RESEARCH ARTICLE

Analysis of Indoleamine 2-3 Dioxygenase (IDO) and EGFR Co-expression in Breast Cancer Tissue by Immunohistochemistry

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Abstract

Background: To determine the amount of co-expression of IDO and EGFR in breast cancer patients. Materials and Methods:In order to obtain the distribution of co-expression of IDO and EGFR in breast cancer, we tested 110 breast cancer paraffin tissue blocks with immunohistochemical methods. Then we investigated the relationship between the diagnostic and pathologic characteristics (tumor size, lymph node status, histologic grade, the gene expression of ER, PR, HER2, p53, Ki67 and PCNA) with the situation of co-expression of IDO and EGFR by reviewing the medical records of 32 breast cancer patients. Results: Among 110 breast cancers, 32 cases demonstrated IDO and EGFR co-expression (29.1%), IDO and EGFR synchronous co-expression being found in 19.1% and asynchronous in 10.0%. Conclusions: IDO and EGFR were co-expressed in breast cancer, including synchronous and asynchronous co-expression. The results suggest that considering IDO and EGFR as two indicators for breast cancer treatment or prognosis analysis provides a potential option of individual treatment for the portion of breast cancer patients with co-expression of IDO and EGFR.

Keywords: EGFR - IDO - co-expression - breast cancer

Asian Pac J Cancer Prev, **15 (14)**, 5535-5538

Introduction

Breast cancer is the most frequently solid malignancy and the main cause of cancer related death in women worldwide, breast cancer is also recognized as a heterogeneous disease increasingly (Cheang et al., 2008; Rakha et al., 2009; Rakha et al., 2009; Kim et al., 2013). This indicated that a multi-target therapy would be more effective in the treatment of breast cancer.As the recurrence and distant metastasis is the reason most cancer deaths, a substantial fraction of breast cancer patients develop distant metastases shortly after diagnosis, and the metastatic breast cancer is associated with poor prognosis with shorter survival time and refractoriness to therapies. Among a variety of breast cancer cells evading the immune attack mechanisms, upregulation of tumor-derived immunosuppression metabolistic enzymes, such as indoleamine 2, 3-dioxygenase (IDO), has shown a crucial role in tumor metastasis (Brandacher et al., 2006; Ino et al., 2002), up-regulation of IDO in primary breast cancer may inhibit local immune surveillance and promote metastasis (Mansfield et al., 2009). EGFR is a member of the ErbB family of receptor tyrosine kinases, activation of the EGFR has been shown to increase mitogenesis and cellular proliferation and to up-regulate factors needed for sustained growth and survival. In addition to proliferative effects, EGFR activation enhances the transcription of genes that regulate processes involved with tumor progression and invasiveness to include such effects on cell motility, cell adhesion, and angiogenesis. EGFR also plays distinct roles in breast carcinomas, the expression of EGFR in basal-like breast cancer is associated with poor prognosis (Cheang et al., 2008; Rakha et al., 2009; Tan et al., 2008; Arteaga, 2003). Compared with Western countries, the incidence of breast cancer in China is lower. The age of peak breast cancer incidence is much earlier and the mortality rate is increasing.Mutations in the EGFR pathway occurred in a small fraction of Chinese breast cancers. Compared with NSCLC and colorectal cancer, anti-EGFR targeted therapy does not produce a dramatic clinical response in breast cancer. Therefore, effective measures to control breast cancer in China are becoming increasingly important. (Abediankenari et al., 2013; Tong et al., 2012; Hu et al., 2014). Although IDO or EGFR was important in breast cancer, but the relationship between IDO and EGFR in breast cancer, that is, whether IDO or EGFR are co-expression in breast cancer, or they influence the outcome of breast cancer was still not be mentioned.In this study, we analyzed the distribution of IDO and EGFR protein expression in 110 samples from breast cancer cases, and the results indicate that EGFR and IDO were co-expressed in breast cancer.

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Materials and Methods

Collection of tumor specimens

For this retrospective analysis, paraffin-embedded tumor specimens were obtained from 110 breast cancer patients who resided in Northeast China. Patients were recruited at the Siping Central Hospital, Siping, Jilin, China between the years of 2010 and 2012. All patients were diagnosed through surgical and pathological methods, signed a consent form for the unspecified use of biospecimens, and completed an interviewer-administered questionnaire. The collection of tumor specimens, survey data, clinical and pathological information, and follow-up data was reviewed and approved by the Siping Central Hospital Institutional Review Board for the participating institutions.

Immunohistochemical staining and scoring of IDO and EGFR

Six slices of paraffin sections were cut from each paraffin tissue of breast cancer, each slice was 2.5μm, three for EGFR detected, the other for IDO detected.Slides were stained according to manufacturer's protocol. Briefly, slides were deparaffinized in Xylene for 20 minutes. For EGFR detected, antigen retrieval method was used in autoclave at 1 min in the antigen retrieval buffer of Tris/EDTA (PH8.0); for IDO detected, antigen retrieval method was used in autoclave at 1 min in the antigen retrieval buffer of citrate (PH6.0). The mouse primary antibody against IDO (cat:mab5412, chemicon, USA) or against EGFR (cat:ZA-0093 Sequoia Jinqiao Biological Technology Co., Ltd., Beijing) was diluted 1:75 with Beijing Sequoia Jinqiao antibody diluent (cat:ZLI-9028)

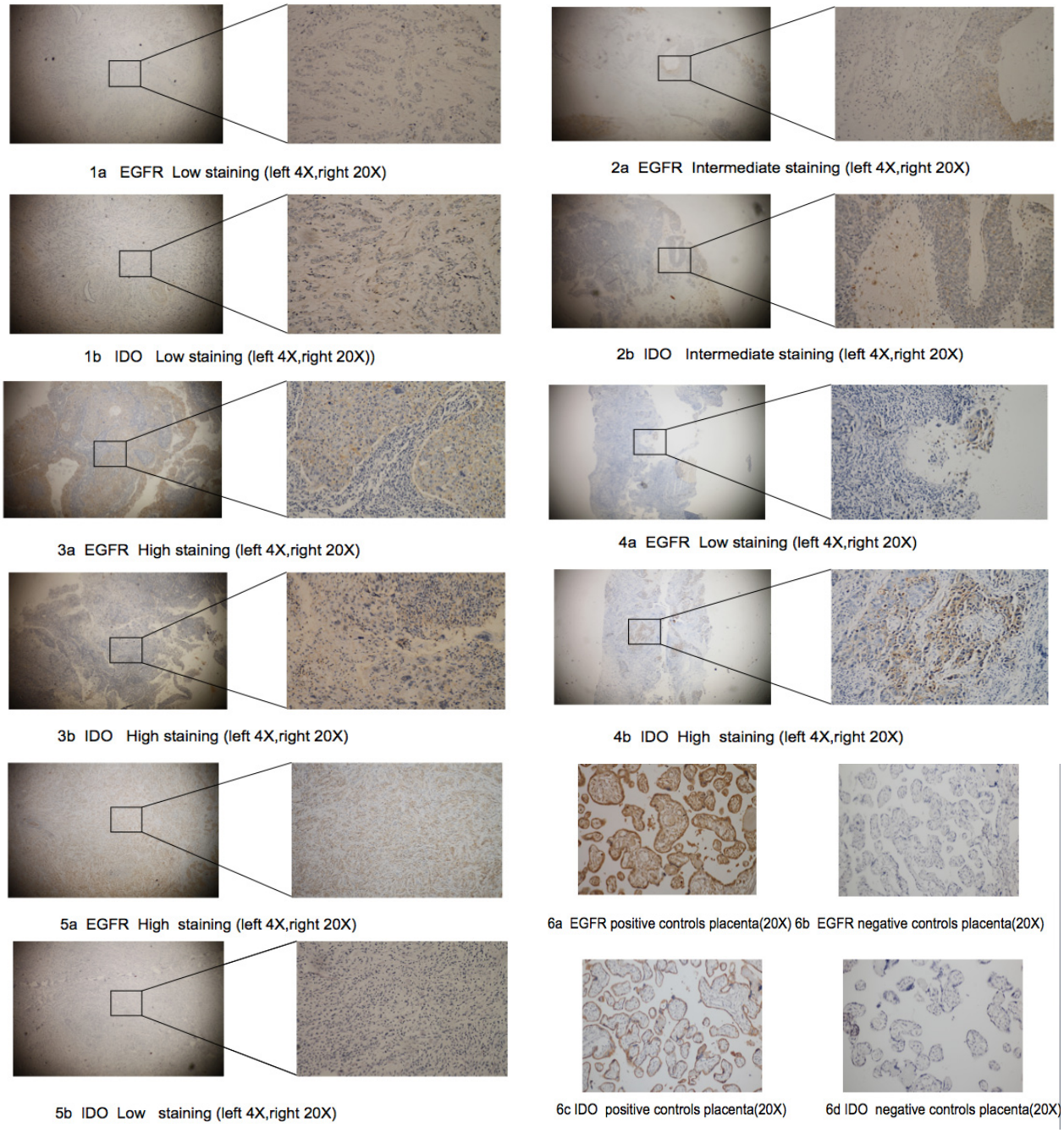

Figure 1. IDO and EGFR Co-expression Immunostaining Photographs. 1 indicated negative and positive controls with placenta; 2 to 5 indicated IDO and EGFR synchronous co-expression in breast cancer; 6 indicated IDO and EGFR difference co-expression in breast cancer

and was incubated overnight at 4°C. The Beijing Sequoia Jinqiao anti-mouse secondary antibody (cat:PV-9000) was incubated for 40 min and counterstained with hematoxylin. Following this, slides were dehydrated and mounted according to normal laboratory protocol. The slides were scored numerically by two pathologists on intensity of IDO or EGFR staining (0, 1, 2, 3). The slides also underwent computer image analysis for IDO or EGFR staining as an additional quality assurance measure (Analytic Microscopy Core, Jilin University). Differences in scores were adjusted between the two pathologists to arrive at a final score. And the scores were stratified as negative = 0, low = 1, intermediate = 2, and high = 3.

Results and Discussion

Co-expression of IDO and EGFR in 110 breast cancers

We used the immunohistochemical double staining method to investigate whether there is the situation of co-expression of IDO and EGFR in breast cancer tissue. There is two methods of the immunohistochemical double staining, that is, the serial sections of double staining method and the immunohistochemical double staining kit (Okamoto H.et al, 2013; Van Noorden S.et al, 1986; Vander Loos CM.et al, 1987), at first we used the immunohistochemical double staining kit to test the paraffin sections, but there are many factors including the incubation temperature and time, dewaxing, rinse time and the dosage of buffer solution leading to non-specific background staining as well as the target antigen staining intensity becoming weak or false negative, at the same time, the antigen repairing methods of the two kinds of antibody should also be the same, but the two kinds of antigen repairing liquid are different, for EGFR detected, the antigen retrieval buffer is Tris/EDTA (PH8.0), for IDO detected, the antigen retrieval buffer is citrate (PH6.0). Then we used the serial sections of double staining method, and this method is the most simple and reliable, for the same immunohistochemical method in two adjacent sections, each antigen is displayed, then compare the two adjacent section immunohistochemical staining results, because each section only once stained, so it will not interfere with each other or false double labeling. Six slices of paraffin sections were consecutively cut from each paraffin tissue of breast cancer, each slice was 2.5μm, three for EGFR, the other for IDO detected. The mode co-expression of IDO and EGFR immunostaining results was displayed in Figure 1, both IDO and EGFR were expressed at the same site of the same tumor tissue, but the ultrastructural regions of IDO or EGFR expression was difference, for IDO, the expression sections was mainly in the cytoplasmic of the malignant ductal cells and mostly undetectable in the adjacent stromal cells. for EGFR, the expression sections was mainly in the cell membrane of the epithelial cells and stromal cells. IDO and EGFR could synchronous co-expressed in breast cancer, and the degree of co-expression was from low to high; meanwhile, IDO and EGFR could also asynchronous co-expressed in breast cancer, such as IDO expressed low degree, and EGFR displayed intermediate or high degree, or EGFR expressed low degree, and IDO displayed intermediate or high degree. The mode medical record information of IDO and EGFR co-expression immunostaining was

Table 1. IDO and EGFR Co-expression Immunostaining Mode Medical Record Information

Serial number	Medical record number	Age	Diagnosis (breast cancer)	EGFR scores	IDO scores
1*	95279	47	right	Low	Low
2*	101567	44	left	Intermdeiate	Intermdeiate
3*	103894	49	right	high	high
4**	94867	44	left	high	low
5**	97590	60	right	low	High

*indicated IDO and EGFR synchronous co-expression; **indicated IDO and EGFR asynchronous co-expression

Table 3. Information of Patient and Tumor Characteristics with 32 cases of IDO and EGFR Co-expression

Variable	Level	IDO and EGFR	
		synchronous co-expression (21)	asynchronous co-expression (11)
Race	Chinese		
Age	<45	5	3
	≥45	16	8
Tumor size	TIS/1/2	10	5
	3/4	11	6
Lymph node metastasis	N	13	8
	P	8	3
Stage	I	4	5
	II	15	4
	III	2	2
ER	N	17	8
	P	3	3
PR	N	18	11
	P	2	0
Her-2	N	16	8
	P	4	3
P53	N	1	0
	P	7	2
Ki67	N	0	0
	P	20	10
PCNA	N	0	0
	P	11	7

N, negative; P, positive

Table 2. The Situation of IDO and EGFR Co-expression in 110 Breast Cancers

Item	Grade	Positive percentage
IDO and EGFR synchronous Co-expression	Low	5(4.5%)
	Intermediate	10(9.1%)
	High	6(5.5%)
IDO and EGFR asynchronous Co-expression	IDO low and EGFR intermediate-high	7(6.4%)
	EGFR low and IDO intermediate-high	4(3.6%)

shown in Table 1. Among 110 breast cancers, 32 cases was detected with IDO and EGFR co-expression, and the total co-expression positive percentage is 29.1%, IDO and EGFR synchronous co-expression is 19.1%, IDO and EGFR asynchronous co-expression is 10.0%, the detailed information was summarized in Table 2.

The relation of patient and tumor characteristics with IDO and EGFR co-expression

The demographics and characteristics of the patient with IDO and EGFR co-expression are summarized in Table 3. Among 32 cases, the median age of patients was 56 (range 37-83), and all participants were female. 9 (28.1%) of participants were stage I, 19 (59.4%)of participants were stage II, and 4 (12.5%) of participants were stage III. In the analysis of categorical variables, it appears that age, tumor size, lymph node metastasis, the expression of her-2 and P53 was entirely unrelated with IDO and EGFR co-expression, but the expression of ki67 and PCNA was positive correlation with IDO and EGFR co-expression, and the expression of ER and PR was an inverse correlation with IDO and EGFR co-expression. All the results indicated that IDO and EGFR were co-expression in breast cancer, and our results suggest that combination of the IDO and EGFR inhibitors or the addition of chemotherapy could provide a potential individual treatment option for the portion of the breast cancer patients with the co-expression of IDO and EGFR. Further studies are warranted to provide a rationale for the use of IDO and EGFR co-expression status as a predictive marker for breast cancer and to identify patients with this breast cancer subtype who are more likely to respond to IDO and EGFR-targeted therapies.

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