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PRECLINICAL STUDIES

Preclinical assessment of histone deacetylase inhibitor quisinostat as a therapeutic agent against esophageal squamous cell carcinoma

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Summary

Esophageal squamous cell carcinoma (ESCC) is one of the most serious life-threatening malignancies. Although chemotherapeutic targets and agents for ESCC have made much progress recently, the efficacy is still unsatisfactory. Therefore, there is still an unmet medical need for patients with ESCC. Here, we report the expression status of HDAC1 in human ESCC and matched paracancerous tissues, and the results indicated that HDAC1 was generally upregulated in ESCC specimens. Furthermore, we comprehensively assessed the anti-ESCC activity of a highly active HDAC1 inhibitor quisinostat. Quisinostat could effectively suppress cellular viability and proliferation of ESCC cells, as well as induce cell cycle arrest and apoptosis even at low treatment concentrations. The effectiveness was also observed in KYSE150 xenograft model when quisinostat was administered at tolerated doses (3 mg/kg and 10 mg/kg). Meanwhile, quisinostat also had the ability to suppress the migration and invasion (pivotal steps of tumor metastasis) of ESCC cells. Western blot analysis indicated that quisinostat exerted its anti-ESCC effects mainly through blockade of Akt/mTOR and MAPK/ERK signaling cascades. Overall, HDAC1 may serve as a potential therapeutic target for ESCC, and quisinostat deserves to be further assessed as a promising drug candidate for the treatment of ESCC.

Keywords Esophageal squamous cell carcinoma · Quisinostat · Histone deacetylase · Cancer therapy

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Introduction

Esophageal cancer (EC) is one of the most common malignant tumors, ranked the sixth cause of cancer death worldwide [1, 2]. Esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC) are two major histotypes of EC, and the former accounts for the vast majority of EC patients [3, 4]. Surgical resection has been considered as the only way for complete disease control of ESCC. However, most sufferers were diagnosed at advanced stages with metastatic disease, and missed the best time for surgical treatment. For these patients, a multimodality treatment is warranted, and chemotherapy is one of the most important systemic therapies [5, 6]. Although chemotherapeutic targets and agents for ESCC have made much progress recently, the efficacy is still unsatisfactory. Therefore, there is still an unmet medical need among patients with ESCC.

Histone deacetylases (HDACs) regulate the acetylation status of histones and many other non-histone substrates.

Over-expression or aberrant activation of HDACs was detected in various types of human cancers, suggesting that they were associated with tumor progress [7–9]. Inhibition of HDACs was considered as a potential strategy for cancer therapy. To date, several HDAC inhibitors including Vorinostat, Romidepsin, Belinostat, Chidamide, and Panobinostat have been approved for the treatment of T cell lymphoma or multiple myeloma in clinic, and there are still a variety of HDAC inhibitors undergoing clinical trials [10–12]. Min Song and colleagues found that knockdown of HDAC1 by lentivirus mediated shRNA could significantly suppress the growth, migration, and invasion of ESCC cells, and enhance the chemosensitivity of ESCC cells to DNA-damaging drugs [13]. The results of a systematic review and meta-analysis also showed that patients with low HDAC1 expression had better overall survival than those with high HDAC1 expression in gastrointestinal malignancy (including ESCC) [14]. These data highlight HDAC1 as a potential target for ESCC therapy.

In this account, we further detected the expression status of HDAC1 in tissue specimens of ESCC patients and case-matched paracancerous tissues. More importantly, the present study comprehensively evaluated the anti-tumor potency of a highly active HDAC1 inhibitor quisinostat in the treatment of ESCC in vitro and in vivo. These data provide a good basis for the application of quisinostat in ESCC treatment.

Materials and methods

Cell culture and reagents

Human esophageal squamous cell carcinoma cell lines were obtained from National Platform of Experimental Cell Resources for Sci-Tech (China). All tumor cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2 according to standard procedures and passaged for less than 3 months after resuscitation. Quisinostat (JNJ-26481585) was obtained from commercial source.

Cell viability assay

Cells were seeded in 96-well plates in growth media supplemented with 10% fetal bovine serum and cultured overnight at 37 °C. The following day, serial dilutions of quisinostat and DMSO vehicle were added to the designated wells, and cells continued to be incubated at 37 °C for 72 h. MTT assay was then performed to determine the cell viability as described previously [15]. IC_{50} values were calculated by dose-response curve fitting using GraphPad Prism v5.0 software.

Colony formation assay

Cells were seeded in 12-well plates at 5000 cells/well and allowed to adhere overnight at 37 °C. The next day, serial dilutions of quisinostat and DMSO vehicle were added to the designated wells. Cells were incubated at 37 °C for 10 days, and the agents were replaced every 3 days. Cells were then fixed with methanol and stained with crystal violet (0.05%, *w*/*v*).

EdU incorporation assay

Cells growing in 96-well plates were cultured in the presence of quisinostat or DMSO vehicle for 24 h. Cell proliferation was then detected using EdU-Apollo DNA incorporation assay according to the manufacturer's instruction (RIBOBIO, China). The pictures were taken using ArrayScan VTI HCS reader (Thermo Scientific, USA).

Flow cytometry for cell cycle and apoptosis analysis

Cells growing in 6-well plates were treated with serial dilutions of quisinostat or DMSO vehicle for 24 h and harvested. For cell cycle analysis, cells were fixed with 70% ethanol overnight and then incubated with 50 μg/mL propidium iodide (PI), 100 μg/mL RNase and 0.1% Triton X-100 for 30 min in the dark, followed by detection with flow cytometry (Becton Dickinson, USA). For apoptosis analysis, cells were detected using the Annexin V-FITC/PI apoptosis detection kit (KeyGEN Bio TECH, China) following the manufacturer's protocol.

Migration assay

Cells were cultured to confluence in 24-well plates and wounded by scratching with a sterilized yellow pipette tip. Subsequently, cells were treated with serial dilutions of quisinostat or DMSO vehicle for 18 h. Images were captured under an OLYMPUS light microscope.

Transwell invasion assay

The Millicell Hanging Cell Culture Inserts (Millipore, USA) were inserted in 24-well plates and pre-coated with 50 μL diluted Matrigel (BD Biosciences, USA). Cells were seeded in the upper chamber in serum-free medium at 50, 000 cells/100 μL, and then treated with serial dilutions of quisinostat or DMSO vehicle. The complete growth medium containing 10% fetal bovine serum was added to the lower chamber. After incubation at 37 °C for 24 h, the invaded cells were fixed with methanol and stained with 0.05% crystal violet (*w*/*v*).

Western blotting

Cells were incubated in medium containing serial dilutions of quisinostat or DMSO vehicle for 18 h, and then lysed in RIPA buffer (Beyotime, China). Cell extracts were separated by SDS-PAGE, and then transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, USA). Immunoblotting hybridizations for the proteins of interest were performed using the corresponding antibodies. The primary antibodies purchased from Cell Signaling Technology and Abcam were used at 1:1000, and the horseradish peroxidase-coupled secondary antibodies (Zhong Shan Golden Bridge Bio-technology, China) were used at a 1:5000 dilution. Specific proteins were detected using the enhanced chemiluminescene system (Millipore, USA).

Xenograft studies

All animal studies were approved by the Animal Care and Use Committee of Sichuan Academy of Medical Sciences and Sichuan Provincial People's Hospital (Chengdu, Sichuan, China). A suspension of 5×10^6/100 μL KYSE150 cells was inoculated subcutaneously into the hind flank region of each NOD/SCID mouse. Mice were randomized ($n = 5$ each) once the mean tumor volume reached ~250 mm^3. Quisinostat was formulated in 20% hydroxypropyl-β-cyclodextrin, and administered at 3 and 10 mg/kg/day by intraperitoneal injection. Tumors and the body weight of mice were monitored every 3 days. Tumor volume was calculated as $(a^2 \times b)/2$ (in which a represents the width and b the length). The inhibition rate of tumor growth was calculated using the following formula: $100 \times \{1 - [(\text{tumor volume}_{\text{final}} - \text{tumor volume}_{\text{initial}})$ for the compound-treated group] / $[(\text{tumor volume}_{\text{final}} - \text{tumor volume}_{\text{initial}})$ for the vehicle-treated group]}.

Tissue microarray and immunohistochemical staining

Esophageal cancer and paracancerous tissue microarray sections were prepared by Wuhan Servicebio Co., Ltd., and used for IHC staining analysis. Moreover, for the immunohistochemical staining of xenografts, tumors were fixed with 4% paraformaldehyde and embedded in Paraffin. Tumor sections were subjected to immunostaining with Ki67 and TUNEL. The representative pictures were captured under a light microscope (Leica, Germany).

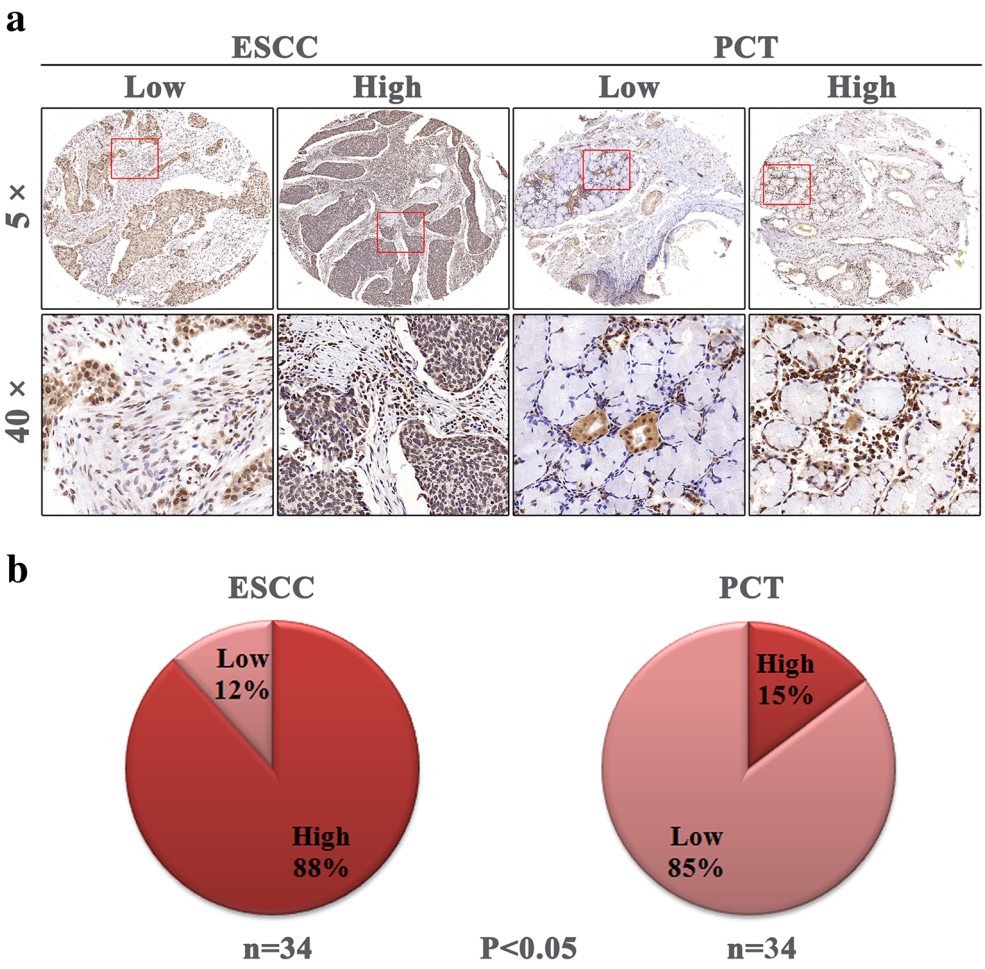

Fig. 1 Expression of HDAC1 in human ESCC and matched adjacent tissues. a Representative cores of HDAC1 staining on tissue array. ESCC, esophageal squamous cell carcinoma; PCT, para-cancerous tissue. **b** Statistical analysis of HDAC1 staining in human ESCC and paracancerous specimens

Statistical analysis

Statistical analysis was performed using GraphPad Prism v5.0 software. Data were presented as mean ± SD, and analyzed by ANOVA and Student's t test. $P < 0.05$ was considered significantly different.

Results

Expression of HDAC1 in esophageal squamous cell cancer and paracancerous tissues

To study the expression status of HDAC1 in human ESCC specimens, a tissue array containing 34 ESCC tissue specimens and matched paracancerous tissues (PCT) were analyzed by immunohistochemistry. The representative images of HDAC1 staining on tissue chip were shown in Fig. 1a. The pattern of HDAC1 expression was statistically different between ESCC and PCT ($P < 0.05$) (Fig. 1b). The high expression rate of HDAC1 in ESCC samples (88%) was significantly higher than that in PCT samples (15%). These results indicated that HDAC1 might be upregulated in tumorigenesis process of esophageal cancer, and might serve as a target for ESCC therapy.

Quisinostat inhibits ESCC cell viability and proliferation in vitro

As a highly active inhibitor of HDAC1, quisinostat (Fig. 2a) is used for further pharmacodynamic study to evaluate whether it is an active agent in the treatment of ESCC. As shown in Fig. 2b, quisinostat potently inhibited the viability of ESCC cell lines KYSE150 and TE-1 with IC_{50} values of 0.115 μM and 0.0728 μM, respectively. To complement the results from short-term treatments, long-term colony formation assay was then performed to visually assess the cytoreductive activity of quisinostat. As indicated in Fig. 2c, both KYSE150 and TE-1 cell colonies were significantly reduced by quisinostat at concentrations higher than 0.1 μM. Similar results were also observed in Edu cell proliferation assay, in which quisinostat at a concentration of 1 μM could markedly decrease the number of proliferating KYSE150 and TE-1 cells (red nuclei) (Fig. 2d). Taken together, these data indicated that HDAC1 inhibitor quisinostat had inhibitory potency against the growth of ESCC cells.

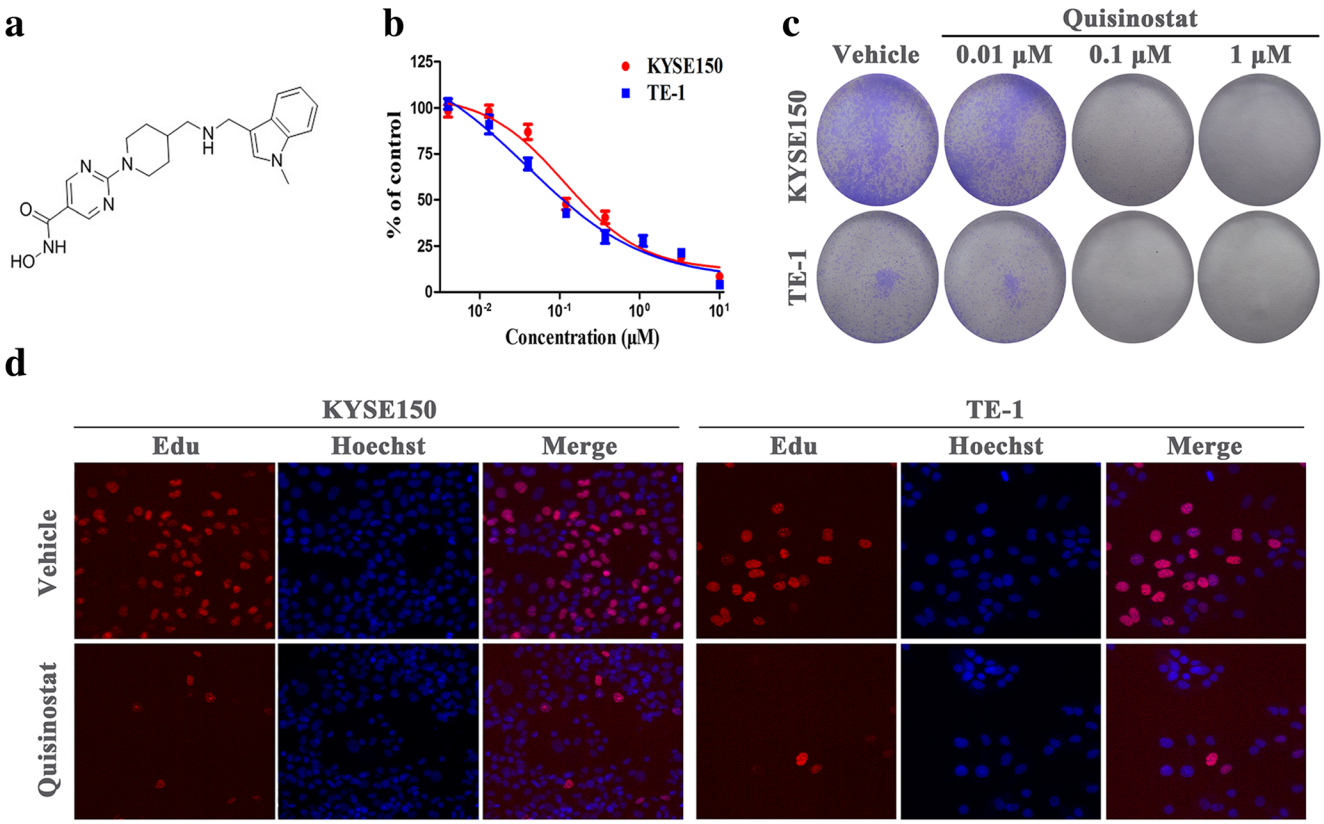

Fig. 2 Quisinostat inhibits the growth of ESCC cell lines in vitro. **a** structure of quisinostat. **b** Anti-viability assay of quisinostat against ESCC cells. Points, mean values; bars, SD. **c** Long-term colony formation assay of quisinostat-treated ESCC cells. **d** Edu cell proliferation assay on ESCC cells after treatment with 1 μM quisinostat for 24 h

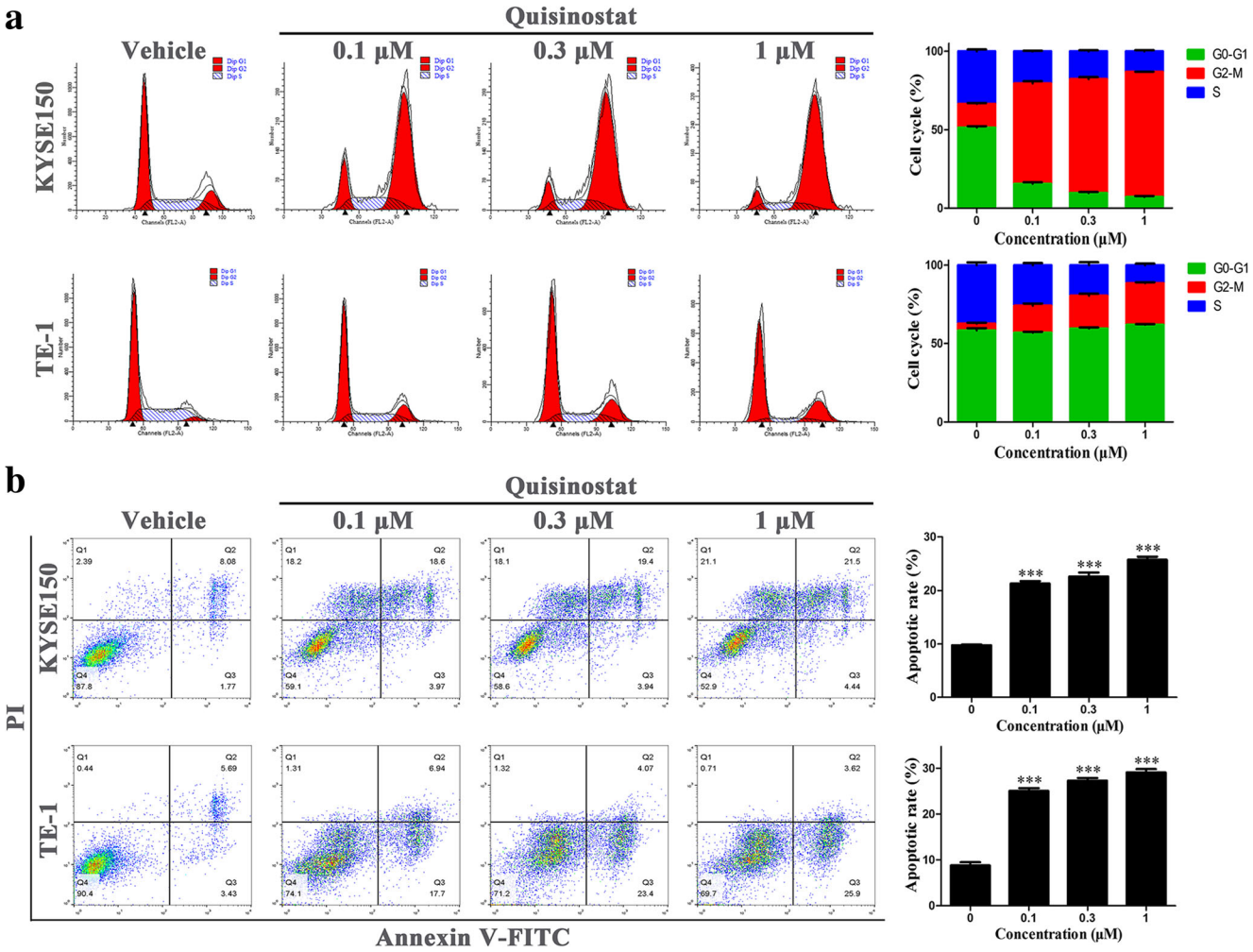

Fig. 3 Quisinostat induces G2/M phase arrest and apoptosis in ESCC cells. **a** Influence of quisinostat on cell cycle progression in KYSE150 and TE-1 cells. The statistical analysis of cell cycle is presented on the right, and data shown in the histogram are means ± SD from three independent experiments. **b** Apoptosis detection was performed using Annexin V/PI co-staining. The assays are performed in triplicate, and the percentage of Annexin V-positive cells is quantified for apoptotic rate statistics. ***, $P < 0.001$ vs vehicle

Quisinostat induces G2/M phase arrest and apoptosis in ESCC cells

We performed flow cytometry on quisinostat-treated ESCC cells to examine its role in cell cycle progression and apoptosis. Cell cycle analysis showed that both KYSE150 and TE-1 cells treated with quisinostat displayed an increased cell ratio in G2/M phase, especially in KYSE150 cells, demonstrating that quisinostat could induce cell-cycle arrest of ESCC cells in G2/M phase (Fig. 3a). Moreover, in apoptosis detection, quisinostat obviously increased the Annexin V-positive populations, with apoptotic rate of 21.3%, 22.6 and 25.8% for 0.1 μM, 0.3 μM, and 1 μM treatment group, respectively, in KYSE150 cells, and 25.0%, 27.3 and 29.1% for 0.1 μM, 0.3 μM, and 1 μM treatment group, respectively, in TE-1 cells (Fig. 3b). To sum up, quisinostat could effectively induce cell-cycle arrest and apoptosis in ESCC cells.

Quisinostat inhibits ESCC cell migration and invasion

HDAC1 is closely related to tumor metastasis. We further assessed the ability of quisinostat to inhibit cell migration and invasion (pivotal steps of tumor metastasis) in ESCC cells. The inhibitory effect of quisinostat against cellular migration was evaluated by wound healing assay. As despicted in Fig. 4a, the number of migrated cells was significantly diminished by quisinostat as compared with vehicle. Meanwhile, in transwell invasion assay, we also observed that quisinostat strongly suppressed the invasion of both KYSE150 and TE-1 cells in a dose-dependent manner, especially at concentrations higher than 0.3 μM ($P < 0.001$) (Fig. 4b). These data demonstrated that quisinostat also had the activity of inhibiting migration and invasion of ESCC cells.

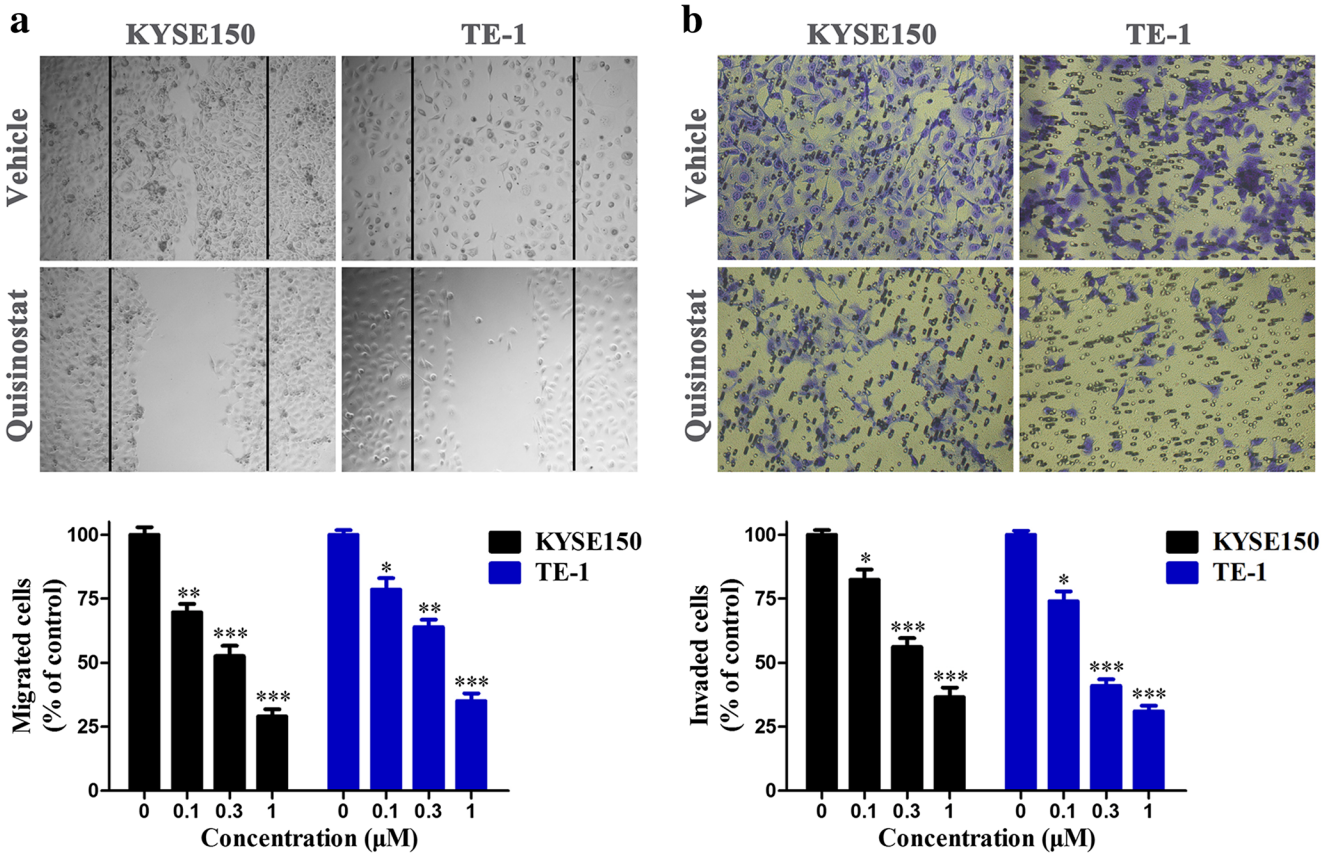

Fig. 4 Quisinostat inhibits ESCC cell migration and invasion in vitro. **a** The representative images (20×) from vehicle and 1 μM quisinostat treated groups in wound healing assay. The index lines in the migration assay indicate the original scratch position. The number of migrated cells is used for statistics and quantified by manual counting. **b** The representative images (20×) from vehicle and 1 μM quisinostat treated groups in transwell invasion assay. The number of invaded cells is counted for statistics. Column, mean; bars, SD ($n = 3$); *, $P < 0.05$ vs. vehicle; **, $P < 0.01$ vs. vehicle; ***, $P < 0.001$ vs. vehicle

Molecular effects of quisinostat in ESCC cells

Western blot analysis was performed to detect the molecular effects of quisinostat, which may account for its anti-tumor activity in ESCC cells. As indicated in Fig. 5, quisinostat significantly induced the acetylation of HDAC substrate H3K9 even at 0.1 μM. The expression of cell cycle inhibitor p21, which is often epigenetically repressed in cancer, was also obviously up-regulated after treatment with quisinostat in both KYSE150 and TE-1 cells. Additionally, quisinostat at concentrations higher than 0.1 μM showed potent inhibitory effect against the phosphorylation of Akt and ERK, the pivotal proteins of PI3K/mTOR and MAPK signaling cascades. These data suggested that quisinostat might exert its anti-ESCC effects mainly through blockade of Akt/mTOR and MAPK/ERK signaling cascades.

Antitumor efficacy of quisinostat in human ESCC xenograft model

We further assessed the in vivo anti-tumor effect of quisinostat in KYSE150 xenograft model. Once daily intraperitoneal injection administration of quisinostat at 3 mg/kg and 10 mg/kg

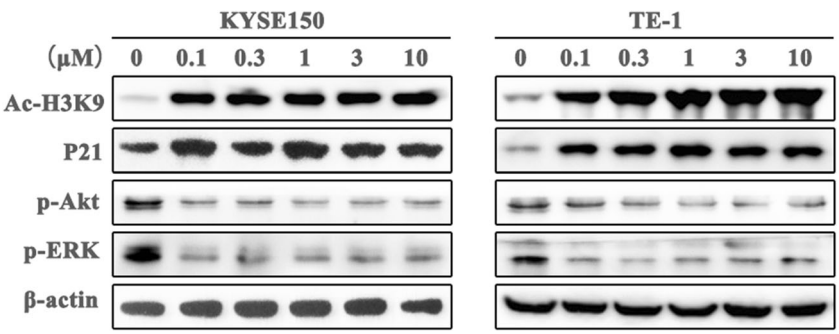

Fig. 5 Molecular effects of quisinostat treatment in ESCC cells. KYSE150 and TE-1 cells were treated with quisinostat or applicable amount of the solvent DMSO for 18 h, then cells were lysed and the proteins were analyzed by immunoblot

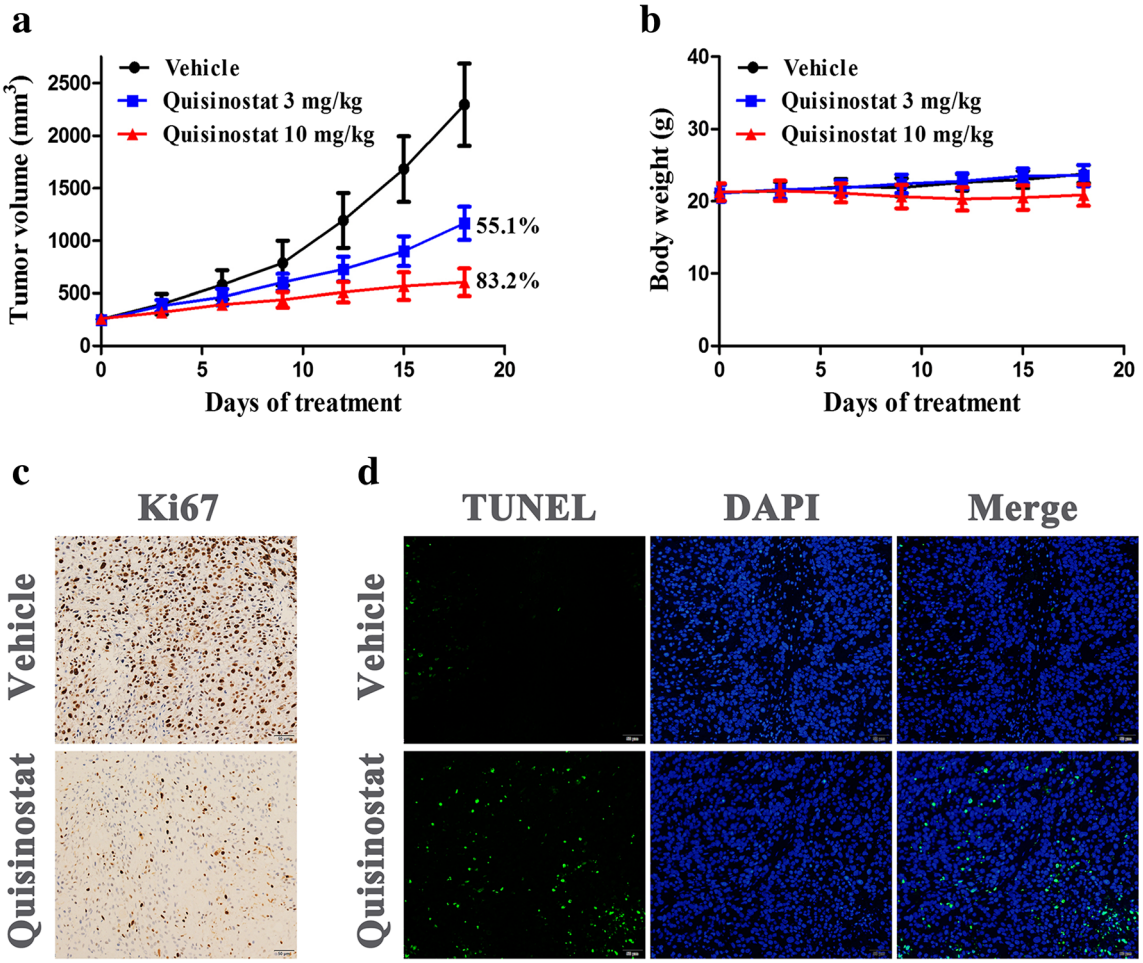

Fig. 6 Pharmacodynamic studies of quisinostat in vivo. **a** Tumor volumes of KYSE150 xenograft model. Points, mean tumor volume; bars, SD ($n = 5$). **b** Body weights of KYSE150 xenograft model. Points, mean body weight; bars, SD (n = 5). **c** Ki67 immunohistochemical staining of the tumors from vehicle control and 10 mg/kg quisinostat treatment groups in KYSE150 model. **d** Apoptosis induction of quisinostat was detected by TUNEL staining in KYSE150 model. Paraffin sections of tumor tissues from vehicle control and 10 mg/kg quisinostat treatment groups were used for analysis

potently restrained the tumor growth in a dose-dependent manner, with tumor growth inhibition rate of 55.1 and 83.2%, respectively (Fig. 6a). No significant weight loss was observed in all treatment groups during the experiment (Fig. 6b). In addition, immunohistochemical staining assays were performed to detect cellular proliferation and apoptosis in vivo. Consistent with in vitro data, quisinostat also led to a significant decrease in proliferating tumor cells (Ki67-positive cells) and substantial increase in apoptotic cells (TUNEL-positive cells) in xenografts (Fig. 6c, d). Taken together, quisinostat could also exert its anti-ESCC effects in vivo through inhibiting proliferation and inducing apoptosis.

Discussion

Accumulating evidence from the literature indicated that epigenetic mechanisms were involved in tumor progression in addition to genetic alterations, and aberrant acetylation in particular has been implicated in carcinogenesis [16–18]. HDACs catalyze the remove of the acetyl group from lysine residues of proteins; such change causes chromatin condensation, and alters the transcription of tumor suppressor genes and oncogenes. Additionally, HDACs also deacetylated various non-histone substrates that regulate a wide range of biological processes in cancer initiation and progression [11, 18]. Aberrant expression of HDACs has been linked to a variety of malignancies. For example, HDAC1 was highly expressed in hormone receptor-positive breast tumor, and HDAC2 and 3 were highly expressed in poorly differentiated and hormone receptor negative breast tumors [19]; HDAC4 was up-regulated in gastric tumor cells compared with adjacent normal tissues, and inhibition of HDAC4 increased the level of cleaved caspases 3 and 9, and exhibited a synergistic effect with docetaxel for gastric tumor treatment [20]. The results of the tissue chip detection in this study showed that the expression of HDAC1 in ESCC tissue specimens was

generally higher compared with the corresponding paracancerous tissues. This result strongly suggested that HDAC1 might serve as a therapeutic target for ESCC.

HDAC inhibitors have shown activity in clinical treatment of hematologic malignancies. However, single agent activity of this class of agents against solid tumor was not impressive due to their transient pharmacodynamic responses and suboptimal potency [21]. Quisinostat, also known as JNJ-26481585, is a novel generation of hydroxamic acid-based inhibitor of histone deacetylase (HDAC) with superior pharmacodynamic properties. Previous research showed that quisinostat had strong inhibitory activity against all class I HDAC enzymes, especially for HDAC1 with IC_{50} value of 0.11 nM [21]. Therefore, it was chosen for further anti-ESCC pharmacodynamic study. The results showed that inhibition of HDACs with quisinostat could effectively suppress cellular viability and proliferation of ESCC cells, as well as induce cell cycle arrest and apoptosis in vitro. The effectiveness was also observed in KYSE150 xenograft model when quisinostat was administered at tolerated doses (3 mg/kg and 10 mg/kg). Furthermore, it is worth mentioning that metastasis is an independent prognostic factor for ESCC, and quisinostat also had the ability to restrain the migration and invasion (pivotal steps of tumor metastasis) of ESCC cells.

The molecular mechanisms of anti-tumor effects of HDAC inhibitors are complex and not uniform in different tumor types and even individual inhibitors; this is attributed to their diverse roles in transcription regulation and protein modification, as well as signal transduction. Previous studies revealed that HDAC inhibitors regulate multiple signaling pathways associated with tumor progression. It was reported that the phosphorylation of ERK and JNK was decreased following the treatment with HDAC inhibitor in leukemia cells [22]. A study by Peng Zhang and colleagues demonstrated that HDAC inhibitors suppressed the proliferation of gallbladder carcinoma cell via inhibition of Akt/mTOR signaling [23]. The similar molecular mechanisms were also observed in quisinostat-treated ESCC cells, in which the activity of pivotal proteins of MAPK and PI3K/mTOR cascades was significantly down-regulated by quisinostat even at a low concentration (0.1 μM). Although these might not be the full mechanisms of action of quisinostat, the results suggested that the anti-ESCC effects of quisinostat involved multiple molecular mechanisms, and blockade of Akt/mTOR and MAPK/ERK signalings might be the main contributors.

In summary, the major finding of our study was that HDAC1 was a potential target for the treatment of ESCC. HDAC1 inhibitor quisinostat was well tolerated, and potently inhibited the growth of ESCC cells mainly through blockade of Akt/mTOR and MAPK/ERK signalings. Meanwhile, it also had the activity to restrain ESCC cell migration and invasion. Taken together, quisinostat deserves to be further assessed as a potential drug candidate for the treatment of ESCC.

Author contributions L Zhong and S Zhou contributed equally to this article.

Conception and design: L Zhong, S Zhou, LY Su, and Q Peng;
Development of methodology: L Zhong, S Zhou, and RS Tong;
Acquisition of data: L Zhong, S Zhou, and JY Shi;
Analysis and interpretation of data: L Zhong, RS Tong, and Q Peng;
Technical and material supports: YX Zhu, L Bai, XM Duan, WZ Liu, and JK Bao; Writing and review of the manuscript: L Zhong, S Zhou, LY Su, and Q Peng;
Study supervision: RS Tong, LY Su, and Q Peng.

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Compliance with ethical standards

Conflicts of interest The authors declare no conflict of interest.

Ethical approval All procedures performed in studies involving animals were in accordance with the ethical standards of the Animal Care and Use Committee of Sichuan Academy of Medical Sciences and Sichuan Provincial People's Hospital (Chengdu, Sichuan, China).

References

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A (2015) Global cancer statistics, 2012. CA Cancer J Clin 65(2):87–108
2. Arnold M, Soerjomataram I, Ferlay J, Forman D (2015) Global incidence of oesophageal cancer by histological subtype in 2012. Gut 64(3):381–387
3. Liu FT, Gao H, Wu CW, Zhu ZM (2017) The association of plasma fibrinogen with clinicopathological features and prognosis in esophageal cancer patients. Oncotarget 8(54):93029–93038
4. Barret M, Prat F (2018) Diagnosis and treatment of superficial esophageal cancer. Ann Gastroenterol 31(3):256–265
5. Shaikh T, Meyer JE, Horwitz EM (2017) Optimal use of combined modality therapy in the treatment of esophageal cancer. Surg Oncol Clin N Am 26(3):405–429
6. Hsu PK (2018) The era of personalized multimodal treatments for esophageal cancer. Ann Transl Med 6(4):75
7. Goey AK, Sissung TM, Peer CJ, Figg WD (2016) Pharmacogenomics and histone deacetylase inhibitors. Pharmacogenomics 17(16):1807–1815
8. Qin HT, Li HQ, Liu F (2017) Selective histone deacetylase small molecule inhibitors: recent progress and perspectives. Expert Opin Ther Pat 27(5):621–636
9. Zibelman M, Wong YN, Devarajan K, Malizzia L, Corrigan A, Olszanski AJ, Denlinger CS, Roethke SK, Tetzlaff CH, Plimack ER (2015) Phase I study of the mTOR inhibitor ridaforolimus and the HDAC inhibitor vorinostat in advanced renal cell carcinoma and other solid tumors. Investig New Drugs 33(5):1040–1047
10. Wahaib K, Beggs AE, Campbell H, Kodali L, Ford PD (2016) Panobinostat: A histone deacetylase inhibitor for the treatment of relapsed or refractory multiple myeloma. Am J Health Syst Pharm 73(7):441–450
11. Yoon S, Eom GH (2016) HDAC and HDAC inhibitor: from cancer to cardiovascular diseases. Chonnam Med J 52(1):1–11

12. New M, Olzscha H, La Thangue NB (2012) HDAC inhibitor-based therapies: can we interpret the code? Mol Oncol 6(6):637–656
13. Song M, He G, Wang Y, Pang X, Zhang B (2016) Lentivirus-mediated knockdown of HDAC1 uncovers its role in esophageal cancer metastasis and chemosensitivity. J Cancer 7(12):1694–1700
14. Cao LL, Yue Z, Liu L, Pei L, Yin Y, Qin L, Zhao J, Liu H, Wang H, Jia M (2017) The expression of histone deacetylase HDAC1 correlates with the progression and prognosis of gastrointestinal malignancy. Oncotarget 8(24):39241–39253
15. Zhong L, Fu XY, Zou C, Yang LL, Zhou S, Yang J, Tang Y, Cheng C, Li LL, Xiang R, Chen LJ, Chen YZ, Wei YQ, Yang SY (2014) A preclinical evaluation of a novel multikinase inhibitor, SKLB-329, as a therapeutic agent against hepatocellular carcinoma. Int J Cancer 135(12):2972–2983
16. Conte M, De Palma R, Altucci L (2018) HDAC inhibitors as epigenetic regulators for cancer immunotherapy. Int J Biochem Cell Biol 98:65–74
17. Vicente-Dueñas C, Hauer J, Cobaleda C, Borkhardt A, Sánchez-García I (2018) Epigenetic priming in cancer initiation. Trends Cancer 4(6):408–417
18. Eckschlager T, Plch J, Stiborova M, Hrabeta J (2017) Histone deacetylase inhibitors as anticancer drugs. Int J Mol Sci 18(7): E1414
19. Muller BM, Jana L, Kasajima A, Lehmann A, Prinzler J, Budczies J, Winzer KJ, Dietel M, Weichert W, Denkert C (2013) Differential expression of histone deacetylases HDAC1, 2 and 3 in human breast cancer–overexpression of HDAC2 and HDAC3 is associated with clinicopathological indicators of disease progression. BMC Cancer 13:215
20. Colarossi L, Memeo L, Colarossi C, Aiello E, Iuppa A, Espina V, Liotta L, Mueller C (2014) Inhibition of histone deacetylase 4 increases cytotoxicity of docetaxel in gastric cancer cells. Proteomics Clin Appl 8(11–12):924–931
21. Arts J, King P, Mariën A, Floren W, Beliën A, Janssen L, Pilatte I, Roux B, Decrane L, Gilissen R, Hickson I, Vreys V, Cox E, Bol K, Talloen W, Goris I, Andries L, Du Jardin M, Janicot M, Page M, van Emelen K, Angibaud P (2009) JNJ-26481585, a novel "second-generation" oral histone deacetylase inhibitor, shows broad-spectrum preclinical antitumoral activity. Clin Cancer Res 15(22):6841–6851
22. Witt O, Sand K, Pekrun A (2000) Butyrate-induced erythroid differentiation of human K562 leukemia cells involves inhibition of ERK and activation of p38 MAP kinase pathways. Blood 95(7):2391–2396
23. Zhang P, Guo Z, Wu Y, Hu R, Du J, He X, Jiao X, Zhu X (2015) Histone deacetylase inhibitors inhibit the proliferation of gallbladder carcinoma cells by suppressing AKT/mTOR signaling. PLoS One 10(8):e0136193