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Downregulated PIRH2 Can Decrease the Proliferation of Breast Cancer Cells

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Background and Aims. We undertook this study to investigate the influence of PIRH2 (p53-induced RING-H2) protein on the proliferation and cell cycle of breast cancer cell lines.

Methods. PIRH2 expression was detected by Western blot analysis, immunohistochemistry (IHC) and Kaplan–Meier curve analysis. Cell proliferation was assessed by cell counting kit-8 (CCK-8). Cell cycle control was analyzed by flow cytometry.

Results. PIRH2 was up-regulated in breast cancer tissues and cell lines and up-regulated PIRH2 was highly associated with tumor size, grade, ER, and Ki-67. Moreover, Kaplan–Meier curve showed that up-regulated PIRH2 was related to the poor overall survival of patients with breast carcinoma. When the expression of PIRH2 was inhibited by siRNA transfection, cell proliferation was reduced. In addition, the number of G0/G1 phase cells was increased, but G2/M cells were not affected significantly.

Conclusion. Decrease of PIRH2 expression in the breast cancer cell line MDA-MB-231 resulted in reduced tumor cell growth via the inhibition of cell proliferation and the interruption of cell cycle transition.

Key Words: Breast neoplasms, Ubiquitin, p53-induced RING-H2 (PIRH2), Proliferation, Cell cycle.

Introduction

Cancer is a leading cause of death in both more and less economically developed countries (1). Breast cancer is one of the most common malignant tumors of women worldwide and is the second highest cause of death of female patients with tumors (2). The incidence of breast cancer results from a combination of hormones, genetic, virus, nutritional factors, environmental factors, and psychological factors (3). A substantial portion of breast cancer cases and deaths could be prevented by the use of early detection tests (4). Thus, markers for poor prognosis of breast cancer have significant clinical applications. Identifying and understanding the mechanisms of these markers will provide new markers for early detection of breast cancer patients, which has important significance for improving patient prognosis.

The ubiquitin-dependent proteasome system plays a critical role in many cellular processes and pathogenesis of various human diseases including cancer (5). PIRH2 (p53-induced RING-H2 protein) was a new member of E3 ubiquitin ligase family, which could target p53 for degradation and thereby repress a diverse group of biological activities regulated by p53 (6,7). The expression levels of PIRH2 was high in human hepatocellular carcinoma, head and neck cancers, prostate cancer, lung cancer, and clear cell renal cell carcinoma. It was proven that PIRH2 promoted tumor progression in lung cancer, hepatocellular

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carcinoma, clear cell renal cell carcinoma and prostatic carcinoma (7−12). It has been previously demonstrated that overexpression of PIRH2 was more frequent in primary breast cancer (7). However, there is currently no evidence that can prove this idea.

In this study we detected the overexpression of PIRH2 in breast cancer tissues and cell lines. Meanwhile, IHC statistics showed the correlation of PIRH2 levels with tumor size, ER, and Ki-67. Interfering PIRH2 could suppress the proliferation and cell cycle of MDA-MB-231 cells. In addition, we also detected the influence of PIRH2 on the protein levels of Cyclin D1. Therefore, we speculated that down-regulation of PIRH2 inhibited breast cancer cells proliferation and cell cycle. PIRH2 was a potential marker for detection of breast cancer. The aim of the present study was to investigate the role of PIRH2 in the development of breast cancer.

Materials and Methods

Tissue Samples

One hundred and five breast cancer sections from patients who underwent surgery between 2003 and 2006 at the Department of Pathology, Nantong University Tumor Hospital were formalin-fixed and paraffin-embedded for histopathological diagnosis and immunohistochemical study. Fresh samples were frozen in liquid nitrogen immediately after surgical removal and maintained at −80°C until used for Western blot analysis. All human tissues were collected using protocols approved by the Ethics Committee of Nantong University Tumor Hospital. Signed informed consent was also obtained from each patient. Clinicopathological factors are shown in Table 1.

Table 1. Expression of PIRH2 in 105 human breast adenocarcinoma tissues

Clinicopathological parameters	Total	PIRH2		p
		Low (n = 39)	High (n = 66)	
Age (years)				
<50	42	16	26	0.869
≥50	63	23	40	
Tumor size				
≤2	32	18	14	0.007[a]
>2	73	21	52	
Histology				
Ductal	88	31	57	0.355
Others	17	8	9	
Grade				
I	27	17	10	0.002[a]
II	33	12	21	
III	45	10	35	
Axillary lymph node status				
N0	42	13	29	0.121
N1	46	16	30	
N2	17	10	7	
ER				
Negative	47	23	24	0.024[a]
Positive	58	16	42	
PR				
Negative	45	16	29	0.771
Positive	60	23	37	
HER-2				
Negative (0−1+)	76	31	45	0.211
Overexpressed (2−3+)	29	8	21	
Ki-67				
Low	39	24	15	0.000[a]
High	66	15	51	

Statistical analyses were carried out with the Pearson χ^2 test.
[a] $p < 0.05$ was considered significant.

Cell Cultures

Human breast cancer cell lines HBL-100, MDA-MB-231 and MCF-7 (kindly provided by the Department of Oncology, Affiliated Tumor Hospital of Fudan University) were used in this study and were maintained in Dulbecco's modified Eagle's medium (DMEM) (Gibco BRL, Grand Island, NY) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mmol L-glutamine, and 100 U/mL penicillin−streptomycin mixture (Gibco BRL) at 37°C and 5% CO_2.

Western Blot Analysis and Antibodies

Western blot assay was performed as previously described (13). From each pair of breast cancer tissues and the adjacent normal tissues, 100 mg was selected; meanwhile, an appropriate amount of cells in the same manner were applied. To tissues and cell samples, 1 mL PIRA (50 mmol Tris-HCL pH 8.0, 150 mmol NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 60 mmol β-glycerophosphate, 0.1 mmol sodium vanadate, 0.1 mmol NaF × protease inhibitor cocktail) was added and then placed on ice for 0.5 h after being fully homogenized. The sample was then centrifuged at 13,000 rpm for 15 min to obtain the supernatant. An appropriate amount of 12% SDS-PAGE buffer (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) was added and the mixture transferred to a PVDF membrane (Millipore, Bedford, MA). The membrane was blocked with phosphate-buffered saline (PBS) containing 0.1% Tween 20 and 5% nonfat milk for 2 h. The membrane was then incubated with the primary antibodies overnight at 4°C. The next day, the membrane was washed with PBS containing 0.1% Tween 20 and then incubated with the secondary antibodies. Immunoreactive bands were visualized by Odyssey Infrared Image. Band density was measured with a computer imaging system (Imaging Technology, Ontario, Canada). Band densities were compared using ImageJ (Wayne Rasband, National Institutes of Health, Bethesda, MD). Antibodies used for Western blot analysis are listed