

## Original Article

# Effects of Scribble on mammary tumorigenesis and metastasis in breast cancer

Yueqing Feng, Zhimin Zhang, Yong Zhou

*Department of Head & Neck, Breast Surgery, Xinxiang Central Hospital, Xinxiang, China*

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**Abstract:** Human Scribble (Scrib) is an evolutionarily conserved cell polarity protein, but it is considered that its precise role in human cancer is controversial. This study aimed to investigate the effects of Scrib on mammary tumorigenesis and metastasis. 12 cases diagnosed with breast cancer and 3 cases with hyperplasia of mammary glands were collected from our hospital. Specimens of breast cancer tissues and the non-breast cancer tissues were selected via surgical resection from those patients correspondingly. And the expression of Scrib in above samples was detected by RT-PCR and Western Blot. Next, recombinant lentiviral vectors of silencing expression and overexpression for Scrib were constructed and transfected into breast cancer cell line MCF-10A, then the effects of Scrib over-expression and knockdown on MCF-10A cell migration were detected by wound-healing assays. The expression of Scrib in all breast cancer samples was down-regulated, and its expression in samples with distant metastasis was decreased sharply. Meanwhile, MCF-10A with Scrib overexpression showed slower cell migration when compared with MCF-10A with Scrib knockdown by wound-healing assays ( $P < 0.01$ ). In conclusion, these evidences support the notion that Scrib may play a crucial role in mammary tumorigenesis and metastasis, indicating that Scrib can be a potentially invaluable therapeutic target for breast cancer.

**Keywords:** Breast cancer, polarity protein, scribble

## Introduction

Breast cancer has been now the most common cancer in Chinese women, and its prevalence is increasing rapidly in recent years. Moreover, Chinese female patients present a younger age at onset of breast cancer when compared with the counterpart of high-income countries, which may be related with differences in genes and risk factors between China and high-income countries. Although Chinese women's risk of breast cancer is lower than women's in high-income countries, yet this risk of today's young Chinese women is higher than that of previous generations. Each year, cases in China account for 12.2% of all newly diagnosed breast cancers and 9.6% of all deaths from breast cancer worldwide [1], and metastasis is the leading cause of death. Breast cancer is considered to originate from epithelial cells of the terminal ductal lobular units (TDLU) in the breast [2, 3]. Each TDLU has multiple small units referred to as acini that consist of a single polarized layer of luminal epithelial cells sur-

rounding a hollow lumen [2]. The establishment and maintenance of polarized organization is critical for normal function of mammary epithelial cells in vivo. Early during initiation and progression of carcinoma, epithelial cells lose their ability to maintain a normal polarized organization, which suggests a critical role for molecules that regulate cell polarity in breast cancer [7].

The cell polarity program is established by at least three interacting protein complexes (Scrib, Crumbs, and Par) in mammal and these complexes mediate several polarization processes, including apical-basal polarity, migration, asymmetric cell division, and planar cell polarity [4, 5]. The apical protein modules on the Crumbs (Pals, PatJ, and Crumbs) and Par (Par3, Par6, and aPKC) complexes act in a mutually antagonistic relationship with the basolateral Scribble module [6]. The Scribble module consists of Scrib, Discs large 1-4 (Dlg1-4), and lethal giant larvae 1/2 (Lgl1/2) [5]. Together, these complexes mediate a complicated series of pro-

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cesses to establish and maintain polarity, including the formation of cell-cell contacts (i.e., adhesion and tight junction) [5].

In the present study, we detected and analyzed the expression of Scrib in breast cancer specimens from the 12 patients, and performed wound-healing assays for breast cancer cell line MCF-10A to explore the effects of Scrib on metastasis.

### Subjects and methods

#### *Patients and specimens*

12 cases diagnosed with breast cancer were collected from our hospital in this study, including 9 cases belonging to primary breast cancer without metastasis and 3 cases with distant metastasis. All of them were in the 45-68 age range with the average of 55.4 years old. Additional 3 cases with hyperplasia of mammary glands were also collected from our hospital as normal controls. Specimens of breast cancer tissues and non-breast cancer tissues were selected via surgical resection from above patients. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Xinxiang Central Hospital. Written informed consent was obtained from all participants.

#### *Construction of retroviral vector with Scrib overexpression*

Recombinant pLXSN-hScrib plasmids were constructed according to conventional molecular cloning methods, and the harvested plasmids were confirmed by DNA sequencing and restriction enzyme. After successfully constructed, they were sent to Shanghai Genechem Co., Ltd. for viral production and titer detection.

#### *Construction of lentiviral vectors with Scrib knockdown*

Recombinant pLV-sh-hScrib plasmids were constructed with lentiviral vector (pLV-Neo). The harvested plasmids were confirmed by DNA sequencing, and then transfected into 293T cells. Finally, recombinant lentiviral vectors of Scrib knockdown were sent to Shanghai Genechem Co., Ltd. for viral production and titer detection.

#### *qRT-PCR*

Total RNA was isolated from above samples using reagent (Trizol; Invitrogen, Carlsbad, California, United States), and the purity and content of the resulting RNA were analyzed at 260 nm and 280 nm. The reverse transcription was performed using random hexamer primers and SuperScript first strand synthesis kit (Invitrogen, Carlsbad, California, United States) with 1.0 µg total RNA as the template. hScrib and β-actin primers were as follows: ScribF, 5'-GCCATGGTGGCAGAGGTTGG, ScribR1, 5'-TGC-TTTCTCCCAGACTCAGG-3'; β-actin-F: 5'-TGACGTGG ACATCCGCAAAG -3'; β-actin-R 5'-CTGGA-AGGTGGACAGCGAGG -3'. The data was gathered on the ABI 7900 sequence detection system (Applied Biosystem, Foster city, California, United States).

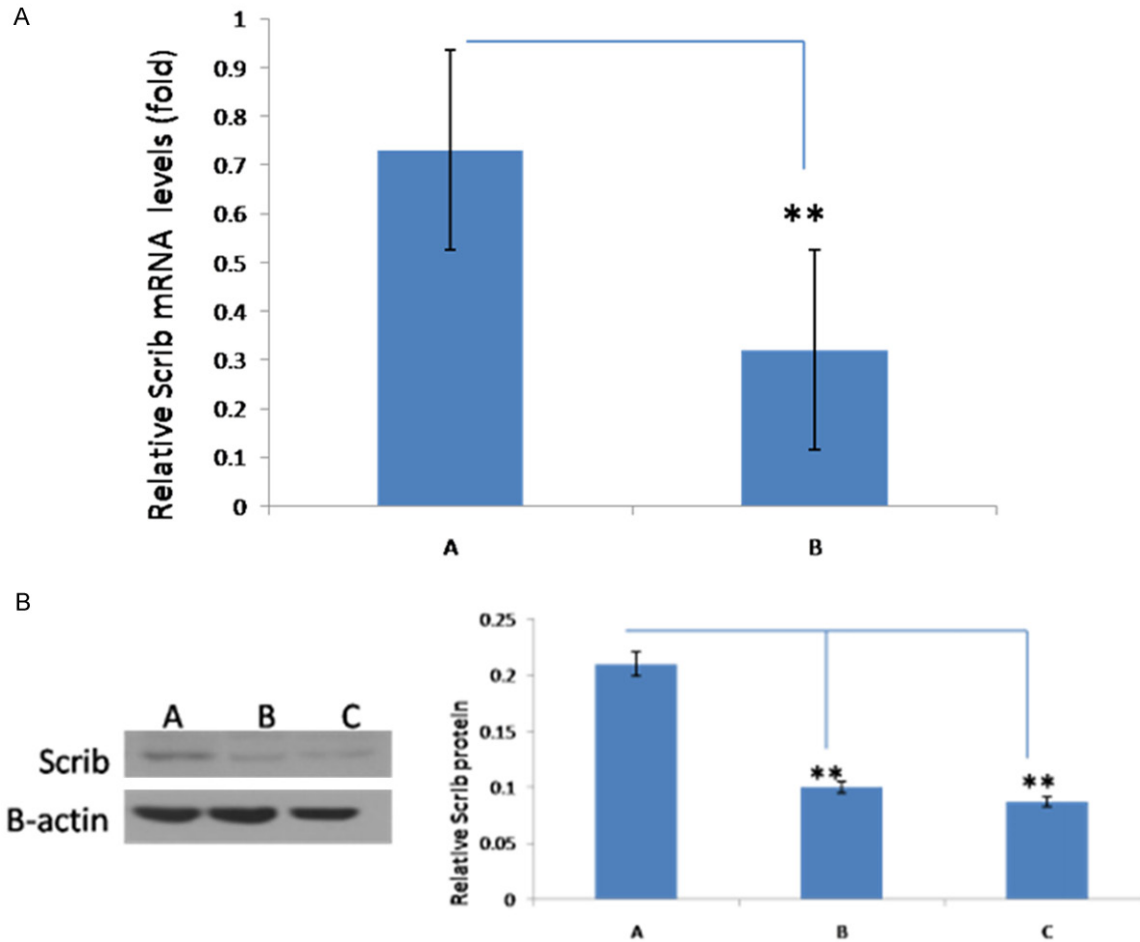
#### *Western blot*

Fresh breast cancer tissue samples were lysed in lysis buffer RIPA (Roche, Swiss). After centrifugation (10 min, 44°C, 16,000 rpm), supernatants were collected and assayed for total protein content using BCA kit (Beyotime, Shanghai, China). 20 µg total protein was performed for 10% SDS-PAGE electrophoresis, and then grafted on 0.22 µm PVDF membrane. Next, they were sealed in TBST (10 mM Tri-HCL, 150 mM NaCl, 0.25% Tween-20, pH 7.5) with 5% skim milk at room temperature for 1 h, and incubated with primary antibody overnight at 4°C. After rinsed with TBST, above PVDF membrane was incubated with goat anti-rabbit IgG-HRP secondary antibody for 1 h. Reactive bands were visualized with ECL, and β-actin was used as the reference. All antibodies were purchased from Santa Cruz Biotechnology (USA).

#### *Transfection of breast cancer cell line MCF-10A with lentivirus*

Breast cancer cell line MCF-10A (70% confluency, purchased from typical species preservation center of Wuhan University) were transfected with recombinant lentiviral vectors of Scrib silencing and overexpression (MOI=100:1). Target cells, which had been transfected with retrovirus, were harvested after 2 days and named as MCF-10AV. siRNA, expressing independent non-targeting Scrib, was used as the control, and the expression of Scrib was detected by Western Blot.

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**Figure 1.** A: The expression of Scrib mRNA in tissue specimens of in situ breast cancer and breast cancer with distant metastasis by quantitative RT-PCR. (A) In situ breast cancer; (B) Breast cancer with distant metastasis. B: The expression of Scrib protein in tissue specimens of non-breast cancer, in situ breast cancer and breast cancer with distant metastasis by Western Blot. (A) non-breast cancer; (B) breast cancer; (C) breast cancer with distant metastasis.

### Wound-healing assays

Breast cancer cell line MCF-10A and MCF-10AV were plated on culture dish with  $1 \times 10^5$  cells/cm<sup>2</sup>, and continuously cultured for 24-36 hours or until cell confluency. Monolayers cells were scratched with a cell scraper, and cell migration at each time point was observed with microscope until wound closure. Quantification of migration distance was determined as the reduction in the wound's gap using computer software.

### Statistical analysis

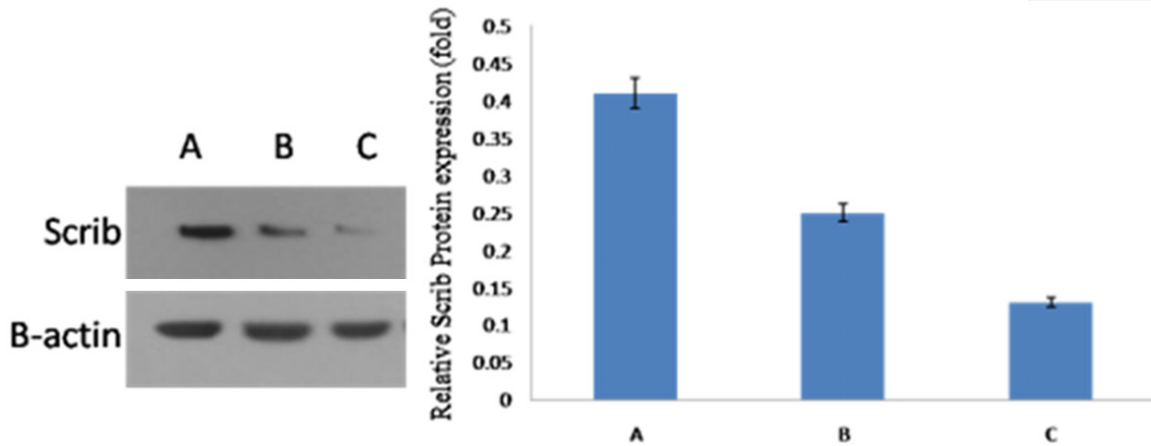
All statistical analyses were performed using SPSS version 7.0 software.  $P < 0.05$  was considered statistically significant.

## Results

### Expression of Scrib in breast cancer

qRT-PCR was applied to analyze the expression of Scrib mRNA in the breast cancer and non-breast cancer tissues, and the results showed that Scrib mRNA expression was down-regulated in the tissue samples of non-breast cancer, primary breast cancer and breast cancer with distant metastasis in proper order, in other words, its expression in breast cancer with more advanced pathological grade was decreased sharply, but there were significant difference in Scrib mRNA expression between primary breast cancer and breast cancer with distant metastasis (**Figure 1A**). Then we used Western Blot to analyze the expression of Scrib

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**Figure 2.** MCF-10A cells with Scrib protein overexpression and knockdown after Lentivirus transfection. A: MCF-10A cells with Scrib overexpression; B: MCF-10A cells without lentivirus transfection; C: MCF-10A cells with Scrib knockdown.

protein in these tissues, and the results showed that Scrib protein expression was down-regulated in the tissue samples of non-breast cancer, primary breast cancer and breast cancer with distant metastasis in proper order, and there were significant difference in Scrib protein expression between breast cancer and non-breast cancer ( $P < 0.05$ ; **Figure 1B**). These results further suggested that Scrib might play a certain role in breast tumorigenesis and metastasis.

### *Expression of Scrib in MCF-10A cell line transfected with lentivirus*

Transfection of breast cancer cell line MCF-10A with Scrib siRNA could effectively silence the expression of target genes, while the control siRNA had no this effect. And transfection of breast cancer cell line MCF-10A with lentivirus for Scrib expressing could up-regulate the expression of Scrib (**Figure 2**). These study demonstrated that the reconstructed recombinant lentivirus could meet the needs of the experiments.

### *Results of wound-healing assays*

To explore the effect of Scrib on breast cancer cell migration, wound-healing assays were carried out using MCF-10A cell line with Scrib overexpression and knockdown, and the results showed that cell migration of MCF-10A with Scrib knockdown was faster than that of normal MCF-10A and MCF-10A with Scrib overexpression, indicating that the decreased expres-

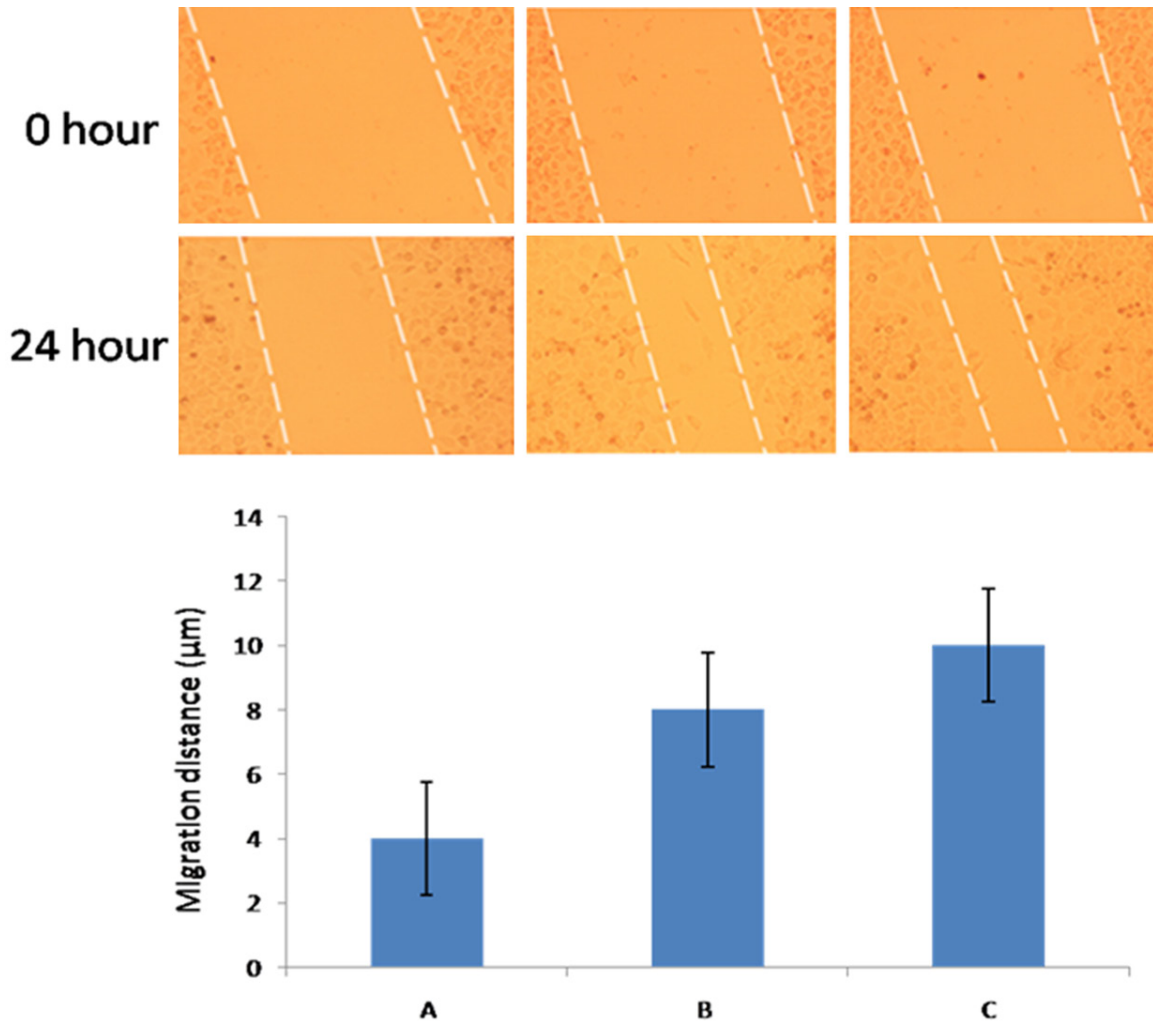
sion of Scrib fastened the cell migration. Meanwhile, the cell migration of normal MCF-10A was faster than that of MCF-10A with Scrib overexpression, but there was no significant difference ( $P > 0.05$ ) (**Figure 3**).

## Discussion

Early during initiation and progression of carcinoma, cells and histological structure are destroyed significantly. However, the role imbalance in cells and histological structure plays in tumorigenesis is not well understood. Recently, it has been reported that Scrib disturbance not only destroy cell structure but also affect the formation of normal cell morphology in vivo and vitro. Meanwhile, Scrib can be a regulator of cell death pathways.

In the present study, we applied quantitative RT-PCR and Western Blot to detect the expression of Scrib in breast cancer specimens of 12 cases and the results showed that its expression in all breast cancer tissues was lower than that in non-breast cancer tissues, and was the lowest in breast cancer with distant metastasis. Zhan et al. [7] showed that Scrib could inhibit the formation of breast cancer, and Scrib imbalance could lead to the destruction of cell morphology and inhibition of cell death. Pearson et al. [8] showed that Scrib expression was deregulated in human prostate cancer, and its deficiency in mice promoted prostate neoplasia. Above these studies demonstrated that imbalance in Scrib (as the cytoskeleton) expression could result in tumorigenesis. In

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**Figure 3.** Wound-healing assays. A: MCF-10A with Scribd overexpressing; B: MCF-10A cells without lentivirus transfection; C: MCF-10A cells with with Scrib knockdown.

epithelial cells, TAZ formed a complex with the cell-polarity determinant Scribble in Hippo signaling pathway, and loss of Scribble--or induction of the epithelial-mesenchymal transition (EMT)--disrupted the inhibitory association of TAZ with the core Hippo kinases MST and LATS, which played a crucial role in the mechanism for breast cancer stem cells (CSCs) to maintain the self-renewal and the initiation of tumor [9]. Moreover, C-terminal end of high-risk human papillomavirus (HPV) oncoprotein E6 presented PDZ-binding motif (PBM), which could interact with PDZ cell polarity proteins, such as Scrib, resulting in Scrib ubiquitin degradation, and this was an important mechanism for high-risk HPV tumorigenesis [10]. These studies were consistent with our results, and Scrib down-regulation might play a role in tumorigenesis,

indicating that Scrib was a tumor suppressor factor. However, other studies had shown Scrib overexpression existed in tumors, which could accelerate the migration of tumor cells [11]. So we speculated that these inconsistent results may be related to cell types in the studies.

Furthermore, metastasis is the leading cause of breast cancer death. In order to study the effect of Scrib on metastasis of breast cancer, recombinant lentiviral vectors were constructed to induce breast cancer cell line MCF-10A overexpression or silencing expression, and wound-healing assays were applied to detect the effects of Scrib on MCF-10A cell migration, and the results showed that the migration of MCF-10A with Scrib knockdown was faster than that of MCF-10A with Scrib overexpression. Our

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results also corroborated by other researchers, and Qin et al. showed that E-Cadherin mediated cell-cell adhesion requires Scrib, and knock-down of Scrib made the cells acquired a mesenchymal appearance, so the migration was enhanced [12]. In addition, Scrib imbalance promoted breast tumor formation, which revealed the role of cell polarity in tumorigenesis [13-15].

In conclusion, our data indicates aberrant over-expression of Scrib is a more complex prelude in tumorigenesis, in which there may be other proteins to make Scrib dislocation, leading to cell-cell contact departure, total destruction of cell migration pathways, and finally causing cell migration, which contributed to the induction of the epithelial-mesenchymal transition (EMT), resulting in tumor distant metastasis in vivo. Therefore, therapeutic targeting of the Scrib pathway may selectively disrupt pivotal mechanisms of tumor cell motility and invasion in tumors, creating concrete new prospects for the development of directed antimetastatic therapies in humans.

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### Disclosure of conflict of interest

None.

**Address correspondence to:** Dr. Yueqing Feng, Department of Head & Neck, and Breast Surgery, Xinxiang Central Hospital, 56 Jinsui Road, Xinxiang 453000, Henan Province, China. Tel: 86-373-2038262; E-mail: yueqingfengcn@163.com

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