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Tivantinib (ARQ-197) exhibits anti-tumor activity with down-regulation of FAK in oral squamous cell carcinoma

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ABSTRACT

Oral squamous cell carcinoma (OSCC) is one of the most common cancers worldwide and the 5 years survival rate of the patients is about 60% in the USA, due to acquired chemotherapeutic resistance and metastasis of the disease. In this study, we found that tivantinib, a selective MET inhibitor, suppresses OCSS cell proliferation and colony formation, however, anti-tumor activities induced by tivantinib are independent of the inhibition of MET signaling pathway. In addition, tivantinib cause G2/M cell cycle arrest and caspases-dependent apoptosis in OSCC cell lines. We also found that tivantinib dose-dependently suppressed the activation and expression of FAK. In all, these data suggested that tivantinib may be developed as a chemotherapeutic agent to effectively treat certain cancers including OSCC.

1. Introduction

Oral cavity cancer, predominantly oral squamous cell carcinoma (OSCC), is one of the most common cancer in the world, with more than 300,000 new cases diagnosed in 2012 [1]. In the past decades, the five year survival rate of the OSCC patients still remains dismally low, only about 60% in the USA, despite the progression in surgery, chemotherapy and radiotherapy [2]. Acquired resistance to conventional chemotherapies and the metastasis have become serious obstacles for the OSCC treatment [3,4].

Tivantinib (ARQ 197) was initially discovered as a non-ATP competitive MET inhibitor and exhibited effective *in vitro* and *in vivo* anti-tumor activities against many human cancers, such as gastric cancer, breast cancer, non-small-cell lung cancer (NSCLC), renal cancer and hepatocellular carcinoma (HCC) [5]. It has been reported that tivantinib might bind to the ATP-binding pocket and prevent MET auto-phosphorylation, which was the initial step for the completely activation of MET [6]. Inhibition of the auto-phosphorylation of MET by tivantinib could result in the suppression of the downstream pathway phosphorylation of AKT and ERK1/2 [5]. Meanwhile, Antonio Calles et al. showed that, compared to other MET inhibitors such as crizotinib and PHA-665752, the anti-proliferative activity of tivantinib was more potent and not restricted to only MET dependent NSCLC cell lines [7]. They also showed that tivantinib surprisingly did not inhibit cellular MET activation or phosphorylation of downstream signaling proteins AKT or ERK1/2 in either MET dependent or independent cell lines. Moreover, Aki Aoyama et al. demonstrated that tivantinib could directly bind to the colchicine binding site of tubulin and disrupt tubulin polymerization [8–10]. Recently, tivantinib was reported to bind and inhibit glycogen synthase kinase 3 (GSK3) alpha and beta in a greater degree than did MET [11]. These studies suggested that the anti-tumor mechanisms of tivantinib remain uncertain and need to be further illustrated [12]. Herein, we showed the anti-tumor effects of tivantinib on two OSCC cell lines, Cal27 and Tca8113, and probe into the potential underlying mechanisms.

2. Materials and methods

2.1. Antibodies and reagents

Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Thermo Scientific (South Logan, UT). Recombinant Human HGF was purchased from R&D Systems (Minneapolis, MN). MTT, propidium iodide (PI), RNase was purchased from Sigma–Aldrich (St Louis, MO). Gentian violet was purchased from Solarbio (Beijing, China). The Annexin VFICT/PI

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apoptosis detection kit were purchased from BD Biosciences (Franklin Lakes, NY). PVDF membrane and chemiluminescent reagents were from Millipore (Billerica, MA). Antibodies to MET, phosphorylated MET(Tyr1234/1235), cyclin B1, phosphorylated histone H3(Ser10), cyclin A, cleaved caspases-3, cleaved PARP-1, FAK and phosphorylated FAK(Try397) were purchased for Cell Signaling Technology (Danvers MA). Antibodies to β-Actin, siRNA specific for FAK and pan-caspases inhibitor z-VAD-FMK were purchased from Santa Cruz Biotechnology (Santa Cruz, MA). Lipofectamine 2000 transfection reagent was purchased from Invitrogene.

2.2. Cell culture

The human OSCC cell lines Cal27 and Tca8113 were purchase from Cell Culture Center of Fudan University (Shanghai, China) and BIOK&KM Cell Bank (Jiangsu, China), respectively. Cells were routinely cultured with DMEM supplemented with 10% FBS, 100 U/mL penicillin and 100 mg/mL streptomycin in a humidified incubator at 37 °C in an atmosphere of 5% CO_2.

2.3. Cell viability assay

Cell viability was carried out using the MTT reagent as described previously [4]. Briefly, at the end of drug treatment, the cultured medium was replaced with 200 mL of fresh medium containing 10% MTT (5 mg/mL in PBS) in each well and incubated at 37 °C for 4 h. Then the MTT-containing medium was discarded and 150 mL of DMSO per well was added to dissolve the formazan crystals newly formed. Absorbance of each well was determined with the micro plate reader at a 490 nm wavelength.

2.4. Colony formation assay

10^3 cells per well were seeded into 6-well plates at a single cell density. 48 h later, the cells were treated with different concentrations of tivantinib or DMSO (as negative control) for additional 48 h. Then the medium was replaced with fresh medium to allow cell growth for 10 days. The cells were then fixed with methyl alcohol for 15 min and stained with gentian violet for 30 min. Colonies consisting of more than 50 cells were counted.

2.5. Flow cytometry analysis

Cells were treated with tivantinib or DMSO for 24 h, and then harvested, washed twice with PBS. For cell cycle assay, the cells were fixed with ice-cold 75% ethanol at −20 °C overnight and stained with 500 mL PI (50 mg/mL) containing 0.1% RNase (1 mg/mL) for 15 min in dark condition at room temperature. For apoptosis assay, the cells were suspended with 100 μL Annexin V-FITC binding buffer and stained with 5 μL Annexin V-FITC and 5 μL PI for 15 min in dark condition at room temperature. After staining, the cells were then analyzed by flow cytometry (Cytomics FC 500 MPL, Beckman Coulter). The results were indicated as mean values from three independent determinations.

2.6. Western blot analysis

Western blot analysis was carried out using standard methods as describe previously [4]. Briefly, at the end of drug treatment, cells were harvested and lysed using RIPA buffer with protease inhibitor cocktail (BestBio, shanghai, China). Protein concentration was determined for all samples using BCA protein assay. The equal volume samples were separated by SDS-PAGE electrophoresis and transferred onto PVDF membrane. After blocking with 5% non-fat dry milk, the membrane was incubated with corresponding primary and secondary antibodies. Immuno-reactive bands were visualized using enhanced chemiluminescence. All experiments were repeated for three times.

2.7. Small interfering RNA (SiRNA) mediated knockdown of FAK expression

FAK siRNA or Control siRNA were transfected into cells using Lipofectamine 2000 according to the manufacturer's protocol. Total cell lysates were prepared 48 h after transfection to assess the knockdown efficiency by western blot analysis. Otherwise, 24 h after transfection, cells were treated with or without tivantinib for additional 24 h and the cell viability was evaluated by MTT reagent.

2.8. Statistical analyses

All experiments were repeated at least three times. Graphpad Prism software was used to analyze all data. Differences between mean values were determined by student's t-test or one-way ANOVA test. P-values of <0.05 were considered to be significant.

3. Results

3.1. Tivantinib suppresses cell growth and colony formation independent of the inhibition of HGF/MET signaling pathway in OSCC cells

The anti-proliferative effect of tivantinib was initially investigated on the two OSCC cell lines Cal27 and Tca8113. Cells were incubated with various concentrations (ranging from 0.031 to 1 μM) of tivantinib for 24, 48, and 72 h, and then the cell viability was determined using MTT assay. As shown in Fig. 1A, the treatment of cells with tivantinib resulted in the decreasing of cell viability in both dose- and time-dependent manner. The half maximal inhibitory concentration (IC_{50}) values of tivantinib in Cal27 cells were 0.68, 0.41 and 0.17 μM for 24, 48 and 72 h, respectively. The IC_{50} values of tivantinib in Tca8113 cells were 0.44, 0.30, and 0.15 μM for 24, 48 and 72 h, respectively. These data suggest that tivantinib exhibited strong cytotoxic effect on OSCC cells at sub-micromolar concentrations.

Next, we determined the effect of tivantinib on the colony formation ability of the OSCC cell lines. Colony formation assay is an *in vitro* cell survival assay widely used to determine the cytotoxicity induced by various chemotherapeutic agents [13]. As shown in Fig. 1B and C, we treated the cells with tivantinib at the gradient concentrations of 0.25, 0.5, and 1 μM for 48 h and found that tivantinib could dose-dependently inhibit OSCC cell colony formation, compared to the controls treated with diluent (DMSO). The above results showed that tivantinib had the obvious inhibition effect on the growth of OSCC cells.

Since tivantinib was previously reported as a selective inhibitor of MET, we also examined whether the cytotoxicity of tivantinib is dependent on the inhibition of HGF/MET signaling pathway. We treated Cal27 and Tca8113 cells with different concentrations of tivantinib for 24 h, and then detected the expression levels of MET and phosphorylated MET by western blot. As shown in Fig. 1D, unexpectedly, the expression levels of MET and phosphorylated MET were not affected by the treatment of tivantinib in both cell lines. Moreover, Cal27 and Tca8113 cells were pre-treated with 10 ng/mL HGF for 6 h and exposed to 0.5 μM tivantinib for an additional 24 h. Cell viability assay showed that pre-treatment with HGF failed to attenuate the anti-proliferative effects of tivantinib in the both cell lines (Fig. 1E). In all, these results indicated that the cytotoxicity of tivantinib against OSCC cells was not obtained by the blockade of HGF/MET signaling pathway.

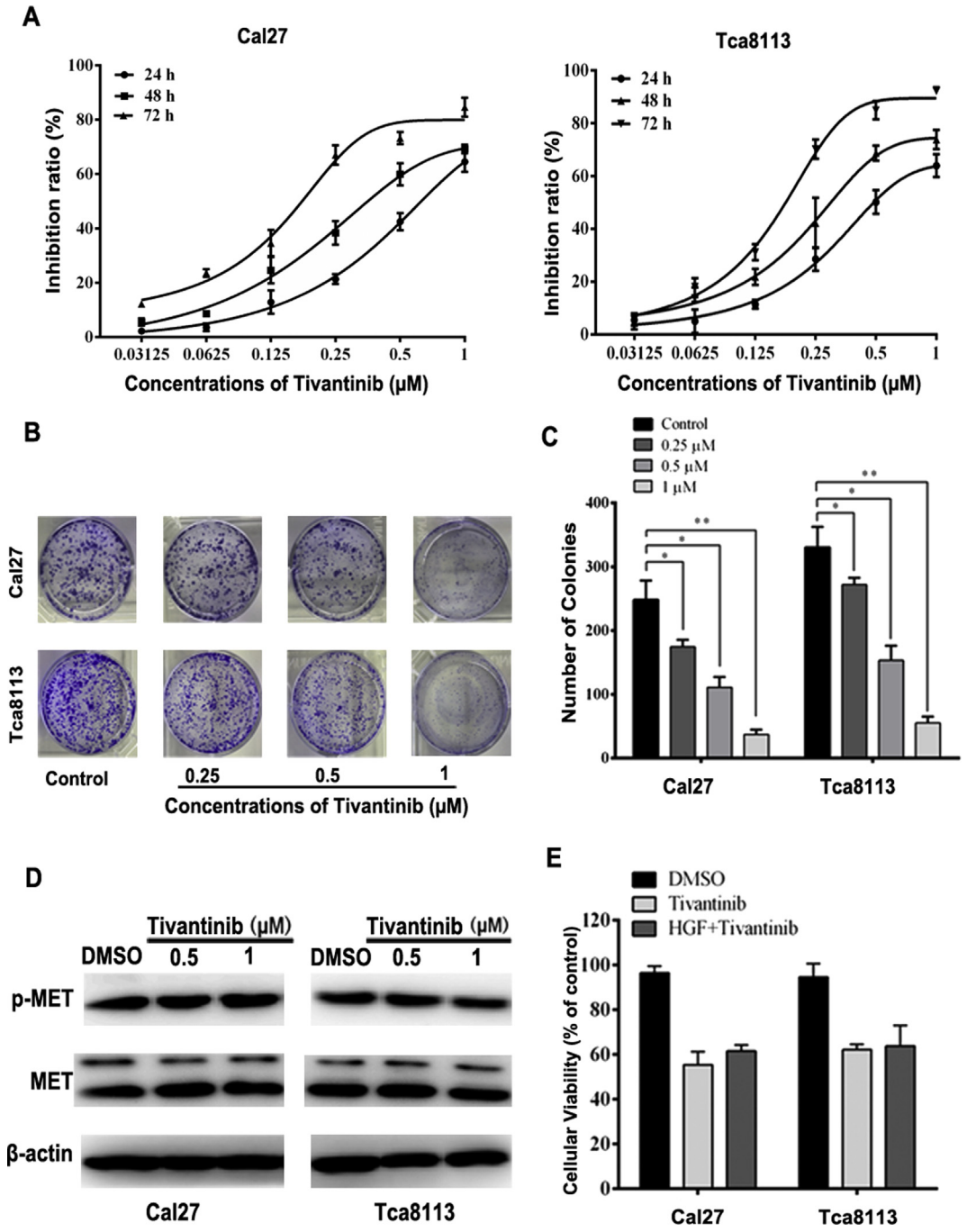

Fig. 1. The effect of tivantinib on cell proliferation and colony formation. (A) Detection of cell proliferation treated by tivantinib by MTT. (B) Representative images of cell colonies after treatment with various concentrations of tivantinib for 48 h. (C) Quantitative analysis of colony formation rate after treatment with tivantinib for 48 h. (D) Immunoblotting analysis of MET induced by tivantinib for 24 h β-actin was used as the loading control. (E) Quantitative analysis of treatment effect of HGF and/or tivantinib on Cal27 and Tca8113 cells. Cells were pre-treated with 10 ng/mL HGF for 6 h and exposed to 0.5 μM tivantinib for an additional 24 h. Data from three independent experiments were analyzed, Error bars = 95% confidence intervals (CIs), $^{*}p < 0.05$, $^{**}p < 0.01$ *vs* control.

3.2. Tivantinib causes G2/M cell cycle arrest

To further explore how the cell growth was inhibited by tivantinib, the cell cycle progression was analyzed by quantitating DNA content using flow cytometry. As shown in Fig. 2A, compared with the control cells treated with the diluent, the proportion of cells in G2/M phase dose-dependently increased in both cell lines when Cal27 and Tca8113 cells were exposure to tivantinib, however, the proportion of cells in S phase concomitantly decreased. To further clarify the effect of tivantinib on cell cycle, we analyzed the expression levels of the regulatory proteins of the G2/M phase [14], including phospho-Histone H3, cyclin B1 and cyclin A using western blotting. As shown in Fig. 2B, the expression levels of cyclin B1 and phosphor-Histone H3 were dose-dependently increased by the treatment of tivantinib for 24 h, but the expression level of cyclin A was decreased. These results suggested that the OSCC cells exited G2 phase and were undergoing mitotic arrest during tivantinib treatment.

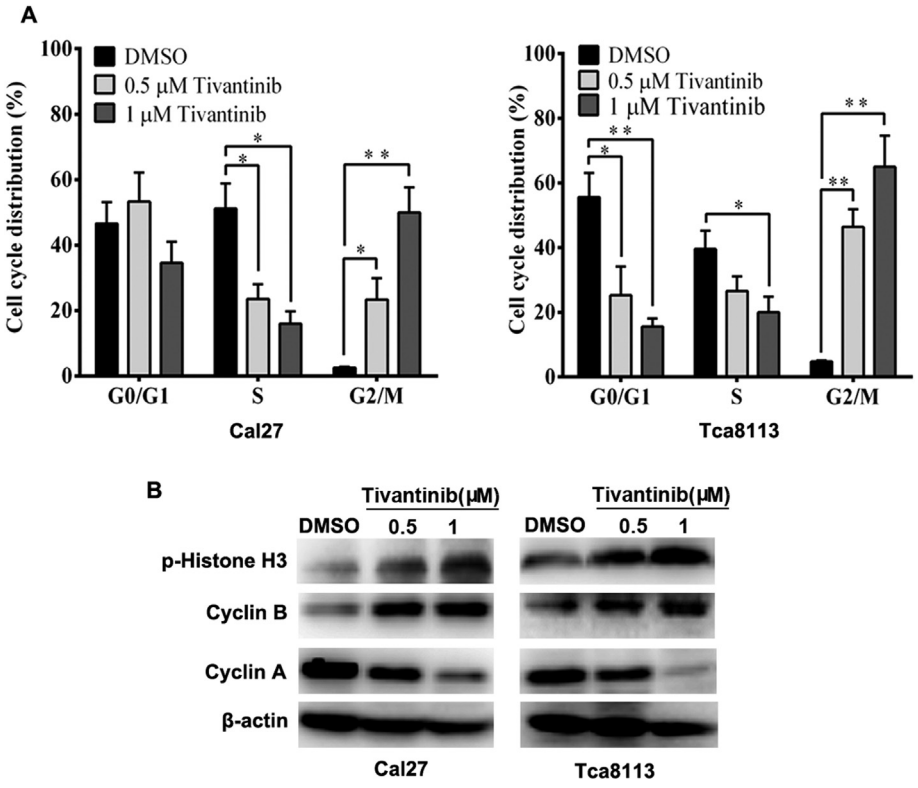

Fig. 2. Tivantinib suppresses cell cycle progression. (A) Statistic histogram of cell cycle distribution after treatment with 0.5 or 1 μM tivantinib for 24 h. (B) Immunoblotting analysis of cell cycle regulatory proteins affected by with 0.5 or 1 μM tivantinib for 24 h β-actin was used as the loading control. Data from three independent experiments were analyzed, Error bars = 95% CIs, $^{*}p < 0.05$, $^{**}p < 0.01$ *vs* control.

3.3. Tivantinib induces cellular apoptosis through the activation of caspase-related apoptotic signaling pathway

To investigate whether tivantinib induces apoptosis in OSCC cells, Annexin-V/PI double staining was applied to quantify the number of apoptotic cells. As illustrated in Fig. 3A and B, the total proportions of Annexin V positive cells were elevated with the increasing concentrations of tivantinib in both OSCC cell lines. Meanwhile, immunoblotting analysis showed that the expression levels of apoptotic-associated cleaved-caspase-3 and cleaved-PARP were up-regulated by the treatment of tivantinib in a dose-dependent manner (Fig. 3C). Furthermore, Cal27 and Tca8113 cells were pre-treated with a pan-caspases inhibitor z-VAD (40 μM) for 4 h, followed by the treatment with 1 μM tivantinib for additional 24 h. As shown in Fig. 3D, the apoptosis induced by tivantinib were significantly attenuated by z-VAD pre-treatment in both cell lines. Correspondingly, the tivantinib-mediated cleavage of caspase-3 and PARP was also blocked by z-VAD, as shown in Fig. 3E. These results indicated that tivantinib could trigger caspases-dependent apoptosis in OCSS cells.

3.4. FAK contributes to the anti-tumor activity of tivantinib

Focal Adhesion Kinase (FAK), a non-receptor tyrosine protein kinase, is significantly overexpressed and hyper-activated in a majority of solid tumors, including OSCC [15–17]. As multifunctional protein, FAK not only regulates extracellular signals from intergrins and growth factor receptors, but also acts as a protein scaffold and contributes to cell mitosis and cytoskeleton organization [18,19]. Therefore, we tested whether tivantinib elicited anti-tumor activities through the inhibition of FAK. As shown in Fig. 4A, tivantinib dose-dependently decreased the total expression and phosphorylation at Tyr397 of FAK in both Cal27 and Tac8113 cell lines. Further, FAK was knockdown through specific siRNA in the both OSCC cell lines, resulting in the corresponsive decreasing of the expression of total FAK and the phosphorylated levels at Tyr397 (Fig. 4B). Cell viability assay showed that knockdown of FAK significantly reduced the cell proliferation in both cell lines (Fig. 4C). In addition, knockdown of FAK could sensitize the cancer cells to tivantinib treatment in both two cell lines (Fig. 4D). Together, these results provided evidences that tivantinib could block the FAK signal pathway, which might be, at least part of, the anti-tumor mechanisms of tivantinib.

4. Discussion

Tivantinib is a promising oral available anti-cancer agent ongoing clinical trial. As monotherapy or combination with other agents, clinical studies showed that tivantinib exhibited encouraging antitumor activities, improved overall survival (OS) and progression-free survival (PFS) in NSCLC [20], advanced HCC [21] and metastatic gastric cancer patients [22]. However, up till now, its anti-tumor effect has not been evaluated in OSCC cells. Except for MET, tivantinib was subsequently found to play a vital role in the disruption of microtube dynamics and GSK inhibition, however, the underlying mechanism still remains uncertain. In this study, we studied the anti-cancer activity and the underlying mechanism of tivantinib in two OCSS cell lines, Cal27 and Tca8113. We found that tivantinib significantly inhibited the proliferation and colony formation of Cal27 and Tca8113 cells. However, the total expression and phosphorylation of MET were not obvious decreased by tivantinib at the cytotoxic concentrations. Moreover, when the cells where treated with HGF, the ligand of MET, the inhibited effect of tivantinib was not attenuated, suggesting that tivantinib may be an

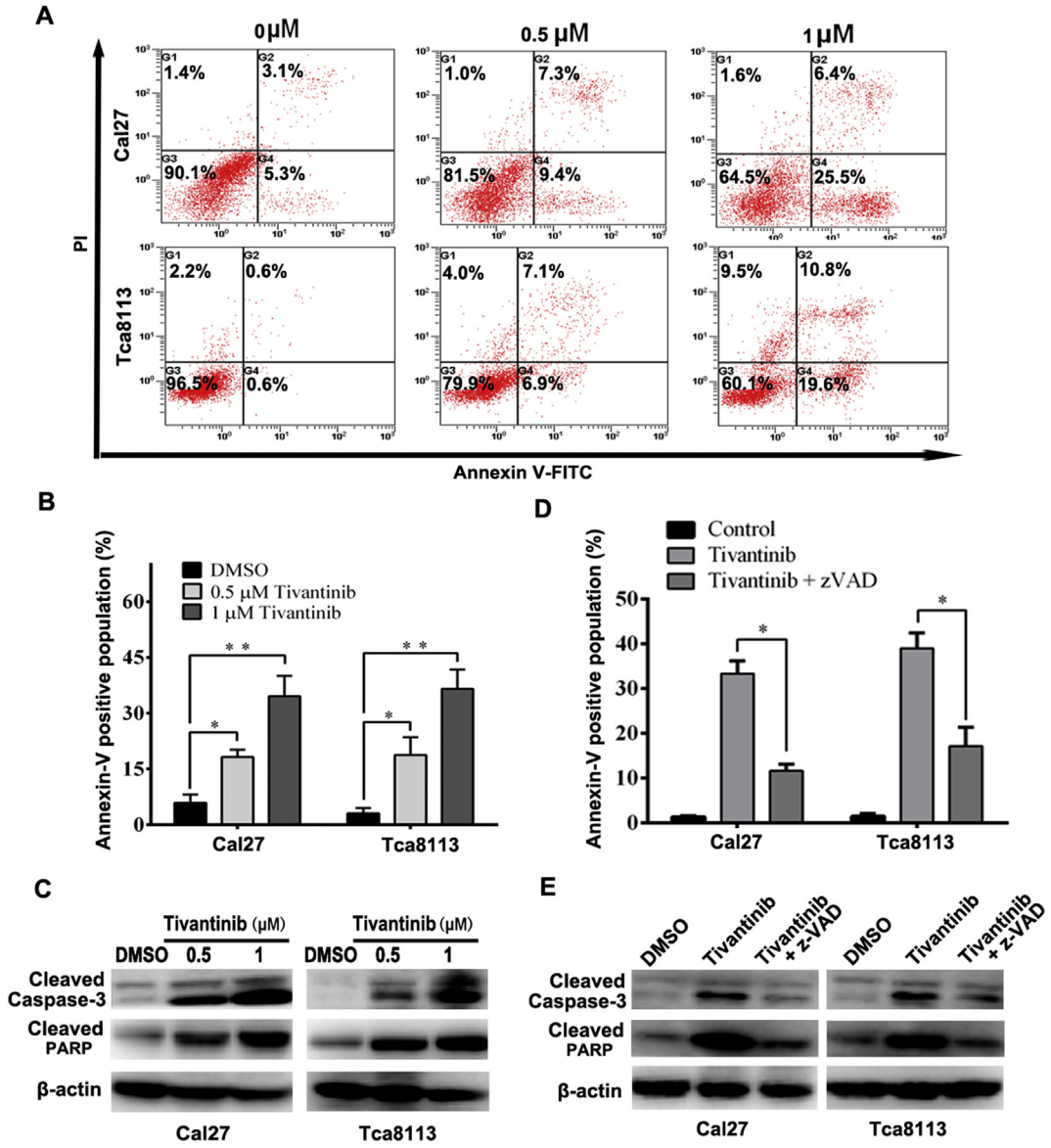

Fig. 3. Apoptosis induced by tivantinib. (A) Apoptosis detected by flow cytometry. (B) Quantitative analysis of apoptotic cells. (C) Immunoblotting analysis of apoptosis-associated proteins. β-actin was used as the loading control. (D) Caspases inhibitor z-VAD attenuates tivantinib-induced apoptosis. Cells were pre-treated with a pan-caspases inhibitor z-VAD (40 μM) for 4 h, followed by the treatment with 1 μM tivantinib for additional 24 h. (E) Western blotting analysis of cleavage of caspase-3 and PARP expression induced by tivantinib and z-VAD. Data from three independent experiments were analyzed, Error bars = 95% CIs, $^{*}p < 0.05$, $^{**}p < 0.01$ *vs* control.

effective chemotherapeutic agent against OCSS cells through MET-independent signaling pathway.

Unlike other MET inhibitors, such as crizotinib and PHA-665752 which induced G0/G1 cell cycle arrest (data not shown), tivantinib caused significant G2/M arrest in the both OSCC cell lines. The increase of cyclin B1 and decrease of cyclin A indicated that the tivantinib treated cells exited from G2 phase and entered into mitotic phase. These results were further confirmed by the up-regulated phosphorylation of histone H3, which is phosphorylated at serine by Aurora B during the cell mitosis [23].

Apoptosis is considered as the principal mechanism by which chemotherapeutic agents elicit their anti-tumor activities. Caspases family plays a vital role in regulating extrinsic apoptosis [24]. Once received apoptotic signaling from death receptor, caspases cascade were cleaved step by step, finally leading to the activation of effector caspases, mainly caspase-3. Subsequently, the activated effector caspases cleave cellular substrates, such as poly-ADP-ribose polymerase (PARP), and amplify the apoptotic signals to induce cell apoptosis [25]. In our study, we found that tivantinib dose-dependently induced the cleavage of caspase-3 as well as PARP-1, corresponding to the induction of cellular apoptosis. More importantly, these effects could be attenuated by the pan-caspases inhibitor z-VAD, indicating the critical roles of death receptor-mediated caspases activation in tivantinib-induced cellular apoptosis.

Increasing studies provide strong evidence that the over-expression and activation of FAK results in increased cell survival, motility, and proliferation, leading to angiogenesis, metastasis, and invasion of tumors [26,27]. Recently, Shalana et al. discovered a small molecular FAK scaffolding inhibitor Y15 which could decrease cancer growth in thyroid cancer and elicit synergy effect with other targeted therapeutics including sorafenib, pazopanib and sunitinib [28]. Another three FAK inhibitors, PF-00562271, VS-4718 and VS-6063, have shown promising clinical activities in patients with selected solid cancers. Thus, the suppression of FAK signaling pathway has emerged as an effective way for cancer therapy [29]. In

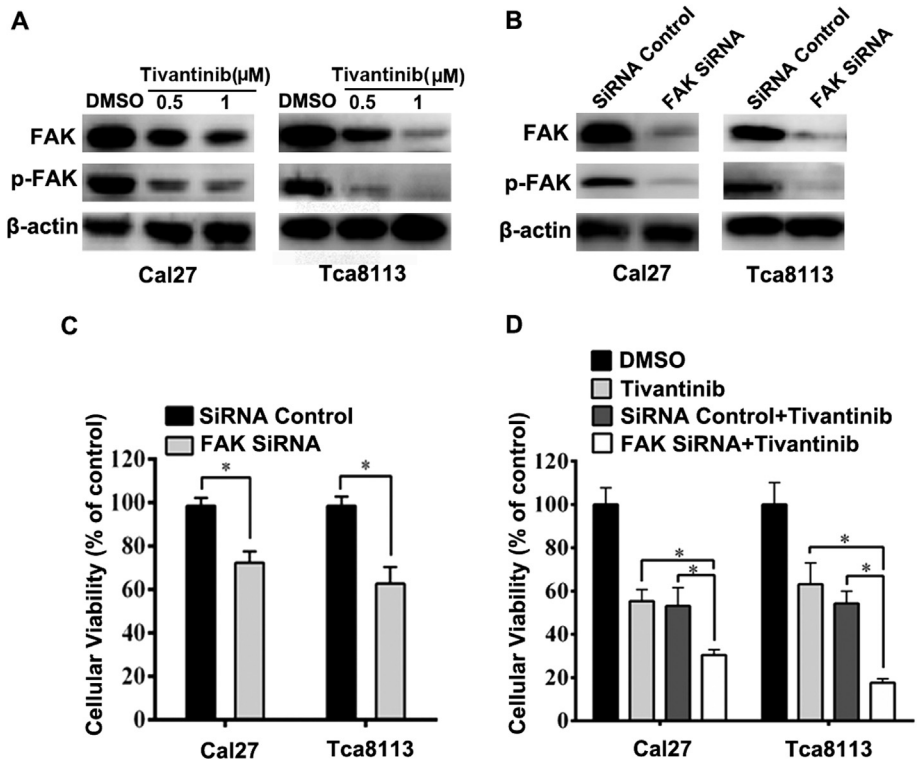

Fig. 4. Tivantinib suppresses FAK signaling pathway. (A) Western blotting analysis of FAK and phosphorylated FAK expression induced by 0.5 or 1 μM tivantinib for 24 h. DMSO was used as negative control. (B) Western blotting analysis of FAK and phosphorylated FAK expression in cells transfected with FAK specific siRNA or control siRNA. (C) Detection of cell proliferation affected by knockdown of FAK by MTT. (D) Detection of cell proliferation affected by knockdown of FAK and tivantinib by MTT. $^{*}p < 0.05$, Error bars = 95% confidence intervals.

the present investigation, we showed that the total expression and phosphorylation of FAK was inhibited by tivantinib in a dose-dependent manner. Meanwhile, silencing of FAK by siRNA decreased the cell proliferation and enhanced the anti-tumor effect of tivantinib, which may indicate that affecting FAK signaling pathway rather than MET signal pathway was accompanied with the anti-cancer effect of tivantinib on OSCC cells. To our knowledge, it was for the first time to describe the inhibition of FAK signaling pathway by tivantinib, which might provide new clues to elucidate the complicated molecular mechanisms of tivantinib. However, whether tivantinib directly binds to FAK and blocked its activation need further investigation in the future.

In conclusion, we provided the new evidence that tivantinib suppresses cell growth, blocks cellular mitosis and induces caspases-dependent apoptosis of human OCSS cells with repressing FAK activation. However, further clinical study may be needed to validate the application of tivantinib in OSCC treatment.

Conflict of interest

The authors have declared that no competing interests exist.

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References

[1] J. Ferlay, I. Soerjomataram, M. Ervik, D. Forman, F. Bray, R. Dikshit, S. Elser, C. Mathers, M. Rebelo, D.M. Parkin, GLOBOCAN, in: Esrtimated Cancer Incidence, Mortality, and Prevalence Worldwide in 2012, 2012 (accessed December 2013), http://globocan.iarc.fr/.

[2] R. Siegel, J. Ma, Z. Zou, A. Jemal, Cancer statistics, 2014, CA Cancer J. Clin. 64 (2014) 9–29.

[3] M. Pérez-Sayáns, J.M. Somoza-Martín, F. Barros-Angueira, P.G. Diz, J.M.G. Rey, A. García-García, Multidrug resistance in oral squamous cell carcinoma: the role of vacuolar ATPases, Cancer Lett. 295 (2010) 135–143.

[4] Y. Qian, Y. Wang, D.S. Li, Y.X. Zhu, Z.W. Lu, Q.H. Ji, G. Yang, The chemokine receptor-CXCR2 plays a critical role in the invasion and metastases of oral squamous cell carcinoma in vitro and in vivo, J. Oral Pathol. Med. 43 (2014) 658–666.

[5] N. Munshi, S. Jeay, Y. Li, C.-R. Chen, D.S. France, M.A. Ashwell, J. Hill, M.M. Moussa, D.S. Leggett, C.J. Li, ARQ 197, a novel and selective inhibitor of the human c-Met receptor tyrosine kinase with antitumor activity, Mol. Cancer Ther. 9 (2010) 1544–1553.

[6] S. Eathiraj, R. Palma, E. Volckova, M. Hirschi, D.S. France, M.A. Ashwell, T.C. Chan, Discovery of a novel mode of protein kinase inhibition characterized by the mechanism of inhibition of human mesenchymal-epithelial transition factor (c-Met) protein autophosphorylation by ARQ 197, J. Biol. Chem. 286 (2011) 20666–20676.

[7] A. Calles, N. Kwiatkowski, B.K. Cammarata, D. Ercan, N.S. Gray, P.A. Jänne, Tivantinib (ARQ 197) efficacy is independent of MET inhibition in non-small-cell lung cancer cell lines, Mol. Oncol. 9 (2015) 260–269.

[8] R. Katayama, A. Aoyama, T. Yamori, J. Qi, T. Oh-Hara, Y. Song, J.A. Engelman, N. Fujita, Cytotoxic activity of tivantinib (ARQ 197) is not due solely to c-MET inhibition, Cancer Res. 73 (2013) 3087–3096.

[9] C. Basilico, S. Pennacchietti, E. Vigna, C. Chiriaco, S. Arena, A. Bardelli, D. Valdembri, G. Serini, P. Michieli, Tivantinib (ARQ197) displays cytotoxic activity that is independent of its ability to bind MET, Clin. Cancer Res. 19 (2013) 2381–2392.

[10] A. Aoyama, R. Katayama, T. Oh-Hara, S. Sato, Y. Okuno, N. Fujita, Tivantinib (ARQ 197) exhibits antitumor activity by directly interacting with tubulin and overcomes ABC transporter-mediated drug resistance, Mol. Cancer Ther. 13 (2014) 2978–2990.

[11] L.L. Remsing Rix, B.M. Kuenzi, Y. Luo, E. Remily-Wood, F. Kinose, G. Wright, J. Li, J.M. Koomen, E.B. Haura, H.R. Lawrence, GSK3 alpha and beta are new functionally relevant targets of tivantinib in lung cancer cells, ACS Chem. Biol. 9 (2013) 353–358.

[12] P. Michieli, F. Di Nicolantonio, Targeted therapies: tivantinib—a cytotoxic drug in MET inhibitor's clothes? Nat. Rev. Clin. Oncol. 10 (2013) 372–374.

[13] N.A. Franken, H.M. Rodermond, J. Stap, J. Haveman, C. Van Bree, Clonogenic assay of cells in vitro, Nat. Protoc. 1 (2006) 2315–2319.

[14] K. Vermeulen, D.R. Van Bockstaele, Z.N. Berneman, The cell cycle: a review of regulation, deregulation and therapeutic targets in cancer, Cell Prolif. 36 (2003) 131–149.

[15] L.J. Kornberg, Focal adhesion kinase expression in oral cancers, Head Neck 20 (1998) 634–639.
[16] K.M. Quesnelle, A.M. Sparano, Y. Wang, G.S. Weinstein, M.S. Brose, Molecular characterization of the role of PTK2/FAK amplification in oral squamous cell carcinoma (OSCC), Proc. Amer. Assoc. Cancer Res. 47 (2006) 798.
[17] J.C. de Vicente, P. Rosado, P. Lequerica-Fernández, E. Allonca, L. Villallain, G. Hernández-Vallejo, Focal adhesion kinase overexpression: correlation with lymph node metastasis and shorter survival in oral squamous cell carcinoma, Head Neck 35 (2013) 826–830.
[18] D.J. Sieg, C.R. Hauck, D. Ilic, C.K. Klingbeil, E. Schaefer, C.H. Damsky, D.D. Schlaepfer, FAK integrates growth-factor and integrin signals to promote cell migration, Nat. Cell Biol. 2 (2000) 249–256.
[19] M.D. Schaller, Cellular functions of FAK kinases: insight into molecular mechanisms and novel functions, J. Cell Sci. 123 (2010) 1007–1013.
[20] D. Gerber, M. Socinski, J. Neal, H. Wakelee, K. Shirai, L. Sequist, R. Rosovsky, R. Lilenbaum, B. Bastos, C. Huang, Randomized Phase 2 Study of tivantinib (ARQ 197) plus erlotinib versus single-agent chemotherapy in previously treated KRAS mutant advanced non-small cell lung cancer (NSCLC), Int. J. Radiat. Oncol. Biol. Phys. 90 (2014) S36.
[21] J. Trojan, S. Zeuzem, Tivantinib in hepatocellular carcinoma, Expert Opin. Inv. Drug 22 (2013) 141–147.
[22] Y.-K. Kang, K. Muro, M.-H. Ryu, H. Yasui, T. Nishina, B.-Y. Ryoo, Y. Kamiya, S. Akinaga, N. Boku, A phase II trial of a selective c-Met inhibitor tivantinib (ARQ 197) monotherapy as a second-or third-line therapy in the patients with metastatic gastric cancer, Invest. New Drug 32 (2014) 355–361.
[23] T. Hirota, J.J. Lipp, B.-H. Toh, J.-M. Peters, Histone H3 serine 10 phosphorylation by Aurora B causes HP1 dissociation from heterochromatin, Nature 438 (2005) 1176–1180.
[24] M.S. Ola, M. Nawaz, H. Ahsan, Role of Bcl-2 family proteins and caspases in the regulation of apoptosis, Mol. Cell Biochem. 351 (2011) 41–58.
[25] A.G. Porter, R.U. Jänicke, Emerging roles of caspase-3 in apoptosis, Cell Death Differ. 6 (1999) 99–104.
[26] T. Miyazaki, H. Kato, M. Nakajima, M. Sohda, Y. Fukai, N. Masuda, R. Manda, M. Fukuchi, K. Tsukada, H. Kuwano, FAK overexpression is correlated with tumour invasiveness and lymph node metastasis in oesophageal squamous cell carcinoma, Br. J. Cancer 89 (2003) 140–145.
[27] G.W. McLean, N.O. Carragher, E. Avizienyte, J. Evans, V.G. Brunton, M.C. Frame, The role of focal-adhesion kinase in cancer—a new therapeutic opportunity, Nat. Rev. Cancer 5 (2005) 505–515.
[28] S. O'Brien, V.M. Golubovskaya, J. Conroy, S. Liu, D. Wang, B. Liu, W.G. Cance, FAK inhibition with small molecule inhibitor Y15 decreases viability, clonogenicity, and cell attachment in thyroid cancer cell lines and synergizes with targeted therapeutics, Oncotarget 5 (2014) 7945–7959.
[29] B.Y. Lee, P. Timpson, L.G. Horvath, R.J. Daly, FAK signaling in human cancer as a target for therapeutics, Pharmacol. Ther. 146 (2015) 132–149, http://dx.doi.org/10.1016/j.pharmthera.2014.1010.1001.