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Original article

KIF26B promotes cell proliferation and migration through the FGF2/ERK signaling pathway in breast cancer

Yan Teng[a,1], Bingling Guo[b,1], Xiaosong Mu[c], Shihong Liu[d,*]

[a] Department of Oncology, Chongqing University Cancer Hospital & Chongqing Cancer Institute & Chongqing Cancer Hospital, Chongqing 400030, China
[b] Department of Hematology and Oncology, Chongqing University Cancer Hospital & Chongqing Cancer Institute & Chongqing Cancer Hospital, Chongqing 400030, China
[c] Integrated Department, Chongqing University Cancer Hospital & Chongqing Cancer Institute & Chongqing Cancer Hospital, Chongqing 400030, China
[d] Department of Palliative Treatment, Chongqing University Cancer Hospital & Chongqing Cancer Institute & Chongqing Cancer Hospital, No 181 Hanyu Road, Chongqing 400030, China

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ABSTRACT

Background: Many studies have suggested that high KIF26B expression is directly linked to poor prognostic outcomes in breast cancer. However, the exact role of KIF26B in breast cancer progression is not fully understood. In this study, we aimed to explore the function and mechanism of KIF26B in breast cancer progression.
Methods: Quantitative real-time PCR and immunohistochemistry analysis were used to detect KIF26B expression in breast cancer cell lines and patient samples. Cell proliferation was assessed by CCK-8 assay, and cell migration and invasion were evaluated by wound healing assay and transwell assay. Western blot analysis was carried out to assess the underlying molecular mechanisms. Tumor formation and metastasis were determined by *in vivo* mouse experiments.
Results: KIF26B levels were significantly increased in breast cancer cells and patient samples. KIF26B level correlated with tumor size, TNM grade, and differentiation in patients with breast cancer. Overexpressing KIF26B *in vitro* promoted breast cancer cell proliferation and migration by activating FGF2/ERK signaling, while silencing KIF26B had the opposite effects. Similarly, KIF26B knockdown repressed tumor formation and metastasis in nude mice.
Conclusion: KIF26B promoted the development and progression of breast cancer and might act as a potential therapeutic target for treating breast cancer.

1. Introduction

Breast cancer, which originates from the ductal epithelium or lobule of the mammary gland, is one of the most common malignancies in women worldwide [1]. Breast cancer development is a complex process involving multiple factors, including environmental and genetic factors [2,3]. The main causes of death in patients with breast cancer are the metastasis and uncontrolled proliferation of breast cancer cells. Therefore, understanding the molecular mechanisms of breast cancer progression and developing suitable drugs for critical targets will play important roles in preventing and treating breast cancer.

KIF26B is one of the kinesin superfamily proteins (KIFs), and is composed of 2108 amino acids [4]. During kidney development, KIF26B is essential for the adhesion and polarization of mesenchymal cells. KIF26B overexpression has been shown to promote the interaction between N-cadherin-dependent cells and MHC class II molecules, thereby increasing cell adhesion [5,6]. Studies have suggested that high KIF26B expression is directly related to the poor prognosis of colorectal and breast cancers [6–8]. However, the exact biological function and regulatory mechanism of KIF26B in cancer development have rarely been reported. Furthermore, the relationship between KIF26B and tumor infiltration is not yet understood.

Fibroblast growth factor 2 (FGF2 or bFGF2), is an FGF family member that plays an important role in promoting tumor angiogenesis [9]. In recent years, multiple studies confirmed that FGF2 plays a vital function not only in tumor angiogenesis, but also in regulating tumor cell migration and invasion, inflammatory responses, and tumor stem cell properties in a variety of solid tumors [10]. Reports showed that FGF2 suppresses breast cancer progression by inhibiting the transforming growth factor-β signaling pathway [11]. However, further studies are necessary to determine the effects of FGF2 in breast cancer progression.

* Corresponding author.
E-mail address: shliu_chongqing@163.com (S. Liu).
[1] These authors contributed equally to this work, they are considered as co-first authors.

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Here, we aimed to determine the biological function and molecular mechanisms of KIF26B in breast cancer. We first measured the expression of KIF26B in breast cancer tissue, and then investigated the function and mechanism of KIF26B in cell proliferation, migration, and invasion.

2. Materials and methods

2.1. Tissue specimens

Breast cancer tissues and adjacent noncancerous tissues (5 cm from the edge of the neoplastic foci) were obtained from patients undergoing mastectomy from October 2017 to April 2018 in Chongqing University Cancer Hospital & Chongqing Cancer Institute & Chongqing Cancer Hospital. The age of the patients with breast cancer, the diameter of the tumor tissues, and the pathological types were available for these samples. No patients received chemotherapy or radiotherapy prior to the operation. In this study, human breast cancer tissue samples and clinical data were collected and approved by the hospital ethics committee and informed consent forms were signed by all patients. All samples were pathologically diagnosed based on the World Health Organization (WHO) classification [12].

2.2. Cell culture and transfection

A normal human breast cell line (Hs 578Bst) and breast cancer cell lines (MCF7, MDA-MB-468, MDA-MB-231, and MDA-MB-415) were purchased from the American Type Culture Collection (Manassas, USA). MCF7 cells were maintained in minimum essential medium (Corning Life Sciences) supplemented with 10% fetal bovine serum. MDA-MB-468, MDA-MB-231, and MDA-MB-415 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. The cells were maintained in a humidified atmosphere at 37 °C with 5% CO_2. Cells were seeded into 6-well plates and cultured to 80% confluence. Then, KIF26B overexpression plasmids (pcDNA3.1-KIF26B) and negative control plasmids (pcDNA3.1), or KIF26B knockdown plasmids (plKO.1-KIF26B) and negative control plasmids (plKO.1), or E-cadherin siRNA and negative control plasmids were transfected into the corresponding cells using Lipofectamine 2000 reagent according to the manufacturer's instructions.

2.3. Immunohistochemistry (IHC)

Tumor tissues obtained from patients with breast cancer and nude mice from *in vivo* experiments were embedded and sliced to 5-μm thickness. The slides were first immersed in 0.01 M citrate buffer (pH 6.0) and boiled for 30 min at 100 °C for heat-induced epitope retrieval. Hydrogen peroxide (3%) was used to block the endogenous peroxidase activity. Then, the slides were incubated with a primary antibody against KIF26B (1:100, Proteintech, USA) or Ki67 (1:100, CST, USA) which was diluted in PBST containing 3% BSA at 37 °C for 2 h. After washing with phosphate buffered saline, the specimens were incubated in the appropriate secondary antibody, which was also diluted in PBST containing 3% BSA conjugated with biotin. This was followed by DAB coloring, hematoxylin re-dyeing, and sealing with neutral gum. In each experiment, tissue slices with known immunohistochemical staining markers were used as positive controls. Phosphate buffered saline was used instead of the primary antibody as a negative control. Tissue sections were photographed with a Nikon TE2000-U camera (Nikon, Tokyo, Japan) equipped with Nikon optics.

Semi-quantitative IHC detection was used to determine KIF26B protein levels. We multiplied the percentage score by the staining intensity score using the H-score method [13]. The percentage of positively-stained cells was scored as "0" (0%), "1" (1–25%), "2" (26–50%), "3" (51–75%), or "4" (76–100%). Intensity was scored as "0" (negative staining), "1" (weak staining), "2" (moderate staining), and "3" (strong staining). The median H-score was chosen as the cutoff point to separate "high KIF26B expression" (H-score > median) from "low KIF26B expression" (H-score ≤ median) tumor samples.

2.4. Quantitative real-time PCR (qRT-PCR)

Total RNA from breast cancer tissues or cell lines was extracted using TRIzol reagent (Invitrogen, USA) and reverse transcribed into cDNA according to the manufacturer's instructions. *KIF26B* and *FGF2* expression levels were assessed using a SYBR Premix Ex Taq kit (Takara, China) on an Applied Biosystems 7500 System (Applied Biosystems, USA). The primer sequences were as follows:

KIF26B forward primers: 5′-GCGTCGGTGGAACCTTTAGA-3′, reverse primers: 5′-TCACCCCGTATTTCTTGCCC-3′;

FGF2 forward primers: 5′-GCAAAAACGGGGGCTTCTTC-3′, reverse primers: 5′-AACGGTTAGCACACACTCCT-3′;

GAPDH forward primers: 5′-ATCACCATCTTCCAGGAGCGA-3′, reverse primers: 5′-ATGGCATGGACTGTGGTCAT-3′.

2.5. Cell proliferation

The effect of KIF26B on breast cancer cell proliferation was determined by a Cell Counting Kit-8 (CCK8, DOJINDO, Japan). Briefly, cells were transfected with pcDNA3.1 or pcDNA3.1-KIF26B, or plKO.1 or plKO.1-KIF26B plasmids for 48 h, then 3000 cells/well were seeded in 96-well microtiter plates (Corning, USA). At the corresponding time point, 10 μL of CCK-8 solution was added (Dojin, Japan) into each well and incubated for 4 h. The absorbance was measured at 450 nm using a SpectraMax M5 microplate reader (Molecular Devices, USA).

2.6. ELISA

Cells (4×10^5) were seeded into 6-well plates and transfected with the corresponding plasmids for 48 h. Then, the culture supernatants were harvested and concentrations of FGF2 were measured through using the DuoSet ELISA Development System (R&D Systems, Japan).

2.7. Wound healing and transwell assays

Wound healing assays were used to determine the migration abilities of breast cancer cells. The breast cancer cells were transfected with the corresponding plasmids, seeded in 6-well plates, and cultured until 90% confluence. After the confluent monolayers were vertically scratched by a 10-μl pipette tip, cells were treated with or without 10 μM U0126. The plates and the related wound width were photographed and measured by Image J software at 0 h and 24 h.

The invasive ability of cells was determined by transwell assays. Cell suspensions at 5×10^4 cells/mL were seeded into the upper filters coated with Matrigel (BD Biosciences, USA). Culture medium containing 20% fetal bovine serum was added to the bottom chamber to stimulate cell invasion. After 24 h, cells in the upper chambers were removed. Invaded cells were fixed in methanol, stained with crystal violet, photographed, and counted. The numbers of invading cells were assessed using a light microscope (Nikon, Japan) by counting 6 fields (magnification: 200×).

2.8. Western blot analysis

Breast cancer cells were washed with cold phosphate buffered saline and lysed in Radioimmunoprecipitation assay (RIPA) buffer with protease inhibitors (Sigma) on ice for 30 min. Total protein was extracted, and protein concentration was measured by a BCA protein assay. Twenty micrograms of protein extracts were resolved by 10% tris-polyacrylamide gels (SDS-PAGE, Bio-Rad, USA), and transferred to PVDF membranes (Whatman, Germany). Membranes were blocked with 5% non-fat milk and probed with rabbit anti-KIF26B (1:1000,