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# Expression of IGF-IR in Breast Cancer tissue before and after Neoadjuvant Chemotherapy

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**Abstract**

Neoadjuvant chemotherapy (NAC) is a common therapeutic schedule in treatment of breast cancer. In order to discover the mechanism of IGF-IR in breast cancer tissue and its influence on cell proliferation and CAM angiogenesis and NAC effect. 160 breast cancer patients in II and III period were selected and tested the expression of IGF-IR. All the patients were diagnosed according to specimen taken with needle biopsy and treated with NAC (Duoxitasai, epirubicin and cyclophosphamide) for 2 months, had mastectomy and tested expression of IGF-IR. The results showed that positive expression rate of IGF-IR in breast cancer patients were73.125% and the expression of IGF-IR is closely related with the periods of tumor and Axillary node metastases (P=0.01) but not related with tumor size (P=0.21). After treatment, IGF-IR in 65 patients had changed from high-expression to low expression (P=0.001) and negative conversion rate was 55.56%. Total effective rate of patients whose IGF-IR is positively expressed was 84.62%, which is obviously lower than that of patients with IGF-IR negatively expressed (P=0.035). IGF-IR before treatment is closely related to VEGF, MVD and ki-67 (P=0.001). NAC can partly inhibit expression of IGF-IR to inhibit tumor growth and treatment with IGF-IR can effectively inhibit proliferation of breast cancer cells and CAM angiogenesis ability.

## 1. Introduction

Insulin-like growth fac-tors-1 (IGF-I) is a kind of cell factor which can effectively promote mitosis and proliferation of cells and angiogenesis. IGF-I plays its part in the onset, development, invasion and metastasis of tumor [1]. Type1 insulin-like growth factor receptor (IGF-IR) is receptor tyrosine kinase which is transmembrane distributed. IGF-IR can induce biological activation of IGF-I and IGF-II [2]. IGF-IR is closely related to the onset and development of many kinds of tumors, especially breast cancer [3]. Some previous studies found out that IGF-I and

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IGF-IR are expressed in specimen taken from mastectomy [4]. Many kinds of malignant tumor cells can secret IGF-I and express IGF-IR. IGF-IR can promote the proliferation of tumor cells by inducing IGF-I. Breast cancer cells own IGF-I~IGF-IR autocrine/by loop. Thus it is a good way to treat breast cancer by messing IGF-I-IGF-IR autocrine and paracrine loop [5,6]. NAC is important treating methods in the advanced stage of breast cancer. It can lower tumor period and difficulty of surgery and increase sensitivity of chemotherapy, but about 8%-10% breast cancer patients also become worse and lost opportunity of surgery even after NAC [7,8]. Therefore, it is important to make clear of curative effect before surgery of NAC. It is a pity that there has no obvious way to evaluate curative effect of NAC [9-10]. This study tested and analyzed the level of IGF-1R, VEGF, MVD and ki-67 before and after experiment on histology level.

**Table 1 relationship between IGF-IR and tumor Clinicopathological data**

| Tumor clinicopathological data | Patients number | IGF-IR positive expression rate （Patients number%） | $X^2$ | P |
|---|---|---|---|---|
| tumor diameter | | | | |
| ＜2cm | 75 | 56(74.67) | 1.45 | 0.128 |
| ≥2cm | 85 | 61(71.76) | | |
| node metastases | | | | |
| yes | 104 | 93(89.42) | 9.85 | 0.001 |
| no | 56 | 24(42.86) | | |
| TNM period | | | | |
| Ⅱ | 67 | 32（47.76） | 8.92 | 0.001 |
| Ⅲ | 93 | 85（91.39） | | |
| ER expression | | | | |
| positive | 64 | 43(67.19) | 2.21 | 0.082 |
| negative | 96 | 74(77.08) | | |
| PR expression | | | | |
| positive | 75 | 56(74.67) | 1.13 | 0.432 |
| negative | 85 | 61(71.76) | | |

## 2. Materials and methods

### *2.1. Agents and materials*

IGF-IR mAb is made by Santa Cruz. Trizol is made by Gibco. MULTISKANMS Automatic enzyme labelling is made by Finland. UMSP microspectrophotometer and KS 400 image analysis system is made by Zeiss in Germany.

### *2.2. Immunocytochemistry testing protein levels of IGF-IR*

Breast cancer tissue was used hematoxylin to staine the slide again and separate color, dehydration, vitrification and made slices. Staining was finished with PBS fluid replacing the first antibody to form the negative control group. Endochylema and membrane of positive cells are brown and yellow

**Table 2 relationship among IGF-IR,breast cancer tissue cell proliferation index (ki-67)and vascular forming index(VEGF,MVD)**

| Indexes | Patients number | IGF-IR positively expressed | IGF-IR negatively expressed | $X^2$ or T value | P |
|---|---|---|---|---|---|
| Ki-67 | | | | | |
| Highly proliferated | 78 | 42(53.85) | 36（46.15） | 13.24 | 0.001 |
| Lowly proliferated | 82 | 75(91.46) | 7（8.54） | | |
| VEGF | | | | | |
| positive | 105 | 85（80.95） | 20（19.05） | 9.64 | 0.002 |
| negative | 55 | 32（58.18） | 23（41.82） | | |
| MVD | | 25.24±7.32 | 14.22±4.66 | 11.58 | 0.001 |

### *2.3. Evaluating methods*

MVD [11] firstly, chose vascular densely distributed area under the microscope. Secondly, selected 10 high densely distributed areas at high magnification for calculation. All brown cells clusters and single endothelial cells were taken as capillary. If lumen is not clear, the branches were also taken as a single vascular. Thirdly, calculated out the average vascular number as the value of MVD. Ki-67 [12]：positive cells≤5% was low proliferation activation, >5% was high proliferation activation.

### *2.4. Statistical methods*

Data in the study were calculated out according to SPSS13.0 software. Relationship among IGF-IR, MVD, Ki-67 and VEGF expression and other clinical data were analyzed with chi-square criterion. The correlation of GF-IR, VEGF and Ki-67 were analyzed with chi-square test of

four-fold table. The relationship between IGF-IR and MVD was analyzed with t test. It has significance when the value of p is lower than 0.05.

## 3. Results

### *3.1. Relationship between IGF-IR and tumor clinicopathological data*

IGF-IR positive expression rate in tissue of breast invasive ductal carcinomas is 73.125%（117/160）. IGF-IR positive expression is closely related with tumor TNM period and axillary node metastases (p=0.001). IGF-IR positive expression rate in patients with node metastases is as high as 89.42%（93/104）and IGF-IR positive expression rate in patients in periodIII is 91.39%（85/93）, which are higher than that of patients with periodII and have no node metastases. The expression of IGF-IR has no close relationship with tumor size, ER and PR expression (p>0.05). IGF-IR expression rate in patients with tumor size above 2cm is 71.76% (61/85) and IGF-IR expression rate in patients with ER and PR positive expressed are 67.19% （43/64）and 74.67%（56/75）respectively (Table1, Figure1) .

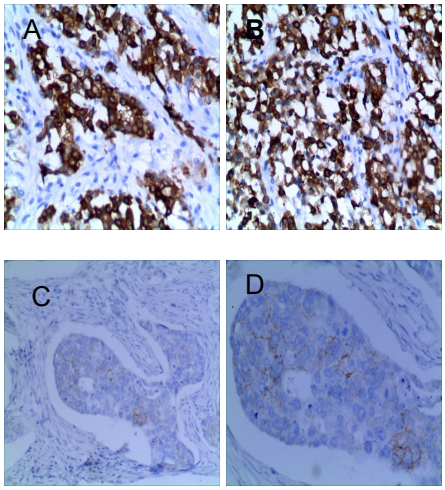


**Figure 1 IGF-IR express in tissues of breast cancer patients**

**A,B:IGF-IR is positively expressed, cytolymph and membrane of cells are brown, A: IHC×200， B IHC×100; C, D: IGF-IR is negatively or weak positively expressed, C: IHC×100 D: IHC×200.**

### *3.2. Relationship among IGF-IR, breast cancer tissue cell proliferation index (ki-67) vascular forming index (VEGF, MVD)*

Before chemotherapy, IGF-IR expression is closely related with breast cancer tissue cell proliferation index (ki-67) and vascular forming index (VEGF, MVD) (P=0.001). IGF-IR expression rate in patients with Ki-67 highly proliferated is 91.46% (75/82) and that in patients with VEGF positively expressed is 80.95% (85/105). MVD number of IGF-IR positively expressed and negatively expressed also has significant difference (P=0.001) (Table2, Fugure2).

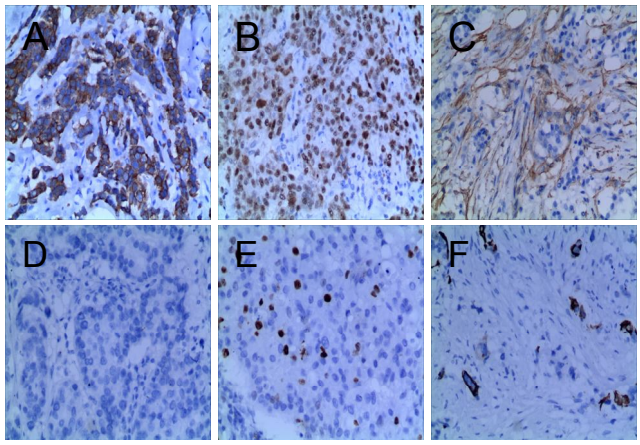


**Figure 2 expression of ki-67, VEGF and MVD in tissues of breast cancer patients, IHC×100**

**A: VEGF positively expressed, cytolymph is brown; B: ki-67 highly expressed; C: MVD positively expressed, vascular is brown; D、E、F: negatively or weak positively expressed VEGF,ki-67 and MVD respectively.**

### *3.3 Relationship between IGF-IR and curative effects of chemotherapy*

IGF-IR in 65 patients has changed from highly expressed to lowly expressed (P=0.001), the negative conversion rate is 55.56% (65/117). Complete symptom relieving rate of IGF-IR positively expressed patients is 20.51% and partly symptom relieving rate is 64.10%. The total curative effect is 84.62%, which is significantly lower than that of the IGF-IR negatively expressed patients (95.35%) (P=0.035) (Table3).

**Table 3 relationship between IGF-IR and curative effects of chemotherapy**

| IGF-R | patients number | Total effective rate（%） |
|---|---|---|
| positive | 117 | 84.62 |
| negative | 43 | 95.35 |
| $X^2$ | | 4.26 |
| P | | 0.035 |

## 4. Discussion

Breast cancer is common cancer in women. Main treating methods for breast cancer now is surgery and chemotherapy. Neoadjuvant chemotherapy (NAC) can minish tumor primary lesion and partly relieve symptoms so as to give convenience to surgery [13]. With the widely using chemotherapeutics, some patients get drug-resistance. It is high time to find out the relative gene connected with onset, development and curative effect so as to use the gene to reach the aim of early diagnosing and treatment. In recent years, many studies study the relationship between insulin like growth factor (IGF) and malignant tumor. IGF-IR is widely expressed on the surface of many kinds of cells. Both of IGF-I and IGF-II have to work with IGF-IR to form autocrine and paracrine to show their functions [14]. Some studies show that after combined with ligands. IGF-IR can improve synthesization of protein and DNA and metabolism of carbohydrate. IGF-IR is closely related to growth and division of cells and development of embryos. Besides, IGF-IR is tumor enhancing factor and it enhances tumor growth by working with signal pathway of other factors such as VEGF after being activated by its petunidin. What is more, with a series of signal transduction, IGF-IR can activate transcription in cells and regulate synthesization of protein to increase cell proliferation and mitosis and induce and retain the phenotype of tumor [15]. IGF-IR can also obviously increase expression of VEGF and matrix metalloproteinase, synthesization of Cadherin and other PECAM in tumor cells, and the invasion and metastasis of tumor cells. Recently, some studies have confirmed over expression of IGF-IR in malignant tumor tissues. But reports about the relationship of IGF-IR and the curative effect of NAC are few [16]. This essay applies immunohistochemical to test expression of IGF-IR on protein level in tissues of breast cancer before and after NAC. The relationship of IGF-IR, MVD, VEGF and Ki-67 is analyzed to confirm the possibility of IGF-IR participating in chemoresistance, tumor growth and CAM angiogenesis. Besides, this essay synthesizes IGF-IR ASODN according to IGF-1RmRNA on cytology level to inhibit expression of IGF-IR on the transcription level. IGF-IR ASODN can restrict the ability of proliferation and surviving in tumor cells. What is more, treatment of IGF-IR ASODN can inhibit growth and transcription of tumor and expression of IGF-IR to enhance sensitivity to radiotherapy and chemotherapy.

IGF-IR is transmembrane protein with activation of tyrosine kinase. It is highly expressed and activated on the membrane of tumor cells of breast cancer, pancreatic cancer, colon cancers and many other cancers [17]. Recently, many basic and clinical studies have confirmed that IGF-IR and its ligand play important role in growth and development of cells, it's over expression can induce cell mitosis and apoptosis to increase mitosis, proliferation and tumor invasiveness [18-19]. Some other studies also prove that IGF-IR is over expressed in breast cancer. IGF-IR ASODN can inhibit growth of tumor in tumor bearing mice and in vitro. It can also inhibit tumor cell proliferation, but the inhibiting effects is weak when using alone (45.13%) [20-21]. With the development of molecular biological techniques, study about the occurrence of tumor has turn to study about abnormity of gene for invasiveness and expansion of breast tumor are closely related with gene abnormity [22-23]. Happerfield and other scholars using immunohistochemistry method to study

tissues of breast tubular adenocarcinoma,Lobular adenocarcinoma and fibroadenoma and normal breast tissues, and have discovered that IGF-IR is highly expressed in malignant cancer tissues and lowly or seldomly expressed in tissues of benign tumor and normal breast [24]. The results of immunohistochemistry in our study show that IGF-IR is highly expressed in breast cancer tissues (73.12%) and IGF-IR. It is closely related to tumor TNM period and axillary node metastases. IGF-IR is highly expressed in 93 patients in 104 patients with node metastases. Further study about the relationship of IGF-IR, VEGF, MVD and Ki-67 confirms that IGF-IR is closely related to tissue proliferation index Ki-67and angiogenesis index VEGF and MVD before chemotherapy. IGF-IR positive expression rate in patients with ki-67 highly expressed tissue is91.46% and IGF-IR positive expression rate in patients with VEGF positively expressed tissue is80.95%.MVD number in patients with IGF-IR positively and negatively expressed is significantly different. The result can preliminarily prove over expression of IGF-IR in the onset and development of breast cancer can induce abnormity of cell proliferation, differentiation and apoptosis. IGF-IR can induce over proliferation, abnormal differentiation, reduced apoptosis and formation of neoplastic vascular so as to aggravate development of malignant tumors. Further analysis of the data before and after NAC has got the result that IGF-IR expression in 65 patients has turned from high expression to low expression and negative converting rate is 55.56%. Total curative effect of IGF-IR positively expressed patients are 84.62%, which is much lower than that of IGF-IR negatively expressed patients. The results show that IGF-IR plays its role in the whole process of chemotherapy and NAC can inhibit the proliferation of breast cancer cells by inhibiting expression of IGF-IR.

## 5. Conclusions

Autocrine and paracrine of IGF-I induced by IGF-IR are very important in growth of breast tumor and angiogenesis. NAC can partly inhibit proliferation of breast cancer cells by inhibiting expression of IGF-IR. Treatment with IGF-IR ASODN can effectively inhibit proliferation of breast cancer cells and angiogenesis ability.

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