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C188-9 reduces patient-specific primary breast cancer cells proliferation at the low, clinic-relevant concentration

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Abstract

Objectives STAT3 is a transcriptional activator of breast cancer oncogenes, suggesting that it could be a potential therapeutic target for breast cancer. Therefore, this study investigated the potential application of C188-9, a STAT3 signal pathway inhibitor, in the treatment of breast cancer through a novel pre-clinical platform with patient-specific primary cells (PSPCs).

Methods PSPCs were isolated from breast cancer samples obtained via biopsy or surgery from fifteen patient donors with their full acknowledgements. PSPCs were treated with C188-9 or other chemotherapeutic agents, and then analyzed with cell viability assay. Western blot assay and real-time quantitative PCR were also used to determine the expression and activity of STAT3 signaling pathway of corresponding PSPCs.

Results C188-9 treatment at normal (experimental) concentration had valid inhibition on PSPCs proliferation. Meanwhile, treatment at a low (clinic-relevant) concentration of C188-9 for an extended period reduced cell viability of PSPCs still more than some of other traditional chemotherapy drugs. In addition, C188-9 decreased expression level of pSTAT3 in PSPCs from some, but not all patient samples. The treatment of C188-9 reduced cell viability of the breast cancer samples through inhibiting the STAT3 to C-myc signaling pathway.

Conclusions In this study, we tested a novel drug C188-9 at a low, clinic-relevant concentration, together with several traditional chemotherapy agents. PSPCs from ten out of fifteen patient donors were sensitive to C188-9, while some of traditional chemotherapy agents failed. This finding suggested that C188-9 could have treatment effects only on those ten PSPC patient donors, indicating the future personalized utilization of PSPCs.

Keywords C188-9, STAT3, Breast cancer, PSPCs

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Introduction

Breast cancer is the most common malignant tumor in females worldwide, and its incidence is still steadily increasing in recent years [1, 2]. Although recent medical technology has significantly increased the 5-year survival rate of many types of breast cancer, there are still 20–30% of patients with recurrent or metastatic diseases, subsequent serious complications, and even death after treatment [3, 4]. According to Dent et al. [5], the 5-year survival rate of patients with recurrent breast cancer is only about 60%. The high mortality is caused by malignant proliferation, distant metastasis, chemoresistance, and recurrence [6]. Therefore, more effective therapeutic regimens are needed for the treatment of breast cancer and the improvement of clinical outcomes.

Transcription factors (TFs) are proteins possessing domains that bind to the DNA promoter or enhancer regions of specific genes. Several TFs are directly involved in the development and progression of breast cancer. One of the most prominent TF families in breast cancer is the signal transducers and activators of transcription (STAT) family, which is comprised of seven structurally similar and highly conserved members, namely, STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6 [7]. Among them, STAT3 plays a crucial role in cell proliferation, dedifferentiation, motility, apoptosis, and tumorigenesis [8]. STAT3 regulates several oncogenes, and affects breast cancer progression, proliferation, apoptosis, metastasis, and chemoresistance. Intriguingly, various upstream regulators and downstream target genes have been newly discovered, suggesting potential targets that could be used for breast cancer therapy [9]. IL-8 and growth regulated oncogene chemokines are found to activate STAT3 and promote inflammatory breast cancer [10]. Notably, several new STAT3 specific inhibitors were found in recent years. Treatments with STAT3 inhibitors alone or combined with other clinical therapeutic drugs may provide promising effects on suppressing or reversing chemoresistance in breast cancer, especially for breast cancer patients suffering from doxorubicin or capecitabine resistance [9]. However, no STAT3 inhibitor has been used clinically thus far.

C188-9, a novel and effective STAT3 inhibitor targeting the SH2 domain of STAT3, was reported to inhibit granulocyte-colony stimulating factor-induced STAT3 phosphorylation, and induce apoptosis of acute myeloid leukemia cell lines and primary samples [11]. C188-9 showed antitumor activity in hepatocellular carcinoma [12], head and neck squamous cell carcinoma [13], non-small-cell lung cancer [14], and pancreatic cancer [15]. In addition, C188-9 alleviated endothelial-mesenchymal transition and liver fibrosis via targeting the STAT3-MKL1-TWIST1 axis [16]. To the best of our knowledge, there were no reports on the treatment of breast cancer with C188-9. In this study, we demonstrated that C188-9 at clinical-relevant concentrations reduced cell viability, and altered related phosphorylated STAT3 (pSTAT3) expression of patient-specific primary cells (PSPCs), freshly isolated via biopsy or surgery from breast cancer patients, in a patient-specific manner. These results indicated the potential use of C188-9 as a novel drug for the clinical treatment of breast cancer.

Materials and methods

Patient samples

Patient tumor samples were obtained via biopsy or surgery from the Cancer Hospital, Medical College of Shantou University with full patient acknowledgments. Based on the choice of the patient and corresponding doctor, if the neoadjuvant therapy needs to be performed, then a needle biopsy was adopted to determine the pathological type before treatment. However, patients who did not need neoadjuvant therapy would be directly treated with surgery, and tumor sampling was collected during the surgery. There was none of drug treatment performed before sample collection. Fresh tumor samples were handled using the PSPC preparation medium provided by Guangdong Procapzoom Biosciences, Inc., Guangzhou, China. The procedure of PSPC generation was showed in Fig. 1A. Briefly, samples were washed five times in washing buffer, then diced into small pieces and mixed with digesting buffer for 15 min at 37 °C. After filtration, the supernatant was then cultured in an incubator at 37 °C with 5% CO_2 using the proprietary medium for 1–2 weeks to obtain PSPCs. PSPCs were cultured in flask (#TCF001025, Jet Bio-Filtration Co., Guangzhou, China) as 2D form. Before seeding PSPCs, the flask needed to be coat with gelatin (#48722, Sigma-Aldrich, Shanghai, China) for 30 min at 37℃.

Reagents and antibodies

C188-9 (432001-19-9) and Romidepsin (128517-07-7) were purchased from Ark Pharm, US. Paclitaxel (33069-62-4), Cyclophosphamide (50-18-0), and Gemcitabine (95058-81-4) were purchased from Aladdin, Shanghai, China; cisplatin was purchased from Innochem, Beijing, China. All drugs were dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich, Shanghai, China) to obtain a stock solution. The stock solution was freshly diluted with the culture medium prior to use. The CCK8 kit (C0039) was provided by Beyotime Biotechnology, Shanghai, China. The Dulbecco's phosphate-buffered solution (DPBS) was provided by Gibco (Thermo Fisher Scientific, China). Western blotting detection reagents were purchased from Millipore (Massachusetts, USA). Primary antibodies included pSTAT3 (GTX118000), STAT3 (GTX104616), Bax (GTX109683) and Bcl-2 (GTX01194) were obtained from GeneTex (Cambridge,

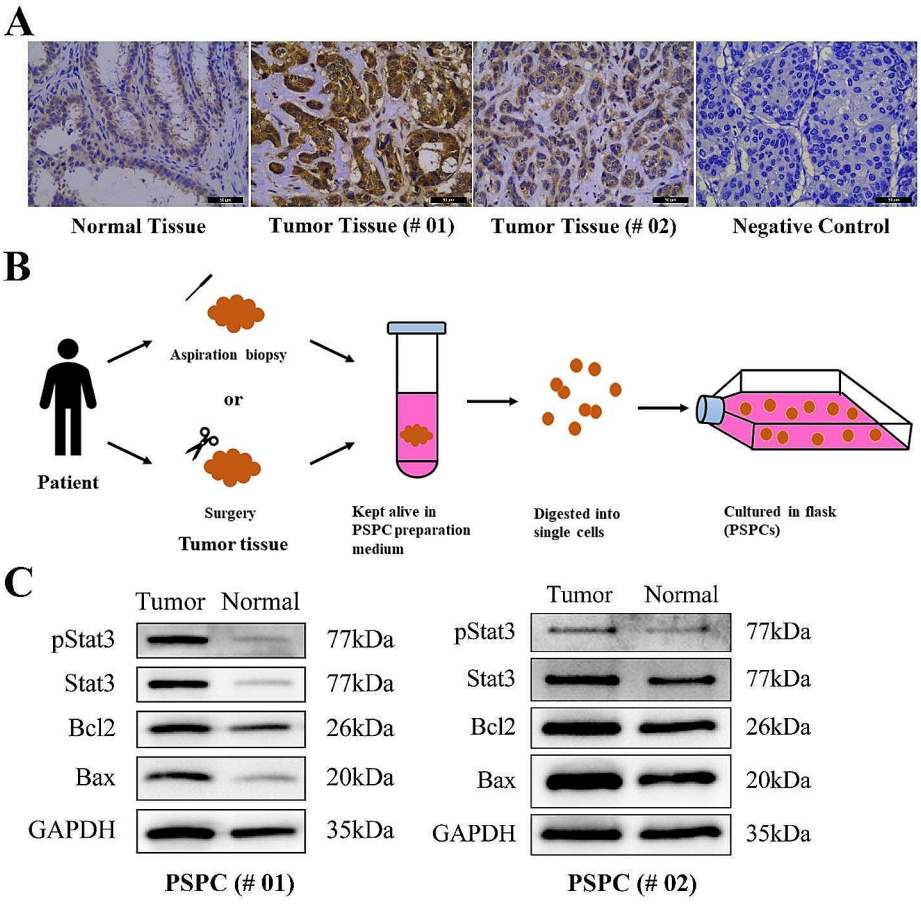

Fig. 1 pSTAT3 expression in breast cancer tissues and correspondinFg PSPCs. (**A**) The immunohistochemical staining of pSTAT3 expression in normal tissue and breast cancer tissues from the patient #01 and #02. (**B**) Procedure of PSPCs generated from the clinical samples and being cultured. (**C**) Western blot assay of phosphorylated STAT3 and other relevant proteins expression of the PSPCs from the two patients

USA). All reagents and solvents used in this study were of analytical grade unless otherwise stated.

Cell viability assays

PSPCs were seeded in a 96-well plate for 4×10^3 cells/well and grown overnight. The cells were changed medium and treated with different drugs for every two days until extended period of 12 days. The cell viability was then analyzed by the CCK8 assay following the instructions of the CCK8 kit. Briefly, after addition of 10 μL CCK8 solution, cells were incubated for two hours at 37 °C in an incubator. The absorbance was then detected with the Microplate Reader PT-3502B (Potenov, Beijing, China) at OD=450 nm.

Western blot

PSPCs were treated with various concentrations of C188-9 or other chemotherapeutic drugs for 48 h, washed twice with ice-cold DPBS, and lysed with RIPA lysis buffer. Protein concentrations were detected with the BCA protein assay kit (Sangon Biotech, Shanghai, China). Equal amounts of protein were applied to 5–20% polyacrylamide gels, and then the electrophoresed proteins were transferred onto polyvinylidene fluoride (PVDF) membranes. After the membranes were blocked, they were incubated with various primary antibodies. Bands were detected using an automatic chemiluminescence image analysis system Tanon 5200 (Tanon, Shanghai, China), and then analyzed and quantified with ImageJ software (https://imagej.en.softonic.com, version v1.53 h).

Quantitative real-time PCR (qRT-PCR)

RNA of PSPCs were harvested with a Total RNA Extraction Kit (#19221ES50, Yeasen Biotech, Shanghai, China), following the step of instructions. qPCR was performed with the Accurate 96 Real-Time PCR machine (DLAB Scientific, Beijing, China) and following the instructions of Hieff® qPCR SYBR Green Master kit (#11203ES08, Yeasen Biotech, Shanghai, China). Each sample was repeated at least four times ($n\geq4$). Target gene expression level was normalized to GAPDH and analyzed by

$2-\Delta\Delta Ct$ method. Primers used for real-time PCR were listed in table S1.

Cell counting

PSPCs were seeded in a 24-well plate for 1×10^6 cells/well and grown overnight. The cells were changed medium and treated with C188-9 at 10 μM for for 48 h. The staining of Hoechst was following the Hoechst 33,258 (#40729ES10, Yeasen Biotech, Shanghai, China) datasheet. Cells were washed twice with DPBS, then added 0.5 mL Hoechst at 5μM in each well and incubated at 37℃ for 20 min. Washing out Hoechst, the PSPCs in wells were observed and photographed under 350 nm excitation spectra. Cell counting was managed on the image (Hoechst stained PSPCs) with the software image J (https://imagej.en.softonic.com, version v1.53 h).

Statistical analysis

All data were expressed as mean±SD, $n=3–6$. One-way ANOVA or two-way ANOVA method with software Prism GraphPad 8 (GraphPad Software Inc, San Diego, CA, USA) was used to compare means among different samples for single variate or multiple variables, respectively. P-values less than 0.05 were deemed statistically significantly different.

Results

Clinical cases presentation

The detailed information of the fifteen patients were listed in Table 1. Briefly, patient #01, #02 and #05 were diagnosed with invasive carcinoma, #06 was squamous carcinoma, and the other majorities were invasive ductal carcinoma. Most of the patient donors were between age 40 to 60. The ratio of cancer location was 53% (8/15) in left breast and 46% (7/15) in right breast. Based on the biomarker expression and the classification indicators for breast cancer, samples #12, #13, and #14 were categorized as luminal A, samples #05, #09, and #10 were luminal B1, samples #02, #04, #07, #08, and #11 were luminal B2, samples #01 and #03 were HER2-positive, and samples #06 and #15 were triple-negative breast cancer. The FISH detecting results for the Her2 (2+) samples were illustrated in the Figure S4.

PSPCs of breast cancer have higher expression level of STAT3 and phosphorylated STAT3 than primary cells from adjacent tissues

Pathological slide demonstrated that pSTAT3 had higher expression level in tumor than normal tissue of those breast cancer patients (Fig. 1A). Correspondingly, their tumor tissues were digested and regenerated as PSPCs. The procedure was showed as the diagrams of Fig. 1B. Two breast cancer samples and two adjacent tissues samples, as the representatives, were obtained from patient

Table 1 Summary of patient's clinical-pathological characteristics

Patient	Age	Tumor size (cm)	L/*R* Breast	Diagnosis	Biomarker expression
#01	58	-	L	Invasive carcinoma	ER (-), PR (-), Her2 (2+), Ki67 (60% +), AR (80% +)
#02	57	4.5×4	R	Invasive carcinoma	ER (> 90% +), PR (> 90% +), Her2 (2+), Ki67 (20% +), AR (20% +)
#03	32	4.5×2.5×6	L	Invasive ductal carcinoma	ER (-), PR (-), Her2 (3+), Ki67 (30% +), AR (> 90% +), P53 (80% +), TopIIa (I)
#04	-	-	L	Invasive ductal carcinoma	ER (90% +), PR (90% +), Her2 (3+), Ki67 (20% +), AR (> 90% +), P53 (1–3% +), TopIIa (I)
#05	57	2×2	R	Invasive carcinoma	ER (70% light+), PR (30% light+), Her2 (-), Ki67 (30% +)
#06	-	-	L	squamous carcinoma	ER (-), PR (-), Her2 (-), Ki67 (40% +), AR (10% light+), P53 (70% +), TopIIa (I), E-cad (light+), CK5/6 (+), P63 (+), P40 (+)
#07	46	0.7×0.7×0.5	R	Invasive ductal carcinoma	ER (80% +), PR (-), Her2 (2+), Ki67 (5% +), AR (90% +), P53 (10% +), TopIIa (I), E-cad (+)
#08	-	Diameter 0.9	R	Invasive ductal carcinoma	ER (> 90% +), PR (> 90% +), Her2 (2+), Ki67 (15% +), AR (> 90% +), P53 (2% +), TopIIa (I)
#09	49	2.5×1×1	R	Invasive ductal carcinoma	ER (90%), PR (90% +), Her2 (2+), Ki67 (20% +), AR (4% light+), P53 (5% +), TopIIa (I), CK5/6 (-), P63 (-), GCDFP-15 (+), S-100 (-)
#10	46	2×1.5×1	L	Invasive ductal carcinoma	ER (90% +), PR (60% +), Her2 (1+), Ki67 (25% +), AR (5% light+), P53 (1% +), TopIIa (I), E-cad (+)
#11	-	Diameter 1.3	L	Invasive ductal carcinoma	ER (> 90%), PR (5% +), Her2 (2+), Ki67 (10% +), AR (90>% +), P53 (1% +), TopIIa (I), EMA (+)
#12	47	1.8×1.5	R	Invasive ductal carcinoma	ER (90% +), PR (80% +), Her2 (-), Ki67 (5% +), AR (90% +), P53 (5–7% +), TopIIa (I), CK5/6 (-), EGFR (-)
#13	58	-	L	Invasive ductal carcinoma	ER (> 90%), PR (90% +), Her2 (-), Ki67 (70% +), AR (90>% +), P53 (-), TopIIa (I)
#14	48	-	L	Invasive ductal carcinoma	ER (> 90%), PR (80% +), Her2 (2+), Ki67 (3% +), AR (90>% +), P53 (3% +), TopIIa (I)
#15	41	-	R	Invasive ductal carcinoma	ER (-), PR (-), Her2 (1+), Ki67 (90% +), AR (-), P53 (1% +), TopIIa (IV), E-cad (+)

01 and # 02, and being used to isolate PSPCs. Proteins harvested from the four samples were analyzed by western blot. As the result, pSTAT3 and other relevant proteins were expressed more in PSPCs of breast cancer samples than in those of adjacent tissue samples in the two patients (Fig. 1C), which was consist with the immunohistochemistry staining in Fig. 1A.

Experimental concentration of C188-9 effectively reduced viability of breast cancer cell line and PSPCs

The breast cancer cell line, MBA-MD-231, could be suppressed by C188-9 at 10μM obviously (Fig. 2A), which was consist with the previous study [12]. Correspondingly, the western blot data revealed that the C188-9 inhibited the pSTAT3 expression (Fig. 2B). The quantification data was showed in the right panel of Fig. 2B. Moreover, the other three cell lines including MCF7, SKBR3 and BT474 for representing Luminal A, Luminal B and HER2-positive breast cancer cells were also treated by C188-9 and presented various sensitivities but general suppression in cell viabilities (Figure S3A) and pSTAT3 expression (Figure S3B). The regent C188-9 reduced the proliferation and migration ability of MBA-MD-231 cells, shown as the Wound Healing assay results, that C188-9 decreased the ratio of Wound Healing area from 71.0 to 46.8%, 28.7% and 0.4% at the concentration of 1μM, 3μM and 10μM (Fig. 2C). As to the PSPCs, still being represent as the sample # 01 and # 02, the number of PSPCs in C188-9 groups had reduced to 17.4% and 27.1%, respectively, when normalized to the number in DMSO (control) groups (Fig. 2D).

C188-9 at low concentrations decreased PSPCs viability

Considering that the experimental concentration of C188-9 will be far higher than that used in clinical condition, we challenged PSPCs of breast cancer with C188-9

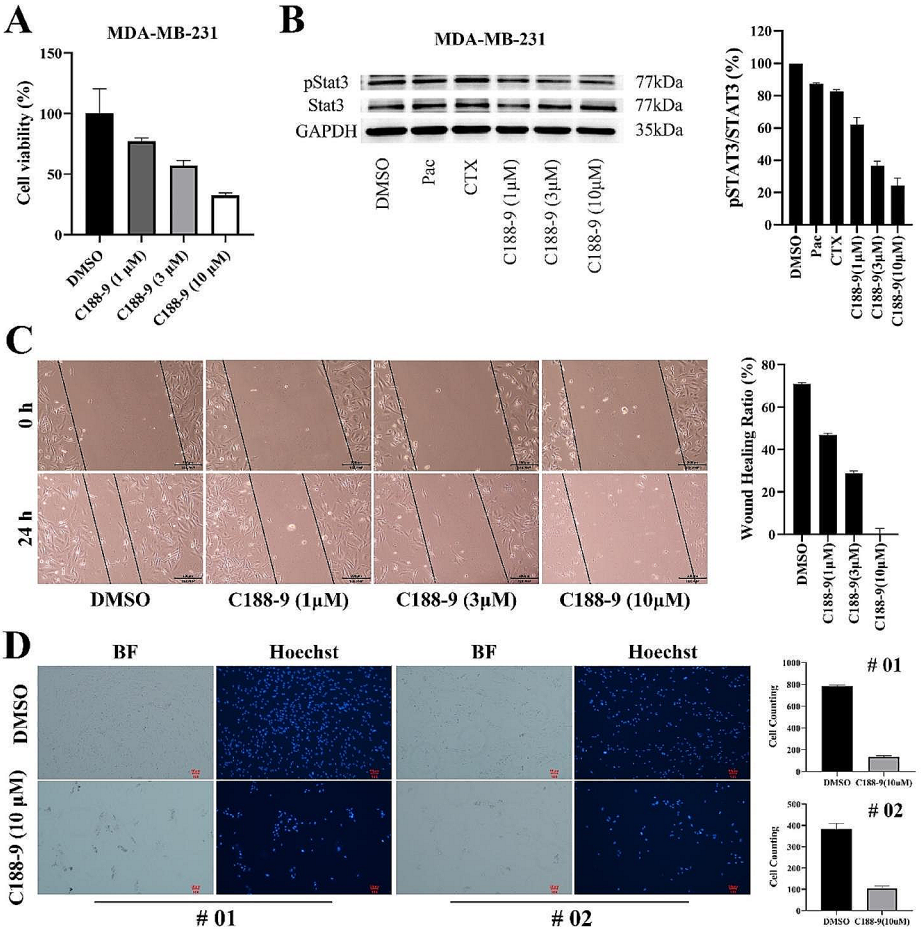

Fig. 2 Cell viability and activity detection of MDA-MB-231 after different concentrations of C188-9 treatments. (**A**) Viability of breast cancer cell line MDA-MB-231 under the treatments of C188-9 at 1, 3 and 10 μM, detected with CCK8 kit. (**B**) pSTAT3 expression (left panel) and pSTAT3/STAT3 ratio (right panel) of MDA-MB-231 cells after C188-9 and traditional chemotherapy drugs treatments. Pac: Paclitaxel, CTX: Cyclophosphamide. (**C**) Wound Healing assay of MDA-MB-231 cells under the treatments of C188-9 at 1, 3 and 10 μM. (**D**) Cell counting of PSPCs after C188-9 treatments at 10 μM. The images were photographed under the bright field or fluorescent field with Hoeshct staining. Pac: Paclitaxel, CTX: Cyclophosphamide

at concentrations 0.5 and 1μM, and extended the C188-9 treatment duration from 1 day to 6 days with multi-times administration, which was mimicking the sustained medication in clinical condition. We observed that PSPCs from different patients showed different sensitivities to C188-9. The sample # 01, # 05 and # 08 represented the sensitive cohort, that their viabilities were down to 59.1%, 54.3% and 56.2% at 0.5μM of C188-9 treatment,

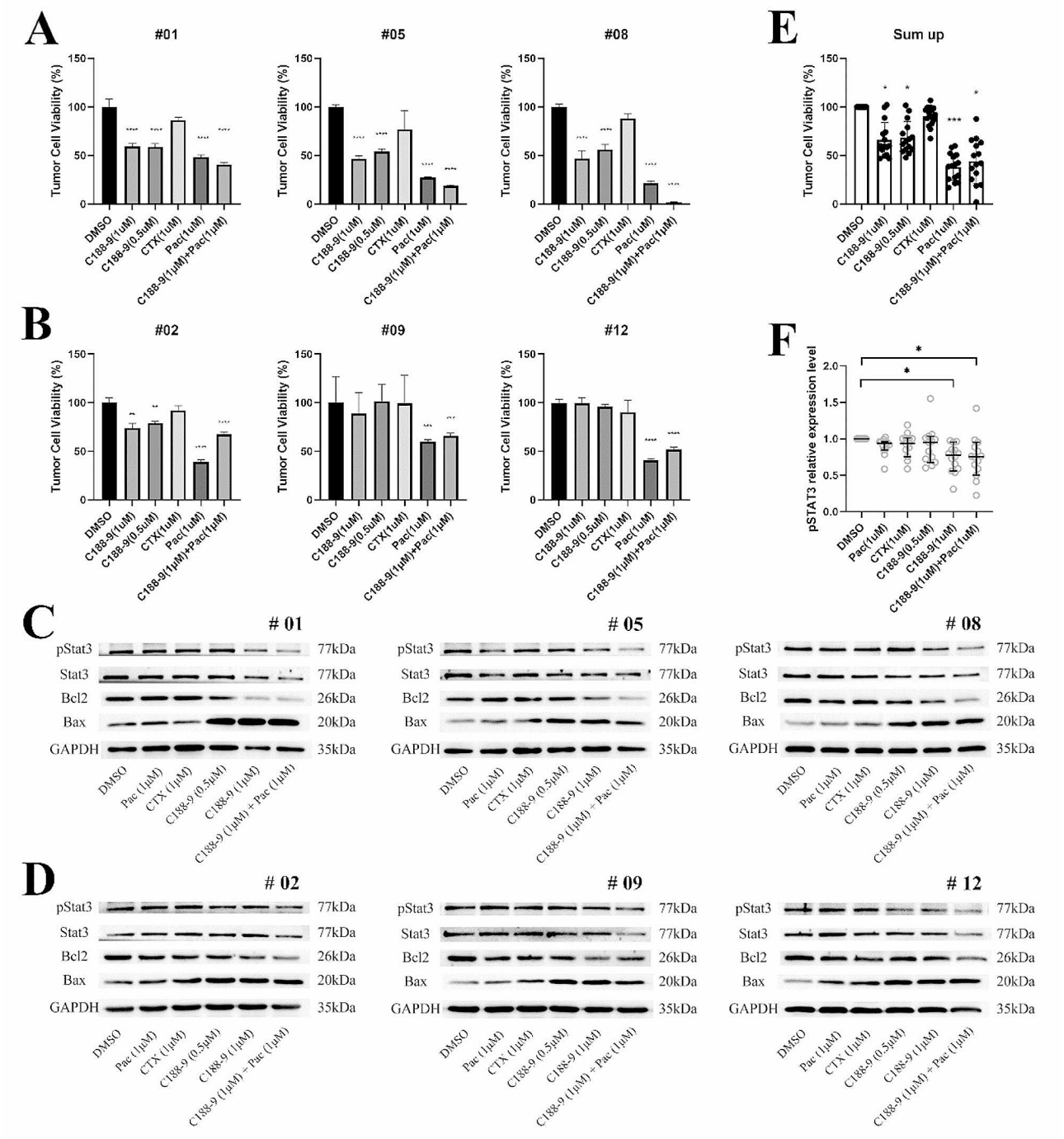

Fig. 3 Results of PSPCs viability and STAT3 signaling pathway inhibition after low concentration of C188-9 treatments. **A, B.** Viability of C188-9 sensitive (A) and insensitive (B) PSPCs measured by CCK8 assay after different drugs treatments. **C, D.** pSTAT3, STAT3, Bcl2 and Bax expression of the sensitive (C) and insensitive (D) PSPCs detected by Western blot assay after the drugs treatments according to the cell viability measurements. **E, F.** Sum up data of PSPCs viability (E) and pSTAT3 relative expression level (F) included all the 15 samples. Each dot represented one sample, for evaluating the general trend. Pac: Paclitaxel, CTX: Cyclophosphamide. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$

respectively, and down to 59.4%, 46.8% and 46.9% at 1μM, respectively (Fig. 3A). While some samples such as # 02, # 09 and # 12 were less affected by the low concentrations of C188-9 treatments (Fig. 3B). The regent Cyclophosphamide and Paclitaxel, as the two commonly used drugs in clinic of breast cancer treatments, were also tested. Cyclophosphamide at 1μM had almost no effect on all 15 samples. While Paclitaxel at 1μM generally reduced all PSPCs viability of the 15 samples (Figure S1). To sum up all of the 15 samples, low concentrations of C188-9 treatment and the combination of C188-9 plus Paclitaxel demonstrated a promising effect of reducing breast cancer PSPCs proliferations (Fig. 3E).

C188-9 inhibited activation of STAT3 signaling pathway

Since C188-9 inhibited STAT3 signaling pathway [12], we further determined the mechanisms of C188-9 functioning on those PSPCs. We treated different drugs to PSPCs from the 15 samples, and evaluated the activation of STAT3 signaling pathway via western blot assay. Compared to DMSO (control) group, treatment with C188-9 at 1μM with or without combination of Paclitaxel had reduced the level of pSTAT3 in the sample #01, #05, and #08 obviously (Fig. 3C). However, the pSTAT3 level didn't reduced and even rose in the sample #02, #09 and #12 after C188-9 treatment (Fig. 3D). As the sum up of quantification results of ratio of pSTAT3 to total STAT3, C188-9 at 1μM alone or combined with Paclitaxel had reduced the ratio of pSTAT3 to total STAT3 to 80.3% and 75.2% in general, respectively (Fig. 3F).

C188-9 induced PSPCs apoptosis trough c-Myc pathway

We subsequentially investigated the down-stream mechanisms of pSTAT3 inhibition when PSPCs treated with C188-9. As shown in the western blot results, Bax and Bcl2, the two major proteins correlated with apoptosis of cells, were upregulated and downregulated, respectively, after C188-9 treated at 0.5μM and 1μM, and with Paclitaxel combination (Fig. 3C and D). The summed quantification results of Bax/Bcl2 level showed the increase of 1.5, 3.0 and 3.8 times in 0.5μM, 1μM and plus Paclitaxel of C188-9 treatment groups, respectively (Fig. 4A). Meanwhile, the flow cytometry data of breast cancer cell apoptosis confirmed this mechanism (Figure S2). Further, the qRT-PCR data demonstrated that c-Myc, the downstream gene related to cellular proliferation, had reduced expression to 0.85, 0.80 and 0.68 times in those three groups (Fig. 4B).

C188-9 reduced the tumor growth in CDX models of breast cancer

Moreover, we verified the efficacy of C188-9 in the in vivo condition. After 36 days of monitoring, the tumor volume in the C188-9 group were smaller than that in the control group in average, and being the smallest in the C188-9 plus Paclitaxel, which was consist with the in vitro experiment results of PSPCs (Fig. 4D). During the whole treatment period, C188-9 did not demonstrate obvious side effects on interfering the physiological condition of the mouse model (Fig. 4C). In addition, the immunohistochemistry assay of the tumors from the CDX models presented that the pSTAT3 expression was indeed decreased in the C188-9 and C188-9 plus Paclitaxel groups (Fig. 4E).

Discussion

Breast cancer is a group of heterogeneous diseases with comprehensive treatment strategies, such as surgery, radiation therapy, chemotherapy, endocrine therapy, targeted therapy, and more recently immunotherapy. New drug discovery from the cellular level to the animal level and beyond the clinical trials is a long and challenging process. Only about a third of highly cited animal research was translated at the level of human randomized trials [17]. Many drug candidates failed in the clinic due to lack of therapeutic effects or severe side effects. As an example, TGN1412, a CD28 monoclonal antibody, induced excellent T-regulatory lymphocytosis in mice model. However, in a phase I trial, TGN1412 resulted in horrific side-effects in six volunteers due to cytokine release storm [18]. This is partly due to the differences between experimental animals and humans. Therefore, it is essential to find appropriate pre-clinical models to predict drug effects.

Aiming to provide insight of clinical utilization, we have chosen to test the concentrations of C188-9 at the clinical-relevant level, which was much lower than that used by *Jung et al.*. [12]. According to the previous studies (Jung et al., 2017 and Brown et al., 2021) [12, 19], C188-9 in patient plasma was between 1.28 μM and 6.17 μM, which was far lower than that in animal model, almost 60 μM (Jung et al., 2017). On the other hand, in the study by Jung et al., 2017, the time-effect curve of C188-9 presented effects at the concentration 1 μM and reached the half lethal dose of 10 μM. Therefore, we initiated our test of C188-9 at 10 μM and confirmed its high efficacy on reducing the viability of breast cancer cell line and PSPCs. Subsequently, we investigated its efficacy on the PSPCs from different breast cancer patients at 1 μM, but extended observation period. At such low concentrations, the different samples of PSPCs showed different sensitivities to the C188-9, which basically reflected the real situation of targeting drugs in clinic. However, the sum up data demonstrated that the PSPCs proliferations could be inhibited by C188-9 generally at the such low concentrations, implying that the underlying molecular mechanism was via upstream regulation, instead of direct cytotoxic effects, consistent with its role as a STAT3

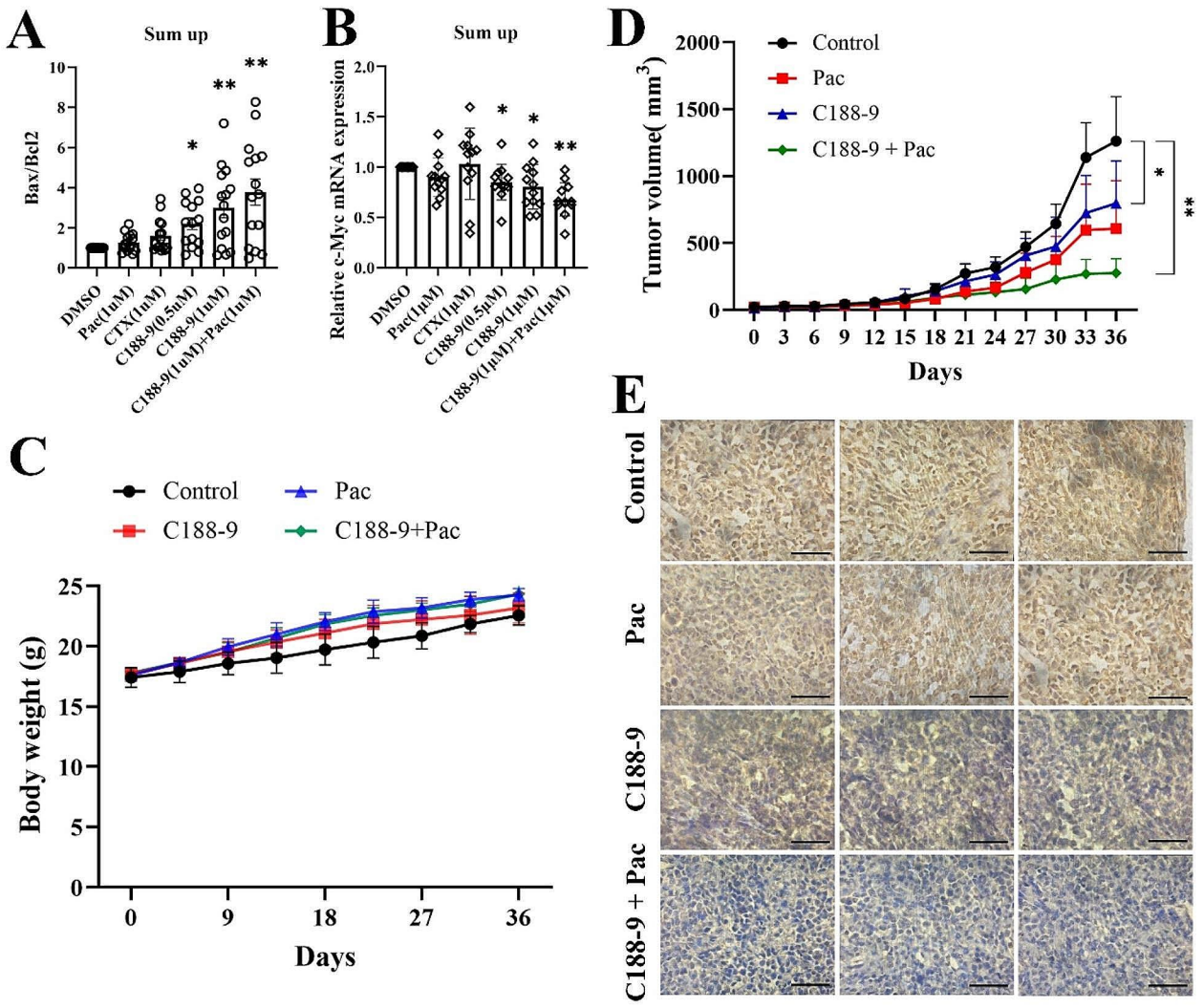

Fig. 4 Exploration of the down-stream changes in STAT3 signal pathway and the in vivo results of C188-9 efficacy. **A, B.** Sum up data of Bax/Bcl2 ratio (A) and c-Myc mRNA level (B) in the rest PSPCs samples from the 12 out of 15 patients. Each dot represented one sample, for evaluating the general trend. **C**. The body weight curves of the mice. **D.** Time curves of tumor volume of the breast cancer CDX models in different treatment groups. **E.** Immunohistochemical staining of pSTAT3 expression in the tumor tissues from the CDX models in different groups. Pac: Paclitaxel, CTX: Cyclophosphamide

blocker. C188-9 downregulated the pSTAT3 expression in the sample #01, #05 and #08 obviously, but not in the sample #02, #09, and #12, mimicking drug effects in real world: treatment was patient-specific, and not all drugs were effective to all patients. Those individual differences of patients could be hardly predicted by animal model, while PSPCs provided the good platform to mimic the pre-clinical condition.

For the relative mRNA expression, since we had limited PSPC samples, only twelve of them were detected. However, they also revealed some mechanisms of C188-9 in reducing tumor cells viability. After C188-9 treatment, the down-stream mRNA c-Myc, regulated by pSTAT3 and related to cellular proliferation, were downregulated in the majorities. It was consistent with the mechanism of C188-9 mainly reducing the pSTAT3 protein level to inhibiting the activation of STAT3 signaling pathway. Moreover, Bax/Bcl-2, another down-stream signal pathway regulated by pSTAT3, being related to mitochondria activity and cellular apoptosis, also showed significant increase after C188-9 treatment in general. It indicated that the mechanism of C188-9 effected multiple down-stream signal pathway in patient. To evaluate the differences between C188-9 responders and non-responders, we sorted the PSPC samples as C188-9 sensitive, C188-9 insensitive and C188-9 tolerant groups according to the cell viability assay results, and further explored the multiple relevant gene expression which might affect the STAT3 signal pathway or side pathway. From the mRNA detection results, we found that the majority of C188-9 sensitive samples had

up-regulatory expression trend of PI3K, an important kinase in the upstream of STAT3 signal pathway (Figure S5). However, whether the PI3K could be a predictor for C188-9 responding needs to be further investigated.

In summary, our study results showed that the C188-9 inhibitor reduced PSPC viability in a patient-specific manner, and indicated that it could be a potential therapeutic drug for certain patients. In addition, we showed that PSPCs could be a useful platform for translational study in the upcoming personalized medicine era. Our finding supported PSPCs as a useful tool for this purpose, as PSPCs from only ten out of fifteen patients were sensitive to C188-9 (Figure S1), implying potential, personalized use of C188-9 only on those ten patients.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12967-024-05542-8.

Supplementary Material 1

Supplementary Material 2

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Author contributions

Haoyu Zeng and Jun-Dong Wu conceived and designed the project. Rongji Zheng, Tian Guan, Chaoqun Hong, Yutong Fang, Chunfa Chen, Huancheng Zeng, Yun Li and Yanchun Lin performed the cellular and molecular experiments. Rongji Zheng, Yao Yao, Wei Huang, Jiman Huang, Hui Lin, Dongmei Chen and Zhechun Ding finished the in vivo experiment and relevant assays. Rongji Zheng, Bing-Feng Chen, Ren-Dong Zhang, and Tian Guan analyzed the data and prepared the figures and tables. Tian Guan wrote the manuscript. Haoyu Zeng and Jun-Dong Wu approved the final version to be submitted.

Declarations

Ethics approval and consent to publish

All of the protocols using human specimens were approved by the Cancer Hospital of Shantou University Medical College, and informed consent was obtained from all of the subjects.

Conflict of interest

The authors declare that they have no competing interests.

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