REVIEWS

The prognostic genes of breast cancer and clinical significance

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Abstract

Breast cancer is one of the most frequent malignant tumors and is receiving more attention due to the increasing incidence and detection rate. Further studies regarding prognosis genes for biology are being conducted. The paper aims to review the prognostic indexes of breast cancer such as p16, Her-2, Ki-67, MCM7.

Key Words: Breast cancer, Related genes, Clinical significance

1 Introduction

Breast cancer is one of the most frequent malignant tumors, whose incidence ranks the top of all of the malignant tumors for females, even more than cervical carcinoma. It is estimated that 1.2 million women suffered from breast cancer and died at the age from 40 to 45 per year.[1] It has been found that occurrence, development, earlier lymph node metastasis, earlier distant organ metastasis such as bone, lung and liver as well as prognosis of breast cancer had a close relation with gene mutation and novel expression with the development of molecular biology and further study of the related genes and protein test. Therefore, a deeper understanding of the clinical significance of related genes and protein expression of breast cancer, co-detection of various gene expression of breast cancer provides basic guideline for therapeutic regime, prognostic assessment and regular follow-up visits. The review aims to analyze the researching process of related genes of breast cancer.

2 Advances and perspectives of relativity research of breast cancer

Tumor suppressor protein p16, also known as multiple tumor suppressor, was first discovered by Kamb, *et al.* at Cold Spring Harbor Lab as an anti-cancer gene. It plays an important role in cell cycle regulation by decelerating cells proliferation, division and progression from G1 phase to M phase. 50% tumor cell strains of human were found homozygous deletion and mutation. Tumor suppressor protein p16 was considered as a more important anti-cancer gene than p53. It was regarded as a brake system that may lead to cells malignant proliferation in case it was out of control. It was a negative regulatory factor of G1 checkpoint. Therefore, p16 acts as a tumor suppressor by binding to CDK4/6 and preventing its interaction with cyclin D, and slows down the cell cycle by prohibiting progression from G1 phase to S phase. Tumor suppressor protein p16, as an inhibitory gene of CDK4, may release the inhibition of Rb phosphorylation and contribute to cells proliferation[2] and tumor formation. The novel expressions of p16 phase, such as homozygous deletion, high methylation, mutation have casual relationship with metastasis and development of breast cancer.[3]

The studies of the association of p16 with breast cancer are of significance for further investigation for its etiology, growth characteristics, diagnosis and treatment. Studies showed that positive rates of p16 protein expression had less correlation with age, histological type and tumor size, but there was close relativity with lymph node metastasis and

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TNM.[4] Tumor suppressor protein p16 is frequently mutated or deleted in a wide variety of tumors, such as breast cancer, colon cancer and esophageal cancer.[5,6] It is a helpful indicator to determine the nature and the treatment regimen of tumor. Some studies also demonstrate the negative relativity between p16 and Ki-67 expression.[7] Combined detection of p16 gene expression and Ki-67 is of clinical significance to the diagnosis of breast cancer prognosis and treatment options. The advantages of p16 lies in the fact that it is of small size (only 1/10 of p53), easier administration for gene diagnosis, and more practical significance to the clinical treatment of tumors. The research of p16 remains a hot topic at present and in the future.

3 Study on relativity between Ki-67 and breast cancer

Ki-67 is a proliferating cell nuclear antigen (PCNA), whose expression protein is strictly associated with cell proliferation. It was originally defined by the prototype monoclonal antibody Ki-67, which was generated by immunizing mice with nuclei of the Hodgkin lymphoma cell line L428 by Gerdes J, *et al.* in 1983. Its molecular weight is between 345,000-395,000, which was a popular index for breast cancer research at present. Ki-67 protein is present during all active phases of the cell cycle (G1, S, G2, and M), but is absent from resting cell phase (G0). It started expression in G1 phase of the cell cycle, increased in S phase and G2 phase, reached a peak in M phase, then quickly disappeared in late cell division. It has been regarded as the most reliable indicator of tumor cell proliferation due to the short half-life period and its expression can also demonstrate rapid malignant tumor growth rate.[8] The level of Ki-67 has a vital significance for the evaluation of tumor proliferation, the research of tumor biological behavior and the estimation of harmful effects to our body. Researches showed that there was significant difference of positive rate of Ki-67 expression in breast cancer and adjacent normal tissue.[9] The results revealed that the positive rate of Ki-67 was of 74.8% in breast cancer, a similar conclusion to foreign countries. Ki-67 was not much associated with age and tumor size. However, there was a casual relationship between tumor grade and lymph node metastasis. The high expression of Ki-67 antigen has a tendency of lymph node metastasis of breast cancer. The positive expression rate of Ki-67 was significantly increased with the clinical progression of breast cancer. Therefore the role of Ki-67 is to justify occurrence, development and prognosis of breast cancer. A lot of studies have confirmed that Ki-67 has prognostic value not only for breast cancer but other tumors such as soft tissue tumor, lung cancer, astrocytic glioma, meningioma, cervical cancer and prostate cancer. A study[10] on positive expression of Her-2 gene in breast cancer has shown there was a positive correlation between Her-2 and Ki-67, which meant the synergistic effect. So it was beneficial for the prognosis of breast cancer to co-detection of Ki-67 and Her-2.

4 Study on relativity between MCM7 and breast cancer

Mini-chromosome maintenance proteins (MCM) group family was a group of protein with highly homology in sequence and closely interaction in function. MCM7 was one of the components of MCM protein compound, and could directly reflect the expression level of MCM. The expression level of MCM protein reached to the peak during the transmitting period from G1 phase to S phase. Cells started differentiation and senescence from G0 phase, and the expression level of MCM decreased and even unmeasurable. The protein encoded by this gene is one of the highly conserved MCM that are essential for the initiation of eukaryotic genome replication. DNA replication disorder would result in genomic instability, and contribute to the malignant cell transformation. Studies of molecular biology showed that MCM was a multifunctional protein which was not only an important part of DNA replication and extension but also more specificity and accuracy as a new nuclear antigen markers of cell proliferation than Ki-67 and PCNA.[11] Studies have reported[12] that the positive rate of MCM7 was 96.4% in breast carcinoma, the positive expression (++)-(+++) of MCM7 was 72.7%, and normal breast tissues or adjacent normal breast tissues had not positive expression of MCM7. In other words, MCM7 can reflect the proliferation activity of tumor cells with an extreme sensitivity and specificity, and is a better cell proliferation marker. It also serves as a reliable biological index for early clinical diagnosis and evaluation of cancer cell proliferation.

Breast cancer is one of the most common malignant tumors in women, whose treatment methods include surgical treatment, chemotherapy, radiation therapy, hormonal therapy, biological target therapy, inhibitors of angiogenesis factors and so on. Neoadjuvant chemotherapy and endocrine therapy have become currently an important part of comprehensive treatment of breast cancer. The new adjuvant chemotherapy is to change administration time of chemotherapy from post-operation traditionally to pre-operation, and to decrease tumor staging, pathological grading by inhibiting tumor cell proliferation, killing tumor cells and lessening the tumor size. Breast cancer can reach a large complete pathological remission (CPR) in some cases, as well as a fully understanding of the sensitivity of chemotherapy drugs to breast cancer and therapeutic regimen adjustment. It is beneficial to therapeutic regimes selection. Factors that contribute to the occurrence and development of breast cancer were multiple. One of factors is associated with novel cells proliferation. The current studies focused on effective cell proliferation markers as the prognosis and therapeutic criteria of neoadjuvant chemotherapy by finding out the identification and evaluation of cells proliferation preneoadjuvant chemotherapy and post-neoadjuvant chemotherapy, and relation with clinical effect. The expession level of MCM7 and Ki-67 pre and post neoadjuvant

chemotherapy and their relationship carry a certain clinical significance as prediction factors of breast cancer. Many studies demonstrate the casual relationship between novel cell proliferation and tumorigenesis, development of tumor. Finding some biological markers for judging accurately the relation of effect of neoadjuvant chemotherapy with development of breast cancer, and the changes of these indicators before and after chemotherapy is beneficial to the clinical effect and prognosis. MCM is a group of DNA replication licensing factor, which can activate the protein family. The family is closely related to DNA replication and cell proliferation. Normal mRNA of MCM changes with cell circle under normal conditions. When the expression of MCM gets to the peak at the transition period from G1 phase to S phase, the expression level of MCM at G0 phase reduced or could not be measured. MCM7 might directly reflect the expression level of MCM protein. Wenbin Dai, *et al.*[13] found that the positive expression rate of MCM7 was 90.19% before chemotherapy, the positive expression of MCM7 protein (++) or (+++) was 72.17%, and the expression level of MCM7 protein was significantly higher prechemotherapy than postchemotherapy ($p < .01$). These studies indicated that neoadjuvant chemotherapy might reduce the expression level of MCM7 protein, and was significantly higher than that of effective chemotherapy group ($p < .01$). The new adjuvant chemotherapy could reduce DNA synthesis rate of cancer cells in breast cancer, has a significant suppression role in tumors, and is closely related to the efficacy of chemotherapy. In other words, it indicates a good result for breast cancer with high expression level of MCM7 and active proliferation of tumor cells. The study was particularly beneficial to patients with high expression level of MCM7 and poor prognosis.

Ki-67, proliferation cell nuclear antigen, was regarded as an important marker to reflect the cell proliferation activity. But it was also reported that decreased Ki-67[14] might be associated with tumor remission. A lower expression level of Ki-67 by neoadjuvant chemotherapy concludes its function to inhibit proliferation of tumor cells. The researches done by Yan Wei, *et al.*[15] did not find the relativity between Ki-67 and tumor response to chemotherapy. Some studies suggested that Ki-67 was not an essential factor for cell proliferation and there was no expression of Ki-67 at early G1 phase. But it plays an important role in the process of ribosome biogenesis.[16] Therefore, Ki-67 may only identify part of proliferating cells. The fact that expression of MCM7 was positively correlated with Ki-67 may be found when analyzing their relationship in breast cancer. Studies have found that the expression level of MCM7 was significantly higher than that of Ki-67 due to MCM7 earlier replication of DNA,[17] cell mitosis involvement and accurate identification of the proliferating cells.[12] MCM7 and Ki-67 can reflect the proliferation activity of tumor cells according to these studies, but MCM7 has higher sensitivity and specificity than that of Ki-67. MCM7 is a better marker of cell proliferation due to its accuracy of cell proliferation status, and has a vital significance in identifying proliferation activity of tumor cells, guiding clinical chemotherapy and assessing the effect of chemotherapy. The relationship between MCM7 and p27 in other endocrine tumors such as thyroid tumors was discussed. Cyclin dependent kinase inhibitor protein 27 could inhibit the abnormal replication of DNA and play a negative regulation to MCM7.[18] MCM7 and p27 inhibited each other at the checkpoint from G1 phase to S phase and both regulated the integration of multiple signal pathways in the process of cell growth. Any abnormal expression may lead to malignant tumor induced by cell transformation.

The abnormal expression of MCM7 and p27 suggested the disorder of cell cycle, leading to DNA replication and extension several times during each cell cycle. The DNA-damaged cells escaped from normal cell cycle and launched directly duplication repeatedly in S, G2, M phase. Moreover, these cells could replicate without any limitation, avoid mechanisms of cells apoptosis and DNA repair, which result in the formation of abnormal duplication and polyploidy. DNA replication and extension leads to genomic instability and contribute to cell malignant transformation. Tumorigenesis is the result of abnormal regulation of some tissue cells in gene level, uncontrolled proliferation and differentiation disorder. Guida, *et al.*[19] analyzed the expression of MCM7 in human normal thyroid tissue, thyroid papillary carcinoma, undifferentiated thyroid carcinoma, which showed that the positive expression of MCM7 in undifferentiated carcinoma was significantly higher than that in normal thyroid tissue and papillary carcinoma. MCM protein can maintain higher proliferation of tumor cells, and become an important molecular marker of the auxiliary diagnosis and histomorphological types. The positive expression level of p27 decreased gradually in the three groups, and the lower expression of p27 was related with histological grade and surgical pathologic stage. And high expression of MCM7 was also associated with histological grade, myometrial invasion, lymph node metastasis and surgical pathologic stage. So the higher expression of p27 and low expression of MCM7 were much associated with tumor recurrence. MCM7 was an ideal marker of cells proliferation with high sensitivity and specificity. It played a crucial role in guiding the effective use of drugs, evaluating the therapeutic effect, improving treatment programs for breast cancer treatment. Co-anti-oncogenes detection was not only for breast cancer but also for other endocrine tumors, which serve as a reliable biological index for early clinical diagnosis and evaluation of cancer cell proliferation.

5 The advances in the study of relationship between VEGF and breast cancer

Vascular endothelial growth factor (VEGF) played a key role in the process of tumor growth. Tumor cells can se-

crete and release a variety of active factors, and these factors might stimulate the growth, migration of vascular endothelium. VEGF was an angiogenesis factor of tumor cells with the strong secretion function and accelerated vascular formation. A lot of tumor vessel with abnormal structure and function was formed. The evolution process of tumor was increased through vascular permeability, caused metastasis then. VEGF was widely used in the study of breast cancer, ovarian cancer, gastric cancer and so on. Many studies have demonstrated the higher expression of VEGF in most tumors. VEGF was related with angiogenesis and played an important role in the process of tumor growth and metastasis. Therefore, VEGF and its receptor as targeting treatment were applied in clinical therapy in recent years. And most studies indicated that VEGF could be a prognostic index of malignant tumors in that patients with positive VEGF had a poor prognosis.

Since more new angiogenesis factors were found at present, VEGF is a highly specific mitogen, which can directly stimulate the formation of new blood vessels. Any other vascular growth factors can induce vascular permeability and degrade fiber protein consequently in the same way as VEGF. Therefore it was considered as a most important and powerful angiogenic factor.[20] VEGF had a dual function: on one hand, its receptor directly stimulated the proliferation of vascular endothelial cells, and induced protein hydrolase, interstitial collagenase and tissue factor to promote the angiogenesis; on the other hand, it caused tumor interstitial edema by increasing vascular permeability, promoting extravasation of plasma protein and fibrinogen, which led to the change of extracellular matrix and provided a suitable basis for the infiltration and metastasis of tumors.

The studies of tumors in other parts of the body showed that the rate of positive expression of VEGF was 52.2% (47/90) in gastric cancer, significantly higher than that of adjacent intestinal metaplasia and atypical hyperplasia tissues ($p < .01$). VEGF had obvious heterogeneity in gastric cancer tissues and showed active angiogenesis due to the positive expression in edge-invasion gastric carcinoma. The positive expression of VEGF in medium and high differentiated gastric cancer was obviously lower than that of low differentiated group, and its positive expression with lymph node metastasis was significantly higher than those without lymph node metastasis, and the positive rate of VEGF was also increased with the depth of invasion, which was in line with the results investigated by Dongbing Liu, Hypdo, *et al*.[21,22] They also found that positive VEGF had a poor prognosis. So the expression level of VEGF reflected the biological characteristics of gastric cancer and carried a significantly prognostic value.

Fisher[22] proposed that breast cancer was a systemic disease in early phase, and this view has been widely accepted. Hematogenous metastasis of breast cancer was another important way except metastasis of lymph nodes and was the premise of hematogenous metastasis. VEGF had been suggested to be one of key growth factors that promoted tumor angiogenesis. Studies showed that VEGF was expressed[23] and detected in serum in breast cancer.[24] The expression of VEGF has been widely regarded as an important mechanism for tumor recurrence and metastasis. The targeting therapy was applied in advanced or recurrent breast cancer by blocking VEGF pathway to stop angiogenesis of tumors. The study on the relationship between the concentration of serum VEGF in breast cancer, micro metastasis and recurrence confirmed that mRNA expression of specific human mammaglobin (hMAM) detected in peripheral blood of breast tissue by nested RT-PCR can identify the micrometastasis of axillary lymph node negative breast cancer (ALNNBC).[25] This study intended to explore the correlation between serum concentration of VEGF and micrometastasis, recurrence in peripheral blood. There was a wide spread agreement that micrometastasis in early breast cancer was earlier than surgery. Micrometastasis could not confirm clinic metastasis only. However, clinical metastasis has closely correlation with micrometastasis. The specific hMAM mRNA was expressed in breast tissue, but not expressed in benign breast disease and normal bodies. Therefore, hMAM mRNA was a specific marker for breast cancer. It indicates the fact that tumor cells may be in the circulation and micrometastasis when hMAM mRNA was detected in peripheral blood. In other words, it showed that a high concentration of VEGF after the surgery had a risk of recurrence and metastasis of breast cancer, and the trend was closely related with micrometatasis in peripheral blood. If the primary tumor with high expression or high secretion of VEGF was resected and the residual tumor still existed in the body, high concentration VEGF could be detected in serum according to the theory. Observation of relationship between VEGF and recurrence, metastasis among patients should be performed to achieve more information, which provides a basis for further investigation. In conclusion, some studies suggested that growth, metastasis and prognosis of tumor was influenced by many factors, one of the more important factors was the formation of new blood vessels in the tumor tissues, which is the morphological basis of tumor growth and metastasis. It not only provided sufficient nutrients to the tumor, but also resulted in growth and metastasis of malignant cells due to the fact that it brought much more tumor cells. Meanwhile more nutrients and oxygen were given through newly vessels, and the weak vessel wall (only one layer of endothelial cells, lack of smooth muscle, thin or incomplete basement membrane) facilitated tumor cells to penetrate, which served an important factor for invasion and metastasis of tumor. Invasion and metastasis of tumors was easier due to angiogenesis since the defective structure of these vessel proved outlet for metastasis of tumor. Moreover, the structure is not complete, which provides export for the metastasis of tumor cells. More evidence indicated that new vessels played an important role in the progression

of solid tumors. VEGF was a vessel growth factor with the strongest secretion function and played an important role in angiogenesis, and its study has become a hot topic along with angiogenesis of tumor. VEGF mainly stems from tumor cells of the body, and cells can secret various active factors which are able to stimulate the growth and migration of endothelia cells.

Most studies illustrated that VEGF might be a prognostic index for malignant tumors due to poor prognosis of positive VEGF carriers. Some reports proved that VEGF was highly expressed in breast cancer, and was associated with histological grade and lymph node metastasis, but it had nothing to do with age, tumor size, menopausal status of the patients with tumor. The positive expression of VEGF had significant difference between benign breast cancer and malignant one. The positive expression of VEGF in breast cancer might not correlate with tumor type, size, but was involved in histological grade, TNM stage, lymph node metastasis with the increasing grade of breast cancer. It further demonstrated VEGF had relativity with infiltration and metastasis of tumor in the progression of breast cancer. The possible mechanism was mainly because the high expression of VEGF increased the tumor angiogenesis so that more oxygen and nutrients were provided to tumor cells to accelerate proliferation, which lead to cancer cell growth and invasion. Meanwhile, hydrolysis of vascular basement membrane caused an increase of vascular permeability and lymph node metastasis since tumor cells from the capillary venous ended into lymphatic vessel. Therefore, VEGF was involved in invasion and metastasis process of breast cancer and its expression might be reference index of biological behavior for identifying the invasion, metastasis of breast cancer.

6 Advances in the study of the correlation between Her-2 and breast cancer

Her-2 gene, known as neu, was a new gene of cancer cloned from DNA of rats with neuroblastoma for the first time by Shih in 1981. After that, Slamon was the first one to isolate Her-1 (epidermal growth factor receptor) from human cDNA library, and this gene is highly homologous with Her-2. Sequence and chromosome spectrum analysis showed that neu and Her-2 are the same gene, generally called Her-2/neu gene or c-erbB-2 gene. Her-2 was the 2nd member of human epidermal growth factor receptor (EGFR) family. The family has 4 members: Her-1, Her-2, Her-3 and Her-4. Her-2 was a more widely expression receptor of EGFR family, and its overexpression of tumor cells was not sensitive to chemotherapy. It was not only involved in inhibiting apoptosis of cells, promoting tumor cell survival but also upregulating VEGF and vascular permeability factor (VPF), promoting tumor angiogenesis, increasing invasiveness of tumor cells and damaging the tissue protection barrier to invasion of tumor cells. Therefore, Her-2 was an important targeting molecular in the study of immunotherapy of tumor. Her-2 gene was located in chromosome 17 q21, encoding p185 transmembrane glycoprotein, and its protein contains extracellular, transmembrane, intracellular segment. The extracellular segment had 40% similarity with human epidermal growth factor receptor. The transmembrane domain contained 23 amino acids which fixed the whole protein molecular on cell membrane, and intracellular segment was regarded as an active function domain of endogenous tyrosine kinase. Her-2 was mainly expressed in the embryonic period, but was less detected and presented in normal tissue in adulthood. Monomer Her-2 had no activity, but its dimer could produce the activation sign.

Her-2 had tyrosine kinase activity, could promote cell division and proteolytic enzyme secretion, so that increasing DNA synthesis, cell development, cell motility, making the invasion and metastasis of the tumor stronger.[26,27] EGFRs were highly homologous, its protein products were closely related to cells growth and played an important role in cell proliferation and differentiation. The positive expression of Her-2 was an independent biological indicator for prognosis of breast cancer. It is more valuable than hormone receptor, tumor size, is also a prognosis index of breast cancer second only to lymph node status. A number of studies have shown that c-erbB-2 expression has positive relativity with histological grading of breast cancer, and the higher the rate of expression is, the worse the prognosis is.[28–30] Overexpression and (or) amplification is also as basic conditions and predicting factor of therapeutical efficacy for Herceptin (trastuzumab) remedy. In recent years, there were a great deal of clinical research data of Herceptin for neoadjuvant therapy, postoperative adjuvant therapy, palliative treatment of metastatic breast cancer with positive Her-2, and providing strong evidence for it.[31–34] NCCN and StGalen guidelines strongly recommended regimens with anthracycline should be chosen in combined chemotherapy for patients with positive Her-2. A lot of studies have revealed that treatment with anthracycline could improve survival more significantly than nonanthracycline treatment,[35,36] but there was little or no benefit for patients with negative Her-2. Whether it was an effective method to treat breast cancer with negative Her-2 using anthracycline as adjuvant chemotherapy remains to be seen. The study of currently sequential anti-Her-2 targeting therapy after the chemotherapy was much conducted, and its efficacy was obvious, considerably improving logivity of patients for 1-3 years,[31,32] though the regime brought much adverse reaction.

Her-2 gene or Her-2/neu gene has tumor transforming activity when it is activated by some factors in vivo or vitro. Moreover, gene amplification and overexpression are its primary way of activation. Overexpression of Her-2 oncogene protein was positively related with progression, metastasis potential of breast cancer. It not only played a key role in the development of breast cancer, but also served as the

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most valuable indicator of prognosis for breast cancer as well. The conclusion of this research Her-2 expression was positive correlated with metastasis of lymph node and tumor stage, and its positive expression rate of axillary lymph node was 88.9%, while the negative expression of lymph nodes was 65.6%, there was statistically difference between them ($p < .05$), the correlation coefficient was 0.286. Her-2 expression was not related with age, tumor size, histopathlogical types and histological grade.

To sum up, patients with breast cancer is a systemic disease, which was widely accepted. The survival extension for patients with breast cancer in the ear of multidisciplinary and comprehensive treatment is mainly due to the adjuvant treatment of preventing recurrence, metastasis and operation mode. It means correct surgical ways, reasonable chemotherapy and endocrine therapy and molecular targeted therapy. The treatment of breast cancer was developed from the beginning of experience medicine, then evidence-based medicine (EBM), and to individualized treatment standard, the developing process required us to establish a reasonable evaluation index. Her-2 of oncology, p16 of tumor suppressor gene, Ki-67 and MCM7 of tumor cell proliferation markers, VEGF of angiogenesis factor were combined detected. On one hand, they were all associated with lymph node metastasis and clinical stage, were valuable prognostic indicators, on the other hand, patients with breast cancer were divided into high-risk and low-risk groups in recurrence tendency through the joint inspection, overexpression of Her-2, negative expression of p16, the positive expression of Ki-67, MCM7, VEGF were regarded as a high-risk groups, conversely, a low risk one. The precaution of the joint assessment of prognosis, taking rational treatment, making follow-up period could not reduce risks but might prevent risks and prolong the survival of patients with breast cancer.

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