EJGO European Journal of Gynaecological Oncology

Immunohistochemistry of phosphatase and tensin homolog and metalloproteinase-9 in breast invasive micropapillary carcinoma

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Summary

Objective: Alteration of phosphatase and tensin homolog (PTEN) and metalloproteinase-9 (MMP-9) expression is involved in carcinogenesis, and both proteins are correlated with malignant cell invasion and metastasis. This study focused on PTEN and MMP-9 expression in the invasive micropapillary carcinoma (IMPC) of the breast and its relationship to clinical pathological features. *Materials and Methods:* The immunohistochemical S-P method was used to detect the expression levels of PTEN and MMP-9 in 49 cases of IMPC (the proportion of IMPC in breast carcinoma is approximately 5–100%) and 30 cases of normal breast tissue. *Results:* In IMPC, PTEN, and MM-9 expression levels were negatively and positively related, respectively, to the histopathologic grade and lymph node metastasis (both $p < 0.05$). The PTEN expression was negatively related to MMP-9 expression ($p < 0.05$). *Conclusion:* These results suggest that lack of MMP-9 and PTEN overexpression are early markers of breast carcinogenesis preceding tumor invasion. Apparently, IMPC carries the risk of progression to a malignant phenotype according to these markers. The clinical importance of these finding is discussed.

Key words: PTEN phosphohydrolase; Matrix metalloproteinase 9; Invasive micropapillary carcinoma.

Introduction

Invasive micropapillary carcinoma (IMPC) is a distinct variant of breast cancer that has a malignant and aggressive behavioral pattern [1]. In the 2003 World Health Organization classification of breast tumors, IMPC is listed as a rare subtype of breast epithelial tumors [2]. It is well known that breast cancer is heterogeneous, and that breast epithelium is capable of differentiating into different histological types of cancer with pure or mixed forms, with different biological behaviors [3]. Some of these tumors such as tubular, papillary, and mucinous carcinoma, have a better prognosis with favorable histological appearance than that of others, such as signet ring cell and inflammatory carcinoma, which have a poor prognosis [4-6]. IMPC has an unfavorable prognosis, with nearly 50% of patients presenting at a later disease stage, associated with widespread lymphatic tumor emboli.

Histopathologically, IMPC is characterized by a nested papillary pattern, consisting of eosinophilic or amphophilic cells within clear, empty spaces mimicking lymphatic vessels. Various definitions by different authors have been used for this pattern, including pseudopapillary (serous-like) [7], reverse polarity [8], reversed apical pattern [9], inside-out growth pattern [10], or morule-like pattern [11]. Lymphovascular invasion is frequently seen in these tumors that are predominantly of histological grade 3. Invasive micropapillary carcinoma can occur in a pure form, which is very rare, or in association with other types of breast cancer. Since the first description of IMPC, numerous studies on this disorder have been published in the literature [2]. The incidence of IMPC varies from 2.6% to 6.0% in some studies, with pure IMPC reported much less than mixed cases [11-16]. The most common histological type of breast cancer associated with IMPC in mixed cases has been reported to be invasive ductal carcinoma (IDC) [12, 14, 15, 17].

Phosphatase and tensin homolog (PTEN) is one of the most commonly mutated tumor suppressor genes, and if not mutated, it is often suppressed or downregulated. PTEN has been discovered to be a crucial factor in various processes that are central to cancer development. Matrix metalloproteinases (MMPs) have been implicated in diverse roles in breast cancer development and progression. The PTEN and MMP-9 expression in IMPC is still unclear. Therefore, this study focused on PTEN and MMP-9 expression in IMPC of the breast and its relationship to clinical pathological features.

Materials and Methods

Forty-nine cases of IMPC of the breast, which were either pure or mixed forms (IMPC pattern was from 5% to 100%) were identified at Zhejiang Province Chinese Medicine Hospital and Wenling No. 1 People's Hospital, between January 2006 and February 2013. Thirty of them had normal tissue close to the cancer tissue, which were used as a control. All the participants were female patients. Patients were to undergo a modified radical mastectomy,

Revised manuscript accepted for publication May 28, 2018

Table 1. — *PTEN and MMP-9 expression and clinicopathologic features in IMPC of breast.*

Clinicopathologic features	PTEN				MMP-9			
	Positive	Negative	x^2	p	Positive	Negative	x^2	p
IMPC								
< 50%	9	8	3.21	0.073	13	2	2.79	0.094
≥ 50%	9	23	28 6					
Age (years)								
< 50	7	15	0.02	0.967	19	2	1.24	0.265
≥ 50	11	16	22 6					
Histological grade								
I	6	3	4.25	0.039	5	4	6.38	0.012
II-III	12	28	36	4				
Lymph node metastasis								
< 4	11	8	5.97	0.014	13	6	5.28	0.022
≥ 4	7	23	28	2				

Table 2. — *Correlation of PTEN and MMP-9 in IMPC.*

MMP-9	PTEN	
	Negative	Positive
Negative	1	7
Positive	30	11
r	-0.445	
p	< 0.05	

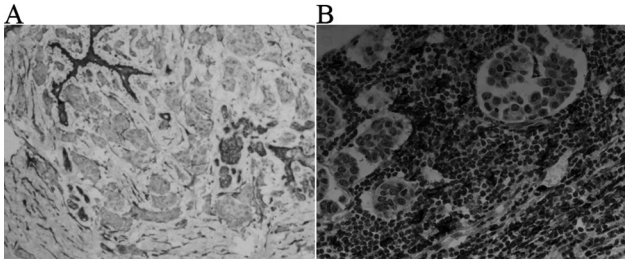

Figure 1. — (A) Typical PTEN negative stain in nuclear and cytoplasm of IMPC tissue (×100). (B) Typical MMP-9 positive stain in nuclear and cytoplasm of IMPC tissue (×200).

and no radiotherapy was conducted before the operation. The average age of patients was 52.2 (36–73) years. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Zhejiang Province Traditional Chinese Medical University. Written informed consent was also obtained from all participants.

Paraffin-embedded sections were deparaffinized in Histoclear and hydrated. Endogenous peroxidase activity was blocked by incubation with 0.3% hydrogen peroxidase for ten minutes, followed by incubation with a protein blocking buffer for ten minutes to prevent any non-specific binding. Primary monoclonal antibodies for PTEN or MMP-9 were added to the specimens, followed by incubation at 4°C in a humidified chamber for 24 hours. Then, the sections were incubated with a biotinylated secondary antibody for one hour following peroxidase with a streptavidin conjugate. The immunohistochemical reaction was then visualized using substrate chromogen 3,3'-diaminobenzidine (DAB). The specimens were counterstained with Hematoxylin and Eosin (H&E).

Two experienced pathologists analyzed the immunohistochemical staining result. The cytoplasmic staining of cells was scored. In the PTEN-positive cells, the cytoplasm and nuclei were yellow or brown stained, otherwise the cell was considered PTEN-negative. In MMP-9-positive cells, the cytoplasm was yellow or brown stained, otherwise, the cell was MMP-9-negative. The following scale was used to score each cell: no staining: 0, light yellow: 1, and deep yellow or brown: 2. A total of 100 cells were scored for each specimen, and positive cells < 10%, 10–50%, and > 50% scored 0, 1, and 2, respectively. The score of each cell multiplied by the score of each specimen was the final expression score. Final score < 2 indicated negative expression whereas ≥ 2 was considered as positive expression.

All the data were analyzed using the statistical package for the social sciences (SPSS) 13.0 software. Comparison between the groups was performed using the chi-square test. The correlation of PTEN and MMP-9 was analyzed using the Spearman correlation analysis. Statistical significance was set at $p < 0.05$.

Results

PTEN and MMP-9 expression and the clinicopathologic features of IMPC of the breast are shown in Table 1. PTEN positivity was less frequently seen in carcinomas tissues, where 18 (36.73%) of the 49 samples showed a positive staining. Typical PTEN-negative staining in IMPC is shown in Figure 1A. In adjacent normal tissues, 24 (80.0%) of 30 samples showed positive staining. The PTEN positivity was significantly higher in normal tissue than it was in carcinomas ($p < 0.05$). PTEN negative expression showed a correlation with higher histological grading ($p = 0.039$) and was more likely to develop lymph node metastasis ($p = 0.014$).

MMP-9 positivity was demonstrated in 41 (83.67%) of 49 carcinoma samples showing positive staining, and typical MMP-9 positive staining in IMPC is shown in Figure 1B. In adjacent normal tissues, only four (13.3%) of 30 samples was MMP-9 positive, which was much lower than that in carcinomas ($p < 0.05$). MMP-9 positive expression showed a correlation with higher histological grading ($p = 0.039$) and was more likely to develop lymph node metastasis ($p = 0.014$).

As shown in Table 2, 30 cases (61.22%) were MMP-9

positive and PTEN negative out of 49 IMPC samples. In the Spearman correlation analysis, MMP-9 and PTEN expression was negatively correlated ($r = 0.445$, $p < 0.05$).

Discussion

The present findings suggest that PTEN negativity was significantly correlated with a higher histological grade and more lymph node metastasis in IMPC of the breast than its positivity was. IMPC is associated with frequent lymph node metastasis and adverse clinical outcome. The molecular mechanisms of IMPC have not been fully studied. Some studies have shown that the loss of the cluster of differentiation 44 (CD44) adhesion molecule and high microvessel density (MVD) may play a significant role in the high incidence of lymphovascular permeation and metastasis in IMPC. E-cadherin (E-Cad) expression is intact in IMPC, which could be because tumor cells travel as clusters and retain their expression of E-Cad [18-21]. PTEN is an important gene for diagnostic and prognostic consideration. In cancer cells, PTEN suppresses the phosphoinositide 3-kinase (PI3K)-AKT-mammalian target of rapamycin (mTOR) pathway through its lipid phosphatase activity and integrates complex feedback loops in this pathway. PTEN is involved in numerous cellular processes such as survival, proliferation, energy metabolism, and cellular architecture. PTEN expression and function are frequently altered in cancer cells including complete disruption, transcriptional regulation, post-transcriptional regulation, post-transcriptional regulation, and protein-protein interactions. In IMPC, the loss or downregulation of PTEN expression could play an import role in carcinogenesis and metastasis, and it is a sign of poor prognosis [22-24].

MMPs have been implicated in diverse roles in breast cancer development and progression. While many of the different MMPs expressed in breast cancer are produced by stromal cells, MMP-9 is produced mainly by the tumor cells themselves. Human breast cancer cell-produced MMP-9 is specifically required for invasion in cell culture and pulmonary metastasis in a mouse model of basal-like breast cancer [25, 26]. In cancer cells, PTEN loss is correlated with MMP-9 overexpression, which was also observed in this study.

Taken together, these results indicate that PTEN and MMP-9 expression in IMPC of the breast were remarkable in invasion and metastasis of IMPC of the breast. Further studies of the expression of PTEN and MMP-9 in the IMPC could provide additional evidence of the molecular mechanisms of invasion and metastasis of IMPC and facilitate advances in tumor-targeting therapy and prognosis of IMPC of the breast.

Acknowledgements

This work was funded by Wenling City, Zhejiang Science and Technology Project (No.2013C31056) and by Zhejiang Provincial Medical and Health Science and Technology Project (2018KY917).

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