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Original article

The diagnostic value of combined detection of genetic markers and serum protein markers on breast cancer

Qiujian Li, Lu Li *

Chinese and Western Medicine Combined Department, Affiliated Cancer Hospital of Zhengzhou University, Zhengzhou 450000, China

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ABSTRACT

Objective: The research is to explore the diagnostic value of several detection methods including separated and combined detection of the related genes and related proteins of breast cancer and combined detection of all genetic markers and serum protein markers on breast cancer. *Method:* The mRNA level expression of the related genes of breast cancer was detected by FQ-PCR technique and the ratio of BRCA-1, Myc, C-erbB2 and β2 micro-globulin was used to express levels of BRCA-1, Myc and C-erbB2; the related proteins of breast cancer were detected through ELISA. Then the research data was analyzed by SPSS19.0 software with t-test as comparison method, and ROC curve was used to calculate the sensitivity, specificity and accuracy of the diagnostic models. *Result:* No difference can be found among the six indexes in the control group and benign breast tumor group while compared with the benign breast tumor group and the control group, the breast cancer group was significantly different from them; combined detection of genes and that of proteins were both superior to their separated detection; all-marker combined detection was superior to separated detection, which is consistent with combined detection of genes and proteins. *Conclusion:* More detection indexes will not necessarily outcome better detection effect. Hence, appropriate detection indexes and number are needed to achieve better diagnosis effect. In order to conduct more specific method, more test samples are needed for further researches.
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1. Introduction

The incidence rate of breast cancer in China is increasing by 3% annually with younger and younger prevalence populations, hence early diagnosis and treatment of the disease is of great significance to the prognosis of breast cancer (Lu et al., 2010). In recent years, with the development of molecular biology technology, many kinds of abnormal gene expression have been found being related to the happening, development and prognosis of breast cancer, such as BRCA-1 (Cox et al., 2011); C-erbB (Révillion et al., 1997); Myc (Yang et al., 2004) and CK19 (Deng et al., 2009). The value of serum tumor markers in the diagnosis of breast cancer has been unanimously approved by many researchers. Currently there are many researches on single gene or serum tumor protein markers at home, and a good number of combined detections of serum tumor biomarkers have been reported, while combined detections of related genes have been reported less, and joint detection which combines the above detections are much less. Detection methods including immunohistochemical staining (Yang et al., 2004) and reverse transcription polymerase chain reaction (RT-PCR) (Révillion et al., 1997) were widely used in previous researches with low sensitivity; poor specificity and complicated operation. Based on conventional PCR, fluorescence quantitative polymerase chain reaction (FQ-PCR) quantitatively analyzed nucleic acid by adding fluorescent probes with simultaneous amplification and detection (Mitas et al., 2001), and this method had the advantages of high sensitivity, high specificity, high accuracy, high efficiency and less pollution. With the use of FQ-PCR, the expression levels of related genes of breast cancer including BRCA-1, c-erbB, Myc were detected by taking β2−micro-globulin as the internal control; through ELISA, three kinds of tumor protein markers including the serum TPS, CEA and CA153 were detected; it was aimed at exploring the value of separated detection and combined detection of genes and that of serum protein markers, and joint detection which combined the above detections for breast cancer diagnosis.

* Corresponding author.
E-mail address: Lilu4123@126.com (L. Li).
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2. Material and methods

2.1. Clinical material

100 cases of breast cancer (breast cancer group) which came to our hospital for the first time during January 2012 and July in 2014 were recruited as research objects, and they were all women aging 31–67 years old with an average age of 44 years old. All patients were confirmed by pathology or cytology. There were 30 cases in benign breast tumor group aging 23–62 years old, and their average age was 37 years old. There were 30 cases in normal control group who were confirmed by our hospital as healthy people aging 28–60 years old, and their average age was 40 years old. There were 34 cases of carcinoma simplex of breast, 46 cases of invasive ductal carcinoma, 16 cases of mucinous carcinoma and 4 cases of atypical medullary carcinoma in the 100 cases of breast cancer. And there were 22 cases of fibroma and 8 cases of lipoma in the 30 cases of benign breast tumor; according to the specimens of the gland tube formation, nuclear atypia and nuclear division, the phase counting of breast cancer patients was divided into level I (30 cases), level II (28 cases) and level III (42 cases). 10 ml fasting blood was taken from patients with breast cancer and benign breast tumor one weeks before the surgery while patients in the control group was taken 10 ml fasting venous blood in the morning among which 5 ml of the blood was centrifuged for backup serum.

2.2. Reagents and instruments

The reverse transcription reaction system, FQ PCR reaction system and the internal control kit of β_2-microglobulin were all from American biotechnology company ABI; 770Sequence Detector analyzer was produced by American biotechnology company ABI; cDNA standards were synthetized by Shanghai Shenyou Bio-Technology Company. And ELISA kit was purchased from Fisher Scientific Thermo in the United States.

2.3. Methods

2.3.1. FQ-PcR

Primers and probes were designed by Primer Express software in PE. FAM fluorescent was taken as the reporter dye to mark probe 5′ end, TAMRA fluorescence as the quencher dye to mark probe 3′ end. FAM fluorescent and TAMRA fluorescence were all synthetized by Shanghai GeneCore Bio-Technologies Company. One-step guanidinium isothiocyanate-alcoholphenyl-chloroform (Chomczynski and Sacchi, 1987) was used to extract the total RNA of cells from 5 ml to venous blood. cDNA was synthesized by reverse transcription reaction and the contents of the standard and specimens including BRCA-1, C-erbB2, Myc and β_2-microglobulin were measured on 7700 Sequence Detector analyzer. After the synthetic standards were diluted 10 times, the changes of correlation coefficient were observed, and the linear range was determined within 10^2–10^6 copy/ml, and based on all the previous procedures, the standard curve was made. Beside, the PCR reaction system and its reaction procedure were set according to the kit, and then according to the standard curve, instrument automatically calculated the copies/ml of BRCA-1, Myc, C-erbB2 and β_2-microglobulin in specimens; and the ratio of BRCA-1, Myc, C-erbB2 and β_2-microglobulin was taken as the expression level of BRCA-1, Myc and C-erbB2.

2.3.2. ELISA method

TPS, CEA and CA153 were detected by ELISA method, and the procedure was carried out according to the operation standard on the kit. The expression levels of TPS, CEA, CA153 were expressed as U/L, ng/ml, U/ml respectively, and their cutting value were 80 U/L, 5 ng/ml, 30 U/ml, respectively.

2.4. Statistical methods

The detection results of BRCA-1, C-erbB2, Myc and β_2-microglobulin and their ratios were expressed by mean value ± 2 standard deviation ($\bar{x} \pm 2$ s). According to statistical analysis (Jin, 1992), the normal control group ($\bar{x} \pm 2$ s) was taken as the range of normal value, if the ratio of Myc/β_2-microglobulin and the ratio of C-erbB2/β2-microglobulin were higher than those in normal control group ($\bar{x} \pm 2$ s), the ratio of BRCA/β2-microglobulin was lower than those in normal control group ($\bar{x} \pm 2$ s), it was determined as positive. If the levels of serum TPS, CEA and CA153 were higher than those of the standard, it was determined as positive. The data was processed by SPSS19.0 software, comparison was made through *t*-test, and the sensitivity, specificity and accuracy of the diagnosis model were calculated by ROC curve. Among them, if $P < 0.05$ m, the difference was statistically significant.

3. Results

3.1. The mRNA expression levels of BRCA-1 in blood of the three groups

The mRNA expression levels of BRCA-1 in blood of the control group, benign breast tumor group, breast cancer group were listed in Table 1. The ratio of BRCA-1 and β2-microglobulin was taken to represent the mRNA expression level of BRCA-1. Compared with the control group, no significant difference in benign breast group group can be found, and mRNA expression level of BRCA-1 in breast cancer group decreased significantly. And the difference of benign breast tumor group and breast cancer group was significant.

3.2. The mRNA expression levels of C-erbB2 in blood of the three groups

The mRNA expression levels of C-erbB2 in blood in the control group, benign breast tumor group, breast cancer group were listed in Table 2. The ratio of C-erbB2 and β2-microglobulin was taken to represent the mRNA expression level of C-erbB2. Compared with the control group, no significant difference in benign breast tumor group can be found, and mRNA expression level of C-erbB2 in breast cancer group increased significantly. The difference of benign breast tumor group and breast cancer group was significant.

3.3. The mRNA expression levels of Myc in blood of the three groups

The mRNA expression levels of Myc in blood of the control group, benign breast tumor group, breast cancer group were shown in Table 3. The ratio of Myc and β2-microglobulin was taken to represent the mRNA expression level of Myc. Seen from the table, there's no significant difference between benign breast tumor group and the control group, and mRNA expression level of Myc in breast cancer group increased significantly. The difference of benign breast tumor group and breast cancer group was significant.

3.4. Serum TPS, CEA and CA153 protein levels in the three groups

Serum TPS, CEA and CA153 protein levels in the control group, benign breast tumor group, breast cancer group were listed in Table 4. Compared with the control group, no significant difference

Table 1
Comparison of mRNA expression levels of BRCA-1 in three groups ($\bar{x} \pm 2$ s).

Groups	n	BRCA-1	β2-microglobulin	BRCA-1/β2-microglobulin
Control group	30	3.08 ± 1.14	3.23 ± 0.10	0.95 ± 0.36
Benign breast tumor group	30	2.99 ± 0.72	3.21 ± 0.12	0.93 ± 0.22
Breast cancer group	100	1.04 ± 0.34	3.22 ± 0.14	0.32 ± 0.10*

Note: * indicates that $P < 0.05$ compared with the control group.

Table 2
Comparison of mRNA expression levels of BRCA-1 in the three groups ($\bar{x} \pm 2$ s).

Groups	n	C-erbB2	β2-microglobulin	C-erbB2/β2-microglobulin
Control group	30	7.01 ± 2.66	3.24 ± 0.14	2.17 ± 0.84
Benign breast tumor group	30	7.18 ± 2.80	3.22 ± 0.12	2.23 ± 0.86
Breast cancer group	100	18.14 ± 6.20	3.23 ± 0.10	5.62 ± 1.94*

Note: * indicates that $P < 0.05$ compared with the control group.

Table 3
Comparison of mRNA expression levels of Myc in the three groups ($\bar{x} \pm 2$ s).

Groups	n	Myc	β2-microglobulin	Myc/β2-microglobulin
Control group	30	4.45 ± 1.12	3.23 ± 0.14	1.38 ± 0.34
Benign breast tumor group	30	4.65 ± 1.08	3.24 ± 0.10	1.44 ± 0.34
Breast cancer group	100	13.01 ± 3.92	3.22 ± 0.12	4.04 ± 1.22*

Note: * indicates that $P < 0.05$ compared with the control group.

Table 4
Comparison of the protein levels of TPS, CEA, CA153 and CYFRA21-1($\bar{x} \pm 2$ s) in the three groups.

Groups	n	TPS level	CEA level	CA153 level
Control group	30	60.14 ± 30.57	2.81 ± 1.03	11.34 ± 6.52
Benign breast tumor group	30	69.21 ± 25.48	3.21 ± 2.52	14.87 ± 7.14
Breast cancer group	100	280.50 ± 33.50*	15.36 ± 6.34*	50.72 ± 22.09*

Note: * indicates that compared with the control group, $P < 0.05$.

in benign breast tumor group can be found, and the levels of the three protein markers increased significantly.

3.5. The diagnostic value of separated and combined detection of every marker on breast cancer

The separated and combined detection of blood genetic markers including BRCA-1, C-erbB2 and Myc, the separated and combined detection of the serum protein markers including TPS, CEA and CA153, and joint detection of all markers, the diagnostic value on breast cancer was shown in Table 5 and Figs. 1–3.

It can be concluded that the area under the ROC curve, the sensitivity and specificity of combined detection of blood genetic markers is higher than that of separated detection. The sensitivity of combined detection of serum protein markers is the same with that of single CEA, CA153 detection, but higher than that of TPS; the specificity is the same with that of TPS but higher than that of single CEA、CA153 detection; as for the area under the ROC curve, combined detection of blood genetic markers is higher than that of separated detection. Compared with the joint detection of gene markers and protein markers, the sensitivity, specificity, and area under the curve of all indexes are all the same.

Table 5
The diagnostic value of separated and combined detection on breast cancer.

Groups	Sensitivity (%)	Specificity (%)	AUC
BRCA-1	86.700	91.000	0.942
C-erbB	90.000	92.000	0.964
Myc	93.300	87.000	0.957
TPS	90.000	99.000	0.958
CEA	96.700	87.000	0.945
CA153	96.700	85.000	0.929
Gene combination	96.700	99.000	0.994
Protein combination	96.700	99.000	0.994
Combination	96.700	99.000	0.994

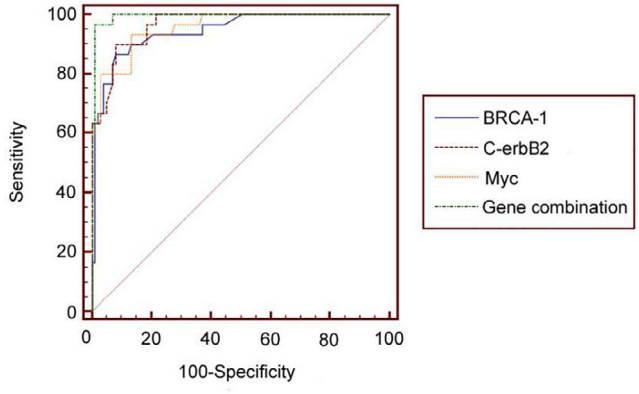

Fig. 1. The ROC curve of separated and combined detection of blood gene markers on breast cancer.

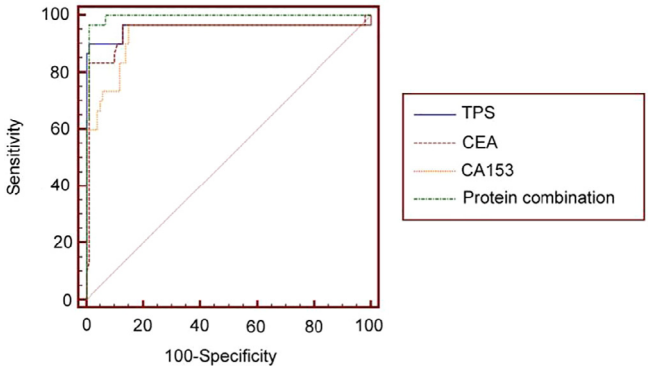

Fig. 2. The ROC curve of separated and combined detection of serum protein markers on breast cancer.

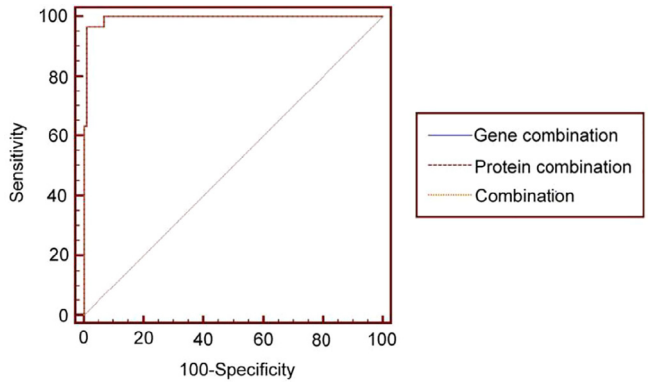

Fig. 3. The ROC curve of combined detection of blood gene markers and serum protein markers, and total joint detection of breast cancer.

4. Discussion

Iconography can't perceive the early development of cancers, but some related genes of tumor in human body will expressed abnormally and some related genes of proteins in blood also express abnormally, hence the detection of related genes and proteins is an important way to detect cancer in its early stage. For malignant tumors, early diagnosis and treatment can significantly improve the efficacy and the survival rate. However, currently no absolutely specific tumor marker can be found to diagnose cancers, hence diagnosis and differential diagnosis of cancer cannot be better performed.

BRCA-1, C-erbB2 and Myc are all related genes of breast cancer, their abnormal expressions are closely related to the occurrence and development of breast cancer. BRCA1 is one of the susceptible genes of breast cancer, it has been positioned between the chromosome 17q21D17s1321-D17s1325, and is playing a crucial role not only in the maintenance of genetic stability, but also in important cellular activities including the inhibition of cell growth, cell cycle regulation, gene transcription regulation, DNA damage and repair and apoptosis (Gu et al., 2013). The oncogene of C-erbB2 is located on the q21 belt of chromosome 17, and it is highly homologous to the receptor gene of epidermal growth factor, its amplification is related to cell malignant transformation, tumor metastasis and prognosis. Myc gene is located on the long arm of chromosome 8, whose coding is related to the related transcription factor of the regulation of cell proliferation, and its abnormal expression is related to many human tumors including lung cancer, gastric cancer, liver cancer and breast cancer. In this research, mRNA levels of the above three genes can be quantitatively and accurately analyzed through FQ-PCR, and it can be concluded that the single detection of the three genes respectively are significantly different from the control group and benign breast tumor group, which shows that separated detection of breast cancer has certain values. Combined detection is superior to separated detection in sensitivity, specificity and the area under the curve, which has a certain referencial value, and the research results are consistent with those of the research conducted by Deng et al. (2011).

The detection of related protein markers of tumor in serum is widely used. TPS, CA153 and CEA are related to protein markers in breast cancer. TPS is a soluble fragments of tissue antigens identified by 18 antibody of cytokeratin, and it expresses highly in epithelial malignant tumors and metastatic tumors, especially in the active proliferation period of tumor cell, accompanied with a large number of blood coming in, which can better reflect the biological behavior of tumors (Tu et al., 1999; Bjorklund and Einarsson, 1996). CA153 is an antigen correlated to breast cancer, it increases differently or over expresses in 30–80% of breast cancer patients, especially in metastasis or recurrence and metastasis in breast cancer patients, and the fluctuation of CA153 level in serum is consistent with the condition changes of breast cancer, which is an important signal (Ebeling et al., 2002) of recurrence and metastasis. CEA is initially considered to be a tumor marker of colon cancer, then high expression of CEA has been found in other malignancies as well, such as pancreatic, lung, breast and gastric cancers (Kong, 2000). In this research, the expression levels of TPS, CA153 and CEA in breast cancer group were significantly higher than those of benign breast tumor group and control group. The sensitivity, specificity, and the area under the curve all indicate that separate detection has certain value too, however considering comprehensively, combined detection is better than separated detection, which is consistent with the research conducted by Zheng and Luo (2005), Yildirim et al. (2017), Khan et al. (2018), Golezar et al. (2017), Altun et al. (2017), Gao et al. (2017).

In this research, separated detection and combined detection of the three genes and the three proteins associated with breast cancer, and the total joint detection of all markers were detected separately, it's found that total joint detection is superior to separated detection, but are not superior to the combined detection of genes and proteins; predictably, the more indexes are, the detection effect is not necessarily better, appropriate detection index and number are needed to achieve better diagnosis effect. The specific information still needs more test samples to conduct further research.

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