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Significance of β-tubulin Expression in Breast Premalignant Lesions and Carcinomas

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OBJECTIVE To explore the expression of β-tubulin in premalignant lesions and carcinomas of the breast, and to observe the relationship of its expression with breast cancer pathological features.

METHODS The expression of β-tubulin was detected immunohistochemically in 50 specimens of premalignant lesions of the breast (ADH and Peri-PM with ADH), 50 specimens of breast in situ ductal carcinomas (DCIS), and 50 specimens of invasive ductal carcinomas (IDC). Thirty specimens of normal breast tissues served as a control group.

RESULTS Immunohistochemical analysis showed that: the differences among the 4 groups (normal breast tissues, breast premalignant lesions, DCIS and IDC, $P < 0.05$) were significant, and there were also statistically significant differences between any 2 groups ($P < 0.05$) except for the β-tubulin positive expression comparing DCIS versus IDC ($P > 0.05$). In addition, β-tubulin was expressed at a higher level in Peri-PM with ADH compared to ADH ($P < 0.05$). Following the degree of breast epithelial hyperplasia involved, and its development into carcinoma, the β-tubulin positive expression displayed an elevating tendency. We also found a significant positive relationship of β-tubulin expression with lymph node metastasis ($P < 0.05$), but no significant correlation with histological grading and nuclear grade.

CONCLUSION Centrosome defects may be an early event in the development of breast cancer and they can also promote tumor progression. Studies of aberrations of centrosomal proteins provide a new way to explore the mechanism of breast tumorigenesis.

KEY WORDS: breast carcinoma, premalignant lesion, β-tubulin, centrosome.

Introduction

The centrosome is a small organelle composed of two cylindrically shaped, β-tubulin-containing centrioles that are surrounded by pericentriolar material. Many studies have found that centrosome defects are a common feature of malignancies such as breast[1] and esophageal tumors[2], bladder[3] and lung cancers[4] etc. The studies also suggest that centrosome defects might contribute to the earliest stages of cancer development through the generation of chromosome instability, and that these defects promote tumor progression[5]. But the involvement of centrosome-associated β-tubulin in epithelial hyperblastosis, breast tumor development and progression is unclear. Therefore the purpose of our study was to explore these relationships.

Materials and Methods

Tissue samples and groups

A total of 180 cases were randomly selected and the pathological sections from their paraffin blocks obtained from the Breast Cancer Pathological Department and Research Laboratory of Tianjin Medical University Cancer Institute and Hospital. The patients were treated during the period from January, 2003 to December, 2006. The pathologic diagnoses were confirmed by two pathologists in reference to the WHO Histopathology Classification[6]. Of these 180 samples, 50 patients were diagnosed with precancerous lesions including ADH and Peri-PM with ADH, 50 patients had DCIS with different nuclear grades, and 50 patients had IDC with different histological grading. In addition, thirty specimens of normal breast tissues were selected as a control group.

Reagents

Anti-β-tubulin multiclonal antibodies were obtained from the Santa Cruz Co. (USA), and a S-P kit was the product of the American ZYMED Co., purchased from the Zhongshan Biotechnology Co.

Staining methods

Immunohistochemical (IHC) staining was performed by the labeled streptavidin-biotin method (S-P). The dilution of the β-tubulin primary antibody was at 1:50, and antigens were restored by high-pressure and boiling water for 1.5 min before the blocking with blood serum. The intensity of the reaction was developed with 3.3'-diaminobenzidine (DAB), and hematoxylin was used to counter-stain the sections. Positive and negative controls were included with every group of tissues.

Standardization assessments of the results

For the results of the immunohistochemical (IHC) staining, positive signals of β-tubulin expression were located in the cytoplasm as indicated by a yellow-brown staining. The scoring method for β-tubulin was modified from that described by Tanaka et al.[7] The mean percentage of positive cells was determined in at least 5 areas at 400-fold magnification, and assigned one of the following five categories: 0, < 5%; 1, 5~25%; 2, 26~50%; 3, 51~75% and 4, > 75%. The intensity of β-tubulin immunostaining was scored as follows: 0, negative; 1+, weak; 2+, moderate and 3+, intense. The scores indicating percentage of positive cells and staining intensity were multiplied to produce a weighted score for each case. We set the following four categories: 0~2, negative (-); 3~4, low expression (+); 5~8, moderate expression (++); 9~, high expression (+++).

Statistical analysis

Analysis of the data was performed with a SPSS 11.5 software package. The methods of statistical analysis were mainly Radit analysis, rank-sum test and non-parameter Kendall Rank Correlation. The level of significance was set to be 0.05.

Results

Expression of β-tubulin in the 4 tissue types

The positive signals of β-tubulin were located in the cytoplasm of the epithelial cells and in tumor cells of the breast. The staining showed a brown-yellow color that could be seen in some cases of normal breast tissues, in most cases of premalignant lesions, and all of the cases of DCIS and IDC (Figs.1~4). The expression of β-tubulin is shown in Table 1.

Table 1. Expression of β-tubulin in the 4 tissue types.

Type	*n*	-	+	++	+++
Normal tissue[a]	30	5	9	12	4
Premalignant lesion[b]	50	2	11	26	11
DCIS[c]	50	0	5	25	20
IDC[d]	50	0	6	18	26
Total	180	7	31	81	61

There was statistically significant differences among the 4 tissue types ($\chi^2 = 25.381$, $P < 0.05$). Comparison of any two groups: *a:b,* $z = -1.992$, $P < 0.05$; *a:c,* $z = -3.807$, $P < 0.05$; *a:d,* $z = -4.161$, $P < 0.05$; *b:c,* $z = -2.469$, $P < 0.05$; *b:d,* $z = -3.120$, $P < 0.05$; *c:d,* $z = -0.909$, $P > 0.05$.

Comparison of β-tubulin expression in the 4 tissue types

There were statistically significant differences among the 4 tissue types ($\chi^2 = 25.381$, $P < 0.05$, Table1). Compared to the normal breast tissue, β-tubulin expression was higher in premalignant lesions ($z = -1.992$, $P < 0.05$), DCIS ($z = -3.807$, $P < 0.05$) and IDC ($z = -4.161$, $P < 0.05$), respectively. In addition, β-tubulin was expressed at a higher level in DCIS ($z = -2.469$, $P < 0.05$) and IDC ($z = -3.120$, $P < 0.05$) compared to the premalignant lesions of the breast. But a significant difference of β-tubulin expression was not observed between DCIS and IDC ($z = -0.909$, $P > 0.05$).

Correlation of β-tubulin expression with different pathological features of breast cancer

Expression of β-tubulin in DCIS with different nuclear grades

The expression of β-tubulin in DCIS with low and high nuclear grades is shown in Table 2. We did not find a statistically significant difference between the two groups ($z = -1.122$, $P > 0.05$), and the β-tubulin expression was not significantly correlated to the nuclear grade of DCIS ($P > 0.05$).

Expression of β-tubulin in IDC with different histological grades

As shown in Table 3, we can see the expression of β-tubulin in IDC with low and high histological grades.

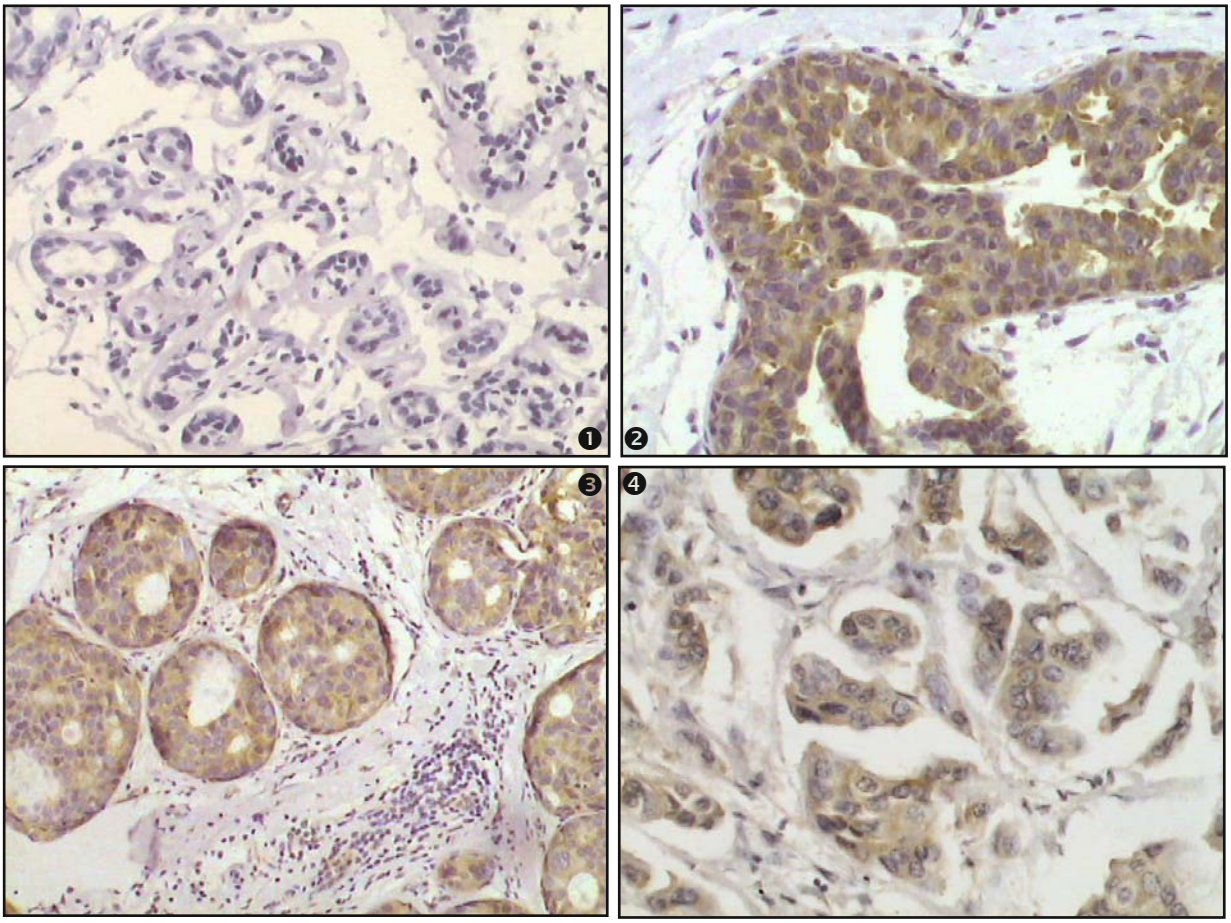

Fig.1. Negative expression of the β-tubulin protein in normal breast tissue(IHC S-P, ×40).
Fig.2. Positive expression of the β-tubulin protein in ADH (IHC S-P, ×200).
Fig.3. Positive expression of the β-tubulin protein in DCIS(IHC S-P, ×100).
Fig.4. Positive expression of the β-tubulin protein in IDC(IHC S-P, ×200).

The difference in β-tubulin expression between the two groups was not significant (z = -0.036, P > 0.05) and there was no relation of β-tubulin expression to the histological grade of IDC (P > 0.05).

Table 2. Expression of β-tubulin in DCIS with different nuclear grades.

Group	*n*	-	+	++	+++
Low grade	26	0	4	13	9
High grade	24	0	1	12	11
Total	50	0	5	25	20

There was not a statistically significant difference between the two groups (z = -1.122, P > 0.05), and the β-tubulin expression was not significantly correlated to the nuclear grade of DCIS (P > 0.05).

Expression of β-tubulin in IDC with and without metastasis of axillary lymph nodes

The β-tubulin expression in IDC with and without metastasis of axillary lymph nodes is shown in Table 4. There was a statistically significant difference between the 2 patient groups (z = -2.0290, P < 0.05). The results also showed that the β-tubulin expression was positively correlated to metastasis of axillary lymph nodes (r = 0.4345, P < 0.05).

Table 3. Expression of β-tubulin in IDC with different histological grades.

Group	*n*	-	+	++	+++
Low grade	35	0	4	13	18
High grade	15	0	2	5	8
Total	50	0	6	18	26

The difference between the two groups was not significant (z = -0.036, P > 0.05) , and there was not a relation of β-tubulin expression to histological grade of IDC (P > 0.05).

Table 4. Expression of β-tubulin in IDC with and without metastasis in axillary lymph nodes.

Group	*n*	-	+	++	+++
Metastasis	27	0	1	14	12
No metastasis	23	0	2	17	4
Total	50	0	3	31	16

A significant difference was observed between the two groups (z = -2.0290, P < 0.05), and the β-tubulin expression was positively correlated to metastasis of axillary lymph nodes (r = 0.4345, P < 0.05).

Expression of β-tubulin in premalignant lesions of the breast

Table 5 shows the expression of β-tubulin in premalignant lesions of the breast (ADH and Peri-PM with

ADH). Significant differences existed between the 2 tissue types (z = -2.4691, P < 0.05).

Table 5. Expression of β-tubulin in ADH and Peri-PM with ADH.

Group	*n*	-	+	++	+++
ADH	39	2	8	24	5
Peri-PM with ADH	11	0	2	2	7
Total	50	2	10	26	12

ADH, atypical ductal hyperplasia; Peri-PM with ADH, peripheral papilloma with atypical ductal hyperplasia. Significant differences existed between the 2 tissue types (z = -2.4691, P < 0.05).

Discussion

The centrosome is the major microtubule-organizing center in animal cells, and it has been shown to play a role in cell polarity, shape and motility. It contributes to mitotic spindle organization and regulates aspects of cytokinesis and cell-cycle progression. Spindle bipolarity is essential for the subsequent separation of the chromosomes into two daughter cells during mitosis. Consequently centrosome defects can lead to the development of multipolar mitotic spindles and a failure to separate chromosomes equally, which may result in aneuploid cells and chromosomal instability (CIN). CIN is thought to be important to promote phenotypic diversity by accelerating accumulation of alleles carrying prooncogenic mutations and loss of alleles containing wild-type tumor suppressor genes. This can lead to acceleration of genomic changes characteristic of carcinoma[8,9]. Therefore centrosome defects may be an early event of cancer development.

The centrosome contains two centrioles with β-tubulin being one of the protein components. As part of the cytoskeleton, tubulins are related to mitosis and stability of centrosomes. Structural and functional studies have suggested that abnormal expression and distribution of cytoskeleton proteins play an important role in regulating the morphologic and phenotypic events of a malignant cells[10,11], and are associated with cancer development, uncontrolled cell proliferation and metastatic progression etc[12].

Studies in recent years have shown that centrosome defects are a common feature of many human cancers such as those found in breast cancers[1]. Although the involvement of β-tubulin in breast cancers has been extensively investigated, its role in precursor lesions still remains controversial. It is important to perform a comprehensive analysis of centrosome defects in preinvasive lesions for several reasons. Some findings have demonstrated that centrosomal defects may occur concurrently with chromosomal instability in the early stages of breast cancer[13]. In addition, breast tumor centrosomes display structural abnormalities characterized by centrosome amplification which is closely related to tumor development[14]. So our study was aimed to investigate the expression of β-tubulin in premalignant lesions and breast carcinomas and to further explore their relationships.

Our results suggested that: compared to normal breast tissues, β-tubulin expression was higher in the other 3 groups (premalignant breast lesion, DCIS and IDC). Furthermore,β-tubulin was expressed at a higher level in DCIS and IDC compared to premalignant lesions of the breast (ADH and Peri-PM with ADH), which suggested that centrosome amplification was present in the early steps of breast tumor development. This alteration might contribute directly to chromosome missegregation and CIN, and through this process, further promote breast cancer development and progression.

Our study also demonstrated that the expression of β-tubulin was not significantly correlated to the nuclear grade of DCIS and histological grade of IDC supporting the hypothesis that centrosome amplification is an early event in breast cancer development. We also suggest that the expression of β-tubulin is positively correlated to metastasis of axillary lymph nodes based on two reasons: *i*) on one hand, the abnormal microtubules may increase the mobility and metastatic potential of tumor cells through cytoskeletal alterations that to some extent affect tissue architecture[15]; *ii*) on the other hand, the high expression of β-tubulin can increase the resistance of tumor cells to anti-tumor drugs[16]. On the basis of our previous work showing that some peripheral papillomas (Peri-PM), particularly those accompanied with ADH, were breast cancer precursor lesions[17]. In addition we showed that β-tubulin expression was higher in Peri-PM with ADH compared to those with only ADH.

In summary, by detecting the expression of β-tubulin in premalignant breast lesions and carcinomas, this study demonstrated that centrosome amplification was an early event in breast cancer development, and that centrosome defects are related to the development and progression of breast cancers. This experimental approach provides a valuable way to further explore molecular mechanisms of breast cancer formation.

Although our study answered the important question of whether centrosomal defects occur in the early steps of breast cancer formation, it also raises a number of unanswered interesting issues. As there are many ways in which centrosomal defects can arise, the fundamental mechanisms responsible for these defects in the earliest stage of breast cancer formation are unclear. Our study also may provide a starting point to define important genes involved in the pathobiology of breast cancer induction so as to find potential targets for breast cancer therapy that involve centrosomes.

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