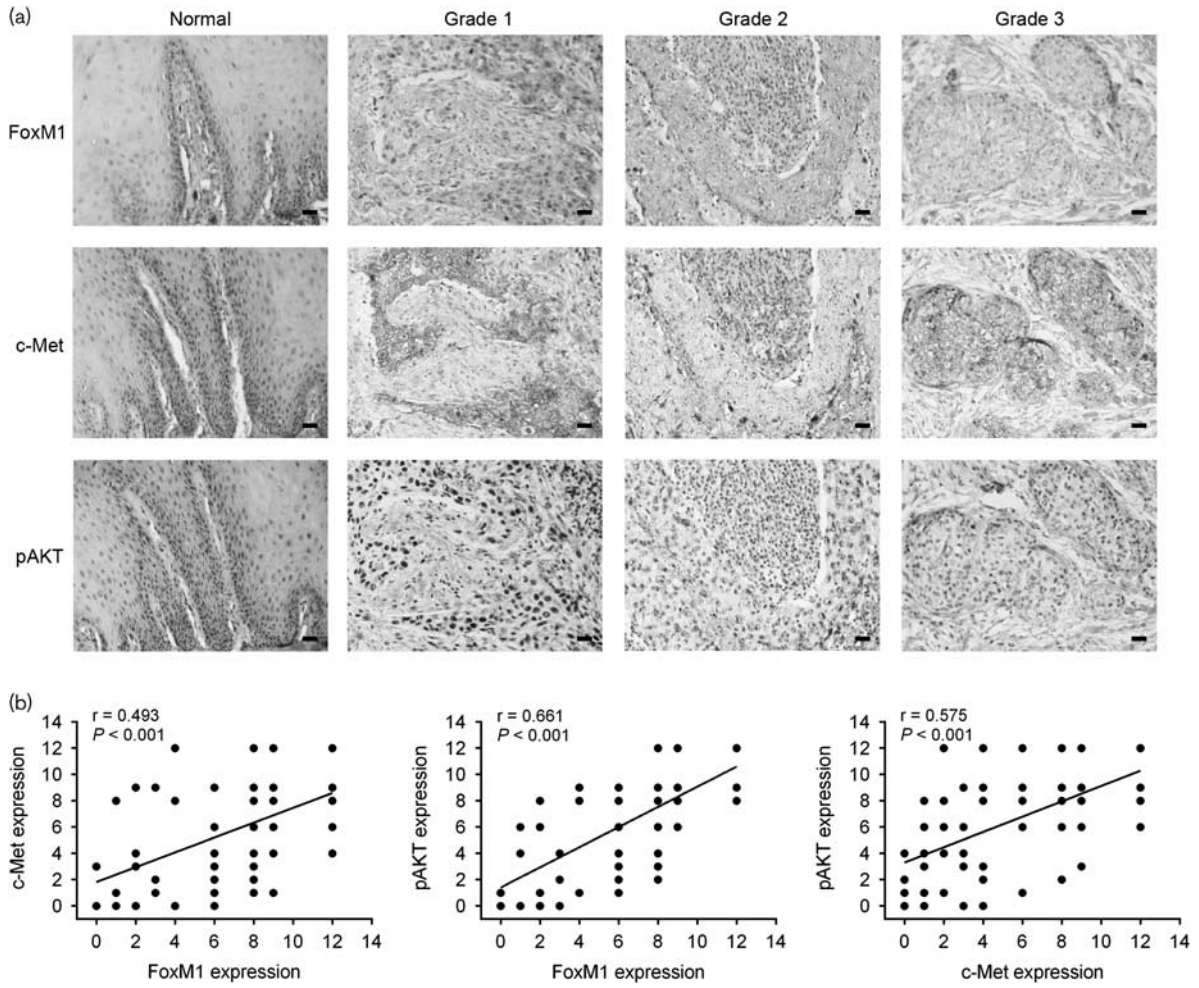
FoxM1 binds to human c-Met promoter and directly enhances its transcription. (a) A putative FoxM1-binding site in the c-Met promoter and construction of reporter plasmids. (b) Chromatin immunoprecipitation analysis of the c-Met promoter using antibodies against FoxM1 in SCC9 and SCC25 cells. (c) The promoter activity of two truncated constructs was measured in SCC9 and SCC25 cells when cotransfected with the control plasmid or FoxM1 shRNA plasmid. (d) The transcriptional activity of FoxM1 on c-Met-luc wild-type (WT) or mutants (MT) was analyzed by luciferase reporter assay in SCC9 and SCC25 cells. (e) The promoter activity of two truncated constructs was measured in SCC9 and SCC25 cells when cotransfected with the control plasmid or pcDNA3.1-FoxM1 plasmid. Promoter activity was examined using a dual luciferase assay kit. The data represent three independent experiments; each bar represents mean ± SD. *P* values were calculated using Student's *t*-test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

Discussion

Diagnosis of TSCC usually occurs when the cancer has already progressed to the advanced stages. Invasion and metastasis are characteristic features and the main factors related to the poor prognosis in patients with TSCC. Therefore, it is important to understand the molecular mechanisms involved in the pathogenesis and progression of metastasis to the development of novel therapies to treat TSCC. In the present study, two TSCC cell lines were studied to systemically address the role of FoxM1 and c-Met in the invasion and migration of TSCC. First, we found that FoxM1 and c-Met promoted the invasion and migration of TSCC cells. Second, we found that FoxM1 enhanced the expression of vimentin, but inhibited the expression of E-cadherin in TSCC cells, thereby promoting EMT in TSCC *in vitro*. Third, FoxM1 binds directly to the promoter regions of c-Met and regulates the expression of c-Met at the transcriptional level. Fourth, FoxM1 was a downstream target of c-Met/AKT signaling, and there was a positive feedback regulation between FoxM1 and the c-Met/AKT signaling pathway in TSCC cells. Finally, we demonstrated that FoxM1, pAKT, and c-Met were concomitantly overexpressed in TSCC tissues, and the expression of these three biomarkers was associated with clinicopathological characteristics of patients with TSCC including tumor stage, tumor size, and lymph node metastasis. These results clearly indicate that the regulatory feedback between FoxM1 and c-Met/AKT pathway may play an important role in the development of TSCC.

FoxM1 is well known for its critical role in cell cycle progression by regulating the transition from G1 to S phase and G2 to M phase progression, as well as to

Fig. 6

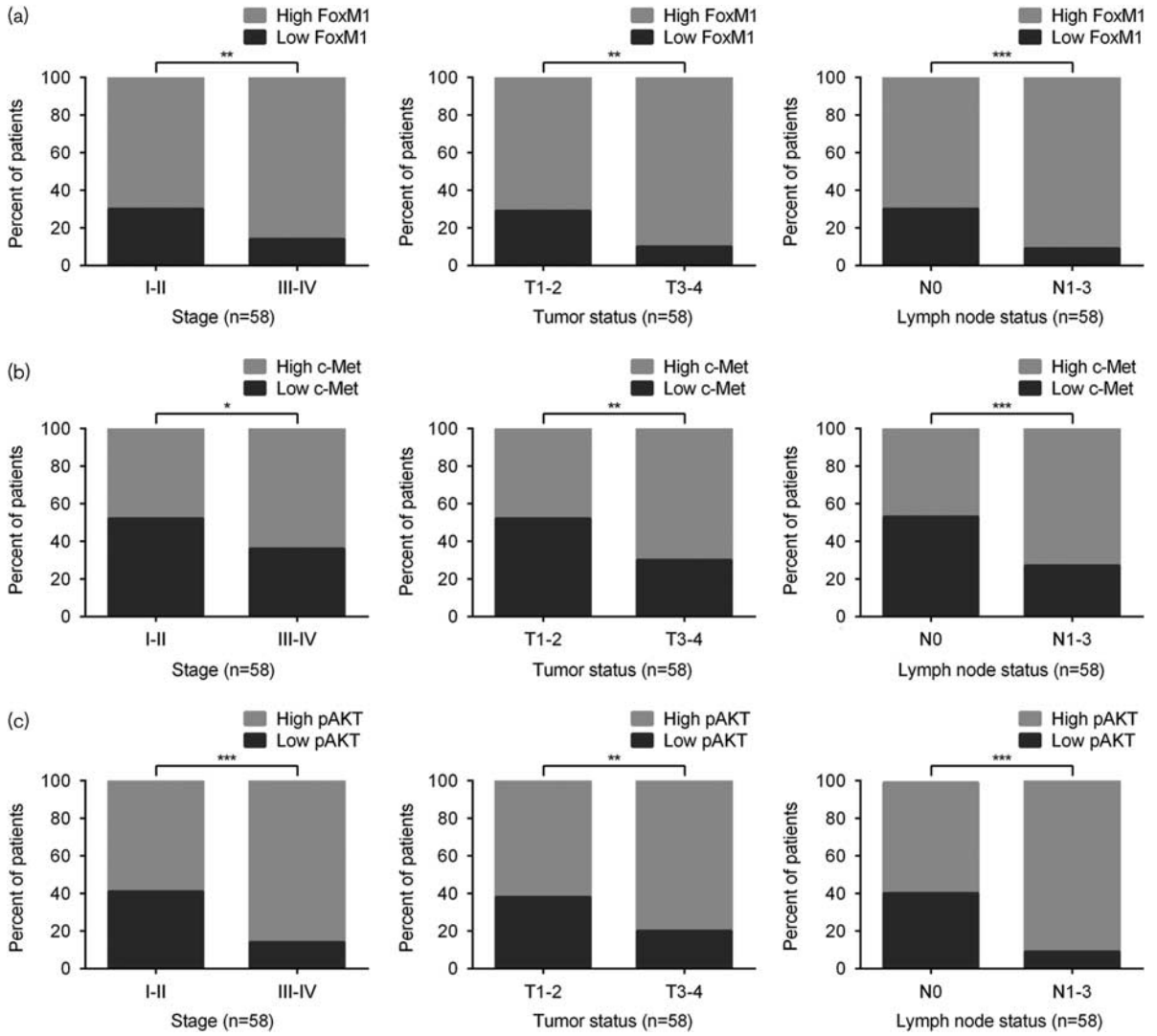


The coordinate expression of FoxM1, c-Met, and pAKT in tongue squamous cell carcinoma tissues. (a) Representative immunohistochemical staining images of FoxM1, c-Met, and pAKT by using consecutive tissue sections from the same patient with tongue squamous cell carcinoma (scale bars, 100 μm). (b) The relationship between the expression of FoxM1, c-Met, and pAKT was analyzed based on immunohistochemical staining. Note that some of the dots on the graphs represent more than one specimen.

mitosis [8]. Besides its essential roles in cell cycle regulation, FoxM1 also emerged as an oncogenic transcription factor with a high expression and functional effect in many types of cancer cells [8–15]. Elevated FoxM1 expression has been shown by a series of recent studies to be associated with aggressive progression and poor outcome in several cancer types [16–20]. Consistent with these studies, the present study confirmed the relationship between FoxM1 expression and the progression of TSCC. To better understand the molecular mechanism by which FoxM1 contributes to the development of TSCC cells, we searched for the potential targets of FoxM1. Signaling through hepatocyte growth factor and its receptor c-Met has a pleiotropic role in cancer development and progression [23–27]. We further investigated whether FoxM1, an oncogenic transcription factor, regulates c-Met expression by transcription in TSCC cells. The results of the present study showed that FoxM1 binds directly to the c-Met promoter regions to promote its transcription.

Like the c-Met signaling pathway, which is also aberrantly activated in TSCC, the AKT pathway is the major downstream signaling routes of c-Met and plays a very important role in TSCC development [23,30]. Furthermore, previous studies have confirmed that FoxM1 was a downstream cellular target of the AKT signaling and had an important role in cancer development [13,31–33]. Therefore, we hypothesized that c-Met might regulate FoxM1 expression by the AKT pathway in TSCC cells. Here we showed that c-Met knockdown inhibited the expression of FoxM1, whereas c-Met overexpression significantly enhanced the expression of FoxM1 in SCC9 and SCC25 cells. However, pretreatment with the PI3K inhibitor LY294002 abolished

Fig. 7



Correlation of the expression of FoxM1, c-Met, and pAKT with clinicopathological characteristics of tongue squamous cell carcinoma. The expression of FoxM1 (a), c-Met (b), and pAKT (c) was positively correlated with disease stage, tumor size, and lymph node metastasis (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). (TNM staging T: size and/or wedge of primary tumor; T1: ≤ 2 cm; T2: > 3 to ≤ 4 cm; T3: > 4 cm; T4: locally invasive tumor, N: regional lymph node; N0: no involvement; N1: a single lateral lymph node metastasis, diameter ≤ 3 cm; N2: lymph node metastasis, diameter ≤ 6 cm; N3: lymph node metastasis, diameter ≥ 6 cm, M: metastasis. I: T1N0M0; II: T2N0M0; III: T3N0M0 or T1/T2/T3N1M0; IV: T4N0/N1M0 or TxN3M0 or TxNxM1).

the effect of c-Met overexpression in the presence of FoxM1, suggesting the involvement of the PI3K/AKT pathway. These data combined with that FoxM1 is a transcriptional activator of c-Met demonstrate that there exists a positive feedback regulation between FoxM1 and the c-Met/AKT signaling pathway in TSCC cells.

Conclusion

Taken together, FoxM1 lies both downstream and upstream of c-Met/AKT signaling pathway and creates a positive feedback loop to promote EMT, invasion, and migration of TSCC cells. Owing to the great importance of the FoxM1/c-Met/AKT axis in TSCC, our results suggest that FoxM1 may serve as a promising therapeutic target for inhibition of TSCC progression.

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W.L., H.W., Y.W., and N.W. are responsible for the study design; H.Y., Y.W., L.W., T.L., and Y.Y. performed the

experiments and drafted the manuscript; M.W., L.Z., and B.W. collected the data; Y.W., M.W., B.W., and W.L. participated in the data analysis and interpretation. All authors read and approved the final manuscript.

Conflicts of interest

There are no conflicts of interest.

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