

STIM2 promotes the invasion and metastasis of breast cancer cells through the NFAT1/TGF- β 1 pathway

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ABSTRACT

A large amount of evidence indicates that the abnormal activation of multiple signal transduction pathways in cells is closely related to the occurrence and development of tumors. TGF- β and NFAT1 signaling pathways can inhibit cell proliferation and promote apoptosis in the early stage of breast cancer, but with the increase of tumor malignancy, the two appear to promote tumor progression and deterioration. Therefore, the study of the relationship between STIM2 and NFAT1/TGF- β 1 is helpful for the discovery and treatment of breast cancer, which is of great significance for improving the survival rate of breast cancer patients. This article focuses on the effect of STIM2 molecules on breast cancer cell migration through the NFAT1/TGF- β 1 pathway and discusses the regulatory mechanism of STIM2 affecting breast cancer cell migration. Experimental data shows that the positive rate of breast cancer NFAT1 is 54%, which is significantly lower than that of benign breast Tissue 85%; the positive expression rate of TGF- β 1 in benign breast tissue is 85%, and the positive expression rate in breast cancer tissue is 49%. The results show that STIM2 protein can promote the invasion and metastasis of breast cancer cells through the NFAT1 / TGF- β 1 pathway.

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Introduction

In 2008, approximately 1.38 million people were diagnosed with breast cancer worldwide, accounting for 23% of new cancer cases among women worldwide, second only to lung cancer, and ranking second among all cancer incidences (1). However, the current research view believes that the occurrence, development, invasion and metastasis of breast cancer are regulated by various factors (including multiple genes, regulatory factors and signaling pathways) (2). In addition to the emergence of drug resistance during treatment, research on the molecular mechanism of breast cancer tumor development and development is more important, but a large amount of evidence indicates that tumor development is closely related to the abnormal activation of multiple signaling pathways in cells (3). Among them, Transforming Growth Factor-beta (TGF- β), Epidermal Growth Factor Receptor (EGFR) family, platelet-derived and insulin-

like growth factors play important roles in tumorigenesis. Role (4-5). Therefore, it is very important to clarify the molecular mechanism of breast cancer invasion and metastasis. Current research has determined that during the development of breast cancer, multiple signaling pathways become abnormally activated and overactivated signaling pathways are closely related to the invasion and metastasis of breast cancer (6). In order to control the invasion and metastasis of breast cancer cells, it is very important to understand its molecular mechanism and metastasis pathway in-depth, and it is also a key issue in the treatment of breast cancer (7).

NFAT1 was originally discovered in T cells as a transcriptional regulator, which can have an important effect on the proliferation and migration of T cells. Jiang et al. Studied and proved that the local immune status is related to the prognosis of gliomas (8). Interleukin 6 (IL6) has been identified as an important

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local immune-related risk marker associated with poor prognosis (9-10). They further investigated the role and regulation of IL6 signaling in gliomas (11). The expression and prognostic value of IL6 and IL6 receptor (IL6R) were explored in the Cancer Genome Atlas (TCGA) and REMBRANDT database and clinical samples (12). The functional effects of gene knockdown and overexpression stimulated by IL6R or IL6 were examined under *in vitro* and *in vivo* tumors, and the effect of nuclear factor of activated T cell-1 (NFAT1) on IL6R and IL6 promoter activity was also examined (13-14). The high expression of IL6 and IL6R is significantly associated with interstitial subtypes and IDH wild-type gliomas and is a predictor of poor survival (15). Inhibition of IL6R *in vitro* reduces cell proliferation, invasion and neurosphere formation, and inhibits tumorigenesis *in vivo* (16). Overexpression of IL6R or IL6 stimulation enhances the invasion and growth of glioma cells (17).

In addition to affecting cell proliferation and differentiation, TGF- β also plays an important role in embryonic development, extracellular matrix formation, bone formation and reconstruction. Li et al. Believe that tumor-associated fibroblasts (CAF) are a major component of the tumor microenvironment and play an important role in interacting with tumor cells to promote ovarian cancer progression (18). Exosomes are nanoscale vesicles that mediate crosstalk between different cell types (19). More and more studies have focused on the fact that tumor cell-derived exosomes affect stromal cells (20). However, the mechanism by which CAF-derived exosomes regulate cancer cells in ovarian cancer remains unclear (21). In order to study the role of CAF exosomes in ovarian cancer, ELISA detected the paired primary, metastatic and normal fibroblast exosomes from patients with stage 7C stage IIIC ovarian cancer (22). They found that in ovarian CAF-derived exosomes, TGF β 1 was upregulated compared to normal omentum fibroblasts (NOF) (23). Ovarian SKOV-3 and CAOV-3 cells absorbed CAF-derived exosomes during co-culture and induced malignant behavior of cancer cells, including enhanced migration and invasion ability (24-25).

In this paper, through the study of breast cancer cell invasion and metastasis, we have a deeper understanding of the molecular mechanism of breast cancer occurrence and development. STIM2 participates in smooth muscle cells, nervous system

cells and immune cells, including cell development, function formation and improvement, and plays an important role in ischemia-induced neuronal damage. Understand the relationship between NFAT1 / TGF- β 1 expression in breast cancer tissues. In order to fully grasp the relationship between their expression in breast cancer and the occurrence, the development of biological behavior and prognosis of breast cancer, provide a strong basis and help for the clinical treatment and prognosis of the disease.

Materials and methods

Experimental materials

MDA-MB-231 and MCF-7 human breast cancer cells were cultured in a DMEM medium containing 10% fetal bovine serum, 5% CO₂, and a 37°C incubator. Passage 2-3 times a week, pass 1: 4, digest with 0.25% pancreatin, and take cells in the logarithmic growth phase for the experiment. Normal breast tissue is normal breast tissue 5 cm away from cancer tissue, adjacent tissue is tissue 2 cm away from the lesion, and cancer tissue is breast cancer tissue confirmed by pathological section.

Main Reagents

Fetal bovine serum, DMEM, Lentivims vectors, TG, CaCl₂, NaCl, YM58483, rabbit anti-STIM2 polyclonal antibody, SYBR Green kit, bovine serum albumin (BSA), Fluo-4 / AM, TGF- β , Hanks, DMSO, Trypsin, reverse transcription kit, DEPC water, chloroform, absolute ethanol, 4% paraformaldehyde fixative.

Preparation of the Main Solution

100 g PBS was dissolved in ddH₂O 500m, and then make up to 2000mL with ddI-120. For Preparation of cell lysate, 30ml100mMTris-HCl (PH7.5), 20ml1MNaCl, 0.825ml600mMMgCl₂ and were mixed well, then 1ml Triton X-100 was added to bring the volume to 100ml, then it was mixed well, and aliquot, and finally it was kept at -20°C. To prepare reagents for immunoblotting experiments, 5.5g of SDS was added to 550ml double distilled water and was stored at room temperature. For the preparation of the blocking solution, 5% skim milk powder was added based on TBST. For preparing antibody diluent, 5% BSA was added on the basis of TBST lotion.

Instruments

CO₂ constant temperature incubator, inverted phase-contrast microscope, fluorescence microscope, protein electrophoresis and transfer system, ultra-clean workbench, desktop centrifuge, refrigerator, ultrapure water machine, pipette, laser confocal microscope, DocGel2000 imaging system, electronic analytical balance, Low-temperature high-speed centrifuge, microplate reader, other equipment used include pH meter, refrigerator, volumetric flask, etc.

Experimental Method

In the process of cell culture, choose the time for cell replacement according to the growth status of the cell. Generally, the medium is changed every 2-3 days. Observe the cell morphology and growth status daily. Wash twice, add 10% serum-containing fresh DMEM culture medium, and place it in the incubator to continue culturing.

Remove the cell cryopreservation tube from the liquid nitrogen storage system, quickly put it in a 37°C constant temperature water bath, shake it constantly to thaw it as soon as possible; centrifuge at 800rpm, room temperature for 2min, discard the supernatant; add 2mL culture medium (containing 10% fetal cattle) Serum DMEM medium) in a centrifuge tube, pipette with a pipette tip to form a cell suspension, add to the Petri dish, then add 3mL culture medium, 5% CO₂, 37 C incubator culture, change the fluid every other day, thereafter every Change the medium once every 2-3 days and pass the cells according to the growth of the cells.

Protein extraction

Remove the cells from the incubator, add the prepared cell lysate according to the cell density, and use the cell planer to plan the cells to fall off, back and forth several times, and rest for 10 minutes to fully lyse the cells. Dilute the protein standard of 1 mg/ml to 0.1 mg/ml, 0.3 mg/ml, 0.5 mg/ml, 0.7 mg/ml in five gradient concentration standards, take the reagent Coomassie brilliant blue 205 μ l and add the standard and sample In the middle, after mixing, incubate at room temperature for 2 minutes, read the absorbance at 560nm wavelength, so that the absorbance of the measured samples are within the standard curve.

RNA extraction

Remove the medium from the cells, wash the cells with pre-chilled PBS, add the TRIZOL reagent with a pipette, repeatedly blow the lysed cells to a uniform and bright liquid, transfer the homogenized sample to a 1.5ml EP tube, and mix upside down, Incubate at room temperature for 5 minutes to completely decompose the ribosome. After centrifugation, the mixture is divided into three layers, the lower layer is red phenol-chloroform containing protein, the middle layer is the DNA layer, the upper layer is the water sample layer, and the RNA is on the upper layer. Generally, 400ul is transferred from the upper layer to a new 1.5ml EP tube with a pipette. Remove the upper suspension, add pre-chilled 75% ethanol 1ml (the reagent was prepared with DEPC water), wash once, and centrifuge at 12000r/ min for 10min at 4 °C.

Cell count

When the cells are in the logarithmic growth phase, digest the cells with an appropriate amount of trypsin and observe the changes in cell morphology under a microscope. When the cells start to round but not float, stop the digestion, add the appropriate amount of fresh culture medium to resuspend the cells, and centrifuge at 1000 rpm for 5 minutes. Remove the supernatant, add 1mL of fresh culture solution, and repeatedly aspirate the cells to resuspend them to ensure the formation of a single cell suspension. Then take out 10 μ L of cell suspension and place it on the counting plate prepared in advance. Do not generate air bubbles and ensure that the suspension fills the entire counting cell. Then record the number of cells in four large squares under the microscope and count the total number of cells.

Statistical Methods

All experiments were repeated three times. The data statistics were analyzed using SPSS22.0 software. The comparison between the two groups was by independent sample t-test, the comparison between multiple groups was by single-factor analysis of variance, and the comparison between groups was by LSD-t test. P <0.05 indicates that the difference is statistically significant.

Results and discussion

STIM2 Expression Mechanism

In order to further explore the molecular mechanism of STIM2's role in breast cancer cell metastasis, in breast cancer, we use genome-wide NFAT1 technology to predict the target gene regulated by TGF-β1 transcription, and the binding peak is located in the promoter region (transcription initiation) Genes from 2000bp upstream to 1000bp downstream are potential genes regulated by SND1. Analysis results show that TGF-β1 can be combined with the promoter of 2592 genes. After statistics, the signal pathway with the most genes participating is shown in Figure 1. Analysis of TCGA's breast cancer IDC database found that 242 genes were associated with SND1 expression. The two data were combined and analyzed, and it was found that 41 genes appeared in both data. The 41 genes were initially considered as the target genes regulated by SND1. Gene ontology analysis of these 41 genes revealed that the main cellular pathways involved in these 41 genes include EMT, SMAD binding and TGF-β signaling pathway.

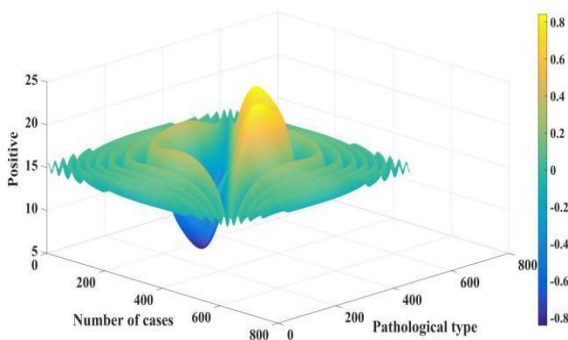


Figure 1. Signaling pathways where genes are most involved after statistics

The expression of STIM2 is shown in Table 1. The positive reaction substances of NFAT1, TGF-β1 and TGF-βRI were localized in the cytoplasm and (or) cell membrane, with occasional nuclear staining. The positive rate of breast cancer NFAT1 is 54%, which is significantly lower than that of benign breast tissue. 85%, the difference between the two is statistically significant; The positive expression rate was 49%, the difference between the two was statistically significant; the positive expression rate of TGF-βRI in benign breast tissue was 75%, and the positive rate in breast cancer tissue was 39%. Statistical significance (P = 0.05).

Adjustment Process of STIM2 to NFAT1

The analysis results showed that the fluorescence intensity of the luciferase reporter gene with NFAT1 / TGF-β1 promoter in the SND1 overexpression group was higher than that of the control group (con) (P <0.01), suggesting that SND1 has transcriptional activation on NFAT1 / TGF-β1 gene. STIM2 adjusts NFAT1 as shown in Figure 2. In MCF-7 cells, we transfected the SND1-expressing plasmid pSG5-SND1 (SND1) and the empty control pSG5 (con). After 48 hours of transfection, the protein was extracted, and the expression of NFAT1 / TGF-β1 and Smurf1 protein was detected by western blot. The results showed that the protein levels of NFAT1 / TGF-β1 and Smurf1 in the SND1 group increased as the SND1 protein level increased compared with the con group. From the above results, we believe that STIM2 can promote the transcription of the NFAT1 / TGF-β1 gene, increase the mRNA level of NFAT1 / TGF-β1 in the cell, and increase the expression of protein in the cell.

Table 1. Expression of STIM2

Pathological type	Number of cases	Positive	Weak positive or negative	Positive rate (%)	X	P
Benign breast tissue	20	15	5	75	8.19	0.004
Non-benign breast tissue	71	28	44	39		
Infiltrating type	21	16	6	72	4.3	0.036
Non-wetting	50	23	27	46		

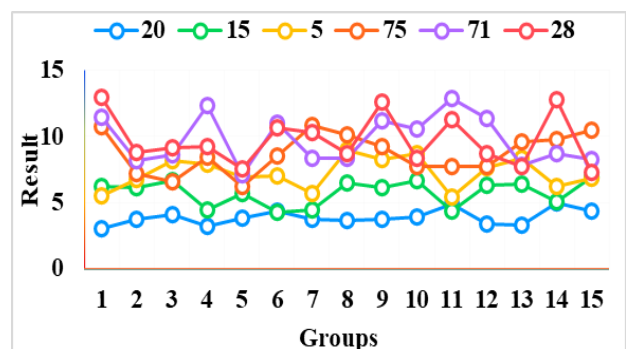


Figure 2. Regulation of NFAT1 by STIM2

Regulation Process of TGF- β 1 on Breast Cancer Stem Cells

In order to study the regulatory effect of TGF β 1 on breast cancer stem cells, the effect of TGF β 1 on the CD44 + / CD24-stem cell population was studied in breast cancer cell line SUM159.MDA and non-sex breast cancer cell line MCF7. Flow cytometry results showed that the triple-negative breast cancer cell line SUM159.MDA after 4 consecutive days of 5ng / ml TGF β 1 stimulation, the CD44 + / CD24-stem cell population in the breast cancer cells of the TGF β 1 group increased significantly, but the non-stem cell population CD44 + / CD24 + The control group decreased significantly (Figures 2A and 2B). The regulatory process of TGF β 1 on breast cancer stem cells is shown in Figure 3. However, TGF β 1 had no similar effect on breast cancer epithelial cell line MCF7. TGF β 1 can specifically increase the proportion of CD44 + / CD24- stem cell population in triple-negative breast cancer.

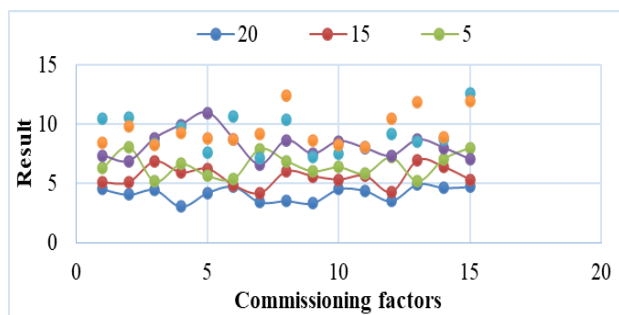


Figure 3. Regulation of TGF β 1 on breast cancer stem cells

Regulatory Process of NFAT1 on Breast Cancer Stem Cells

Recombinant human NFAT1 (10ng / ml) was added to detect the effect of propofol on the migration and invasion of STIM2 cells after overexpression of NFAT11. Western blot results showed that compared with the propofol-treated group, the addition of recombinant human NFAT1 could reverse the inhibitory effect of propofol on the expression levels of TGF- β 1, MMP-2 and MMP-9 in STIM2 cells. The results of NFAT1 regulation of breast cancer stem cells are shown in Figure 4. The results of cell function tests were obvious: the number of transmembrane cells (180.3 ± 9.6) in the Transwell migration experiment (180.3 ± 9.6), the number of transmembrane cells (172.0 ± 10.3) in the Transwell migration experiment (172.0 ± 10.3). (107.3 ± 8.6)

compared with the propofol-treated NFAT11 cells group (100.7 ± 4.9), (57.3 ± 5.7), (50.7 ± 5.8), the difference was statistically significant. The effect of NFAT1 on STIM2 cells on the transfer ability of STIM2 cells, the results show that TGF β 1 down-regulates the MMP-2 and MMP-9 protein expression levels in STIM2 cells, prevents breast cancer cells from passing through the transwell cell, and reduces the tumor cells passing through the matrigel Ability to inhibit tumor cell-induced neovascularization, these results indicate that NFAT1 can inhibit breast cancer metastasis. At the same time, it was found that NFAT1 can down-regulate the expression level of TGF- β 1 protein in breast cancer STIM2 cells in a dose-dependent manner, as well as the down-regulation of TGF- β 1 expression confirmed by research.

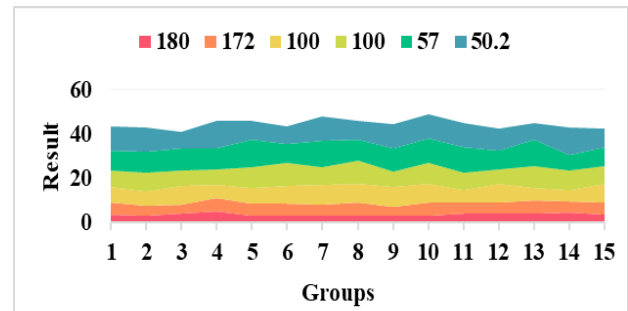


Figure 4. Regulation of TGF β 1 on breast cancer stem cells

Breast cancer is a malignant tumor that occurs in the epithelial tissue of the breast glands. In my country, the incidence of breast cancer ranks first among female malignancies. Due to changes in breast cancer cells and loss of normal cell phenotype and function, cancer cells are prone to fall off, enter the blood and lymph fluid and spread to the whole body, causing distant metastasis and increasing the mortality of breast cancer. Surgical removal of tumor tissue is one of the main treatments for many solid tumors including breast cancer. In addition to the tumor marker level, clinical stage, pathological grading, residual lesion size, lymph node metastasis and clearance, tumor growth, recurrence and metastasis have gradually become one of the hot topics of research.

This paper further validates the role of STIM2 in breast cancer cell migration on breast cancer cell line models and initially demonstrates the role of STIM2 molecule in breast cancer cell migration, and its involvement in the regulation of non-SOCE-

dependent external calcium influx and internal process of calcium influx caused by the emptying of the plasma reticulum calcium reservoir provides a new experimental basis for elucidating the molecular mechanism regulating breast cancer cell migration.

STIM2 promotes the invasion and metastasis of breast cancer cells through the NFAT1 / TGF- β 1 pathway. NFAT1 is overexpressed and activated in GBM. Under normal conditions, NFAT1 regulates GBM cell invasion without significantly affecting proliferation. PMA and I α excessively activate NFAT1, which in turn up-regulates Fas / FasL expression and induces GBM cell death, which has important clinical significance. The TGF- β superfamily plays an important role in the development of vertebrates by regulating cell proliferation, differentiation, migration and apoptosis. TGF- β 1 is a proliferation inhibitory factor for many types of cells. The expression of TGF- β 1 is often reduced or absent in malignant tumor cells. The combination of TGF- β 1 and its corresponding receptor affects the occurrence and development of malignant tumors as well as its biological behavior and prognosis.

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Interest conflict

None.

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