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The Significance of Minimally Invasive Core Needle Biopsy and Immunohistochemistry Analysis in 235 Cases with Breast Lesions

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OBJECTIVE To evaluate core needle biopsy (CNB) as a minimally invasive method to examine breast lesions and discuss the clinical significance of subsequent immunohistochemistry (IHC) analysis.

METHODS The clinical data and pathological results of 235 patients with breast lesions, who received CNB before surgery, were analyzed and compared. Based on the results of CNB done before surgery, 87 out of 204 patients diagnosed as invasive carcinoma were subjected to immunodetection for p53, c-erbB-2, ER and PR. The morphological change of cancer tissues in response to chemotherapy was also evaluated.

RESULTS In total of 235 cases receiving CNB examination, 204 were diagnosed as invasive carcinoma, reaching a 100% consistent rate with the surgical diagnosis. Sixty percent of the cases diagnosed as non-invasive carcinoma by CNB was identified to have the presence of invading elements in surgical specimens, and similarly, 50% of the cases diagnosed as atypical ductal hyperplasia by CNB was confirmed to be carcinoma by the subsequent result of excision biopsy. There was no significant difference between the CNB biopsy and regular surgical samples in positive rate of immunohistochemistry analysis (p53, c-erbB-2, ER and PR; $P > 0.05$). However, there was significant difference in the expression rate of p53 and c-erbB-2 between the cases with and without morphological change in response to chemotherapy ($P < 0.05$). In most cases with p53 and c-erbB-2 positive, there was no obvious morphological change after chemotherapy.

CONCLUSION CNB is a cost-effective diagnostic method with minimal invasion for breast lesions, although it still has some limitations. Immunodetection on CNB tissue is expected to have great significance in clinical applications.

KEY WORDS: breast carcinoma, breast lesions, core needle biopsy, immnuohistochemistry.

Introduction

Core needle biopsy (CNB)[1] is a minimally invasive diagnostic method for breast lesions, which has been widely applied in many other countries. Recently, it has also been employed in the early diagnosis of breast lesions in China, and it is replacing the traditional intraoperative frozen section method gradually [2]. In this research, CNB results of 235 cases with breast lesions are summarized and analyzed, and the significance of immunohistochemistry (IHC) examination in 87 cases is emphasized.

Materials and Methods

Clinical data

A total of 276 CNB specimens were received from Tianjin Medical University Cancer Hospital from Jun. 2001 to Dec. 2004. In the total of 276 patients, 235 cases receiving the CNB before surgical excision were included in this research. These tissue samples were all from female patients with breast lesion. The median age of the patients was 53 years old ranging from 26 to 81 years old. One hundred ninety-one cases (81.28%, 191/235) were diagnosed as breast carcinoma by clinicians referring the results of the clinical examinations before CNB, while the remaining 44 cases (18.72%, 44/235) were unable to be categorized due to the difficulty in demonstrating the benign or malignant potential nature by clinical and mammography examinations.

Collection and check of specimen

i) Core needle biopsy gun was used in 225 patients when the mass of lesion was palpable, clear or superficial, and easy to fix. Breast tissue was drawn into a concave groove located in the needle core under negative pressure, and then it was taken out after severed by a severing needle. *ii*) Ultrasound guided biopsy was applied in 6 patients when the lesion was tiny (< 1 cm) or not easy to be targeted in a deep location. *iii*) X-ray guided stereotactic biopsy was performed in 4 patients when the lesion was not clearly palpable, but the clusters of microcalcified particle were detected through mammography. At least 4 sequential samples were obtained in each case.

Section preparation and pathological diagnosis

Sample strips were fixed with 10% AF solution (alcohol-formalin mixed solution) (formalin: 95% ethanol 1:9) soon after collection, and then they were treated through a series of dehydration and paraffin immersion for 20 h. Subsequently, the sample strips were embedded into paraffin, and then sectioned at 4 μm thick followed by the procedure of staining with hematoxylin and eosin (H&E). The pathological diagnosis was established by two senior pathologists through light microscopy[3].

Immunohistochemistry and results evaluation

The immunohistochemical assay for the protein expression of p53, c-erbB-2, ER and PR was conducted on 87 paraffin embedded CNB tissue samples. Monoclonal antibody against p53 and c-erbB-2 were purchased from Zemyed Corporation, and monoclonal antibody against ER and PR were obtained from Santa Cruz Corporation. PV-9000 Polymer Detection System IHC kit was from Zhongshan Biotechnology Limited Co. Primary antibody dilutions were as the following: p53 at 1:50, c-erbB-2 at 1:100, ER at 1:100, and PR at 1:75. The 4μm thick sections of paraffin embedded CNB tissues were mounted on slides followed by a series of procedures: dewax, rehydration, and dip in 3% methanol at 60°C overnight in order to make sections adherent to the slides firmly. Then the sections were incubated with H_2O_2 to block the activity of endogenous peroxidase and heated with microwave in EDTA buffer to recover the antigens. Primary antibody, secondary antibody and third antibody (streptavidin HRP conjugate) were respectively added (in where) in order and incubated at 37°C for 20 min. Afterwards, reaction products were visualized with freshly prepared 3,3'-diaminobenzidine (DAB), and the slides were counterstained with hematoxylin. Positive and negative controls were included in every batch of slides.

Result evaluation standard[4,5]: positive signals of p53, ER and PR proteins were located in cancer cell nuclei, while positive signals of c-erbB-2 were located in cancer cell membrane, and all of the positive markers displayed a brown yellow color. If the percentage of the positive cellular nucleus was ≥ 20%, the case was considered as p53 positive, if the percentage ≥ 15%, it meant ER and PR positive, and if that ≥ 30%, it meant c-erbB-2 positive. The intensity of positive signal for c-erbB-2 was graded as 1+ (weak), 2+ (moderate) and 3+ (intense) depending on staining extent.

Examination of surgically excised specimens

Routine pathological examination[3] and immunostaining were also performed on paraffin sections of surgically excised cancer tissues, and the results were compared with those of CNB samples. When routine sections of the cases receiving preoperative chemotherapy were examined by microscopy, the change of morphology in cancer tissues was observed and then assigned to 3 stages: stage I, degeneration and necrosis; stage II, granuloma formation, and stage III, fibrosis generation[6].

Statistic analysis

The data analysis was performed with the SPSS10.0 software package and Concise Statistics. The cross table χ^2 test and R × C table χ^2 test were used to compare the percentage difference between results of CNB and the surgical specimens. The level of significance was set at 0.05.

Results

Pathological diagnosis of CNB

In 235 patients, 204 of them were diagnosed as breast carcinoma, including 119 cases of invasive carcinoma (Fig.1a) and 5 cases of non-invasive carcinoma (Fig.1b). The remaining 31 cases were diagnosed as the following: 8 cases of atypical ductal hyperplasia (Fig.1c), 3 cases of phyllodes tumor (Fig.1d), 2 cases of fibroadenoma (Fig.1e), 5 cases of other benign lesion, 7 cases of normal tissue and 6 (2.55%) cases of having uncertain

results because of unsuccessful CNB section preparation or/and uncertain pathological diagnosis, as a result, the 6 cases were suggested to undergo intra-operative frozen section diagnosis.

Comparison of pathological diagnosis results coming form CNB and from surgical excision specimen

All of 235 cases had surgery and different patterns of the surgery were chosen for different patients depending on the results of their clinicopathologic examinations (radical mastectomy, modified radical mastectomy, quadrantectomy and lump extensive resection). Comparison of pathological diagnosis results between CNB and surgical excision specimen is presented in Table 1.

Concordance rate of pathological diagnosis results for invasive carcinoma between CNB and surgical specimens was 100%, and false positive rate of CNB was 0. Nevertheless, 60% non-invasive carcinoma diagnosed by CNB was found to have the presence of invading elements in surgical speci-

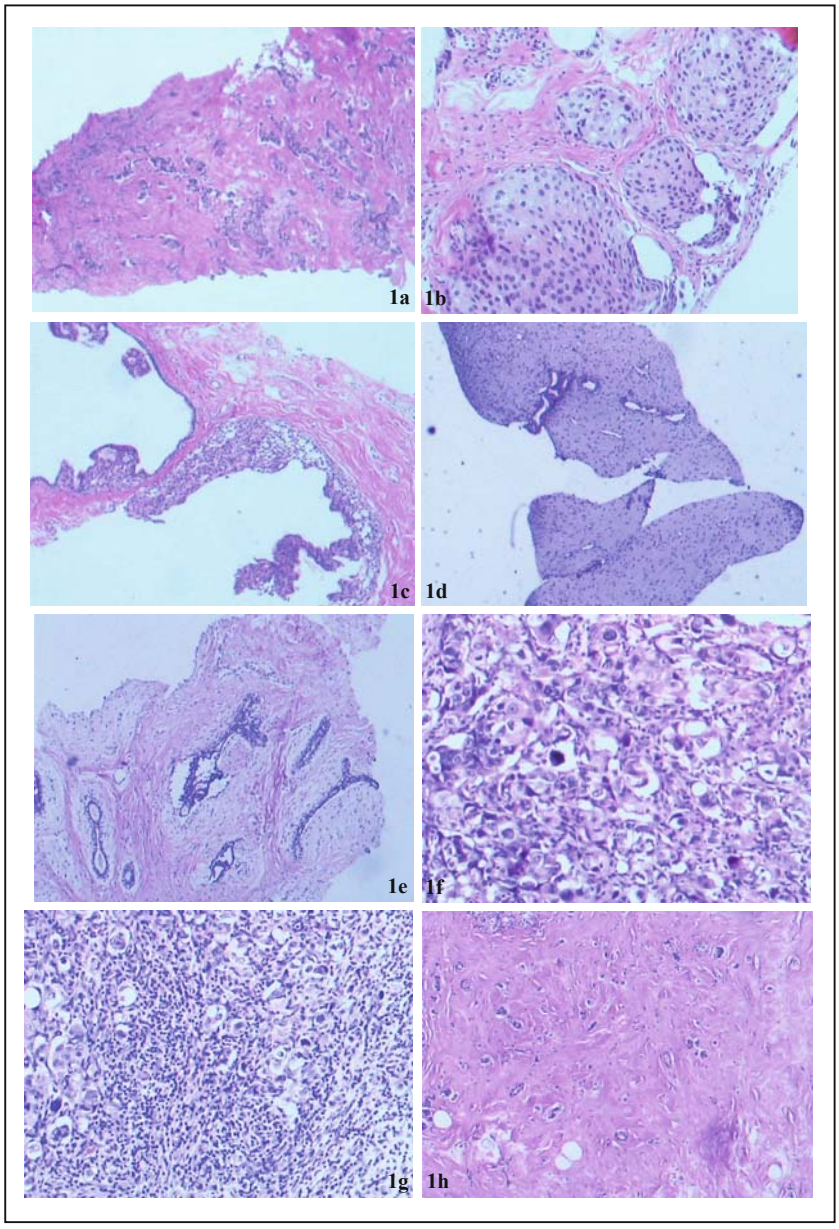

Fig.1a. Breast invasive carcinoma in CNB section (H&E×40); Fig.1b. Breast DCIS in CNB section (H&E×100); Fig.1c. Breast atypical ductal hyperplasia in CNB section (H&E×100); Fig.1d. Breast phyllodes tumor in CNB section (H&E×40); Fig.1e. Breast fibroadenoma in CNB section (H&E×40).Fig.1f. Morphological changes stage I in regular section (H&E×200); Fig.1g. Morphological changes stage II in regular section (H&E×200); Fig.1h. Morphological changes stage III in regular section (H&E×100).

Table 1. Comparison of pathological diagnosis outcomes between CNB and surgery.

Pathological diagnosis of CNB		Pathological diagnosis of surgery			
Pathological diagnosis	Cases	Consistent with CNB	Cases	Not consistent with CNB	Cases
invasive carcinoma	199	invasive carcinoma	199	benign lesion	0
non-invasive carcinoma	5	non-invasive carcinoma	2	invasive carcinoma	3
atypical hyperplasia	8	atypical hyperplasia	4	carcinoma	4
phyllodes tumour	3	phyllodes tumour	3	other lesion	0
fibroadenoma	2	fibroadenoma	2	other lesion	0
benign lesion	5	benign lesion	0	carcinoma	5
normal tissue	7	benign lesion	4	carcinoma	3
Total	229	total	214	total	15

Six cases were not included in Table 1 due to unsuccessful section preparation or uncertain diagnosis, among which, 4 cases and 2 cases were pathologically testified to be carcinoma and benign lesions, respectively after operation.

mens. Fifty percent of atypical ductal hyperplasia diagnosed by CNB was demonstrated to be carcinoma by subsequent excision biopsy. Few cases diagnosed as benign lesion by CNB were proven to be carcinoma in surgical specimen because of the paucity of tissues or the inaccuracy of sampling.

Morphological responses to chemotherapy

Preoperative chemotherapy of 1 to 3 weeks was administered to 89 patients right after diagnosed as invasive carcinoma by CNB. Among them, 14, 19, 54 and 2 individuals underwent chemotherapy for 1, 2, 3, and more than 3 weeks respectively before the operation. Combined chemotherapy regimens were administered, including cyclophosphamide, adriamycin and 5-Fu or cyclophosphamide, amethopterin and 5-Fu, and paclitaxel and taxotere. Morphological changes in response to chemotherapy were observed on 54 cases (Fig.1f, 1g, 1h), among which 36, 15 and 3 cases were assigned to stage I, stage II and morphological changes stage III, accordingly. Positive morphological response was not observed in 35 cases.

IHC results of breast carcinoma

IHC assay was performed for 87 individuals who were diagnosed as invasive carcinoma by CNB and underwent preoperative chemotherapy (2 samples were not examined because after the second round of section, the paraffin embedded cancer tissue was too few). The results of the expression of p53, ER, PR and c-erbB-2 in 87 cases receiving preoperative chemotherapy are presented in Fig.2a~h. The same IHC assay was also conducted on surgical specimens of the 87 patients. The comparison of positive rate of 4 markers between CNB and the corresponding surgical specimens is shown in Table 2. There was no significant difference between the results of CNB and the surgical specimens, $P > 0.05$.

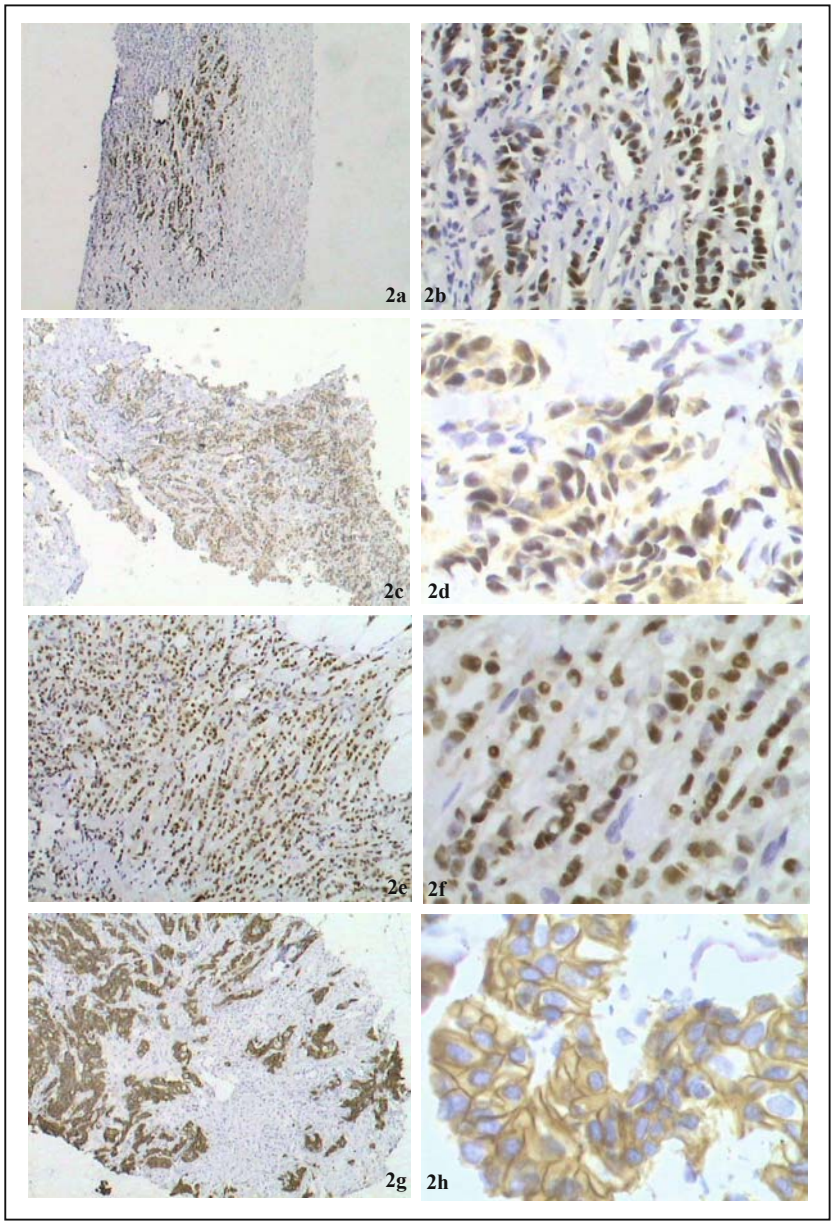

Fig.2a. Immunohistochemistry for p53 (+) in CNB section (× 40); 2b. Immunohistochemistry for p53 (+) in CNB section (× 200); 2c. Immunohistochemistry for ER (+) in CNB section (× 40); 2d. Immunohistochemistry for ER (+) in CNB section (× 200); 2e. Immunohistochemistry for PR (+) in CNB section (× 40); 2f. Immunohistochemistry for PR (+) in CNB section (× 200); 2g. Immunohistochemistry for c-erbB-2 (+) in CNB section (× 40); 2h. Immunohistochemistry for c-erbB-2 (+) in CNB section (× 200).

Table 2. Comparison of IHC outcomes between pre-chemotherapy and post-chemotherapy (%, *n*).

	Positive rate in CNB (pre-chemotherapy)	Positive rate in surgical section (post-chemotherapy)	χ^2	*P*
p53	33.33 (29/87)	31.03 (27/87)	0.112	> 0.05
ER	64.36 (56/87)	66.67 (58/87)	0.102	> 0.05
PR	56.32 (49/87)	64.36 (56/87)	1.177	> 0.05
c-erbB-2	41.37 (36/87)	45.98 (40/87)	0.374	> 0.05

The positive intensity of c-erbB-2 expression in CNB was summarized as follows: 17 cases + (weak), 13 ++ (moderate), and 6 +++ (intense). The 3 positive groups accounted for 47.22%, 36.11% and 16.67% of all positive cases (36), respectively. The positive intensity of c-erbB-2 expression in surgical specimen was displayed as follow: 19 cases +, 14 ++, and 7 +++. The 3 positive groups accounted for 46.34%, 35.00% and 18.66% of all positive cases (40), respectively. For all the 3 positive groups with different intensities, there was also no significant difference regarding to their percentage distribution in the whole positive population between the results of CNB and surgical specimen (χ^2 =5.864, χ^2 =7.820, χ^2 =9.282, $P > 0.05$).

Each IHC result of CNB was correspondingly compared with that of surgical specimen side by side. The concordance rate in the specimens taken from the two ways for p53, ER, PR and c-erbB-2 expression was 88.51% (77/87), 85.06% (74/87), 86.21% (75/87) and 93.10% (81/87), respectively.

P53, c-erbB-2 expression and morphological responses to chemotherapy

The positive rates of p53, c-erbB-2 expression in cases with (sensitive) and without (insensitive) morphological change in response to chemotherapy are presented in Table 3. There was significant difference in positive rate for p53 or c-erbB-2 between the two groups, χ_{p53}^2=38.345, P_{p53}<0.05; $\chi_{c\text{-}erbB\text{-}2}^2$= 22.839, $P_{c\text{-}erbB\text{-}2}$<0.05, whereas there was no significant difference in positive rate for p53 or c-erbB-2 among chemotherapy-sensitive groups with different stages of morphological responses, χ_{p53}^2=0.450, P_{p53}>0.05, $\chi_{c\text{-}erbB\text{-}2}^2$= 0.415, $P_{c\text{-}erbB\text{-}2}$>0.05. P53 and c-erbB-2 double positive were observed in 2 cases (3.70%, 2/54) with morphological responses, whereas similar events were observed in 20 cases (57.14%, 20/35) without morphological responses. Therefore, p53 and c-erbB-2 double positive result was very likely to appear in the cases without morphological responses.

Table 3. Comparison of p53, c-erbB-2 expression between the cases with morphological changes and without (%, *n*).

		Positive rate	
Morphological change	Cases	p53	c-erbB-2
Visible	54	11.11 (4/36)	27.78 (15/54)
Stage I	36	12.96 (7/54)	25.00 (9/36)
Stage II	15	13.33 (2/15)	33.33 (5/15)
Stage III	3	0 (0/3)	33.33 (1/3)
Without	35	57.14 (20/35)	71.43 (25/35)

Discussion

The fine needle aspiration used for cytology test is a conventional method of diagnosis for breast lesions. However, it is often difficult to establish an authentic diagnosis as a result of cytology's limitation, so clinicians have less and less trust on cytological diagnosis. Instead, intra-operative frozen section is performed as a common way of diagnosis for almost every patient, and the operation time is prolonged correspondingly. However, CNB for breast lesion[7] aroused attentions of medical professionals again in recent years as a minimally invasive diagnostic method, and its sampling and diagnostic techniques have been greatly improved. CNB has been widely applied to ordinary patients with breast lesions in Europe and America, and it is even replacing the intra-operative frozen section diagnosis. Likewise, CNB research and application is also under way in China now. Advantages of breast CNB are demonstrated in the following aspects: *i*) CNB is merely a minimally invasive method for patients; *ii*) it can establish pathological diagnosis before operation and make the timely preoperative therapy possible; *iii*) it eliminates the necessity of frozen section examination in the surgery. Our studies show that the results of pathological diagnosis from CNB are highly consistent with that from surgical specimen, and false positive case does not occur. False negative cases are mostly due to sampling error and the paucity of tissues although the limitation of CNB technique is also a main factor. CNB often has some difficulty in differentiating atypical ductal hyperplasia from low grade ductal carcinoma in situ (DCIS), DCIS from invasive carcinoma, and benign from malignant papillary lesions[8,9]. In fact, because it is very easy to make mistake in diagnosing atypical hyperplasia and papillary lesions as false-negative, immediate surgical resection biopsy is warranted in such situation to confirm if or not the presence of cancer.

Another advantage of CNB is that it can help us obtain breast carcinoma tissue before operation, so we might make full use of these precious materials to do further work. The aim of our research was to detect the oncogenic protein expression in CNB regular sections by the means of IHC. Some foreign publications[10,11] reported that the positive rate of oncogenic protein expression in CNB is in parallel with that in surgical specimen. The positive expression rate of oncogenic protein ER, PR, p53 and c-erbB-2 in CNB and surgical specimen in our research is in concordance with that reported by foreign literature. Furthermore, our research also compare the expression intensity of the oncogenic protein in CNB with that in surgical specimen, and the results demonstrate a fair consistency, thus providing some evidence for the accuracy of preoperative IHC assay performed on CNB tissues. IHC examination in CNB section will have more applications. For example, ER and PR detection will help to determine whether or not to chose endocrine therapy for the patients in late stages, and not suitable for surgery.

Neo-adjuvant chemotherapy for breast carcinoma[12,13] has become a prevalent therapeutic method in China

and worldwide. Chemotherapy before and after surgery has been applied in our country for 20 years, and the regimen usually includes preoperative medication for 3 weeks and postoperative medication for 4 weeks. For tumor sensitive to such treatment, the lump can be partially relieved (PR) or even clinically completely relieve (CR) after preoperative chemotherapy, whereas for tumor insensitive to chemotherapy, the lump does not diminish or become soft based on clinical observation. Morphological changes of carcinoma tissue in response to chemotherapy observed under light microscope after operation can be assigned to three different grades. The absence of morphological changes in some tissues after chemotherapy may reflect that some tumor tissues are either insensitive to the medicine or have already acquired medicine resistance. Some studies[14,15] have demonstrated that certain alteration of oncogenes, such as p53 mutation and expression abnormality, or c-erbB-2 over-expression, can induce tumor cells to develop medicine resistance, therefore, the sensitivity of tumor tissues to chemotherapy is correspondingly reduced. In this research, pre-operative IHC assessments (p53 and c-erbB-2) is conducted on CNB tissue samples from the patients who were diagnosed as breast carcinoma by CNB prior to operation, and the same IHC assays is also performed on regular specimens taken from the patients who underwent the operation. When the pathological results of the samples taken from the two ways were compared, a considerably high consistency was observed. The discussion above implies that preoperative IHC examinations for chemotherapy-related oncogene expression in CNB tissue samples can predict the individual's sensitivity to chemotherapy and provide suggestions for physicians to formulate personalized medication regimens. Besides, our research suggests that: *i*) the protein expression of other chemotherapy respond related genes such as p21, MDM2 and p170, may also be determined in CNB regular section for dynamic supervision of medicine resistance variation before and after chemotherapy. *ii*) The inference of p53, c-erbB-2, and other genes influencing the chemotherapy effect should be further explored.

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