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Original Article

Clinical significance of CCN5 and mutant p53 in primary and recurrent lesions of breast cancer

Guofeng Zhou, Xingxing Gui, Wei Qu

Department of Pathology, The Third Hospital of Nanchang, Nanchang, Jiangxi Province, China

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Abstract: Objective: To analyze the expression and significance of CCN5 and mutant p53 proteins in primary and recurrent lesions of breast cancer (BC) patients. Methods: The expression of CCN5 and mutant p53 proteins in 20 normal breast tissues, 60 primary and chest wall recurrent lesions were detected by streptavidin peroxidase conjugated (SP) method. Results: The differences in CCN5 and mutant p53 expression is significant among normal breast tissue, primary lesion, and recurrent lesions (X^2=18.380 and X^2=30.549, $P < 0.05$), and the expression of CCN5 protein was higher and the expression of mutant p53 protein was lower in all primary lesions than in recurrent lesions ($P < 0.05$). CCN5 expression was higher in the group without lymph node metastasis (LNM) than in the group with LNM in BC patients, while mutant p53 protein expression was higher in the group with LNM than in the group without LNM (X^2=9.775, X^2=7.102, $P < 0.05$). There was a negative relationship between CCN5 and mutant p53 protein expression in BC tissues (r_p=-0.013, $P < 0.001$). Conclusion: CCN5, mutant p53 protein expression may play different regulatory roles in BC recurrence and LNM and have important implications in BC development and prognosis.

Keywords: Breast cancer, primary foci, recurrent lesion, CCN5, p53

Introduction

Breast cancer (BC) is a malignancy that seriously threatens women's health, and its incidence is increasing year by year since the 1970s [1]. Lymph node metastasis (LNM) and local recurrence are important factors influencing the prognosis of BC. The growth, invasion and metastasis of malignant tumors is a multi-step complex process, in which mitosis, intercellular adhesion, induction of apoptosis, production of extracellular matrix, inhibition of growth and migration of multiple cells play critical roles. CCN5 is a newly identified apoptosis suppressor protein and may be a potential proto-oncogene associated with malignant transformation of cells [2]. CCN5 may inhibit infiltrative metastasis of tumors by suppressing the process of epithelial mesenchymal transformation [3, 4]. The mutant p53 protein not only lacks the tumor suppressor activity of wild-type p53 but also gains new oncogenic functions. It also alters gene expression by affecting cytokinesis and exocytosis in BC cells [5]. It has been reported that CCN5 expression is negatively correlated with mutant P53 proteins in pancreatic cancer while P53 protein can inhibit the expression of CCN5 [6]. Therefore, mutant P53 gene overexpression in progressive BC may be related to silencing of CCN5 gene [7].

CCN5 and p53 proteins can also be used as indicators for determining the recurrence and metastasis of BC patients [8]. In this study, we detected CCN5, p53 protein expression in primary foci, recurrent foci, normal tissues and BC tissues with and without LNM in BC patients using SP method and analyzed the correlation between them.

Materials and methods

Source of specimens

Female patients with BC who underwent surgery from January 1, 2015 to December 30, 2019 at the Third Hospital of Nanchang City were enrolled. They aged 25 to 80 years, with a median age of 40 years. All patients underwent modified radical mastectomy after initial diagnosis. The pathological type was invasive duc-

tal carcinoma, 60 cases had primary and recurrent lesions, 19 of them were those with lymph node metastases, and 33 without lymph node metastases. 20 samples of normal breast tissues were collected. The recurrent BC was confirmed by live biopsy or coarse-needle biopsy, and complete medical history and paraffin sections were accessible in all cases. The time to recurrence ranged from 3-48 months, with a median recurrence time of 10 months. This study has been approved by the Ethics Committee of the Third Hospital of Nanchang. All study participants provided written informed consent before participating in the study.

Inclusion criteria: ① The diagnosis of breast cancer was confirmed by histopathology; ② After chemotherapy for breast cancer in women over 18 years old; ③ The patient knew his condition and was willing to participate in this study; ④ No serious heart, lung, liver, kidney and other diseases.

Exclusion criteria: ① Those who have received radiotherapy or neoadjuvant chemotherapy before surgery; ② Non-primary breast cancer or breast cancer recurrence. ③ Those with mental disorders or language and communication disorders; ④ Those with breast cancer IV.

Main reagents

The concentrated rabbit anti-human CCN5 polyclonal antibody (Ab38317) was purchased from Abcam, USA, and diluted at 1:100. The p53 monoclonal antibody (ready-to-use working solution), secondary antibody and DAB staining kit were purchased from Maixin, Fuzhou, China. Other reagents include xylene, gradient ethanol (100%, 90%, 85%), 3% H_2O_2, PBS, hematoxylin, etc.

Study methods

All specimens were fixed using 10% formalin and dehydrated to prepare paraffin-embedded sections, followed by HE staining. Immunohistochemistry was performed using the SP method, with PBS instead of primary antibody as the negative control, and positive tissue slices of BC as the positive controls. The procedures of immunohistochemistry were as follows: (1) bake the sections in an oven at 60°C overnight; (2) dewax and hydrate the tissue sections; (3) antigen retrieval with citrate solution in a pressure cooker (4) incubation with 3% H_2O_2 deionized water for 10 min to block endogenous peroxidase; (5) dropwise addition of primary antibody, placed in the refrigerate at 4°C overnight; (6) dropwise addition of secondary antibody, incubated at room temperature for 18 min; (7) dropwise addition of DAB solution for color development; (8) re-staining, dehydration, transparent, and neutral gum sealing of the sections.

Determination of results

CCN5 protein positive expression was determined as the appearance of brownish-yellow granules in the cytoplasm or in the nucleusas of p53 proteins. The staining results were observed using a double-blind method and the intensity of staining and the proportion of positive cells were evaluated: 1) no positive staining was scored 0, light yellow was scored 1, brownish-yellow was scored 2, and brownish-brown was scored 3. 10 high magnification fields were randomly selected to calculate the percentage of positive in 200 cells, and the results were averaged. The ratio of positive cells < 5% is 0, 5%-25% is 1, 25%-50% is 2, 50%-75% is 3, > 75% is 4. The two scores are added together: ≤ 3: negative; > 3: positive.

Statistical methods

With SPSS 19.0, the differences in CCN5, p53 protein expression were compared between tissues using non-parametric test, and the relationship between CCN5 and p53 expression was analyzed using Spearman's correlation. Olympus BX63 microscope software were used for catching the immunohistochemical pictures. Differences were considered statistically significant at $P < 0.05$.

Results

CCN5 and p53 expression in each group

As shown by immunohistochemical results (**Figure 1**), CCN5 was mostly localized in the cytoplasm and p53 was mainly localized in the nucleus. CCN5, p53 expressed differently among recurrent lesion, primary lesion and normal breast tissue (X^2=18.380, P=0.000; X^2=30.549, P=0.000, **Table 1**).

Effects of LNM on CCN5, p53 expression

The positive expression rate of CCN5 in the group without LNM was 72.73% (24/33), signifi-

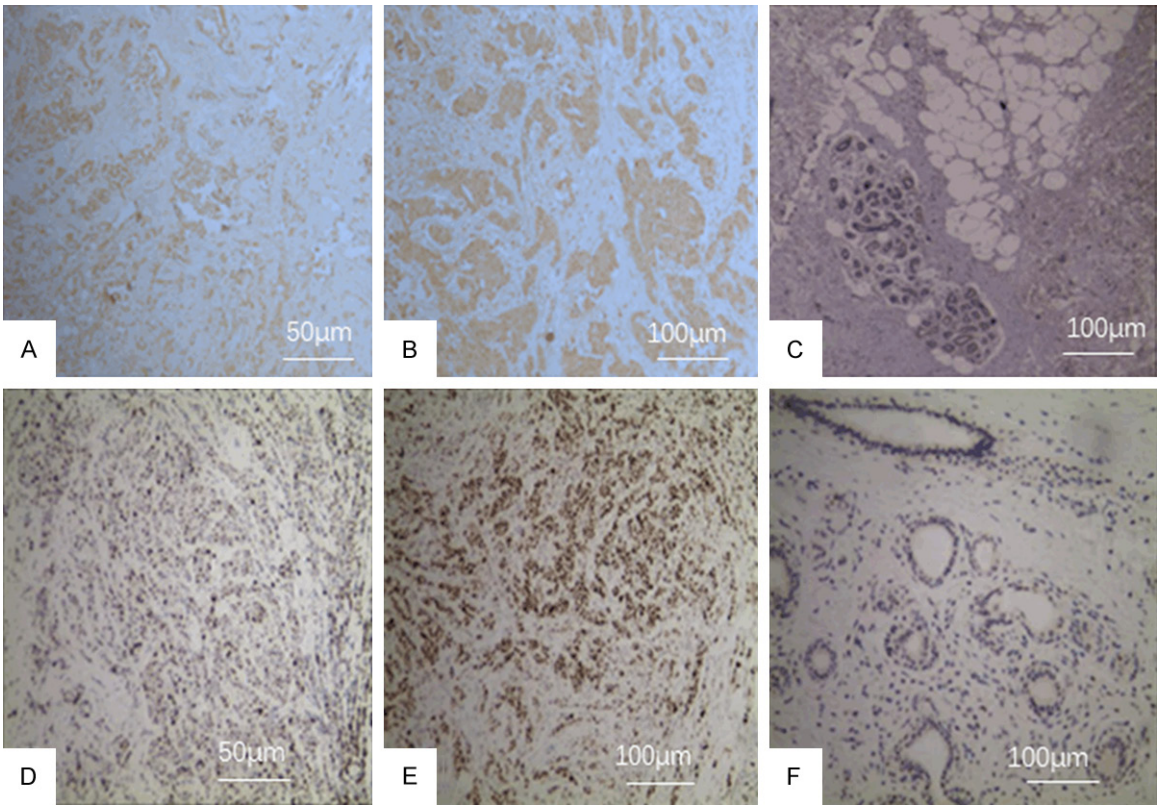

Figure 1. Expression of CCN5 and p53 in each group. A: CCN5 is expressed in the primary lesion (×400); B: CCN5 expression in recurrent lesion (×100); C: CCN5 expressed in normal tissue (×40); D: p53 expressed in primary lesion (×100); E: p53 expressed in recurrent lesion (×200); F: Expression of p53 (×100).

Table 1. Expression of CCN5, p53 in 3 breast tissues (%)

Type	Case	CCN5	p53
Normal tissue	28	4 (14.29)	6 (21.43)
Primary lesion	52	33 (63.46)	21 (40.38)
Recurrent lesion	52	21 (40.38)	42 (80.20)
X^2		18.308	30.549
P		0.001	0.002

cantly higher than that of 36.84% (7/19) in the group with LNM (X^2=9.775, P=0.003); The positive expression rate of p53 was 45.46% (15/33) in the group without LNM, which was significantly lower than 68.42% (13/19) in the group with LNM (X^2=7.102, P=0.010, **Table 2**).

Correlation between CCN5 and p53 expression levels in breast tissue

The expression levels of CCN5 in breast tissue were negatively correlated with p53 expression levels (r=-0.013, P < 0.01, **Table 3**).

Discussion

Significance of CCN5 expression in BC

WISP-2 (Wnt-1 induced secreted proteins-2), also known as CCN5, is a member of the cysteine-rich 61/connective tissue growth factor/nephroblastoma-overexpressed (CCN) family, which is involved in a variety of critical pathophysiological processes such as cell proliferation, differentiation, migration, apoptosis, angiogenesis, and tumor formation [2, 9]. Studies have shown that CCN5 protein plays a role in the development of various tumors [10, 11]. CCN5 has the ability to inhibit or promote cell proliferation, depending on the cell type and the specific microenvironment [12]. For example, the overproduction of EGF or IGF-1 by CCN5 is caused by mitogenic effect of estrogen receptor-positive, non-invasive breast tumor cells [13]; It plays the role of growth arrest-specific gene in vascular smooth muscle cells and prostate cancer cells [14]. CCN5 protein is a bidirectional regulatory signaling molecule, pri-

Table 2. Expression ofCCN5, p53 in breast cancer patients with and without lymph node metastasis (%)

Type	Case	CCN5	p53
Group with lymph node metastasis	19	7 (36.84)	13 (68.42)
Group without lymph node metastasis	33	24 (72.73)	15 (45.46)
X^2		9.775	7.102
P		0.003	0.010

Table 3. Relationship between CCN5, p53 protein expression in primary lesion tissue

CCN5	p53		r	*P* value
	Positive	Negative		
Positive	54	64		
Negative	40	50	-0.013	0.001

marily inhibiting aggressive behaviors of cancer cells, such as epithelial-mesenchymal transitions (EMT) [4, 15]. However, the clinical significance and molecular mechanisms of down-regulation of CCN5 proteins during disease progression remain unclear. CCN5 protein expression is negatively associated with the production of excessive mutant P53 proteins in pancreatic cancer [16]. Gene display and mathematical modeling studies found that P53 protein can eliminate CCN5 expression. Therefore, the overexpression of mutant P53 genes in progressive BC may be related to silencing of CCN5 gene [7].

Significance of p53 expression in BC

The transcription factor p53 is a tumor suppressor. It is essential for the regulation of cellular physiology [17, 18]. It responds to multiple cellular stress processes, including DNA damage, hypoxia, heat shock, ionizing radiation, and oncogenic stimuli through pathways relating to the cell cycle, apoptosis, and other cellular process-associated molecules. Mutant p53 functions by mutations or deletions was commonly seen, particularly in BC [19, 20]. Studies have shown that mutations that prolong the half-life of this protein not only inhibit the tumor suppressor function of the gene, but also acquire new pro-cancer functions through inactivation of DNA damage response genes or trans-activation of target genes associated with cell proliferation, apoptosis, tumorigenesis and tissue invasiveness [21, 22]. Therefore, mutant p53 is a gene of bidirectional regulatory function in cancer progression [23].

The relationship between CCN5 and p53 expression in BC

The p53 gene is prone to mutation in BC, and its mutation correlates with tumor malignancy as well as prognosis [24]. Mutant p53 is present in approximately 23% of BCs, and up to 80% in basal-like BCs [25]. Mutant p53 is associated with BC progression. CCN5 expression is negatively correlated with p53 expression in some types of cancer. It was shown [8] that the expression of mutant p53 in pancreatic and BC tissue specimens or cell lines was negatively correlated with the expression of CCN5/WISP-2 mRNA. CCN5/WISP-2 was always overexpressed in non-invasive breast tumor cells and cell lines without mutant p53, while the expression of CCN5 was decreased or undetectable in most cells expressing mutant p53. Thus, the oncogenic function of mutant p53 may inhibit CCN5 function in breast tumor cells. The results of this study showed that CCN5 and mutant p53 were expressed antagonistically in BC, suggesting that they may play a distinct regulatory role in the recurrence and metastasis of BC, and the specific molecular mechanism needs to be further investigated.

In summary, downregulation of CCN5 expression and upregulation of mutant p53 expression in BC tissues with primary and recurrent lesions and lymph node metastases further indicate that they are important biological markers in BC development, progression, and metastasis. The expression of combined CCN5 and mutant p53 showed clinical significance in determining the recurrence, metastasis and prognosis of BC.

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Disclosure of conflict of interest

None.

Address correspondence to: Guofeng Zhou, Department of Pathology, The Third Hospital of Nanchang, 1268 Jiuzhou Street, Chaoyang New Town, Xihu District, Nanchang 33000, Jiangxi

Province, China. Tel: +86-15083558231; E-mail: zhouguofeng1984@sina.com

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