

Correlation between Expression of P38 MAPK-Signaling and uPA in Breast Cancer

Yanchun Han¹
Luying Liu¹
Dongxia Yan¹
Guihua Wang²

¹ Department of Pathology; ² Department of Physiology, Binzhou Medical University, Binzhou 256603, Shandong Province, China.

Correspondence to: Yanchun Han
Tel: 86-535-6913 215
E-mail: hanxinyu78@hotmail.com

Received October 18, 2007, accepted February 28, 2008.

CJCO <http://www.cjco.cn>
E-mail: 2008coccr@gmail.com
Tel (Fax): 86-22-2352 2919

OBJECTIVE To study the expression of phosphorylated p38 mitogen-activated protein kinase (p-p38) and uPA and the correlation of their expression with breast cancer clinicopathological characteristics, and to investigate the role of the p38MAPK-signaling pathway in regulating uPA expression in breast cancer cells.

METHODS Immunohistochemistry (S-P) was used to test the expression of p-p38 and uPA in 60 specimens of breast cancer tissues. Western blots were adopted to detect expression of the p-p38 and uPA proteins in MDA-MB-231 and MCF-7 breast cancer cells, and uPA expression after treatment with SB203580, a specific inhibitor of p38 MAPK.

RESULTS The positive rate of the p-p38 protein and uPA protein expression in the breast cancer tissues was 56.7% and 60.0%, respectively. The expression of p-p38 was positively related to the expression of uPA ($r = 0.316$, $P < 0.05$). The expression of p-p38 and uPA was related to lymph node metastasis and the TNM stage ($P < 0.05$), but it was not related to the patient's age or tumor size ($P > 0.05$). The expression of p-p38 and uPA in MDA-MB-231 cells was higher than that in MCF-7 cells. SB203580 inhibited the p38 MAPK pathway and reduced uPA protein expression.

CONCLUSION The p38 MAPK-signaling pathway promotes breast cancer malignant progression by up-regulating uPA expression, and it may be an important process in breast cancer invasion and metastasis.

KEY WORDS: p38 MAP kinase, uPA, breast cancer.

Copyright © 2008 by Tianjin Medical University Cancer Institute & Hospital and Springer

Introduction

Although combined therapy of breast cancer has advanced over the last several decades, over 30% of breast-cancer patients die due to a recurrence and metastasis. Therefore an understanding of the detailed mechanisms of invasion and metastasis in breast cancer would be helpful in improving treatment. Since breast cancer invasion and metastasis are multifactorial processes, studies related to these processes have received current emphasis.

uPA is a serine protease and, when bound to its receptor, uPA initiates the activation of MMPs as well as the conversion of plasminogen to plasmin. uPA confers the ability for cells to degrade the extracellular matrix. Up to now, a growing body of evidence has shown that over-expression of the urokinase-type plasminogen activator (uPA) can be detected in various malignant tumors, and that it plays a key role in tumor invasion and metastasis. However, there is a need to demonstrate the molecular regulatory mechanisms for uPA expression. p38 MAPK is an important member of mitogen-activated

protein kinases (MAPKs). The MAPKs have been shown to transduce extracellular signals into cellular responses and play important roles in cell proliferation, apoptosis, differentiation, cell migration, and cytoskeleton remodeling. Recently, it has been suggested that the p38MAPK-signaling pathway is involved in the process of tumor invasion and metastasis by a positive regulatory mechanism. Therefore, we investigated the expression of p-p38 and the uPA protein in breast cancer tissues and cells, as well as the effect of the p38 MAPK-signaling pathway on the expression of uPA. Our studies suggest that an inhibitor of p38 MAPK might be a potential therapeutic target for anti-breast cancer treatment.

Materials and Methods

Specimens

A total of 60 paraffin-embedded breast cancer tissues were obtained from surgical resections at the Pathology Department of the First Affiliated Hospital of China Medical University from 2001 to 2003 (Shenyang, Liaoning Province, China). According to the 2003 World Health Organization breast carcinoma histological classification criteria, all specimens were invasive ductal carcinomas. Human MDA-MB-231 and MCF-7 breast cancer cells were obtained from the Pathology Department of the China Medical University.

Reagents

DMEM and RPMI 1640 were purchased from Gibco. The first antibodies were mouse monoclonal antibody to phosphorylated-p38 and rabbit polyclonal antibody to uPA. SB203580, a specific inhibitor of p38MAPK, was purchased from Calbiochem.

Immunohistochemistry

IHC was performed according to the indirect streptavidin-biotin-hyperoxidase method, following the manufacturer's protocol. For the negative controls, the primary antibody was replaced by RIPA buffer, but all incubation steps were identical. Previously identified strongly staining tumor tissue sections were used as positive controls. We used an intensity-adjusted scoring system to

evaluate the immunostaining indices. The sections were scanned by light microscopy. Five fields were randomly selected and 100 tumor cells in each field were counted. The staining intensity was graded 0–2, corresponding to weak, moderately strong, and intense staining, respectively. With respect to the invasive cell count, < 10% positive cells were scored as 0, 10–50% were scored as 1, and > 50% were scored as 2. By multiplying these two factors, an immunoreactive score ≥ 2 was considered to be positive.

Western blots

The cells were lysed in RIPA buffer, sonicated and centrifuged at low temperature. The protein concentration was measured by the Bradford method and cell lysates electrophoresed in 12% polyacrylamide SDS gel followed by transfer onto polyvinylidene difluoride membranes, protein bands were blocked and incubated with the first and second antibody and visualized with a DAB kit. Protein contents were calculated by densitometry.

Statistical analysis

All statistical calculations were carried out using SPSS 10.0 statistical software. The results were compared using the chi-square and Pearson tests. $P < 0.05$ was considered statistically significant.

Results

Correlation between p-p38 and uPA protein expression in breast cancer tissues Immunohistochemical results showed that the p-p38 protein was detected in the nucleus of the breast cancer cells. It showed dark staining with a positive rate of 56.7% (34/60) (Fig.1). The uPA protein was expressed in the cytoplasm with dark staining, with a positive rate of 60.0% (36/60) (Fig.2). The uPA protein was expressed positively in 25 of 34 cases, which were also positive for p-p38 protein expression, and 15 cases showed negative expression of the uPA protein in 26 cases with p-p38 negative expression. Statistical analysis suggested a positive relationship between expression of p-p38 and uPA ($r = 0.316$, $P < 0.05$).

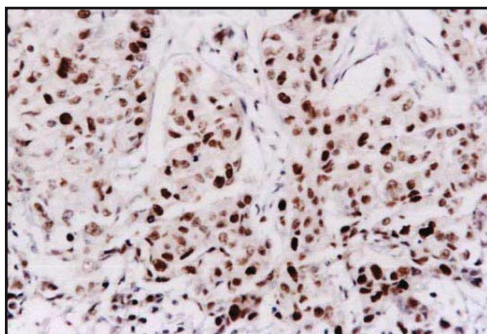


Fig.1. Expression of p-p38 in breast-infiltrating duct carcinoma showing nuclear staining (S-P method, $\times 200$).

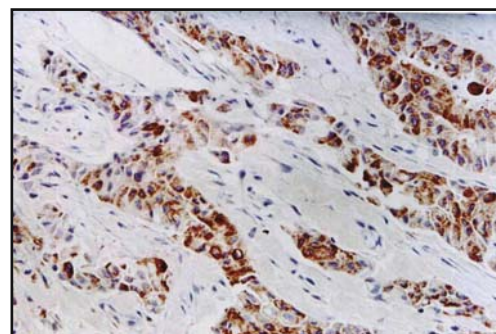


Fig.2. Expression of uPA in breast-infiltrating duct carcinoma showing cytoplasmic staining (S-P method, $\times 200$).

Table 1. Correlation of p-p38 and uPA expression with clinicopathological characteristics of breast cancer.

Characteristic	Cases	Positive cases (%)		<i>P</i>	
		p-p38	uPA	p-p38	uPA
Clinical stage				0.021*	0.004**
I-II	29	12 (41.4)	12 (41.4)		
III-IV	31	22 (71.0)	24 (77.4)		
Tumor size (cm)				0.121	0.428
< 3	19	8 (42.1)	10 (52.6)		
≥ 3	41	26 (63.4)	26 (63.4)		
Age, years				0.750	0.451
≤ 45	24	13 (54.2)	13 (54.2)		
> 45	36	21 (58.3)	23 (63.9)		
Lymphatic metastasis				0.016*	0.022**
No	22	8 (36.4)	9 (40.9)		
Yes	38	26 (68.4)	27 (71.1)		

*, $P < 0.05$; **, $P < 0.01$.

Relationship between p-p38 and uPA protein expression and clinical pathological characteristics

There was a significant correlation between the level of uPA and p-p38 expression and lymph node status and clinical stage ($P < 0.05$). The level of uPA and p-p38 expression was not significantly related to the patients' age or tumor size ($P > 0.05$) (Table 1).

Expression of the p-p38 and uPA protein in two different metastatic breast cancer cells

Western blot analysis indicated that the expression of p-p38 and uPA in the highly metastatic MDA-MB-231 cells was higher than that in lowly metastatic MCF-7 cells (Fig.3).

The change of uPA protein expression after the p38 MAPK-signaling pathway was blocked by SB203580

The expression level of the uPA protein in MDA-MB-231 cells decreased after the addition of SB203580, a specific inhibitor of p38 MAPK, was added into the

culture medium. SB203580 treatment of the cells reduced uPA protein expression in a concentration-dependent manner (Fig.4).

Discussion

The MAPKs are serine/threonine kinases, which generally are found in various cells. MAPKs have been shown to transduce extracellular signals involving cell proliferation, differentiation and malignant transformation, and play important roles in tumor generation and development. p38 MAPK is an important member of the MAPK family. p38 MAPK undergoes phosphorylation at both tyrosine and threonine sites and can be activated by a wide spectrum of stimuli, including inflammatory cytokines, growth factors and cellular stress. The p38MAPK-signaling pathway has been implicated in cell growth, apoptosis, cellular motion with an invasive phenotype that mediates cell migration, tumor invasion and metastasis^[1-4]. Urokinase plasminogen activator

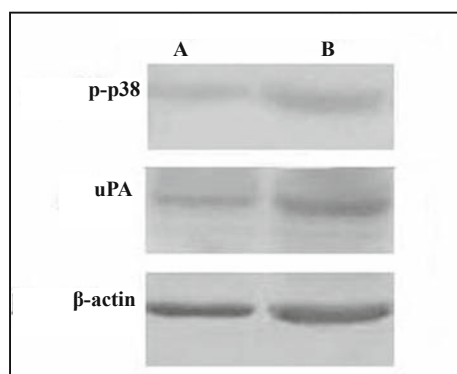


Fig.3. Expression of p-p38 and uPA in breast cancer MDA-MB-231 and MCF-7 cells. A, MCF-7 cells; B, MDA-MB-231 cells.

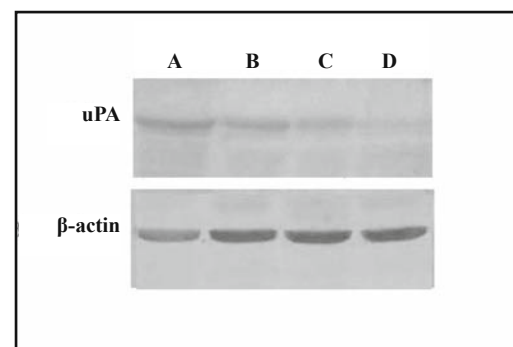


Fig.4. The change in uPA protein expression in MDA-MB-231 cells 24 h after adding SB203580 (0, 2, 5, 10 $\mu\text{mol/L}$) into the culture medium. A, Control; B, C, D, SB203580 2, 5, 10 $\mu\text{mol/L}$ respectively.

(uPA) is a serine protease that initiates the conversion of plasminogen to plasmin. These proteases confer cellular ability to degrade the extracellular matrix. uPA has been clearly demonstrated to be essential in the maintenance of invasive and metastatic phenotypes^[5–9].

At the present time, only a few studies have demonstrated the relationship between uPA expression and a signal-transduction pathway. Recent research has indicated that the p38 MAPK-signaling pathway participates in regulating uPA expression in gastric, lung, leukemic and breast cancer cells^[10–13]. Our studies suggest that there is a significant correlation between the level of uPA and p-p38 expression and lymph node status and the clinical stage of breast cancer, and we demonstrated that the expression of p-p38 and uPA in the highly metastatic MDA-MB-231 cells was higher compared to lowly metastatic MCF-7 cells. Our results indicate that over-expression of p-p38 and uPA might be related to breast cancer malignant progression, invasion and metastasis. Immunohistochemical results showed a positive relationship between the expression of p-p38 and uPA, suggesting that uPA protein expression might be regulated by the p38 MAPK-signaling pathway. We also investigated the relationship between uPA expression and the p38 MAPK-signaling pathway. Western blot analysis showed that SB203580 treatment of the MDA-MB-231 cells decreased uPA protein expression in a concentration-dependent manner, demonstrating that the p38 MAPK-signaling pathway might be involved in regulating uPA expression.

In summary, we suggest that the p38 MAPK-signaling pathway promotes breast cancer malignant progression, invasion and metastasis. Although the precise mechanism remains unknown, an inhibitor of the MAPK-signaling pathway represents a novel target for cancer intervention strategy. Our study suggests that the p38 MAPK-signaling pathway may be a potential therapeutic target for breast cancer treatment.

References

- 1 Widmann C, Gibson S, Jarpe MB, et al. Mitogen-activated protein kinase: conservation of a three-kinase molecule from yeast to human. *Physiol Rev* 1999; 79: 143–180.
- 2 Zarubin I, Han J. Activation and signaling of the p38MAP kinase pathway. *Cell Res* 2005; 15: 11–18.
- 3 Kim MS, Lee EJ, Kim HR, et al. p38 kinase is a key signaling molecule for H-ras-induced cell motility and invasive phenotype in human breast epithelial cells. *Cancer Res* 2003; 63: 5454–5461.
- 4 Huang X, Chen S, Xu L, et al. Genistein inhibits p38 map kinase activation, matrix metalloproteinase type 2, and cell invasion in human prostate epithelial cells. *Cancer Res* 2005; 65: 3470–3478.
- 5 Reuning U, Schmitt M, Lubert B, et al. Methods to analyze the effects of the urokinase system on cancer cell adhesion, proliferation, migration, and signal transduction events. *Methods Mol Med* 2006; 120: 427–440.
- 6 Tan X, Egami H, Nozawa F, et al. Analysis of the invasion-metastasis mechanism in pancreatic cancer: involvement of plasminogen cascade proteins in the invasion of pancreatic cancer cells. *Int J Oncol* 2006; 28: 369–374.
- 7 Pappot H, Pedersen AN, Brunner N, et al. The complex between urokinase (uPA) and its type-1 inhibitor (PAI-1) in pulmonary adenocarcinoma: relation to prognosis. *Lung Cancer* 2006; 51: 193–200.
- 8 Schoppmeyer K, Fruhauf N, Oldhafer K, et al. Tumor cell dissemination in colon cancer does not predict extrahepatic recurrence in patients undergoing surgery for hepatic metastases. *Oncol Rep* 2006; 15: 449–454.
- 9 Manders P, Tjan-Heijnen VC, Span PN, et al. Complex of urokinase-type plasminogen activator with its type 1 inhibitor predicts poor outcome in 576 patients with lymph node-negative breast carcinoma. *Cancer* 2004; 101:486–494.
- 10 Shin BA, Yoo HG, Kim HS, et al. p38MAP kinase pathway is involved in the urokinase plasminogen activator expression in human gastric SNU-638 cells. *Oncol Rep* 2003; 10: 1467–1471.
- 11 Behren A, Binder K, Vucelic G, et al. The p38 SAPK pathway is required for Ha-ras induced in vitro invasion of NIH3T3 cells. *Exp Cell Res* 2005; 303: 321–30.
- 12 Montero L, Nagamine Y. Regulation by p38 mitogen-activated protein kinase of adenylate- and uridylylate-rich element-mediated urokinase-type plasminogen activator (uPA) messenger RNA stability and uPA-dependent in vitro cell invasion. *Cancer Res* 1999; 59: 5286–5293.
- 13 Esteva FJ, Sahin AA, Smith TL, et al. Prognostic significance of phosphorylated p38 mitogen-activated protein kinase and HER-2 expression in lymph node-positive breast carcinoma. *Cancer* 2004; 100: 499–506.