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Correlation between the Expression of Aquaporin 1 and Hypoxia-inducible Factor 1 in Breast Cancer Tissues

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Summary: The correlation between aquaporin 1 (AQP1) and hypoxia-inducible factor 1 (HIF 1) in breast cancer tissues was preliminarily studied. In 155 cases of breast cancer, the expression levels of AQP1 were detected by immunohistochemisty in HIF1-positive group or HIF1-negative group, and the correlation between AQP1 and HIF1 was analyzed. The overexpression of AQP1 and HIF1 were observed in 155 cases of breast cancer tissues. The expression level of AQP1 in HIF1-positive group was significantly higher than that in HIF1-negative group. The positive expression rate of AQP1 was 296.55±24.67 and 168.37±37.53 in HIF1-positive group and HIF1-negative group respectively with the difference being very significant between them (P<0.001). It was concluded that AQP1 was overexpressed in the HIF1-positive group and there were some correlations between AQP1 and HIF1, suggesting they interact each other and regulate the oncogenesis of breast cancer.
Key words: aquaporin 1; hypoxia-inducible factor 1; breast cancer; correlation

Hypoxia is a common feature of solid tumor microenvironment. Hypoxia-inducible factor 1 (HIF 1), which is produced by the cells under hypoxic condition, is a nuclear factor that generally resides in mammalian animal and human body. Many studies[1–3] had confirmed that HIF1 was an import molecule in tumor signal transferring network and regulated the process of oncogenesis, angiogenesis and cancer metastasis. Aquaporin1 (AQP1) is a transmembrane protein which regulates the transportation of water transmembrane and AQPs generally reside in plant, animal and microorganism. Recent studies[4–6] had revealed that cell migration, cancer cell invasion and cancer metastasis were powerfully regulated by AQP1 function. Is there any correlation between AQP1 and HIF1? In this study, the expression of AQP1 in HIF1-positive group or HIF1-negative group was detected in 155 cases of breast cancer tissues in order to preliminarily study the correlation between AQP1 and HIF1 and provide a research fundament about interaction of both of them.

1 MATERIAL AND METHODS

1.1 Materials

From January 2003 to December 2004, 155 cases of breast cancer with complete case documents, who had received radical mastectomy or simple lumpectomy, were randomly sampled. All of tumor tissues were fixed by 10% formalin, embedded with paraffin and cut into 4 μm-thick section. All the cases without chemotherapy or radiotherapy history before operation were pathologically confirmed.

Of them, there were 133 cases of invasive ductal adenocarcinoma, 6 cases of invasive lobular adenocarcinoma, 2 cases of mucinous adenocarcinoma, 10 cases of medullary carcinoma, 3 cases of ductal carcinoma in situ, and 1 case of squamous carcinoma. Among them, 11 were at stage Ⅰ (UICC TNM stage, 2002), 96 at stage Ⅱ, 48 at stage Ⅲ. Their age ranged from 23 to 73 y old (mean 46.3).

1.2 Main Reagents

Anti-AQP1 monoclonal antibody was the product of ADI Company (USA) and diluted to 1:100 in the experiment. Anti-HIF1 monoclonal antibody and the kits of immunohistochemistry were purchased from Wuhan Boster Biological Technology Ltd. (China).

1.3 Immunohistochemistry

AQP1 or HIF1 was stained by S-P method. The breast cancer tissues were fixed with 10% formalin, embedded in paraffin, sliced into 4-μm sections, roasted at 65°C and deparaffinized with dimethyl benzene, 95% alcohol, 85% alcohol and distilled water. Endogenous peroxidase was inactivated with 3% H_2O_2, the antigen was repaired with microwave, and the nonspecific background was blocked out with 10% natural goat serum. After the addition of the primary antibody of AQP1 or HIF1, the sections were left in working liquid wet box for 30 min at 37°C, rinsed with PBS, made to react with the second biotinylated antibody for 30 min at 37°C, disposed for 30 min at 37°C with streptomycintropin labeled with horseradish peroxidase. After rinsing with TBS, the sections were visualized with diamidobianilin (DAB). Sections were counterstained with haematoxylin, dehydrated and mounted for microscopic examination.

In the negative control sections, phosphoric acid

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buffer solution was substituted for the first antibody.

1.4 Criteria for Evaluation of Positive Results

Criteria for AQP1 positive tumor cells: Those with buffy or brown particle in cytoplasma or on cell membrane were regarded as AQP1 positive tumor cells. Pathological sections with clean background view were used to calculate positive tumor cell and do statistical analysis. Positive tumor cells and positive rate, which stands for the mean level ($\bar{x}\pm s$), were calculated among 1000 tumor cells in 5–10 high power (10×40) fields randomly selected[7].

Criteria for HIF1 positive tumor cells: Those with buffy particle in cytoplasma or cell nucleus were regarded as HIF1 positive tumor cells. 1000 tumor cells were counted in 5—10 high power (10×40) fields randomly selected. Score zero: without buffy strain; score 1: <1% buffy coloration in cell nucleus; score 2: 1%—<10% buffy coloration in cell nucleus or weak coloration in cytoplasm; score 3: 10%—<50% buffy coloration in cell nucleus; score 4: ≥50% buffy coloration in cell nucleus. Scores 0–1 were regarded as HIF1 negative tumor group, and scores 2–4 as HIF1 positive tumor group[8, 9].

1.5 Statistical Analysis

The data were expressed as $\bar{x}\pm s$ and the experimental results were analyzed by *t*-test. $P<0.05$ was considered to be statistically significant.

2 RESULTS

The overexpression of AQP1 and HIF1 was detected in 155 cases of breast cancer tissues. All cases were divided into two groups: HIF1 positive group (n=124) and HIF1 negative group (n=31) according to the expression level of HIF1 detected by immunohistochemistry. The expression level of AQP1 in HIF1 positive group was significantly higher than that in HIF1 negative group. The positive expression rate of AQP1 was 296.55±24.67 and 168.37±37.53 in HIF1 positive group and HIF1 negative group respectively with the difference being very significant between the two groups ($P<0.001$).

Table 1 Correlation of the expression of AQP1 and HIF1 in 155 female breast cancers

HIF1expression status	Positive rate of AQP1 ($\bar{x}\pm s$)	*P*
HIF1 negative (n=31)	168.37±37.53	<0.001
HIF1 positive (n=124)	296.55±24.67	

3 DISCUSSION

Breast cancer is one of the most common malignant neoplasms and its oncogenesis, proliferation, tumor invasion, angiogenesis and metastasis are regulated by signal transduction network.

Hypoxia is a common feature and fundamental distinction in solid tumors. Cell hypoxia in microenvironment can lead to malignant transformation and promote the process of angiogenesis and metastasis[8, 10]. HIF1, a nuclear factor first extracted by Semenza in 1992, is released by hypoxic cells of human being or animals under hypoxic condition. Many target molecules, such as VEGF, EPO, endotheltin-1 and glycolytic enzyme etc., are regulated by HIF1. Accumulating evidence had confirmed that HIF1 played an important role in energy metabolism, angiogenesis, proliferation, invasion and metastasis of tumor cells[3]. Researchers had paid more attention to how HIF1 worked in breast cancer[2, 10, 12].

In most tumors, vascular permeability and the pressure of interstitial cell fluid in tumor tissue are elevated, which can result in local hypoxia in tumor tissue, directly causing the increased expression of HIF1. Local hypoxia also interferes with energy metabolism and the function of ion channel on the cellular membrane, subseqeuntly influencing the function of water channel proteins (AQPs) on cellular membrane. AQPs can effectively regulate the local hypoxia and indirectly affect the expression of HIF1 by adjusting the transportation of water transmembrane and the fluid pressure of local tumor tissue. To sum up, it is clear that there is some complicated pathophysiological relation between AQPs and HIF1. Our study showed that there was overexpression of AQP1 and HIF1 in tumor cells and positive expression rate of AQP1 in HIF1 positive group was significantly higher than that in HIF1 negative group, suggesting AQP1 might be related to HIF1 and both of them might functionally interact with each other. A study by Yamamoto *et al*[13] showed that there was the increased expression of AQP4 and AQP9 mRNA in peripheral cortex of cerebral ischemia infarction, suggesting that hypoxia can regulate the AQP4 expression. Their further study[14] revealed that there was a HIF1 binding site in AQP4 gene promotor domain. More evidence of the study[13, 14] confirmed that hypoxia can down-regulate the expression of mRNA and protein of AQP4 and AQP9 in cultured rat astrocytes under hypoxic condition, but the expression of AQP4 was elevated rapidly during reoxygenation. Another study[15], which was about human brain with infarction, demonstrated that the expression of AQP4 was enhanced by hypoxia. Increasing evidence showed that different types of AQPs have different functions. By now, the report has been skimpy concerning the relation between HIF1 and AQPs. In fact, AQP1 played a more important role in cancer than its analogs. Now questions may be raised: is there some correlation between AQP1 and HIF1, and is there some analogical mechanism on regulating each other. Accumulating evidences showed that AQP1 or HIF1 could powerfully regulate each step of cancer processes and new approach may works on such molecular target.

With much more results of the study on the regulating mechanism of ER, PR and Her-2 in signal transduction network, the treatment which aimed directly at these molecular targets in breast cancer had made a rapid progress and become a powerful approach. Not only these molecules became independent prognosis factors, but some treatment strategies were dependent on the expression level of these molecules as well. Therefore, our study aimed to primarily study the correlation between AQP1 and HIF1. There are some pending questions to solve in our next step: how do AQP1 and HIF1 interfere with each other and what is the signal transduction network on AQP1 and HIF1. Solved such problems not only

benefit to breast cancer treatment, but also benefit to prognosis determination.

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