Journal of Clinical and Nursing Research

Research Article

Expression and Clinical Significance of Hypoxia-related Factors HIF-1a, Gli-1 and MMP9 in Breast Cancer

Chen Hong[1#], Donghong Xu[2#], Haizhi Qiao[1], Jinmei Li[1], Jinku Zhang[1*]

[1]Department of Pathology, Baoding First Central Hospital, Baoding 071000, Hebei Province, China;

[2]The First Department of Breast Surgery, Baoding First Central Hospital, Baoding 071000, Hebei Province, China

[#]Hong Chen and Donghong Xu both are the first authors and contributed equally to this work

Abstract: ***Objective:***To investigate the expression and clinical significance of hypoxia inducible factors HIF-1α, Gli-1 and MMP9 in breast cancer. ***Methods:*** Eighty patients with invasive ductal carcinoma of the breast and 40 normal tissues adjacent to cancer were selected. Immunohistochemical methods were used to detect the expression of HIF-1α, Gli-1 and MMP9 in breast cancer and normal tissues adjacent to cancer, and their relationship with clinicopathological features of breast cancer and prognosis was explored. ***Results:*** The positive rates of HIF-1α, Gli-1 and MMP9 in breast cancer tissues were significantly higher than those in normal breast tissues. HIF-1α, Gli-1 and MMP9 expressions are positively correlated in breast cancer. ***Conclusion:*** HIF-1α, Gli-1 and MMP9 proteins are involved in the pathogenesis of breast cancer.

Keywords: Breast cancer; Hypoxia-inducible factors; MMP9; Tumor treatment

Publication date: January, 2021

Publication online: 31 January, 2021

[*]***Corresponding author:*** Jinku Zhang, 843561234@qq.com

1 Introduction

The most important feature of tumors is the growth of tumor cells that are difficult to regulate. The increasing number of cells leads to increase in cellular oxygen consumption, which can easily lead to the formation of a hypoxic microenvironment within the tumor, making tumor cells more aggressive. This study explored the effects of HIF-1α, Gli-1 and MMP9 on the invasion and metastasis of breast cancer cells under hypoxia in order to find out the best parameters for evaluating breast angiogenesis and provide some insights for the diagnosis and treatment of breast. The results are reported as follow.

2 Materials and Methods

2.1 Study Subjects

From 2010 to 2019, 80 patients with invasive ductal carcinoma of the breast were first diagnosed in our hospital; 40 patients with adjacent normal tissues were followed up for more than 5 years. Complete clinical and pathological data. The histological classification and clinical stage of each case are in line with the breast cancer diagnosis and treatment standards established by the Ministry of Health in 1988.

2.2 Methods

Breast cancer MDA-MB231 cells were cultured under hypoxia, and normal oxygen culture was used as a control. The invasion ability of MDA-MB cells in each group was tested by Transwell invasion assay; the expression levels of HIF-1α, Gli-1 and MMP9 protein were detected by Western blot. Breast cancer cells were stably transfected with shRNA, and the effect of hypoxia on the invasion ability of breast cancer cells was detected by the Transwell invasion assay again. The expression levels of HIF-1α, Gli-1 and MMP9 proteins were detected by Western blot to study the effect of hypoxic environment on the invasion and metastasis of breast cancer.

2.3 Results Assessment

Under the microscope, MMP9, Gli-1 and HIF-1α were observed as brown particles. The positive parts of the three were observed with a 100x microscope and a 200x microscope. The staining level was evaluated based on the staining intensity and the percentage of positive cells. Colorless or light yellow is 0, brownish-yellow or brown is positive, and transparent brown particles in the cell membrane are positive: 1 point for staining only stronger than negative, 2 points for clear staining, 3 points for strong staining; 2 points for 10%-50% of the total number of cells being positive, 3 points for 51%-80% positive, and 4 points for >80% positive. The two scores were added together, and the staining intensity is (-) as long as the number of positive cells is less than 10%; 3 points is considered as weak positive (+); 4 to 5 points as medium positive (++) and 6 to 7 points as strong positive (+++)[1].

2.4 Statistical Processing

SPSS software system was used to carry out the correlation analysis of x2 test. Spearman correlation analysis was used.

3 Results

Expression of HIF-1α, Gli-1 and MMP9 Proteins in Breast Tissue. The positive expression rates of HIF-lα protein in normal breast tissue, dysplastic breast tissue and breast cancer tissue were 0%, 28% and 60.53%, respectively, and the differences between the groups were statistically significant (P<0.05). The positive expression rates of Gli-1 protein in normal breast tissue, dysplastic breast tissue, and breast cancer tissue were 0%, 27.4%, and 52.29%, respectively, and the difference between the groups was statistically significant (P<0.05). The positive expression rate of MMP9 protein in normal breast tissue and dysplastic breast tissue and breast cancer tissue were 0%, 26.51% and 55.83%, respectively, and the difference was not statistically significant (all P>0.0167).

Table 1. The relationship between expression of HIF-lα and Gli-1 proteins and the clinicopathological features of breast cancer

Clinicopathological Features	*N*	HIF-la		*P*	Gli-1		*P*
		(+~+++)	(-)		(+~+++)	(-)	
Tumor Size				>0.05			>0.05
≤2cm	43	27	16		16	27	
>2cm	37	25	12		22	15	
Clinical Stage				<0.05			<0.05
Stage Ⅰ, Ⅱ	49	31	18		20	29	
Stage Ⅲ	31	19	12		23	8	
Tissue Grading				<0.05			<0.05
Gl + G2	48	29	19		20	29	
G3	32	21	11		18	13	
Lymph Node Metastasis				<0.05			<0.05
Positive	59	48	11		40	19	
Negative	21	16	5		7	14	
Androgen Receptor				<0.05			>0.05
Positive	57	45	12		26	21	
Negative	23	17	6		12	11	
Progesterone receptor				>0.05			>0.05
Positive	49	31	18		26	23	
Negative	31	20	11		14	17	

MMP9 immunohistochemical staining showed that the positive expression of MMP-9 protein was mainly located in the cytoplasm, and the positive expression was pale yellow to brownish-yellow particles. Under the microscope, brown microgranular cells are benign cells, and interstitial cells do not contain positive cells[2]. At high magnification, five fields were randomly selected, the staining concentration area was selected to determine the number of cells and the result, and the ratio of positive cells to the total number of cancer cells is calculated. The number of positive cells < 10% is considered negative, 10-25% is +, 25-50% is ++, >50% is +++, where +++ is strong positive expression. In this study, ++ and +++ were regarded as positive expression, and the rest were considered negative expression.

4 Discussion

HIF-lα is the main transcriptional regulator, which mediates the adaptive response of cells to the hypoxic microenvironment composed of subunits and β subunits, one of which is considered to be a specific oxygen regulatory subunit and the activity of HIF-lα. HIF plays a key role in the adaptive response of tumor cells to hypoxia and resistance to radiation damage. HIF1 is a heterodimer composed of HIF-1α and hIf-1b subunits, while HIF-1α is more sensitive to hypoxia and indirectly reflects tissue hypoxia by detecting the expression of HIF-1α. Local hypoxia and hypoglycemia microenvironment formation are common phenomena during rapid tumor growth. In this hypoxic state, HIF-1α induces the expression of enzymes involved in glycolysis, increases glycolysis, and to a certain extent can improve the imbalance of energy supply and energy consumption caused by tumor hypoxia[3].

HIF-1α is not expressed in most normal human tissues, but high expression of HIF-1α can be detected in many tumor tissues. The expression of HIF-1α is driven by the continuous growth and volume increase of tumor cells, which induces the expression of various genes and proteins related to the malignant transformation of tumor cells, and promotes the angiogenesis of tumor tissues. The results showed that the positive expression of HIF-1α protein in normal breast tissue and hyperplastic breast tissue increased, and the abnormal expression of HIF-1α may be an early factor in breast cancer, indicating that HIF-1α gene expression is upregulated in breast cancer[4].

The glucose transporter Gli-1 is the most widely distributed transporter among known glucose transporters. In the hypoxic response mediated by hili-1α, Gli-1 expression is up-regulated. In this study, the expression of Gli-1 in normal breast cancer, proliferative breast cancer and breast cancer was significantly different, indicating that Gli-1 can be used as a marker for early breast disease. In breast cancer, the expression of Gli-1 is related to lymph node metastasis and tumor differentiation. The expression rate of tumors with lymph node metastasis is higher than that of non-metastatic tumors, and the expression rate of moderately differentiated cancer tissues is higher than that of well differentiated tissues. At the same time, the slower the tumor stage, the faster the positive expression rate[5]. It is suggested that the high expression of Gli-1 protein may be a characteristic event of breast cancer, and can be used as an indicator of the malignancy and prognosis of breast cancer.

Studies have shown that the expression of HIF-1α and Gli-1 is positively correlated. HIF-1α plays an important role in the regulation of breast cancer and the expression of glycometabolism-related proteins in tumor tissues. HIF-1α transcription activation induces Gli-1 expression. Under normoxic conditions, some tumor genes and growth factors can induce the expression of HIF-1α and Gli-1, suggesting that the synergistic effect of HIF-1α and Gli-1 may play an important role in the occurrence of breast cancer.

MMP9 and tumor blood vessels: MMP9 can participate in various physiological and pathological processes of the human body. The positive expression of MMP9 in small cell tissues was significantly higher than that of normal tissues and benign disease tissues, and the difference was statistically significant, but there was no significant difference between normal tissues and benign tissues. It has also been found that the expression of MMP9 is related to the clinical stage, pathological grade and lymph node metastasis of non-small cell carcinoma[6]. The most basic step of tumor neovascularization is the degradation and destruction of extracellular matrix and vascular basement membrane by cancer cells.

In summary, HIF-1α, Gli-1 and MMP9 can be used as indicators to evaluate the malignant degree of breast cancer, and the combined detection of these three indicators can more accurately reflect the biological characteristics of breast cancer. With the in-depth study of HCa and Ghu, treatments targeting them may become an important means of tumor treatment and prevention.

Acknowledgement

Key Laboratory of Molecular Pathology and Early Diagnosis of Tumor in Hebei Province

References

[1] Liu B, Li F, Han XD, et al. Expression and clinical significance of HIF-1αin breast cancer [J]. Chinese Journal of Endocrine Surgery, 2016, 10(2): 135-138+143.

[2] Hua JR, Zeng D, Pan M. The expression and clinical significance of MMP9 in type Ⅰ and type Ⅱ endometrial carcinoma [J]. Modern Hospitals, 2019, 19(11): 1697-

1699+1702.

[3] Xu SX, Zhang L, Zhang LQ, et al. The expression and clinical significance of E-cadherin and vimentin in invasive breast cancer [J]. Maternal and Child Health Care of China, 2019, 34(1): 174-176.

[4] Wang HS, Li Z, Cao YJ, et al. The expression and clinical significance of SRC-3 and MMP-9 in breast cancer [J]. Heilongjiang Medical Science, 2017, 40(4): 125-126+128.

[5] Sun WZ, Yu LB, Zhang QY. Expression of H1F-1α and Glut1 in breast cancer and its clinical significance [J]. Chinese Journal of Gerontology, 2007(11): 1061-1062.

[6] Hua B, He SR, Wang Z, et al. The expression and clinical significance of androgen receptor in breast cancer[J]. Chinese Journal for Clinicians, 2017, 45(2): 32-35.