The Expressions of HIF-1α, CA153, CEA and CA125 in Breast Cancer and Significance with Chemotherapy

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Abstract. Biomarker in tissue and serum plays significant roles in patient management and treatment and can be used as prognostic factors. For the diagnosis, treatment, prognosis study of breast cancer, we examined the expressions of Hypoxia inducible factor 1α (HIF-1α) in 128 cases mammary carcinoma tissues, and 43 cases serum levels of Cancer antigens153 (CA153), Cancer antigens125 (CA125) and CEA before and after neoadjuvant chemotherapy and analyze their relationship with clinicopathological factors such as metastasis, recurrence and chemotherapy. Our results demonstrated that the positive rates of HIF-1α were 68.75%, which were higher than that in DCIS, ADH, UDH ($P<0.01$, respectively). There was posive correlation in over-expression of HIF-1α with the level of ER or PR, histological grade, lymph node metastasis and distant metastasis of carcinomas ($P<0.01$, respectively), and the expressions of HIF-1α were not related with age (≤50 yr vs 50yr) and size of tumor (≤5cm vs >5cm) ($P>0.05$). Before and after therapy, the expression of HIF-1α had decreased significantly ($P<0.05$). The chemosensitivity of HIF-1α, CA153, and CEA positive group were worse than those of nagetive groups ($P<0.05$), however, there was no significant difference of chemosensitivity of CA125 before and after neoadjuvant chemotherapy. Biomarkers such as HIF-1α can be used as prognostic predictors in many malignancy and CA153 and CEA can be used as followed up potential factors of breast cancer chemosensitivity to help following up undergoing individualized chemotherapy.

Introduction

Breast carcinoma is a serious threat to women's health [1]. In China, the incidence of breast cancer is relatively high, and the peak incidence is in advance, young breast cancer has become a major clinical type of breast cancer, a large number of patients died of breast cancer complications or serious organ metastasis each year [2,3]. Many studies have found that certain biological indicators by detecting abnormal expression of molecules, such as ER, PR, HER2, etc, can guide clinical diagnosis and treatment activities prognosis [4,5]. There are several other molecular biomarkers, such as hypoxia inducible factor 1α (HIF-1α), Cancer antigens153 (CA153), Cancer antigens

125 (CA125) and CEA have been confirmed to participate in the evolution of malignancy. In recent years, neoadjuvant chemotherapy has additionally become an option for patients with operable tumors who desire breast conservation therapy [6-8]. Breast carcinoma, like most other forms of malignancy, neoadjuvant chemotherapy with agents such as doxorubicin and cyclophosphamide had historically been offered to patients with locally advanced disease with a goal of reducing tumor size to enable surgical resection. So far, there is no evidence for efficacy of screening with serum and tissue biomarkers in breast cancer. To validate whether combined detaction biomarkers in tissues and serum may improve accuracy and sensitivity of predicting recurrence, metastasis and chemotherapy prognosis of breast cancer, we composed a panel of potential biomarker HIF-1α in breast cancer tissues, CA153, CA125 and CEA in serum in order to explore the above markers expressions and significance with chemotherapy.

Patients and Methods

Patients

Permission was obtained from the Local Ethical Committee to collect breast cancer tissues and all patients signed informed consents to the research. 128 patients diagnosed breast invasive ductal carcinoma (IDC), 57 cases of atypical ductal hyperplasia(ADH), 89 cases of ductal carcinoma in situ(DCIS), were collected during excision surgery at Rizhao people's Hospital, and a complete clinical and follow-up data were confirmed by surgery and pathology, and 60 cases of usual ductal hyperplasia(UDH) were selected as a control group. 52 cases of breast invasive ductal carcinoma patients were treated by the clinical protocol as neoadjuvant chemotherapy. The breast cancer patients had not been treated with hormone endocrine therapy, anti-neoplastic chemotherapy or radiotherapy during the last six months before needle core biopsy diagnosis. The age of mammary carcinoma patients mean 47.5±5.3 years. Neoadjuvant chemotherapeutic regimens for treated patients included combination anthracyclines, taxanes, and alkylating agents. The expression of HIF-1α in breast cancer were detected and their relationship with clinical-pathological parameters including the histological grade, region lymph node metastasis, distant metastasis and recurrence on files.

Pathology Study

The pathological reading was determined for each biopsy slide with an overall pathological diagnosis determined for each subject. The tumor grade was obtained according to the modified Bloom-Richardson score by summing the scores and was then divided into three grades (I–III). Immunohistochemical *Ultra Sensitive* [TM] *S-P* method (Maixin-Bio, Fuzhou, Fujian, China) was used according to the manufacturer's instructions to detect differences in tumor tissue HIF-1α expression. Tissue samples were fixed in 10% neutral buffered formalin and embedded in paraffin. Tissue sections were deparaffinized and rehydrated using standard procedures. Signals were visualized using the DAB substrate, which stains the target protein yellow. The negative controls were used. The primary antibody was replaced with PBS, containing 0.1% bovine serum albumin at the same concentration as the primary antibody. The positive controls were tissues known to express the antigen being studied. For HIF-1α, the immunoreactive score (IRS) was obtained by multiplying the percentage of positive cells and the staining intensity. Immunostaining was recorded according to stain intensity (intensity score) and percentage of cancerous cells that stained positively

(quantity score). Briefly, a proportion score was assigned that represented the estimated proportion of positive tumor cells on the entire slide. For each histological section, the percentage of positive cells was scored as 0 (<5%), 1 (6%–25%), 2 (26%–50%), 3 (51%–75%), and 4 (>75%), and the staining intensity was scored as 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). Immunohistochemical results with an IRS of 0 were considered negative, an IRS of 1-4 was considered weakly positive, an IRS of 5–8 was considered moderately positive, and an IRS of 9–12 was considered strongly positive. Assessment of the staining was evaluated by two independent pathologists without knowledge of the clinical status of the patients.

Serum Biomarker Measure

For CA153, CA125 and CEA analysis, 3ml heparinized blood was drawn from each individual in 43 cases breast cancer. The biomarkers were detected with electroc-hemiluminescence method (Roche E-601, Germany) in the clinical laboratory in Rizhao People's Hospital.

Evaluate Therapeutic Effect

HIF-1α expression was detected before and after chemotherapy in 52 cases breast cancer patients to neoadjuvant chemotherapy. According to the current International Union against Cancer TNM classification of solid malignant tumors standard and American Joint Committee on Cancer Cancer Staging Manual to evaluate curative effect of therapeutic effect [9,10]. Complete remission (CR): all known lesions disappeared at least up to 4 weeks; Partial response (PR) : measurable lesions, the total volume by 50% at least more than 4 weeks and no progression or other lesions; stable condition (SD): one or more of the measurement volume reduced less than 50% of the lesions or increased less than 25%, the time for at least four weeks; Disease progression (PD): one or more measurable lesion volume increases more than 25% or the emergence of new lesions. The total effective rate was CR and PR.

Statistical Analysis

SPSS version 17.0 statistical software (SPSS Inc.: Chicago, IL, USA) was used to analyze the data. Enumeration data with χ^2 test, while measurement data between groups was compared with t test. The P value was considered to be significant if less than 0.05.

Results

HIF-1α Express and Relationship with Clinicopathological Factors

In mammary cancer tissues, HIF-1α immunoreactivity expression the percentage of cancer cells showed nuclear reactivity. The study demonstrated that the positive rates of HIF-1α were 68.75%, which were higher than that in DCIS (43.8%), ADH(31.6%), UDH (9.4%) (χ^2=13.442, 22.272, 52.789, P<0.01, respectively). There was no significance in DCIS and ADH (P=0.139). There was a significantly difference in the mean express of HIF-1α frequency between histoligical grade (χ^2=4.85, P=0.03), lymph node metastasis (χ^2=3.89, P=0.04), distant metastasis (χ^2=5.48, P=0.02) and recurrence (χ^2=6.06, P=0.01) (Table 1). There was posive correlation in over-expression of HIF-1α with the level of ER or PR, and the expressions of HIF-1α were not related with age (≤50 yr vs 50yr) and size of tumor (≤5cm vs >5cm) (P>0.05)(not shown).

Table 1. HIF-1α express relation with biological parameters in breast cancer.

Biological parameters	Number	Negative	Positive	χ^2	P Value
Grade					
I or II	96	35	61	4.85	0.03
III	32	5	27		
Lymph node metastasis					
Absent	45	19	26	3.89	0.04
Present	83	21	62		
Distant metastasis					
Absent	91	34	57	5.48	0.02
Present	37	6	31		
Recurrence					
Positive	22	2	20	6.07	0.01
Negative	106	38	68		

Serum Biomarker Levels Relation with HIF-1α Express and Chemotherapy

The present study investigated the value of CA153, CEA and CA125 potential serum markers in the diagnosis and prognosis of 43 cases breast cancer. In this study, the follow-up duration of serum biomarkers ranged from 3 months to 2 years, and the levels of the above indicators were detected through dynamic blood draw samples. The CA153, CEA and CA125 serum levels in HIF-1α positive and negative groups in 43 cases of mammary cancer patients were shown in Fig. 1. The serum CA153, CEA and CA125 levels in HIF-1α positive patients were significantly higher than those in negative patients ($P<0.05$, respectively) (Fig. 1A). There were significant difference of chemosensitivity of CA153 and CEA before and after neoadjuvant chemotherapy, however($P<0.05$, respectively), there was no significant difference of CA125 before and after chemotherapy($P>0.05$) (Fig. 1B). CA153 and CEA can be used as followed up potential factors of breast cancer chemosensitivity to help following up undergoing individualized chemotherapy.
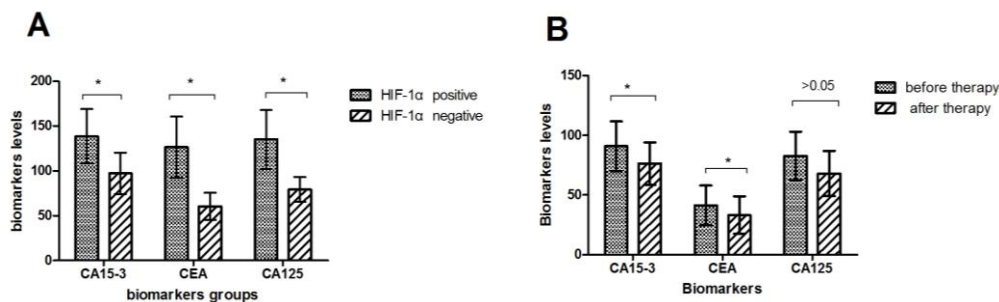

Figure 1. Serum biomarkers levels among the diffrent groups.

HIF-1α Express with Chemotherapy

Immunohistochemical UltraSensitive^{TM} S-P method was used to detect differences in tumor tissue HIF-1α expression and the situation before and after chemotherapy in 52 cases of mammary cancer to neoadjuvant chemotherapy. Before and after chemotherapy, the expression of HIF-1α has changed significantly from 46.15% down to 21.15%, $P< 0.05$. Before and after chemotherapy, the expressions of HIF-1α are positive correlation, $P<0.05$, in Table 2.

Table 2. HIF-1α express with chemotherapy (n=52).

Chemotherapy	HIF-1α		χ^2	P Value
	Negative	Positive		
Before	28(53.85%)	24(46.15%)	7.278	0.007
After	41(78.85)	11(21.15)		

For HIF-1α expression, the patients were divided into negative and positive two groups. Considering only the HIF-1α expression, survival was found to be longer in negative group than that in positive group ($P <0.05$) , in Table 3.

Table 3. Chemosensitivity in HIF-1α positive and negative express groups (n=52).

HIF-1α	Number	CR+PR	SD+PD	Efficient	χ^2	P Value
positive	24	14	10	58.33%	4.924	0.026
negative	28	24	4	85.71%		

Before neoadjuvant chemotherapy, the chemosensitivity of HIF-1α positive expression was worse than the chemosensitivity of HIF-1α nagetive expression ($P<0.05$). HIF-1α can be used as a predictor of mammary cancer chemosensitivity to help develop individualized chemotherapy.

Discussion

In this study, we analyze the expressions of HIF-1α in mammary cancer, and the relationship between the HIF-1α expression and clinicopathological parameters including stage, grade, lymph node metastasis, distant metastasisand recurrence, and the combined detecting CA153, CEA and CA125 serum levels and achieved a better application effect. Hypoxia is a common feature of various cancers. Solid tumors are characterized by regions of low oxygen tension, which play a central role in tumor progression and resistance to therapy [11,12]. Cells under hypoxic conditions develop numerous adaptive responses to hypoxic stress concurrently with altered expression of hundreds of genes that are regulated by hypoxia inducible factors [13,14]. Low oxygen tension affects mitochondrial function and for the cells to survive, mitochondria must functionally adapt to low oxygen tension to maintain the cellular bioenergetics [11,13,15-16]. HIF-1α is an important cellular survival protein under hypoxic conditions, regulating the cellular response to low oxygen tension via recruitment of a transcriptional co-activator, induces expression of multiple genes involved in cell survival, proliferation, angiogenesis, and tumor development [13-15]. Mammary cancer is one of the commonly-encountered solid malignant tumors, like most other forms of malignancy, occur as a result of HIF-1α of the effects of environmental and heritable factors. Cells under hypoxic conditions develop numerous adaptive responses to hypoxic stress concurrently with altered expression of hundreds of genes that are regulated by hypoxia inducible factors. In our study, the over-expression rates of HIF-1α in mammary cancer was 68.75%, which was significantly higher than benign leisions($P<0.01$). The cell of immunostaining for HIF-1α overexpression is more common in invasive mammary cancer than mammary cancer benign leisions. Our previous research shows there is no significance difference of HIF-1α expression between ADH and DCIS, and ADH as DCIS represent intraepithelial neoplasias, which indicates that ADH and DCIS are likely to represent precursor, albeit not obligate, to invasive mammary cancer [2,17]. We also detect differences in HIF-1α expression situation before and after chemotherapy of mammary cancer to neoadjuvant chemotherapy. In recent years, the incidence of mammary carcinoma is signifcantly on the rise all over the world [18], hence, how to treat mammary carcinoma, effectively

assess the therapeutic effect, correctively evaluate the prognosis and find the postoperative recurrence of patients with mammary carcinoma have been paid attention by more and more scholars all over the world [19-20].In our study, before and after chemotherapy, the expression of HIF-1α has reduced significantly. Our results implicate the importance of HIF-1α can be used as a predictor of breast cancer chemosensitivity to help develop individualized chemotherapy. However, further study is needed to understand the exact pathogenic mechanism. In Breast carcinoma, abnormal expresses of HIF-1α is associated with poor differentiation, recurrence, metastasis and chemotherapy. Our results suggest that in breast cancer, over-expressions the abnomlally expressions of HIF-1α seem to as event in carcinoma development. Meanwhile, the abnomlally expression of HIF-1α rate in the patients with metastasis, recurrence and prognosis is also conspicuously higher than those without. The over-express HIF-1α is correlated with worse prognosis. Furthermore, combined detecting HIF-1α expression and serum CA153 and CEA also had a certain clinical signifcance in predicting metastasis and prognosis of breast cancer, and can be used as followed up potential factors of breast cancer chemosensitivity to help following up undergoing individualized chemotherapy, which suggested that HIF-1α inhibitors could be an alternative for treating and controlling advanced breast neoplastic disease in females. Future studies should therefore preferentially select a broader target set of potential biomarkers.

Conflict of Interest

The authors declare no conflicts of interest.

Acknowledgements

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