The TGF- β /Smad Pathway Inhibitor SB431542 Enhances The Antitumor Effect Of Radiofrequency Ablation On Bladder Cancer Cells

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Hong-qing Zhou^{1,*}
Ming-sheng Liu^{1,*}
Ti-bin Deng¹
Ping-bo Xie¹
Wei Wang¹
Tao Shao¹
Yao Wu¹
Peng Zhang²

¹Second Ward of Urology, Qujing Affiliated Hospital of Kunming Medical University, Qujing City 655000, Yunnan Province, People's Republic of China;

²Department of Urology, State Key Laboratory of Kidney Diseases, Chinese People's Liberation Army (PLA) General Hospital/Chinese PLA Medical Academy, Beijing 100853, People's Republic of China

*These authors contributed equally to this work

Correspondence: Ming-sheng Liu
Second Ward of Urology, Qujing Affiliated Hospital of Kunming Medical University, Huayuan Road, Qilin District, Qujing City 655000, Yunnan Province, People's Republic of China
Tel +86 874 3326975
Fax +86 874 3311977
Email 18908741981@189.cn

Peng Zhang
Department of Urology, State Key Laboratory of Kidney Diseases, Chinese People's Liberation Army (PLA) General Hospital/Chinese PLA Medical Academy, No. 28 Fuxing Road, Haidian District, Beijing City 100853, People's Republic of China
Tel +86 10 6693 8008
Fax +86 10 6693 8008
Email alwaysjumper@126.com

Background: Despite progress achieved in bladder cancer (BC) treatment, the prognosis of patients with advanced BC (ie, metastasized from the bladder to other organs) is poor. Although mortality in cases of low-grade BC is rare, the treatment, such as a radical cystectomy, often has a serious impact on the quality of life. Thus, research is needed to identify more effective treatment strategies and this work is aiming to examine the potential application of combination of radiofrequency ablation (RFA) and SB431542, a inhibitor of transforming growth factor β (TGF β)/Smad pathway.

Methods: BC cells were transplanted into nude mice (thymusdeficiency Bal B/c) to form subcutaneous tumors. The mice with subcutaneous tumors were then treated with RFA and oral administration of SB431542, an inhibitor of TGF β /Smad signaling pathway. The antitumor effect of RFA was measured by tumor proliferation curves and micro-positron emission computed tomography (micro-PET). The effect of SB431542 on epithelial–mesenchymal transition (EMT) related regulators in subcutaneous tumor tissues formed by BC cells were examined by quantitative real-time polymerase chain reaction (qPCR) experiments.

Results: The SB431542 treatment enhanced the antitumor effect of RFA on subcutaneous growth of BCs. SB431542 also decreased EMT-related regulators in subcutaneous tumor tissues formed by BC cells in nude mice.

Conclusion: SB431542 enhances the effect of RFA on BC.

Keywords: bladder cancer, radiofrequency ablation, SB431542, epithelial–mesenchymal transition

Introduction

Bladder cancer (BC) is one of the most common human cancers and is associated with high morbidity and mortality rates in the absence of optimal treatment.¹ For primary BC or nonmuscle-invasive BC, the first treatment choice is complete resection of BC tissues (ie, radical treatment).² Induction and maintenance immunotherapy with the Bacillus Calmette–Guérin vaccine or chemotherapies may prevent BC recurrence or lengthen the time to recurrence.^{2,3} Although radical treatments may prolong survival in cases of nonmuscle-invasive BC,⁴ they affect patients' quality of life. In advanced BC, systemic cisplatin-based chemotherapies or immunotherapies may not control the progress of the disease. As a result, the prognosis of patients with advanced BC remains poor.⁵ Therefore, more effective treatment strategies for BC treatment are urgently needed.

Radiofrequency ablation (RFA), which is a kind of interventional therapeutic strategy, involves local ablative therapy to selectively destroy tumor tissues and minimize damage to tissues surrounding the tumor.⁶ RFA is a promising therapeutic strategy and has been widely used in human cancer treatment, such as advanced hepatocellular carcinomas.^{7,8} Although RFA selectively destroys tumor tissues or decelerates the progress of human cancers, the potential for rapid and aggressive recurrence of tumors after incomplete RFA treatment is a major obstacle.^{7,8} It is also impossible to infinitely increase the temperature during RFA, as this would result in serious organ damage. Therefore, research aimed at the development of adjuvant treatment strategies for BC that can improve the safety and effectiveness of RFA therapy is needed.

The ability of RFA to directly target tumor tissue for destruction makes it important in the treatment of BC.⁹ RFA can ablate BC tissues and avoid urinary system damage induced by extensive resection of bladder tissue in radical treatment of BC. There are only a few reports on the application of RFA in BC treatment.¹⁰ Previous studies confirmed that the epithelial–mesenchymal transition (EMT) played an important role in RFA resistance or tumor recurrence after RFA treatment.^{11–13} A combination of RFA with various molecular targeting agents (eg, sorafenib or apatinib) enhanced the antitumor effect of RFA by inhibiting the EMT process.^{7,13} Therefore, inhibition of the EMT is a promising approach to combine with RFA. The transforming growth factor beta (TGFβ)/Smad signaling pathway plays a central role in the EMT process in human cancer cells.^{14,15} In the present study, we used SB431542,¹⁶ an inhibitor of the TGFβ/Smad signaling pathway, to enhance the effect of RFA on BC and examined its antitumor effect in combination with RFA using in vivo and in vitro models.

Materials And Methods

Cell Lines And Agents

Clinical specimens of BC were obtained from five patients with bladder urothelial carcinomas during surgery as part of normal medical care. The specimens were preserved in our lab until used. The collection of the clinical specimens and protocols were in compliance with the Helsinki Declaration. The methods and research were approved by the ethics committee of the First People's Hospital, Qujing City, Yunnan Province, People's Republic of China. Informed written consent was obtained from all the patients. The

represented pathological analysis image of clinical specimens of BC tissues was shown as [Supplemental Figure 1](#). The antitumor agent SB431542 (Cat. No. S1067) was purchased from Selleck Corporation, Houston, Texas, USA.

Cell Culture And Survival Analysis

The BC cell lines, T24, 5637 or RT4, were purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, People's Republic of China), a Chinese government organization containing typical biological samples. The BC cells were cultured in DMEM (Thermo Fisher Corporation, Waltham, MA, USA) with 10% FBS (Thermo Fisher Corporation). SB431542 was initially dissolved in dimethyl sulfoxide and then diluted with DMEM without FBS. To determine the impact of SB431542 on BC cell survival/inhibition, the cells were treated with the following concentrations of SB431542 for 48 hrs: 10 μmol/L, 3 μmol/L, 1 μmol/L, 0.3 μmol/L, 0.1 μmol/L, 0.03 μmol/L, and 0.01 μmol/L. MTT assays were then performed, and the optical density of the cell samples was examined. The inhibition rates of SB431542 on BC cells' survival were calculated using the methods described previously.^{17,18}

Luciferase Experiments

The luciferase reporters (the vectors of luciferase reporters) of TGFβ/Smad signaling pathway, the SRB-Luc or the 3TP-Luc reporters, were gifts from Dr Fan Feng in Research Center for Clinical and Translational Medicine, the 302nd Hospital of Chinese PLA, Beijing, 100039, People's Republic of China. The BC cells, which were transfected with luciferase reporters, were treated with indicated concentration of SB431542. Then, cells were harvested for luciferase activation-examination by using a kit purchased from Promega Corporation (Madison, Wisconsin, USA) following methods described by Yang et al (2013) or Lu et al (2013).^{19,20} The inhibition rate of SB431542 was calculated by using luciferase activation and the IC₅₀ values of SB431542 were calculated based on the inhibition rates.

Quantitative Real-Time Polymerase Chain Reaction (qPCR) Experiments

mRNA was extracted from the BC cells and subcutaneous tumor samples and subjected to reverse transcription or the qPCR in accordance with methods described previously.^{21,22} The primers used in the qPCR experiments are listed in [Table 1](#).

Table I Primers Used in This Work

Targets	Primers	Sequences (5'-3')
β-Actin	Forward Sequence	CTCCATCCTGGCCTCGCTGT
	Reverse Sequence	GCTGTCACCTTCACCGTTCC
E-cadherin	Forward Sequence	AAGGCACGCCTGTCTGAAGCA
	Reverse Sequence	ACGTTGTCCCGGGTGTATCCT
N-cadherin	Forward Sequence	CCTCCAGAGTTTACTGCCATGAC
	Reverse Sequence	GTAGGATCTCCGCACTGATTC
Vimentin	Forward Sequence	AGGCAAAGCAGGAGTCCACTGA
	Reverse Sequence	ATCTGGCGTTCCAGGGACTCAT
Snail	Forward Sequence	TGCCCTCAAGATGCACATCCGA
	Reverse Sequence	GGGACAGGAGAAGGGCTTCTC
Twist	Forward Sequence	GCCAGGTACATCGACTTCCTCT
	Reverse Sequence	TCCATCCTCCAGACCGAGAAGG

Subcutaneous Tumor And RFA Experiments

The BC cells were cultured and harvested to prepare a cell suspension. The cells were then injected into nude mice to form subcutaneous tumors. When the tumor volumes reached 1200–1500 mm³, the mice received RFA treatment.^{7,8} The RFA was performed in accordance with previous methods.⁷ RFA was performed at 65–70°C for 3–5 mins to attenuate the subcutaneous growth of these cells. Subsequently, the mice were treated with different concentrations of SB431542 or a solvent control (control) via oral administration. The antitumor effect of RFA was measured based on the tumor volumes and tumor weights. The volumes were calculated based on tumor length and width in accordance with the methods described previously.^{23,24}

In Vivo Animal Micro-Positron Emission Computed Tomography (micro-PET) Experiments

The BC cells were cultured and then seeded into nude mice to form subcutaneous tumors. The tumors were

then treated by RFA, and the nude mice were analyzed by micro-PET (positron emission computed tomography). The absorbance of ¹⁸F-FDG (fluorodeoxyglucose) by the subcutaneous tumors formed by the BC cells was examined using previously described methods.^{25,26}

Western Blot

The subcutaneous tumor tissues formed by BC cells were harvested for Western blot experiments. The expression level of EMT-related proteins was examined by their antibodies (Abcam Corporation, Cambridge, CB2 0AX, UK). The images of Western blot were quantitative examined by Image J Software (National Institutes of Health, Bethesda, Maryland, USA). GAPDH was chosen as a loading control.

Transwell Experiments

The BC cells were separated from the tumor tissues. The methods have been described previously.^{8,23} Single cells were then separated and analyzed by transwell experiments as described in our previous study.²⁷ SB431542- and RFA-induced inhibition of in vitro invasion or migration was calculated as follows: relative invasion/migration cell number in the control group – relative invasion/migration cell number in the SB431542/relative invasion/migration cell number*100% in the control group.

Ethics Statement

For the usage of cell lines or clinical specimens, the methods, research, protocol or collection of the clinical specimens were approved by the ethics committee of the First People's Hospital, Qujing City, Yunnan Province, People's Republic of China. All experiments were performed in compliance with the Helsinki Declaration. The collection of clinical specimens were the consent of patients by written consent from all the patients. For animal experiments, the methods, protocol or usage of animals were approved by the ethics committee of the First People's Hospital, Qujing City, Yunnan Province, People's Republic of China. All animal studies were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986 and associated guidelines.

Statistical Analysis

Statistical analysis was performed by Bonferroni correction, with or without a two-way analysis of variance using SPSS Software (IBM Corporation, Armonk, NY, USA). The IC₅₀ or EC₅₀ values (50% effective concentration on agents'

inhibition rate or 50% effective concentration on agents' effective rate) were calculated using Origin software, version No 6.1 (OriginLab Corporation, Northampton, MA, USA). A P -value of <0.05 was considered statistically significant.

Results

SB431542 Inhibited The EMT Process In Cultured BC Cell Lines

To examine the effect of SB431542 on BC cells, a patient-derived BC cell line was cultured and treated with the indicated concentrations of SB431542. As shown in Figure 1, SB431542 inhibited the survival and EMT of BC cells. The effect of the SB431542 treatment on the BC

cell lines from the five patients is shown in Table 2. SB431542 inhibited the survival of the BC cells in a dose-dependent manner. In addition, the SB431542 treatment inhibited the EMT process in these cells (Table 3). The effective dose of SB431542 in terms of the EMT in BC cells was much lower than the effective dose of SB431542 on BC cell survival (Tables 2 and 3). To examine the specificity of SB431542, the activation of 3TP-Luc or SRB-Luc, two luciferase reporters of TGF β /Smad was examined. As shown in Supplemental Table 1 and Supplemental Table 2, SB431542 repressed the activation of the two reporters in a dose-dependent manner in BC cells, both in current cell lines or PDCs. Therefore, SB431542 could be used in BC treatment.

Figure 1 The effect of SB431542 on BC cells. A patient-derived BC cell line (patient no. 1) treated with the indicated concentrations of SB431542. The BC cells were harvested for MTT experiments (A) or qPCR experiments (B–D). The inhibition rates or effective rates of SB431542 on BC cell survival (A), E-cadherin (B), N-cadherin (C) and vimentin (D) are shown as the mean $\pm$ SD. * P <0.05 versus a solvent control with SB431542.

Abbreviations: BC, bladder cancer; qPCR, quantitative real-time polymerase chain reaction.

Table 2 The IC₅₀ Values Of SB431542 On BC Cells' Survival

PDC	IC ₅₀ values (μmol/L) of SB431542 on cells' survival
No. 1	4.37±0.35
No. 2	2.52±0.22
No. 3	5.55±0.44
No. 4	3.33±0.10
No. 5	2.67±0.66
Averaged data	3.69±1.27

Abbreviations: PDC, patients derived cells; BC, bladder cancer; IC₅₀, half-effect concentration of inhibition rates.

Table 3 The IC₅₀ Values Of SB431542 On BC Cells' EMT Process

PDC	E-Cadherin	N-Cadherin	Vimentin
	IC ₅₀ Or EC ₅₀ values of SB431542 on cells' EMT		
No. 1	0.33±0.01	0.21±0.02	0.25±0.05
No. 2	0.26±0.01	0.28±0.04	0.30±0.03
No. 3	0.51±0.05	0.32±0.02	0.46±0.04
No. 4	0.25±0.04	0.25±0.04	0.25±0.04
No. 5	0.38±0.03	0.38±0.03	0.38±0.03
Averaged data	0.35±0.10	0.29±0.06	0.33±0.09

Abbreviations: PDC, patients derived cells; BC, bladder cancer; IC₅₀, half-effect concentration of inhibition rates; EC₅₀, half-effect concentration of effective rates; EMT, epithelial-mesenchymal transition.

SB431542 Inhibited The EMT Of BC Cells In Subcutaneous Tumors

To further examine the effect of SB431542 on BC cells, the BC cells obtained from the five patients were injected into nude mice to form subcutaneous tumors. In this patient-derived tumor xenograft model, the mice were treated with various concentrations of SB431542. As shown in [Figure 2](#) and [Table 4](#), SB431542 inhibited subcutaneous growth of BC cells in a dose-dependent manner. Furthermore, SB431542 decreased the expression of N-cadherin or vimentin and enhanced the expression of E-cadherin in subcutaneous tumors formed by the BC cells ([Figure 3](#)). The SB431542 treatment inhibited the EMT in the BC cells in these subcutaneous tumors. Among the various concentrations of SB431542, concentrations of 0.3 mg/kg, 1 mg/kg, 3 mg/kg and 10 mg/kg inhibited the EMT process in the BC cells in subcutaneous tumors ([Figures 2 and 3](#), [Tables 4 and 5](#)). A concentration of 1 mg/kg of SB431542 which could significantly inhibit the EMT process of BC cells was selected for use in subsequent experiments.

SB431542 Enhanced The Antitumor Effect Of RFA And Inhibited The EMT In BC Cells In Subcutaneous Tumors

The BC cells were subcutaneously injected into nude mice. The results are shown in [Figure 4](#). As depicted in [Figure 4](#), the RFA treatment resulted in shrinkage of the subcutaneous tumors formed by the BC cells. Administration of a 1 mg/kg dose of SB431542 enhanced the antitumor effect of RFA. The expression of EMT-related factors in subcutaneous tumor tissues was then examined by qPCR experiments and similar results were obtained from Western blot ([Supplemental Figure 2](#) and [Supplemental Figure 3](#)). The images of Western blot are shown as [Supplemental Figure 2](#) and the results from quantitative analysis are shown as [Supplemental Figure 3](#). Moreover, as shown in [Figure 5](#), the RFA treatment induced the EMT process of cells in subcutaneous tumors, and the SB431542 treatment inhibited the EMT process induced by RFA. To further examine the antitumor effect of RFA on tumor tissues, micro-PET screening was performed to determine the vitality of the BC cells in the tumor tissues. As shown in [Figure 6](#), the RFA treatment significantly inhibited the vitality of these cells, and a dose of 1 mg/kg of SB431542 enhanced the effect of RFA.

To further examine the antitumor effect of SB431542, the cells were isolated from the subcutaneous tumors formed by the BC cells ([Figure 4](#)) for transwell assays. As depicted in [Figure 7](#), both the SB431542 treatment and RFA treatment attenuated in vitro invasion or migration of single cells separated from the tumor tissues, and SB431542 enhanced the effect of RFA. Next, the in vivo growth of the single cells separated from the tumor tissues seeded into nude mice was examined. As presented in [Figure 8](#), the RFA treatment decreased the subcutaneous growth of the cells, and the SB431542 treatment enhanced the effect of RFA on the BC cells. Thus, SB431542 enhanced the antitumor effect of the RFA treatment.

Discussion

The important role of RFA in local therapy for advanced HCC treatment is well known.²⁸ Recently, RFA has also been used to treat other cancers, such as non-small-cell lung cancer.²⁹ However, previous studies demonstrated tumor recurrence after RFA treatment, as well as incomplete ablation, which can induce cellular stress and lead to pathological changes, such as the EMT.^{11–13} The same studies reported that EMT was related to RFA resistance or

Figure 2 The effect of SB431542 on subcutaneous growth of BC cells. A patient-derived BC cell line (patient no. 1) was injected into nude mice to form subcutaneous tumors. The mice were treated with the indicated concentrations of SB431542 via oral administration. Results of effect of SB431542 on BC cells' subcutaneous growth were shown as photographs (A), tumor volumes (B) and tumor weights (C). * $P < 0.05$.

Abbreviation: BC, bladder cancer.

Table 4 The IC₅₀ Values Of SB431542 On Subcutaneous Tumor Formed By BC Cells

PDX	IC ₅₀ values (mg/kg) of SB431542 on cells' survival
No. 1	1.24±0.45
No. 2	0.93±0.22
No. 3	1.10±0.30
No. 4	1.46±0.67
No. 5	1.02±0.35
Averaged data	1.15±0.20

Abbreviations: PDX, patient-derived tumor xenograft; BC, bladder cancer; IC₅₀, half-effect concentration of inhibition rates.

recurrence of tumors after RFA. The TGF β signaling pathway is one of the foremost mediators of the EMT,^{15,16} and the TGF β /Smad signaling pathway plays an important role in many important physiological processes, such as neuromuscular regulation and bone metabolism.^{30–32} The specificity of SB431542 was examined by luciferase experiments: treatment of SB431542 inhibited the activation of 3TP-Luc or SRB-Luc in a dose-dependent manner. The 3TP-Luc or SRB-Luc is the common and widely accepted tools to examine the activation of TGF β /Smad pathway.^{33–35} The findings of the present study suggest that inhibition of

Figure 3 The effect of SB431542 on the EMT process in BC cells in subcutaneous tumors. A patient-derived BC cell line (patient no. 1) was injected into nude mice to form subcutaneous tumors. The mice were treated with the indicated concentrations of SB431542 via oral administration. Tumors were harvested for qPCR experiments. The inhibition rates or effective rates of SB431542 on tumor weights (A), E-cadherin (B), N-cadherin (C) and vimentin (D) are shown as the mean $\pm$ SD. * P <0.05.

Abbreviations: BC, bladder cancer; EMT, epithelial–mesenchymal transition; qPCR, quantitative real-time polymerase chain reaction.

Table 5 IC₅₀ Values Of SB431542 On Subcutaneous Tumor Formed By BC Cells' EMT

PDX	E-Cadherin	N-Cadherin	Vimentin
	IC ₅₀ or EC ₅₀ values (mg/kg) of SB431542 on cells' EMT		
No. 1	0.11 $\pm$ 0.00	0.20 $\pm$ 0.03	0.15 $\pm$ 0.02
No. 2	0.15 $\pm$ 0.02	0.25 $\pm$ 0.01	0.22 $\pm$ 0.03
No. 3	0.25 $\pm$ 0.03	0.16 $\pm$ 0.02	0.24 $\pm$ 0.05
No. 4	0.08 $\pm$ 0.01	0.11 $\pm$ 0.02	0.10 $\pm$ 0.01
No. 5	0.13 $\pm$ 0.02	0.21 $\pm$ 0.04	0.13 $\pm$ 0.02
Averaged data	0.14 $\pm$ 0.06	0.19 $\pm$ 0.05	0.17 $\pm$ 0.06

Abbreviations: PDX, patient-derived tumor xenograft; BC, bladder cancer; IC₅₀, half-effect concentration of inhibition rates; EC₅₀, half-effect concentration of effective rates; EMT, epithelial–mesenchymal transition.

activation of the TGF β /Smad signaling pathway is a promising approach to enhance the antitumor effect of RFA.

In the present study, we established an RFA model based on subcutaneous growth of BC cells derived from BC patients. Subsequent treatment with SB431542 inhibited the EMT of BC cells and enhanced the antitumor effect of RFA in multi-models, eg, subcutaneous tumors. These findings point to the potential importance of SB431542 as an adjuvant in antitumor therapy. A urothelial carcinoma, also known as a transitional-epithelial tumor carcinoma, is the most common pathological subtype of BC.^{36–38} Given the histological features (epithelial source/origin) of urothelial carcinomas, the EMT may be

Figure 4 The effects of a combination of SB431542 and RFA on subcutaneous growth of BC cells. A patient-derived BC cell line (patient no. 1) was injected into nude mice to form subcutaneous tumors. RFA of the tumor tissues was performed, and the mice were treated with SB431542 at a dose of 1 mg/kg via oral administration. The results are shown as photographs (A) of subcutaneous tumors, tumor-growth curves (B) or tumor weights (C) at the end of the experiment. * $P < 0.05$.

Abbreviations: BC, bladder cancer; RFA, radiofrequency ablation.

closely related to the occurrence and progression of BC. The EMT may also play a role in BC cell tolerance to treatment.^{39,40}

Radical treatment for BC, such as a cystectomy, can markedly prolong the survival of BC patients.^{1–3} However, both total and partial bladder resection affect the quality of life of the patient.^{1–3} In advanced BC, where metastasis or invasion has occurred, surgical treatment may be contraindicated.^{1–3} In this setting, RFA can play a role by precisely targeting and destroying local tumor tissue and reducing damage to tissues surrounding the tumor.^{7,8} There are few reports on the application of RFA in BC. The present study established a relevant research model that not only contributes to RFA-related research on BC but also to clinical treatment selection.

The methodological approach used in this study is transferable to the clinical setting. We also determined the weights of the subcutaneous tumors and generated

tumor-growth curves, which quantitatively reflected the antitumor effects of RFA on tumor tissues. Furthermore, we used micro-PET, which is an effective in vivo imaging technique, to visually reflect the effect of RFA on the viability of tumor tissues. Further isolation/separation of cells from the tumor tissue and subsequent analysis further confirmed the antitumor effect of RFA and SB431542. It is reported that, some molecular targeting agents, including aorafenin or apatinib, could inhibit the EMT of cancer cells.^{41–45} However, these agents were targeting to the receptor tyrosine protein kinases represented by vascular endothelial growth factor receptor.^{46–52} Therefore, the inhibitor of TGF/Smad pathway may be a more useful or specific strategy to enhance the efficiency of RFA via repressing EMT process.

It is worth mentioning that the RFA conditions used in this study are similar to those of incomplete ablation. Thus, RFA can change the characteristics of tumor cells

Figure 5 The effects of a combination of SB431542 and RFA on the EMT in subcutaneous tumor cells. A patient-derived BC cell line (patient no. 1) was injected into nude mice to form subcutaneous tumors. RFA of the tumor tissues was performed, and the mice were treated with SB431542 at a dose of 1 mg/kg via oral administration. Tumors were then harvested for qPCR experiments. The mRNA levels of E-cadherin (A), N-cadherin (B), vimentin (C), ZEB1 (D), twist (E) and Snail (F) in tumor tissues are shown as scatter diagrams. * $P < 0.05$.

Abbreviations: BC, bladder cancer; RFA, radiofrequency ablation; EMT, epithelial-mesenchymal transition; ZEB1, zinc finger E-box binding homeobox 1.

Figure 6 Results of the micro-PET analysis of the combined effect of SB431542 and RFA on subcutaneous growth of BC cells. A patient-derived BC cell line (patient no. 1) was injected into nude mice to form subcutaneous tumors. RFA of the tumor tissues was performed, and the mice were treated with SB431542 at a dose of 1 mg/kg via oral administration. The results of micro-PET at particular time points, showing (A) images of micro-PET, (B) tumor areas and (C) tumor intensity. In the micro-PET images, the arrows indicate the location of the tumor tissues (A). * $P < 0.05$ versus control group with RFA treatment group; # $P < 0.05$ versus RFA treatment group with RFA+SB431542 treatment group.

Abbreviations: BC, bladder cancer; qPCR, quantitative real-time polymerase chain reaction; RFA, radiofrequency ablation; PET, positron emission computed tomography.

and induce the EMT process in tumor cells while inducing tumor cell injury and delaying the growth of tumor tissues. The tumor model established herein was a subcutaneous

tumor model. Future research will focus on how to establish bladder in situ tumors or simulate the characteristics of bladder organs.

Figure 7 In vitro invasion or migration of BC cells separated from subcutaneous tumors. The subcutaneous tumor tissues (Figure 4) were harvested, and single cells were separated. Transwell experiments were then performed to determine in vitro invasion or migration. Photographs of in vitro invasion (A) and migration (B). The results are the mean $\pm$ SD. * P <0.05.

Abbreviation: BC, bladder cancer.

Figure 8 Subcutaneous growth of single cells separated from subcutaneous tumors. Single cells were separated from subcutaneous tumors (Figure 4). The cells were seeded into nude mice to form subcutaneous tumors. Photographs showing the (A) tumor volumes (B) and tumor weights (C). * $P < 0.05$.

Abbreviations: BC, bladder cancer; RFA, radiofrequency ablation.

Conclusion

Only a few previous studies have reported antitumor effects SB431542 on BC.^{53–55} In the present study, SB431542 inhibited the effects of the RFA-induced EMT and upregulated the antitumor effect of RFA. Thus, SB431542 enhanced the effect of RFA on BC. The results indicate that SB431542 combined with RFA is a new and promising treatment strategy for BC.

Author contributions

All authors made substantial contributions to the design and conception, acquisition, analysis or interpretation of data. All authors took part in either drafting or revising the manuscript. All authors also gave final approval of the version to be published and agree to be accountable for all aspects of the work in ensuring that questions related to

the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Disclosure

The authors report no conflicts of interest in this work.

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