Receptor for activated C kinase 1 (RACK1) promotes the progression of OSCC via the AKT/mTOR pathway

XUEFENG ZHANG[1*], NA LIU[2*], DANHUA MA[1], LING LIU[1], LU JIANG[1],
YU ZHOU[1], XIN ZENG[1], JING LI[1] and QIANMING CHEN[1]

[1]State Key Laboratory of Oral Diseases, West China Hospital of Stomatology, Sichuan University, Chengdu, Sichuan;
[2]Department of Oral Diseases, First Affiliated Hospital of Anhui Medical University, Hefei, Anhui, P.R. China

Abstract. Our previous study suggested that receptor for activated C kinase 1 (RACK1) contribute to the progression of oral squamous cell carcinoma (OSCC). The aim of this study is to elucidate the mechanism by which RACK1 regulates cell growth in OSCC using *in vitro* and *in vivo* models. The effects of RACK1 knockdown with lentivirus based shRNA in stable cell lines were evaluated by Q-PCR and western blot analysis. RACK1 silencing effects on the cell cycle in OSCC cells were detected by flow cytometry and western blot analysis. The effect of RACK1 silencing on inhibiting the progression of OSCC was illustrated using a xenografted mouse model. RACK1 and relevant signaling pathways were investigated in tissues and cells using immunohistochemistry and/or western blot analysis. Stable silencing of the RACK1 gene resulted in a distinct G1 and G2 phase arrest by downregulating Cyclin B1 and Cyclin D1. Depleted RACK1 led to markedly decreased tumor volume and the expression of Ki67, CD34, and VEGF *in vivo*. The expression of RACK1 and p-AKT has a parallel pattern in different stages of oral carcinogenesis tissues. In addition, the protein level of RACK1 was positively correlated with p-AKT in OSCC tissue samples and cell lines. We found specific transient knockdown of RACK1 could downregulate the protein levels of p-AKT, p-mTOR, and p-S6 in a dose-dependent manner. This study demonstrates that RACK1-dependent OSCC growth and survival may be related to the increased activation of the AKT/mTOR/S6 pathway.

Introduction

Oral squamous cell carcinoma (OSCC) is a highly lethal disease, owing to lack of effective diagnostic biomarkers and

Correspondence to: Dr Jing Li or Professor Qianming Chen, State Key Laboratory of Oral Diseases, West China College of Stomatology, Sichuan University, Chengdu, Sichuan, P.R. China
E-mail: lijing1984@scu.edu.cn
E-mail: qmchen@scu.edu.cn

*Contributed equally

therapeutic targets (1,2). Despite recent advances in diagnosis and treatment, the 5-year survival rate of patients with OSCC is ≤50% (3,4). Over the past two decades, numerous prognostic and predictive markers for the clinical outcomes of OSCC have been proposed (5), however, few have been applied in clinical practice owing to the lack of understanding of the mechanism and the non-reproducibility of the initial findings (2,6).

RACK1, characterized by highly conserved internal WD-40 repeats (Trp-Asp) (7) was originally identified as an anchoring protein for the conventional protein kinase C (PKC) (8). In recent years, the understanding of RACK1 function has increased remarkably. First identified as an anchoring protein for activated PKC, it is now known to serve as an anchoring or adaptor protein in various crosstalk signal pathways. RACK1 has been broadly approved as a multifaceted scaffolding protein involved in multiple biological events via interaction with different partners (9,10), including cell migration (11), and angiogenesis (12). RACK1 also modulates kinases, phosphodiesterases, and phosphatases by regulating their activities, subcellular distributions, and association with substrates, contributing significantly to the regulation of the signal transduction network (13).

RACK1 exerts dual functions in cell growth. For example, RACK1 could promote cell growth via PKCβII/eIF4E (14), MKK7/JNK (15), SHH signaling (16), PI3K and GSK3β (17), but inhibits cell growth by promoting β-catenin (18) and ΔNp63 (19) degradation and inhibition of Src activity (20,21). RACK1 also has opposing roles in apoptosis. It could promote degradation of pro-apoptotic molecules, such as Fem1b and BimEL (22-24) or E1A to reduce apoptosis levels (25). It could increase apoptosis by dissociation of the Bax/Bcl-XL complex (22), advance Bax oligomerization, and inactive AKT and enhance the expression of pro-apoptotic Bim (26). Thus, RACK1 was reported to be a cancer inhibitor with low expression in gastric cancer (18), but a cancer promoter with high expression in multiple kinds of cancer, including non-small-cell lung cancer (NSCLC) (16), pulmonary adenocarcinoma (27,28), hepatocellular carcinoma (HCC) (15), glioma, esophageal squamous cell carcinoma (ESCC) (29), and OSCC (30).

In our previous studies, RACK1 was found abnormally overexpressed in OSCC by comparative proteomics and it is an excellent predictor for poor clinical outcome (30,31). In addition, knockdown of the RACK1 gene could inhibit

the proliferation, and motility of OSCC cells and induced by decreased protein levels of pEGFR, HER2, and MMP2/9 (11). However, to our knowledge, there has been no prior study on the mechanism of RACK1 regulation of cell growth and the effects of RACK1 on the behavior of OSCC cells *in vivo*.

The aim of this study was to elucidate the mechanism by which RACK1 regulates cell growth in OSCC using *in vitro* and *in vivo* models. We selected OSCC cells stably infected with lentivirus based RACK1-sh, to investigate the antitumor efficiency of RACK1 depletion, and employed a xenograft mouse model and clinical cohort to uncover the potential mechanism. We provide the basis for the potential use of RACK1 as a novel therapeutic target for OSCC in the future.

Materials and methods

Clinical tissue samples. The Institutional Review Boards of the West China Hospital of Stomatology, Sichuan University approved this study. The study was approved by the ethics committee both of the West China Hospital of Stomatology and the Guangdong Provincial Stomatological Hospital and was conducted in agreement with the Helsinki Declaration.

Four normal tissues from plastic surgery, 8 oral leukoplakia tissues and 15 excised primary OSCC specimens were included in this study. The only selection criterion was epithelial continuity for normal and premalignant tissue (Table I).

Cell lines. The cell lines 293T and Cal-27 were purchased from American Type Culture Collection (Manassas, VA, USA). HSC-3 and HSC-4 cells were purchased from the Cell Bank of Japanese Collection of Research Bioresource (JCRB, Shinjuku, Japan). UM1 and UM2 were provided by Dr Xiaofeng Zhou (Center for Molecular Biology of Oral Diseases, College of Dentistry, University of Illinois at Chicago). The human immortalized oral keratinocyte cell line HOK16E6E7 was provided by Dr Xuan Liu (Charles R. Drew University of Medicine and Science). Five oral squamous cell carcinoma cell lines (HSC-3, CAL-27, UM1, UM2 and HSC-4) and 293T cells were maintained in Dulbecco's modified Eagle's medium (Invitrogen) containing 10% fetal calf serum (Invitrogen), 100 U/l penicillin, and 10 mg/l streptomycin. HOK16E6E7 cells were cultured in keratinocyte growth medium containing 0.15 mM calcium and supplemented with epidermal growth factor (Invitrogen).

shRNA plasmids and transfection. Three lentiviral-based shRNA plasmids were used: RACK1-specific shRNAs pLV-sh-RACK (RACK1-sh1/2 target the sequence TCGAGATAA GACCATCATCAT and CAAGCTGAAGACCAACCACAT of RACK1, respectively), and negative control pLV-sh-NC (NS-sh) were purchased from Chengdu Bomei Biotechnology Inc.(Chengdu,China).Transfection regent Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA), was used according to the manufacturer's instructions. Plasmid transfection was performed as previously described (32).

Stable cell line generation. Lentivirus delivering RACK1-sh or NS-sh was prepared used 293T cells. HSC-3 cells were infected with either RACK1-sh or NS-sh. Selective culture medium containing puromycin was used to select cells with stable expression of sh-RACK1. The expression of RACK1 was detected by Q-PCR and western blot analysis.

Q-PCR. The nucleotide sequences of the sense and antisense primers used for RACK1 amplification were 5'-GCTCTG CCATAAACTTCTAGCGTGTGC-3' and 5'-CTGTGCTTCT GGAGGCAAGGATGGCCA-3', respectively. The primers for GAPDH amplification were purchased from Invitrogen. Q-PCR was performed as previously described (30,31).

Western blot analysis. The protein expression levels of RACK1, AKT, p-AKT, mTOR, p-mTOR, S6, and p-S6 in the OSCC cells were examined by western blot analysis. RACK1 antibody was purchased from Santa Cruz (Santa Cruz, CA, USA) and AKT, p-AKT (Ser-473), mTOR, p-mTOR (Ser-2448), S6, and p-S6 (Ser235/236) antibodies were purchased from Cell Signaling (Danvers, MA, USA), western blot analysis was done as previously described (30,31).

Cell cycle analysis. Cell cycle was assessed as previously described (33). Briefly, $2x10^6$ HSC-3 cells of each group were harvested, washed twice, and fixed with cold 70% ethanol at 4˚C overnight. The cells were then washed and digested with RNase, then stained with 800 μl propidium iodide (50 μg/ml) at room temperature for 30 min. Cell cycle analysis was done by using FACS Aria flow cytometry system (BD Biosciences). Data were analyzed using BD FACS Diva software.

In vivo tumor-formation assay. Animal studies were approved by the Animal Care and Use Committee, State Key Laboratory of Oral Diseases, in compliance with the Guide for the U.S. Public Health Service's policy on humane care and use of laboratory animals. The *in vivo* tumor-formation was done as previously described (34). Briefly, female BALB/c nude mice 6 weeks of age weighing 20-22 g were used in the study of tumor formation. The animals were monitored every 3 days for tumor development. All the mice were sacrificed after the last measurement. Tumor tissues were cut into 4-mm sections, deparaffinized, rehydrated, and treated with a peroxidase block, before processing for H&E and immunohistochemistry. Results of animal experiments were expressed as mean ± SD of five tumors analyzed.

Immunohistochemistry assay. Tumor tissues were cut into 4-mm sections, deparaffinized, rehydrated, and treated with a peroxidase block. After heat-based antigen retrieval, sections were incubated with 5% normal goat or horse serum (Zymed, San Francisco, CA, USA) to block non-specific sites before incubation with anti-RACK1 immunoserum diluted 1:100 or monoclonal antibody Ki67 diluted 1:200 (from Dako and Abcam, respectively), or mouse CD34 monoclonal antibody diluted 1:200 (BD Biosciences, San Jose, CA, USA), or anti-VEGF antibody (R&D Systems, Abingdon, UK), or anti-RhoA antibody (Cell Signaling Technology, Beverly, MA, USA), anti-mLYVE-1 antibody (R&D Systems), or anti-E-cadherin antibody (Cell Signaling Technology) was incubated at 4˚C overnight. Sections incubated without primary antibodies were used as negative controls.

Table 1. Clinicopathological factors in 27 patients with normal oral mucosa, oral leukoplakia, and OSCC.

Characteristic	Normal oral mucosa (N=4) No.	Oral leukoplakia with mild to moderate epithelial hyperplasia (N=4) No.	Oral leukoplakia with severe epithelial hyperplasia (N=4) No.	OSCC (N=15) No.
Age				
<60 years	2	2	3	6
≥60 years	2	2	1	9
Gender				
Male	1	2	4	10
Female	3	2	0	5
Smoking history				
Never	3	2	1	7
Ever	1	2	3	8
Drinking history				
Never	3	2	0	5
Ever	1	2	4	10
Cell differentiation				
High	NA	NA	NA	6
Moderate or low	NA	NA	NA	9
Primary site				
Ventral tongue/floor of mouth	2	1	3	4
Buccal mucosa	1	3	0	6
Gingiva	1	0	1	3
Others[a]	0	0	0	2
Tumor stage				
T1 or T2	NA	NA	NA	7
T3 or T4	NA	NA	NA	8
Nodal stage				
N0	NA	NA	NA	11
N1-3	NA	NA	NA	8
Clinical TMN stage				
I or II	NA	NA	NA	9
III or IV	NA	NA	NA	6
Surgical method				
Local	4	3	3	3
Unilateral or bilateral or other	0	1	1	12

[a]Others included hard palate, mandibular and lip mucosa. NA, not applicable.

For clinical tumor tissues, the RACK1 and pAKT staining were determined based on the staining intensity (scale, 1-3) and percentage of tumor staining (scale, 1-3) as previously described (34). The total staining was expressed as a product of the two numbers (resulting in a staining scale of 1-9). The evaluation was performed by two independent investigators.

Statistical analysis. The values given are mean ± SEM. The significance of difference between the experimental groups and controls was assessed by Student's t-test. The difference was considered significant at the P-value of <0.05.

Results

RACK1 silencing induces G1 and G2 phase cell cycle arrest in OSCC cells. Two shRNAs (RACK1-sh1 and RACK1-sh2) designed against different regions of RACK1 were transfected into 293T. RACK1-sh2 had proven effective in the inhibition of RACK1 expression, as confirmed by both RT-PCR and western blotting. Thus, RACK1-sh2 (RACK1-sh) plasmid and stably transfected RACK1-sh cells were utilized for the following experiments (Fig. 1A). Then we successfully screened for stable RACK1 low expression HSC-3 and HSC-4
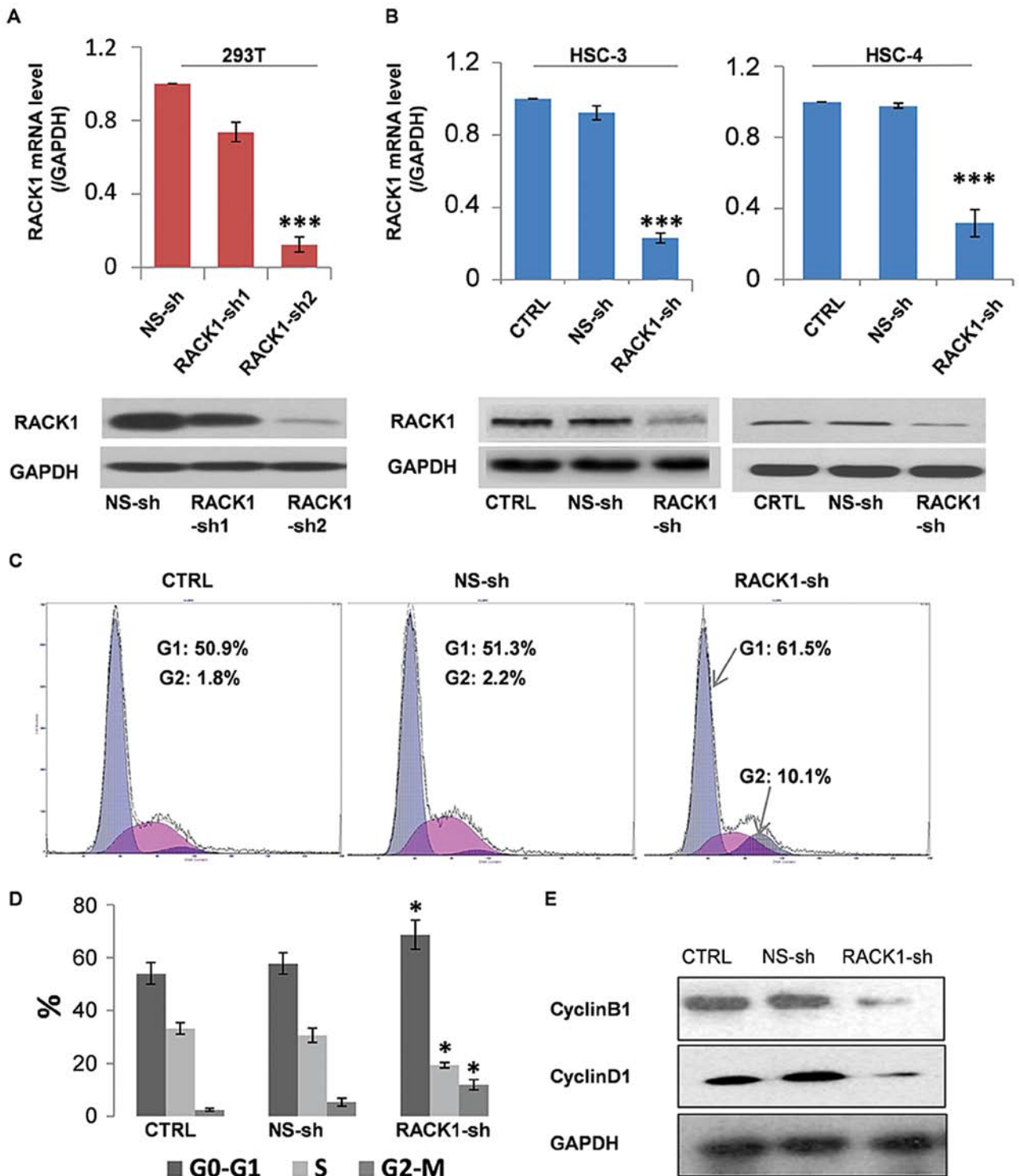
Figure 1. RACK1 silencing induces G1 and G2 phase arrest though downregulating Cyclin B1 and Cyclin D1 in OSCC cells. (A) The expression level of RACK1 was markedly decreased in RACK1-sh2 group. Q-PCR and western blot analysis detected RACK1 expression in 293T cells (***P<0.001). Data are representative blots of three independent experiments. (B) Q-PCR and western blot analysis detected RACK1 expression in RACK1 stably silencing OSCC cells (***P<0.001). Data are representative blots of three independent experiments. (C) Cell cycle in HSC-3 cells was detected by flow cytometry. (D) Quantitative analysis of cell cycle. All data are represented as the mean ± SD from three independent experiments (*P<0.05). (E) Cell cycle regulators were detected by western blot analysis. RACK1-sh group has a lower protein level of Cyclin B1 and Cyclin D1 than two control groups. Data are representative blots of three independent experiments.

cell lines with RACK1-sh (Fig. 1B). RACK1 expression in HSC-4 cells is lower than HSC-3 cells, and HSC-4 cells were hard to conform to a mouse model in our preliminary experiments (date not shown). Therefore, we selected HSC-3 cells for the next experiments.

To study the potential mechanisms by which RACK1 silencing inhibits HSC-3 cell growth, the effect of RACK1 shRNA on cell cycle was evaluated using flow cytometry. As shown in Fig. 1C, the percentages of G0-G1 phase cells in two control groups were 50-55%, whereas that in the RACK1
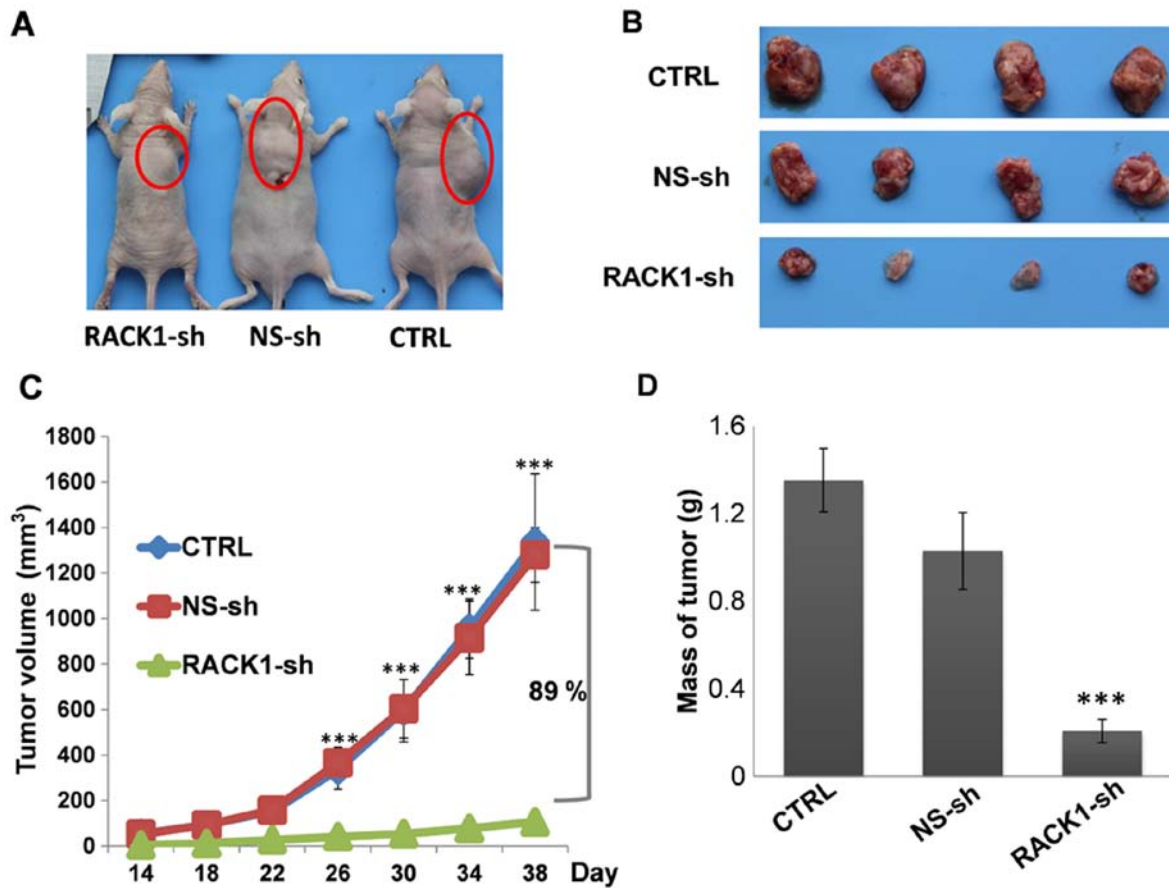
Figure 2. Loss of RACK1 inhibits the tumor progression of OSCC *in vivo*. (A) The final tumor image of HSC-3 cell xenografts *in vivo*. (B) Tumor growth curves. (C) The tumor volumes (mean ± SD) of RACK1-sh group were significantly smaller compared to the other two groups, 38 days after cell injection (n=6, [***]P<0.001). (D) Average tumor weight (mean ± SD, n=6, [***]P<0.001).

shRNA transfected group was 65%. Approximately 20% of the cells in the RACK1 shRNA-transfected group were in S-phase, compared with 30-35% of cells in the two control groups. Approximately 15% of cells in the RACK1 shRNA-transfected group were in G2-M phase, compared with 1-2% of cells in the two control groups (Fig. 1D). Therefore, RACK1 silencing may arrest the cell cycle at the G1 and G2 phase by inhibiting the G1→S and G2→M transition in HSC-3 cells. Since cell proliferation is normally controlled by cell cycle regulatory proteins, we performed western blotting to investigate the effect of RACK1 silencing on cell cycle regulators. The expression levels of Cyclin B1 and Cyclin D1 were downregulated (Fig. 1E). These results indicate that RACK1 silencing might regulate the cell cycle in HSC-3 cells by modulating the expression of cell cycle regulators, thus inhibiting the processes of oral carcinogenesis.

RACK1 contributes to the progression of OSCC in vivo. Next, we investigated the effect of RACK1 on the tumorigenicity of OSCC cells using an *in vivo* xenograft model. As shown in Fig. 2, tumors formed in the mice transplanted with RACK1-sh silenced HSC-3 cells were smaller in both size and weight than the tumors in mice transplanted with control cells. The average tumor volume in the RACK1-sh group was reduced by 89.1%. To investigate the potential mechanisms underlying the effects of RACK1 silencing *in vivo*, we examined tumor cell prolif-

eration. Marked reduction in the proliferation marker Ki-67 expression was found in the RACK1 silenced group (Fig. 3A). Angiogenesis in tumors was detected by microvessel density (MVD) CD34 staining, VEGF staining was also used as a marker for angiogenesis in tumors. As shown in Fig. 3A, these analyses showed significant decrease in the average number of microvessels per vascular hot spot and, VEGF expression in the RACK1-sh group compared with those in the two control groups. Additionally, the expression of RhoA decreased, and E-cadherin increased in the RACK1 silenced group (Fig. 3B). Both proteins are important for tumor metastasis, this is basically consistent with our above results. Taken together, these results suggest that RACK1 contributes to the progression of OSCC *in vivo*.

Cell line and clinical relevance of RACK1-induced AKT activation in human OSCC. Our previous study showed that activated AKT correlates with poor clinical outcomes for OSCC (35), and single-nucleotide polymorphisms (SNPs) in AKT1 (rs3803300) are associated with progression-free survival time of OSCC patients (36). AKT is considered as an important key protein regulating tumor progression in various types of human cancer. Peng *et al* showed knocking down RACK1 *in vitro* decreased AKT activity in glioma (37). We further investigated whether RACK1 expression and AKT activation are relevant in human OSCC cell lines and clinical samples. As shown in Fig. 4A,
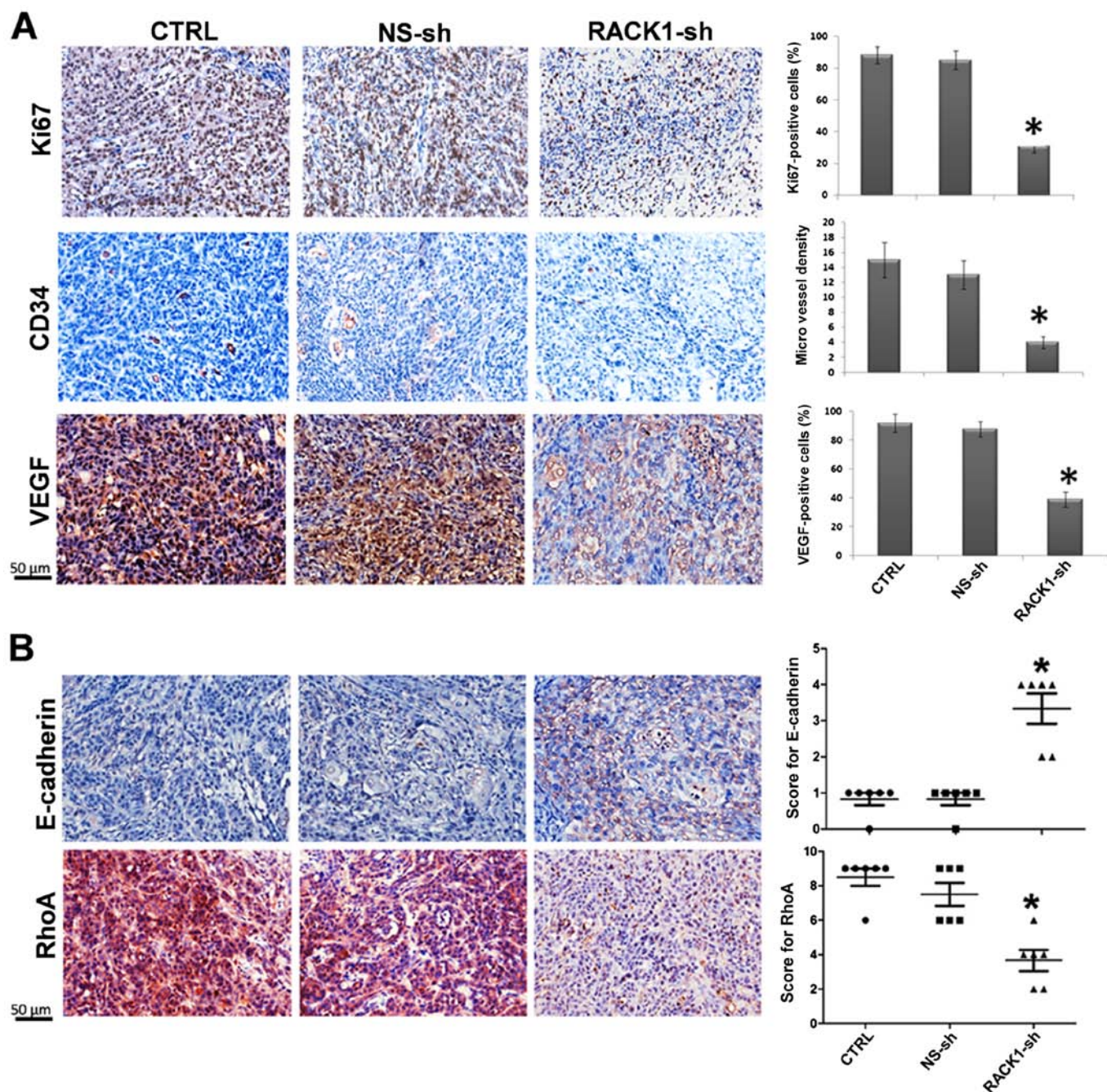
Figure 3. RACK1 silencing changes proteins expression levels of tumor malignant progress makers in OSCC *in vivo*. (A) Percentages of Ki67-positive nuclei in RACK1-sh group were significantly lower than those in the two control groups (mean ± SD, *P<0.05). Angiogenesis in tumors was detected by CD34 and induced VEGF. The average number of microvessels and VEGF expression were significantly lower in RACK1-sh group tissues compared with those in the two control groups (mean ± SD, *P<0.05). (B) RACK1 silence could decrease the expression of RhoA and increase the expression of E-cadherin of OSCC *in vivo* (mean ± SD, *P<0.05).

RACK1 and p-AKT showed similar expression in different stages of oral carcinogenic tissues. Furthermore, the level of RACK1 expression in 15 collected clinical OSCC samples correlated positively with p-AKT expression (Fig. 4B, R = 0.6748, P<0.05). To further confirm the result, OSCC cells were harvested from 6 OSCC cell lines (HSC-3, HSC-4, SCC-5, CAL-27, SCC-25, and SCC-9), together with HOK, an immortalized oral keratinocyte cell line, and then equal amounts of cell lysates were examined using western blot analysis. Consistently, expression of p-AKT had a positive correlation with the level of RACK1 in OSCC cell lines (Fig. 4C).

RACK1 contributes to activation of AKT/mTOR in OSCC. A growing body of evidence demonstrates that the AKT/mTOR signaling pathway plays a central role in both cell cycle and angiogenesis in various types of human cancer, including OSCC (38). According to published data, the mTOR pathway is aberrantly activated in most OSCC tumors (69.5%), and in turn phosphorylates and activates proteins causing aberrant signals, leading to the translation of proteins required for tumor progression. Therefore, we investigated whether RACK1 is involved in regulation of the AKT/mTOR signaling pathway in OSCC. As shown in Fig. 5A, silencing of RACK1 reduced the
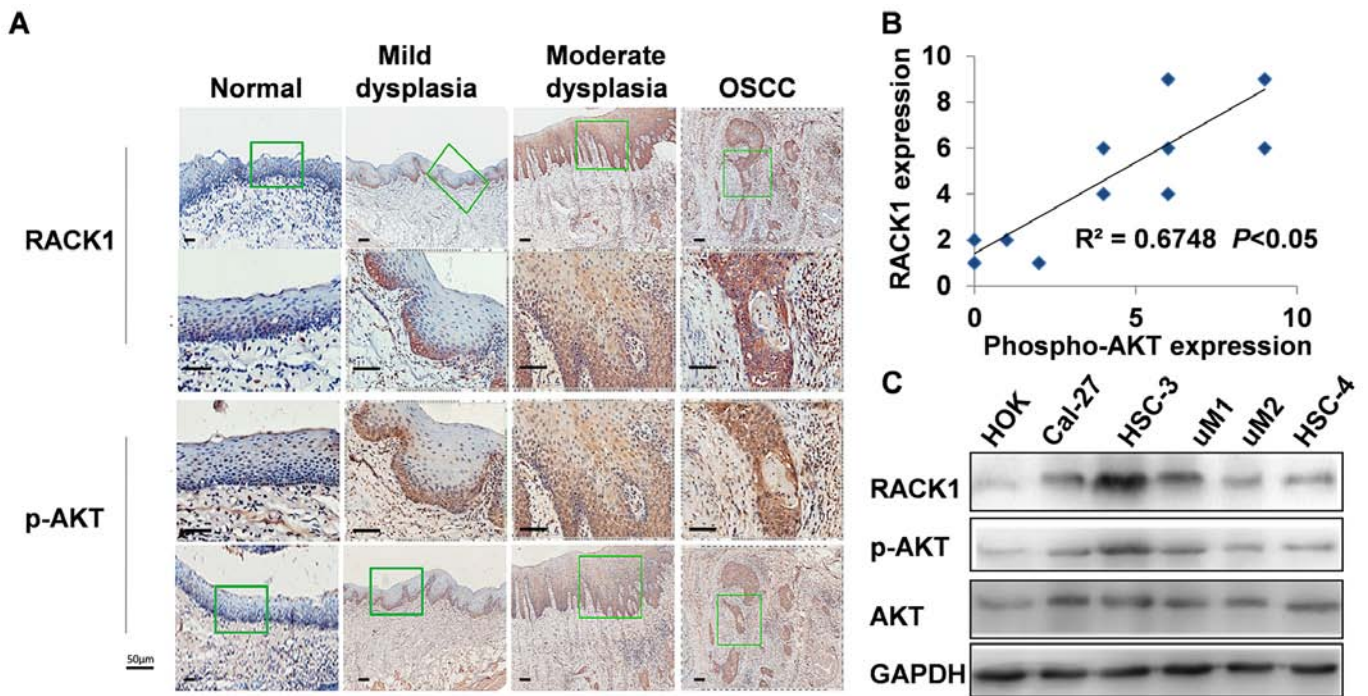
Figure 4. RACK1 expression was positively correlated with p-AKT in OSCC tissues and cells. (A) Protein level of RACK1 and p-AKT have a similar tendency in different stages of oral carcinogenesis tissue. (B) RACK1 expression was positively correlated with p-AKT in OSCC tissues. (C) RACK1 expression was positively correlated with p-AKT in OSCC cells.
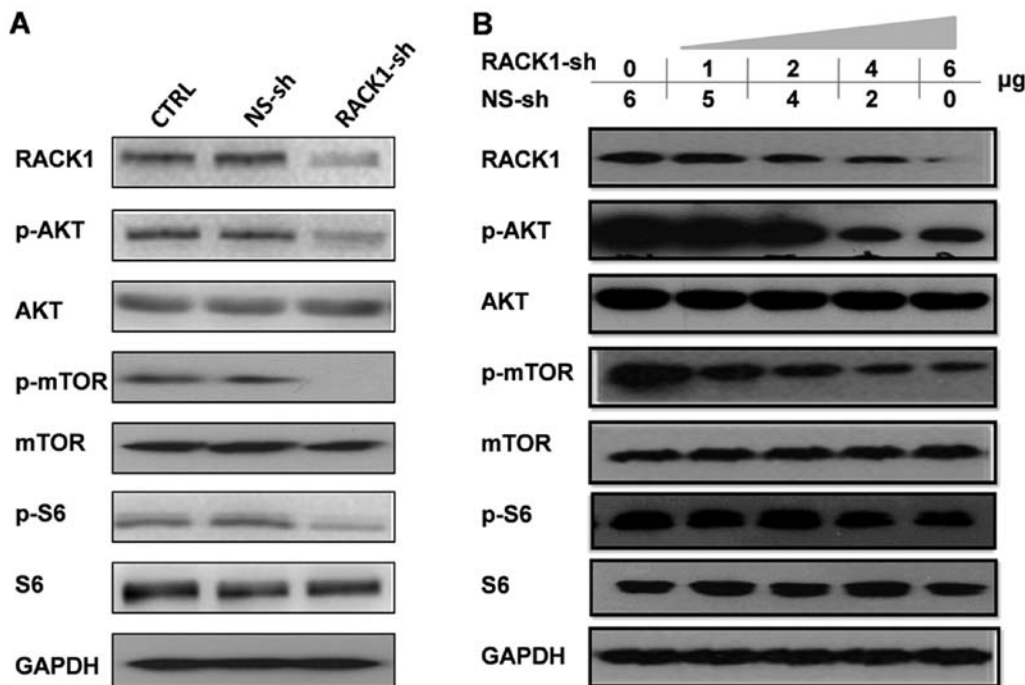

Figure 5. RACK1 silencing downregulates the phosphorylation of AKT/mTOR/S6 *in vitro*. (A) The effect of RACK1 stable silence on the expression of AKT, p-AKT, mTOR, p-mTOR, S6 and p-S6 in HSC-3 cells. (B) The effect of transient knockdown of RACK1 on the expression of AKT, p-AKT, mTOR, p-mTOR, S6 and p-S6 in 293T cells. GAPDH was used as an internal control for protein equal loading. Data are representative blots of three independent experiments.

phosphorylation of AKT and mTOR, as well as the well-characterized mTOR downstream protein S6, but did not reduce Pen AKT, mTOR, and S6 in OSCC cells. Moreover, there was a dose-dependent effect on the decreased phosphorylation of the AKT/mTOR pathway in transient RACK1 silencing in 293T cell lines (Fig. 5B). Additionally, depleted RACK1 could significantly reduce the protein levels of p-AKT, p-mTOR, and p-S6 in OSCC xenografted tumors *in vivo* (Fig. 6A), which was consistent with our previous findings *in vitro*. Additionally, the effect of RACK1 on the lymphangiogenesis in OSCC was also
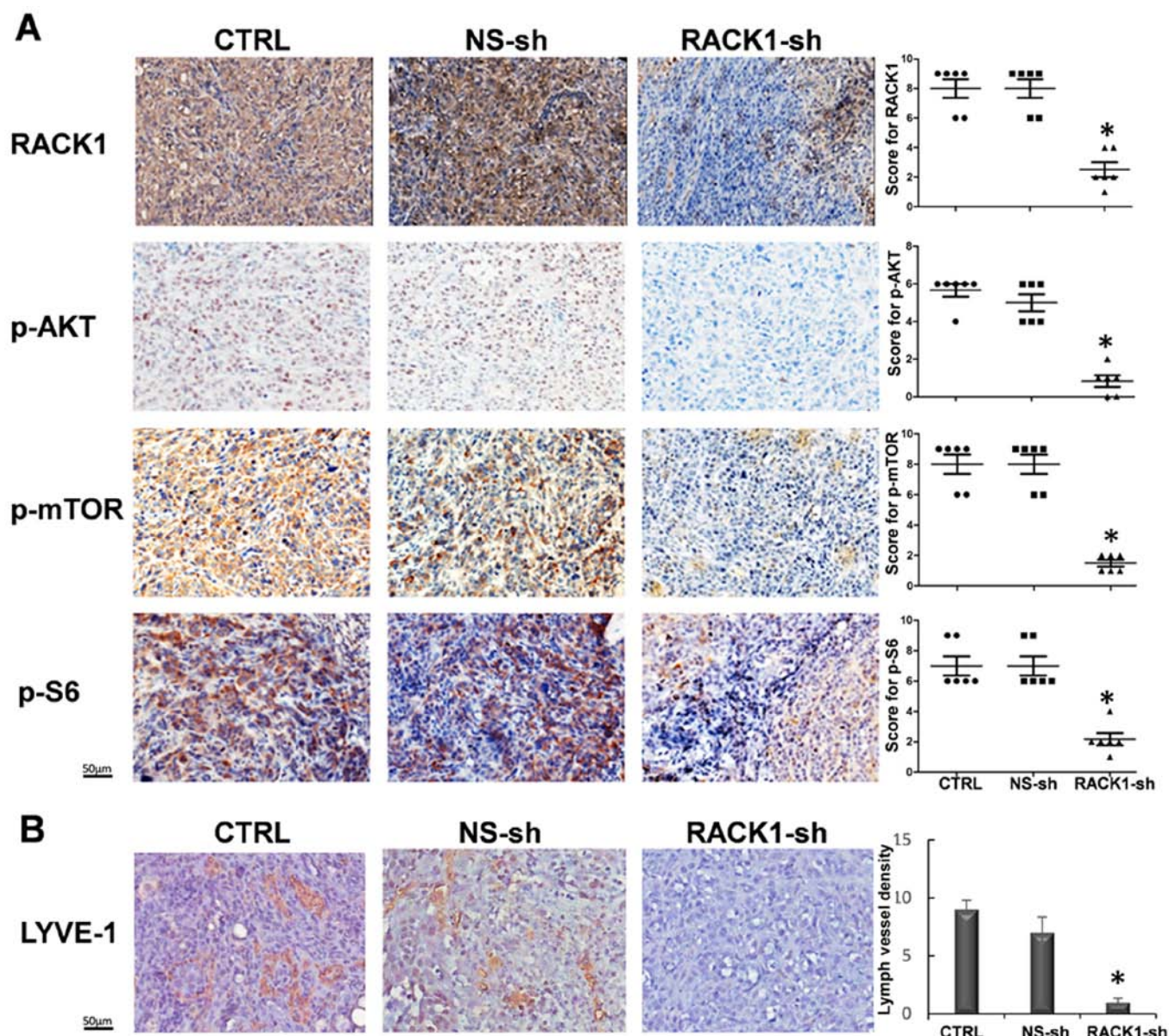
Figure 6. RACK1 silencing downregulates the phosphorylation of AKT/mTOR/S6 *in vitro*. (A) RACK1 stable silence downregulates the expression of p-AKT, p-mTOR and p-S6 in comparison with two control groups (mean ± SD, *P<0.05). (B) The average number of lymph vessels was significantly lower in RACK1-sh group compared with those in the two control groups (mean ± SD, *P<0.05).

assessed, as the mTOR function on the lymphangiogenesis was widely described (39,40). We investigated the expression of LYVE-1 in the RACK1 silenced group, which was diminished greatly comparing with the two control groups (Fig. 6B), hinting RACK1 promotes lymphangiogenesis of OSCC. Collectively, these results indicate that RACK1 contributes to the activation of AKT/mTOR in OSCC.

Discussion

OSCC is the most common malignancy of HNSCC, and has a poor survival rate (1,2). Current treatments for OSCC patients are not satisfactory, and novel therapeutic strategies are urgently required. Biotherapy was wildly proposed, and it became the fourth method of treating HNSCC in the last decade (41,42). Among these biotherapies, gene therapy is the one that has been most rapidly developed. To date, several gene therapy drugs have already been used in the clinic to achieve the desired results (43,44). In this study, we found that specific targeting of RACK1 could inhibit the cell cycle by decreasing Cyclin B1 and Cyclin D1. In a mouse xenograft model of OSCC, an 89% decrease in tumorigenicity was found in the RACK1 silenced group, and intratumoral expression of Ki67, CD34, and VEGF in RACK1 stably silenced group was significantly decreased when compared with the two control groups. Moreover, the expression of RhoA was decrease, whereas E-cadherin was increased in the RACK1 stably silenced group. These proteins are associated with cell motility, consistent with our previous report that silencing RACK1 by RNA interference could effectively inhibit cell motility ability in OSCC (11).

AKT, or protein kinase B (PKB), is a serine-threonine kinase which functions as a downstream target and effector of phosphatidylinositol 3-kinase (PI3K). Abnormal activation

or expression of AKT can perturb cellular signaling cascades, resulting in the occurrence of human diseases (45-47). Recently, AKT has been considered an important oncogene because it is abnormally activated in several types of human cancers, especially those that have poor prognosis (48). In our previous OSCC tissue microarray study, p-AKT was highly expressed in OSCC tissues. The expression of p-AKT correlated with lymph node metastasis and recurrence and the 5-year survival rate (35). Both RACK1 and p-AKT were suggested as predictive markers. We hypothesized that there may be a positive relationship between RACK1 and p-AKT in OSCC. Further, we postulated that RACK1 might increase cell growth and angiogenesis through p-AKT and its downstream pathway. Thus, we analyzed the expression of RACK1 and p-AKT in a panel of OSCC cell lines and immortalized oral keratinocyte cells.

To explore the mechanism of RACK1 silencing in OSCC cell growth inhibition both *in vitro* and *in vivo*, we quantitatively re-assessed expression of RACK1 and p-AKT in a large precancerous and cancer patient cohort. We detected a similar tendency between RACK1 and p-AKT in different stages of oral carcinogenetic tissues. We deduced that the expression of p-AKT is positively correlated with the level of RACK1 in OSCC cell lines based on western blot analysis.

Next, we aimed to identify the downstream pathway of AKT involved in the RACK1 silencing effect. mTOR is one of the major targets of activated AKT, which in turn regulates a number of downstream molecules, such as ribosomal protein pS6. AKT is the key regulator of the AKT/mTOR/S6 pathway, which ultimately controls fundamental cell processes such as cell cycle, cell proliferation, and angiogenesis (49,50). The AKT/mTOR/S6 pathway was suggested to contribute to the premalignant potential of OSCC (38,51). Recent findings indicate that multiple genetic and epigenetic alterations converge on the persistent activation of AKT/mTOR signaling in most HNSCC lesions. Therefore, we investigated whether the AKT/mTOR/S6 pathway was influenced in this case. We found that specific transient knockdown of RACK1 upregulated the levels of p-AKT, p-mTOR, and p-S6 in a dose-dependent manner. Moreover, RACK1 knockdown inhibited the phosphorylation of AKT, mTOR, and S6 *in vivo*. In addition, mTOR inhibitors exerted a remarkably increased antitumor activity, particularly in HNSCC cells and inhibition of mTOR diminished lymphangiogenesis in the primary tumors (39,40). Our results also support these previous studies. In other words, RACK1 might play a significant role in lymphomagenesis with potential mTOR effect. Thus, we propose that RACK1 may have potential as a target for therapy in a range of tumor types. Our results indicate that targeted RACK1 therapy in OSCC cells results in cell growth inhibition *in vitro* and OSCC xenografts suppression *in vivo*.

In conclusion, this study demonstrates that RACK1 promotes cell growth of OSCC, regulating cell growth and enhancing the progression of OSCC *in vivo*, at least in part via activation of the AKT/mTOR/S6 signaling pathway. This study reveals a novel mechanism by which RACK1 contributes to the poor prognosis of OSCC, and suggests a potential novel therapeutic target.

Acknowledgements

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