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Collagen expression alterations and the clinical pathology significance in stroma following neoadjuvant chemotherapy of Taxotere in Breast Cancer

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Abstract: The quantitative alterations of collagen expression in the context of neoadjuvant chemotherapy and the relationship between it and chemotherapy effectiveness of clinic and pathology are investigated in this article. Seventy-one patients with breast cancer in general surgery department during January 2018 to February 2022, were enrolled and evaluated. Thirty-one of which received neoadjuvant chemotherapy, the others are not which are chosen as control. All the clinical and pathological information was collected. Pathological responses to neoadjuvant chemotherapy were performed on the basis of the Miller-Payne (MP) criteria, by which the efficiency and inefficiency of chemotherapy outcome was discriminated. The expression of collagen proteins was quantitatively evaluated by using morphometric analysis of Masson Trichrome Staining.The expression levels of collagen in breast cancer's extracellular matrix after neoadjuvant chemotherapy were up-regulated significantly, Compared with non-treated group.Collagen expressions in breast cancer 's extracellular matrix were affected by neoadjuvant chemotherapy. And it will provided more theoretical basis for improving the efficacy of Taxotere chemotherapy in breast cancer in the future.

Keywords: Breast cancer; Neoadjuvant chemotherapy; Collagen

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1. Introduction

Breast cancer is one of the most common malignancies in women worldwide, and it seriously endangers the health of female patients[1]. Chemotherapy (preoperative neoadjuvant chemotherapy and postoperative adjuvant chemotherapy) is one of the important strategies for the comprehensive treatment of breast cancer. Neoadjuvant chemotherapy can increase the chance of breast-conserving surgery by shrinking or down-staging the tumor, or making an inoperable tumor resectable[2]. Although the effectiveness rate of breast cancer chemotherapy can be as high as 50% to 84%, a considerable proportion of breast cancer patients still fail chemotherapy due to primary or secondary drug resistance, followed by tumor recurrence and metastasis, affecting the survival and quality of life of patients[3]. In clinical practice, we can often observe that patients with breast cancer of the same type and stage have differences in sensitivity and efficacy of the same regimen of neoadjuvant chemotherapy, but the relevant mechanism is unclear[4].

Studies have shown that the extracellular matrix (ECM) is an important component of the microenvironment[5-7], which not only provides skeletal support for cell adhesion and tissue development, but also plays an important role in regulating tumor cell growth, promoting tumor proliferation, infiltration, metastasis, and affecting treatment sensitivity[8-11].

The expression of extracellular matrix collagen content not only affects the function of tumor microenvironment, promotes tumor growth, infiltration, metastasis and vascularization, but also plays a non-negligible role in the penetration of chemotherapy drugs and the effect of chemotherapy efficacy[12-14]. Jain, Tannock, et al. noted in the study[15, 16]: The extracellular matrix can affect the survival of tumor cells by preventing drug penetration to reach the tumor site.

To this end, it is of great significance to explore the relationship between the tumor stroma collagen expression changes and chemotherapy efficacy and pathological changes after neoadjuvant chemotherapy. In this paper, the changes of tumor stroma collagen after docetaxel neoadjuvant chemotherapy in breast cancer patients are observed by Masson three-color method.

2. Materials and Methods

Study Population

1. Inclusion criteria for patients: all selected

cases were punctured by coarse needle and the histopathological diagnosis was as invasive breast cancer. There are definite chemotherapy indications for chemotherapy Patients in the neoadjuvant chemotherapy group. They agree to receive neoadjuvant chemotherapy and sign an informed consent form. Before neoadjuvant chemotherapy, all patients had not been treated with any chemotherapy drugs, such as local or systemic chemotherapy, local radiation therapy, endocrine therapy etc. The chemotherapy regimens were selected by docetaxel 75mg/m2, 21 days/cycle, 4 cycles. And after 2 cycles of chemotherapy, breast ultrasound and MRI were used to evaluate the efficacy of chemotherapy. 4 cycles of treatment are followed by surgical treatment, and 4 cycles of epirubicin + cyclophosphamide chemotherapy are continued after surgery. What's more, radiotherapy, endocrine therapy or targeted therapy are carried out according to NCCN guidelines after surgery.

2. Clinical characteristics of enrolled patients: breast cancer patients admitted to the Department of Breast Surgery of the First Affiliated Hospital of Shandong First Medical University between July 2018 ~ February 2022 in the neoadjuvant chemotherapy group (experimental group) and non-neoadjuvant chemotherapy group (control group) were selected.

Among them, 31 patients (experimental group) were selected for neoadjuvant chemotherapy, all of whom were women. Criteria for enrollment of patients: (1) patients with locally advanced breast cancer (mass >3cm or with lymph node metastasis at diagnosis), young patients with breast conservation willingness and some stage IV breast cancer patients; (2) Neoadjuvant chemotherapy was not preceded by local or systemic chemotherapy, local radiation therapy, endocrine therapy and other systemic treatments; (3) Chest X-ray, abdominal ultrasound, bone scan, etc. are excluded or confirmed without distant metastasis;

The non-neoadjuvant chemotherapy group (control group) was selected for 30 patients with invasive breast cancer at the same time, all of whom were female, and their age and sex were matched with the neoadjuvant chemotherapy group. All the patients in this group received surgery directly after being diagnosed without neoadjuvant chemotherapy and any systemic therapy. Histopathological information on both groups is shown in (Table 1).

Table 1. Clinicopathological features of neoadjuvant chemotherapy group and non-neoadjuvant chemotherapy group

	Neoadjuvant chemotherapy group (N=31) (%)	Non-neoadjuvant chemotherapy group (N=30) (%)
Gender		
Female	31	30
Age†		
≤50 years	16/31(51.6)	13/30(43.3)
> 50 years	15/31(48.4)	17/30(56.7)
Median age and interval	50(26-67)	53(38-82)
Type of histology		
IDC	26/31(83.9)	27/30(90)
ILC	2/31(6.4)	1/30(3.3)
Mixed and Others	3/31(9.7)	2/30(6.7)
Histological grading†		
Grade 1	2/31(6.5)	2/30(6.7)
Grade 2	16/31(51.6)	21/30(70)
Grade 3	13/31(41.9)	7/30(23.3)
pT†		
pT0	3/31(9.7)	0
pT1	9/31(29)	17/30(56.7)
pT2	14/31(45.2)	10/30(33.3)
pT3	5/31(16.1)	3/30(10)
pT4	0	0
Lymph node metastasis		
Positive	24/31(77.4)	10/30(33.3)
Negative	7/31(22.6)	20/30(66.6)
IHC receptor status‡		
ER	21/31(67.7)	28/30(93.3)
PR	19/31(61.3)	25/30(83.3)
HER-2	16/31(51.6)	7/30(23.3)
TNBC	3/31(9.7)	1/30(3.3)

Postoperative paraffin tissue samples from patients in the experimental group and control group were selected as the research objects, and the expression of collagen in the tumor stroma was evaluated by Masson staining.

Image processing and statistical processing of data

Images acquired by using ImagePro Plus 6. Image analysis software performs histomorphological quantification to reflect collagen expression with measured cumulative optical density values (IODs). The SPSS (statistics package for social science) for Windows 19.0 was applied to statistically analyze the data, and the statistical results were expressed as M (P25, P75). Independent sample rank sum test was used to compare the difference in collagen expression between the experimental group and the control group. And it was also used to compare the differences in collagen expression between the clinical and pathological treatment with effective group, ineffective group and the control group. The difference between the groups was compared. The statistical results are statistically different with $P<0.05$.

3. Results

1. Clinical and pathological remission assessment of neoadjuvant chemotherapy patients

According to the Miller and Payne (MP) pathologic response criteria, 5 specimens achieved Grade 5 (pathological complete response) and 7 patients achieved Grade 4 (tumor cytopenia> 90%), 10 cases reached Grade3 (tumor cell reduction of 30%-90%), 5 cases reached Grade2 (tumor cell reduction of about 30%), and 4 cases reached Grade1 (no significant reduction of tumor cells); That is, there were 22 cases in the treatment response group (MP3-5) and 9 cases in the treatment response group (MP1-2).

The criteria for determining the efficacy of neoadjuvant chemotherapy patients are as shown:

Pathologic evaluation is independently assessed by 2 pathologists (ZTT and YF).

Pathological treatment effective group (RESP): MP3-5 (Miller and Payne system grade 3-5);

Treatment Failure Group (NON-RESP): MP1-2 (Miller and Payne system grade 1-2)

2. Pathological changes in total collagen expression after neoadjuvant chemotherapy

A total of 31 cases were measured in the experimental group (neoadjuvant chemotherapy group), and the cumulative optical density IOD value was measured as 7.9×105 (3.2×105, 1.0×106), a total of 30 cases in the control group (non-neoadjuvant chemotherapy group), the cumulative optical density IOD value measured by experiment was 2.8×105 (1.5×105, 6.1×105), and the statistical analysis results showed that the collagen expression in the extracellular matrix was significantly increased (P=0.001) compared with the control group, which was statistically different. Typical microscopic observations of extracellular matrix content expression in the experimental and control groups are shown in (Fig.1).

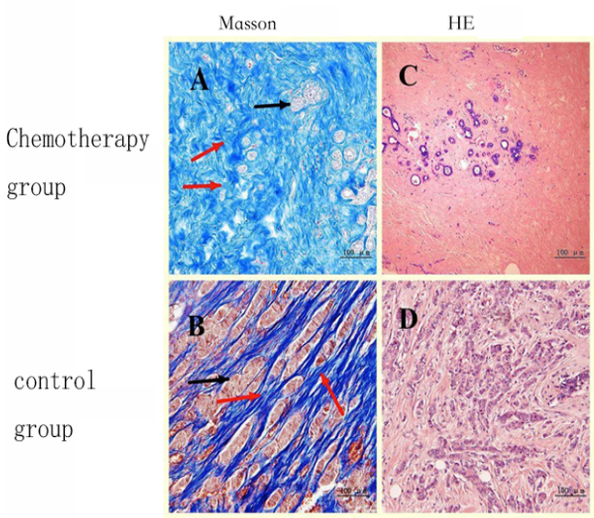
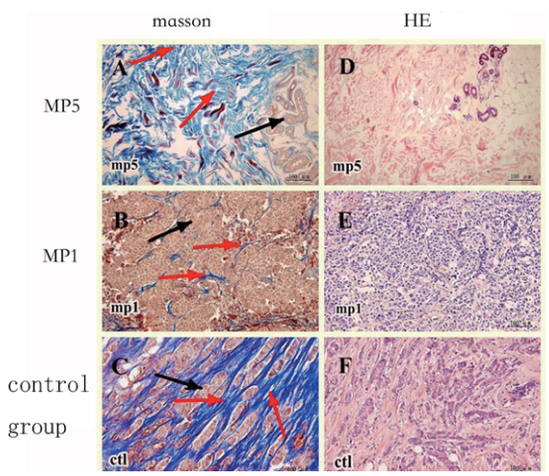


Fig. 1 Pathological changes in collagen expression after neoadjuvant chemotherapy (20×). Illustrations (A and B) are Masson collagen staining, (C and D) are H&E staining; The black arrow is the tumor cells, and the red arrow is stained collagen fibers; Compared with Figures (A and C) and (B and D), the expression of collagen in the extracellular matrix increased significantly after chemotherapy.

3. The change of collagen expression in extracellular matrix after neoadjuvant chemotherapy is related to the efficacy of chemotherapy

According to the Miller and Payne (MP) evaluation criteria to evaluate the efficacy of pathological changes, the experimental group (neoadjuvant chemotherapy group) was divided into chemotherapy active group (MP3-5), with a total of 22 cases, and chemotherapy ineffective group (MP1-2), a total of 9 cases. The cumulative optical density IOD value of the chemotherapy effective group was 8.2×105 (6.1×105, 1.2×106), and the cumulative optical density IOD value of the chemotherapy ineffective group was 3.4×105 (2.0×105, 8.6×105), and the statistical analysis results showed that the expression of collagen in the extracellular matrix after chemotherapy was increased between the chemotherapy effective group and the control group (non-neoadjuvant chemotherapy group)

(P value <0.001, there was a statistical difference, and there was no significant difference in collagen expression between the chemotherapy ineffective group and the control group (P=0.271). Compared with the chemotherapy ineffective group, the expression of extracellular matrix collagen in chemotherapy effective group was increased (P=0.049), which was statistically different. The results showed that after neoadjuvant chemotherapy with different degrees of pathological changes, there was a difference in the expression of stroma collagen in tumors. That means, the collagen expression between the chemotherapy effective group was higher than that between the chemotherapy ineffective group and the control group. Meanwhile there was no statistically significant difference in collagen expression between the chemotherapy ineffective group and the control group. Typical microscopic manifestations of collagen expression in the extracellular matrix of tumors with different pathological changes



(Fig. 2).

Fig. 2 Pathological manifestations of extracellular matrix collagen expression with different pathological changes after neoadjuvant chemotherapy (20×). (A, B, C) stained for Masson collagen, (D, E, F) stained for H&E; Figures A, D and B, E were pathologically complete response (MP5) and pathologically unresolved (MP1), respectively, and the black arrow was tumor cells; The red arrow shows the indigo-dyed collagen fibers.

4. The change of collagen expression in extracellular matrix is not related to the clinical remission efficacy of chemotherapy

According to the Response Evaluation Criteria for Solid Tumors (RECIST), the experimental group (neoadjuvant chemotherapy group) was divided into the chemotherapy effective group RESP (CR+PR) and the chemotherapy ineffective group NON-RESP (SD+PD), the non-neoadjuvant chemotherapy group was as control group. The RESP group had a total of 22 cases, the cumulative optical density IOD value measured experimentally is 8.0×105 (4.5×105, 1.0×106). There were 9 cases in the NON-RESP group, and the cumulative optical density IOD value measured in the experiment was 7.8×105 (1.7×105, 1.4×106); statistical analysis results showed that the expression of collagen in the extracellular matrix of RESP were increased in the chemotherapy-effective group (P<0.001), compared with control group. There was no significant difference in the expression of collagen of the NON-RESP group, compared with control group (P=0.064). (Fig.3). However, there was no significant difference in the expression of extracellular matrix collagen between the chemotherapy-effective group RESP and the chemotherapy-ineffective group NON-RESP (P=0.892). The results showed that after neoadjuvant chemotherapy, the expression of collagen in the tumor stroma increased in the chemotherapy-effective group. There was no significant difference in collagen expression between the chemotherapy-ineffective group and the control group. There was no significant difference in collagen expression between the chemotherapy-effective and ineffective groups in different clinical remission situations

4.Discussion

This study retrospectively analyzed the changes of collagen expression in breast cancer tumor extracellular matrix after neoadjuvant chemotherapy, and evaluated the correlation between neoadjuvant chemotherapy efficacy and collagen expression in tumor stroma according to selected criteria. The results showed that the expression of total collagen in the extracellular matrix of breast cancer tumors after neoadjuvant chemotherapy was significantly higher than that of non-neoadjuvant chemotherapy (p=0.001). And the results were similar to those after chemotherapy in other tissues[17-19]: Tokes et al found that After anthracycline neoadjuvant chemotherapy, the content of collagen in the tissue of the patients was significantly increased, and the collagen had been increased in the patients after clinical remission compared with the patients without remission[18]. The difference was statistically significant. The research of scholars such as Rintoul and Sethi pointed out that the components in tumor stroma is able to regulate the surrounding microenvironment[20], as to achieve the potential to produce a protective extracellular matrix that is beneficial to the tumor cells themselves. Because the extracellular matrix components have the ability

to be produced in tumor cells , we can speculate that tumor cells can prolong their survival by producing extracellular matrix that is beneficial to their growth and survival. It means that the tumor cells can selectively produce extracellular matrix components that can resist the penetration of tumor drugs, which is happened after tumor cells receive the effect of chemotherapeutic drugs, In the process of coordinating the microenvironment and resisting the killing of tumor drugs, and eventually leading to an increase in collagen content. At the same time, the secondary chemoresistance seems to be explained accordingly when the extracellular matrix is remodeled. In our previous study, we also found that the expression of collagen in triple-negative breast cancer cells was significantly increased after chemotherapy with paclitaxel, and the COL12A1 gene induced collagen synthesis was also increased, which also supports the above conclusion[21-24].

Secondly, this article showed that there was no significant difference in collagen expression between the clinically effective and ineffective groups. However, in pathological remission Compared with the effective and ineffective groups, the expression level of collagen in tumor stroma was significantly increased. Therefore, neoadjuvant chemotherapy affects the expression of stroma collagen in breast cancer, and its changes are more remarkable in the chemotherapy-effective group. Its mechanism of action can be explained by the theory of self-protective secretion of extracellular matrix by tumor cells. Besides, it is worth that with the increase of collagen expression, will that further affect the penetration of chemotherapeutic drugs and lead to secondary resistance? This trial is neoadjuvant chemotherapy, and the time node is 4 cycles. Imagining whether the emergence of different degrees of drug resistance caused by the prolongation of the adjuvant chemotherapy cycle is related to the increase in collagen content of tumor stroma caused by chemotherapy, and how to determine the corresponding time node to more effectively intervene drug resistance. It is worth further exploration.

Thirdly, pathological evaluation, as the gold standard for guiding diagnosis and treatment in clinical practice, has a high level of reliability and guiding significance. Because clinical evaluation relies on auxiliary examinations or artificial measurements, statistical errors are more likely to occur when the sample size is small. Therefore, in this experiment, pathological evaluation was used as the diagnostic standard. In addition, it is worth noting that in the process of sorting out clinical data and pathological section analysis of this study. it was found that a small number of cases had inconsistencies between clinical remission assessment and pathological remission assessment. It was speculated that the pathology was complete remission, but the clinical physical examination and imaging showed disease progression. At the meantime, in the case of complete elimination of tumor cells, due to the influence of stroma hyperplasia or post-chemotherapy response, errors in clinical remission assessment may be caused by physical examination and ultrasound measurement, which may mislead clinical diagnosis and treatment. Therefore, clinical evaluation of the efficacy of neoadjuvant chemotherapy may be unreliable if it relies merely on changes in tumor size.

This study mainly based on the quantitative content of collagen in the extracellular matrix after chemotherapy, and was insufficiency of further analyzing its related mechanism. Due to the limitation of the sample quantity, further confirmation by large-scale studies will be needed and Gene expression lines go deeper. Through our research on CAFs in tumor stroma, it will provided more theoretical basis for improving the efficacy of Taxotere chemotherapy in breast cancer in the future.

Conflict of Interest

The authors declare that they have no conflict of interest.

Informed consent

Informed consent was obtained from all individual participants included in the study

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