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STUDIES ON THE HISTOLOGICAL CLASSIFICATION OF PROLIFERATIVE DISEASE OF BREAST AND ITS RELATION WITH BREAST CARCINOMA

Zhang Jizeng[1] 张继增 Liu Huafu[1] 刘华福 Liu Bin[1] 刘斌 Zhang Chenguang[2] 张晨光

1 Department of Pathology, General Hospital, Lanzhou Military Area, PLA Lanzhou, 730050
2 Department of Pathology, Institute of Endemic Disease, Gansu Province, 730030

Objective: Proliferative disease of breast (PDB) always exists compositely, it is difficult to be classified during pathologic diagnosis. For probe into the relationship between PDB and breast carcinoma in the cyto-biologic level, a new classification method was studied.

Materials and methods: Specimen slices of 439 PDB in females and 176 breast carcinomas were studied in contrast. The degree of fibroplastic proliferation was taken as the classifying index of the consecutive progress of PDB. Then the PDB cases were divided into three types: *the lobular hyperplastic type (LHT), the fibroadenosis* type (FAT) and the fibrosclerosis type (FST). Each type was once again separated into simple and compound groups. A part of these slices were stained with monoclonal immunohistochemical stain to demonstrate the hyperplastic activities of PDB cells as well as the *encirclement of periductal myoepithelial cells and base* membrane.

Results: PCNA express level was increased successively along with the progress of pathologic changes in all the three types of PDB. And especially, it was almost parallel with the cancerous express in FST. ASMA *staining showed atrophy and rupture of periductal* myoepithelial cells and base membrane in FST and serious untypical hyperplastic PDB cases.

Conclusions: In FST and serious untypical hyperplastic PDB cases, the ductal epithelial cells proliferated vigorously, it is closely related with the *growth of carcinomas. The imcomplete encirclement of* periductal myoepithelial cells and base membrane are also a sort of dangerous factor.

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Owing to the various nomenclatures of the complex mammary dysplastic lesions various have appeared in the literatures, Page, et al.,[1] (1990) suggested comprehensively the term as proliferative breast disease (PDB). In (1981) WHO,[2], classified PDB into three types:duct hyperplasia, Lobular hyperplasia and fibrosclerosis. In histopathologic pattern, the cystic, lobular and duct hyperplasia are not individualized, which mixed in different patterns in pathological diagnosis. The close relationship between PDB and breast carcinoma (BC) has been reported previously, but seldom reported from cytobiologic bases and intrinsic regularity of these lesions. We try to simplify the histopathological classification according to the degree of fibroplastic proliferation, incidence age of patients and immunohistochemical expression of proliferative cell neuclear antigen/Cyclin (PCNA), and its rational corelationship with BC.

MATERIAL AND METHODS

Histologic Classification

From 1977-1992, 439 cases of the PDB biopsy sections and 176 cases of specimen sections after radical mastectomies for BC were examined and put their ages for comparasion. According to the degree and progression of fibroplastic proliferation as classification index, it has the following types:

Lobular hyperplasia type (334 cases): No fibroplastic proliferation in lobular, the lobular expanded by increasing proliferated terminal ducts instead. This ductular epithelial cells were less than 4 layers with several dilated terminal ducts, with diameters <45 μm. When the terminal ducts increase in size, the lobular coalescence seems to be an appearance of tubular adenoma.

Fibroadenosis type (68 cases). On the basis of lobular hyperplasia, the proliferated fibrous tissue intersected into lobules, separated terminal ducts incompletely, depicted a picture of focal fibrosis but much like adenomatoid adenosis, which consist of proliferative fibrous tissue and epithelial cells. The hyperplastic changes of duct epithelium might assume papillary, solid or in cribrifirm. The glandular tubes irregularly dilated, tortuously deformed and accompanied with proliferation of fibrous tissue.

Fibro-sclerosis type (37 cases): On the basis of fibrodenosis, the terminal ducts compressed by extensively the proliferated fibrous tissue. The narrowed terminal duct deformed in narrow cordlike strips.

Of the above 3 types, each type may divided into 2 subgroup, i.e., simple (243 cases) and complex (196 cases). The later contained atypical hyperplasia of tubular epithelial cells (64 cases), cystic apocrine metaplasia (69 cases) and fibroadenoma formation (63 cases)

Immunohistochemical Investigation

Tissue of 100 cases of PDB and 35 cases of breast carcinoma were taken randomly, treated by routine formaldehyde fixing, paraffin embeded and immunostaining followed by ABC method processing.

PCNA: The first antibody was anti-mouse monoclonal antibody PC10 (Dako Co.) diluted at 1: 20; the secondary antibody was biotinated anti-mouse IgG diluted at 1: 200; the third was ABC complex (Vector Co.) diluted at 1: 100. Phosphate-buffered salt solution (PBS) and normal mouse serum were used as negative control to substitute the first antibody. Positive cellular nucli showed dark-brown staining. Degree I<25%; degree II>25%; degree III>75%. Radil method were used for analysis. Negative cellular nucli were not stained.

Alpha-Smooth muscle cell actin(ASMA). The test have been used to observe the surrounding ductular condition of myoepithelium and base membrane. The first antibody was mouse anti-human monoclonal antibody (Dako Co.) diluted at 1: 60; the secondary antibody was biotinated horse anti-mouse serum (Dako Co.) diluted at 1: 200; and the third antibody was ABC (Vector Co.) diluted at 1: 120. Control slices with PBS to substitute first antibody. Positive markers showed dark-brown stain and vascular wall displayed positive reaction, too.

RESULTS

The Statistic Study of Patient's Incidental Age and Light Microscopic Findings

Proliferative disease of breast

The mean age of PDB was 37.46 with its Peak 31-40. The mean incidence age of the lobular hyperplasia, fibroadenosis and fibrosclerosis types were 35.74, 38.75 and 40.92 years old respectively. Among the above 3 type of patients, 64 cases (14. 58%) exhibited atypical hyperplasia of epithelial cells depicted in intralobular, interlobular, extralobular ducts. Of the 64 cases, 22 cases (34.38%) expressed severe degree with single or multiple ducts transforming into circular expanded form and closely approached each other. These cells were large, heteromorphic with evidence of mitosis. Their regular polar arrangement and double layer structures (Figure 1) no longer existed. The periductal positive ASMA markers depicted a complete cuff-like distribution, though some were thin and showed linear breakages (Figure 2). Cysts (single or multiple) were covered with atrophic flat epithelium. Positive ASMA markers were not seen in highly expanded cysts. Some metaplastic apocrine glands (MAG) crowded together. Capsules were rarely seen in PDB associated with fibroadenoma. Various Complex lesions were present in all above mentioned types. Positive ASMA markers in fibrosclerosis type were compressed, distorted and ruptured (Figure 3).

Breast Carcinoma

Of 178 cases of breast carcinoma patient with mean age of 47.38 (peak age, 41-50), 6 cases (3.4%) have been diagnosed as mammary cystic hyperplasia 4 — 6 years ago, 96 cases of Infiltrative duct

carcinoma (54. 55%), 25 cases of predominant in infiltrative lobular carcinomas (14. 04%), 12 cases of lobular carcinomas *in situ* (6. 74%) and 10 cases of intraductal carcinomas (5. 62%), and other carcinomas were 35 cases (19. 66%) ASMA reactions were absent in cancerous tissues and its peripheral tissues of infiltrative carcinoma. Periductal positive ASMA markers showed disruptions at early stage. Carcinoma *in situ* showed macronucli and obvious nucleoles, mitosis and occasionally necrotic mucli cells. Their Periductal positive ASMA markers assume similar pictures of atypical hyperplasia of ductular changes in PDB. There were 51 cases (28.9%) of various types of hyperplastic PDB which were discovered around the cancerous tissue. Their mean ages were 42.18. The mean age of complex PDB and these of carcinoma patients associated with PDB were put into comparison. The ages of patients with PDB including the complex type of fibroadenosis ($P>0.05$), the simplex type ($0.057>P>0.01$) and complex type ($P>0.05$) of fibrosclexosis approximate to that of patients with pericarcinomatous tissues associated with proliferative lesions (Table 2).

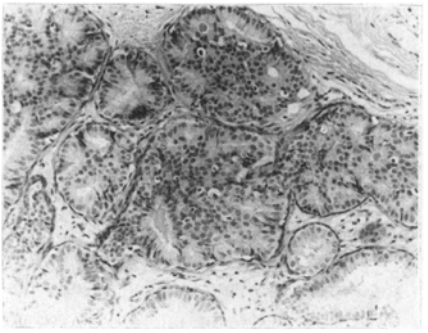

Fig 1. Proliferative disease of breast, lobular hyperplasia type. Hyperplasia of terminal ducts in lobular. A part of epithelium showsatypical hyperplasia, degree I-II. HE × 200

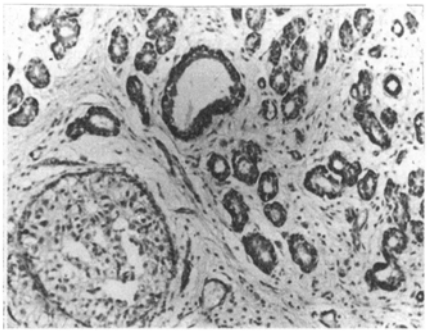

Fig 2. Lobular hyperplasia type, atypical hyperplasia of extra lobularducts, degree III. Positive ASMA markers are compressed. It appearsatroph and have disappeared partly. ABC × 100

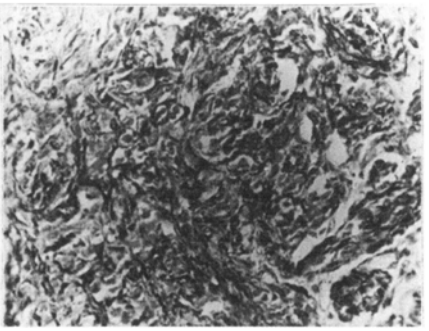

Fig 3. Fibrosclerosis type, positve ASMA markers appear to be tortuousities and break. ABC × 200

Immunohistochemical expression of PCNA

As shown in Table 2 and Figure 4, expression of PCNA have been further strengthened with the advancement of lesions. The Ridit analysis: BC 0. 53 — 0. 79; LHT 0. 25 — 0. 43; FAT 0. 36 — 0. 56; FST 0. 51 — 0. 73. showed the active expression of PCNA increasing in advancement of the above 3 kinds of lesions. The positive expression level went parallelly with that of BC.

Table 1. Histologic classification of 439 cases of PDB (%)

Lesions	Cases	LHT	TAT	FST
Simple	243	193 (79. 42)	30 (12. 45)	20 (8. 23)
Complex				
AH of DEC	64	47 (73. 44)	10 (15. 63)	7 (10. 94)
AMG and/or Cyst	69	41 (59. 42)	23 (33. 33)	5 (7. 25)
Fibroadenoma	63	53 (84. 13)	5 (7. 94)	5 (7. 25)

Table 2. Expression of PCNA in PBD and BC tissues

Lesions	cases	Positive degree (cases %)		
		I	II	III
BC	35	7 (20. 00)	6 (17. 14)	22 (62. 86)
PBD				
LHT	40	26 (65. 00)	9 (22. 50)	5 (12. 50)
FAT	32	14 (43. 75)	8 (25. 00)	10 (31. 25)
FST	28	5 (17. 86)	7 (25. 00)	16 (57. 14)

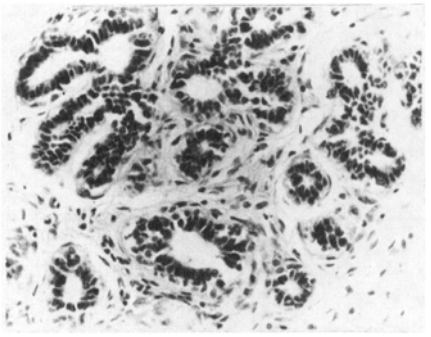

Fig 4. Lobular hyperplasia type, the PCNA staining shows strong positive reaction (+++). ABC × 400

DISCUSSION

PDB is a progressive hyperplastic disease of both ductal epithelium and interstitial fiberes. In this paper, the histologic classification is simplified on the basis of the degree of fibrous tissue proliferation, make it easier to grasp the rational classification during pathologic diagnosis. Since PDB of the cystic and ductular epithelium hyperplasia, though a complex event, but not a separate entity, it has no need to be nominate separately. When pathologically diagnosis. Among PDB, the cystic or ductular epithelium hyperplasia is a complex event, not a separate entity, it not need to nominate separately. During pathologic diagnosis the type of PDB must to be put first, follows by subtypes, simple or complex. In complex lesions, atypical hyperplasia should recorded first. The mean age and the peak period of BC patients were about 10 years older than PDB patients. There were 28.97% PDB lesions around the cancerous tissue and their ages approach to that of fibrosclerosis type of patients, It implies that PDB complex is closely related to breast carcinoma. Severe atypical hyperplasia offers their morphologic manifestations similiar to those of BC, though the former is depicted no cellular necrosis.

In the meeting which discussed mainly fibrocystic disease of the breast held in New York[5] (1985), several authors pointed out that the risk of developing carcinoma in non-atypical hyperplasia were 1. 5 — 2 times, but those with atypical hyperplasia lesions were 5 times more. Therefore, besides the age factor, the atypicasl hyperplasia of ductal epithelium might regarded as a dangerous phenomena.

PCNA positive nuclei appears in "S" stage of cell cycle. It symbolize their proliferative activity, which has been used in various cancer research work.[6,7] PCNA immunohistochemical staining have not yet been used in comparison between PDB and BC up to day. Our experimental results demonstrated progressive increase of PCNA activity in the 3 types of PDB, from LHT to FST, illustrated their relationship between the degree of proloferation and active expression of PCNA in PDB. The expression levels of PCNA activity in the FST of PDB are parallel with the BC. The periductular myoepitheliums are tortuous and their base membrances are breaked, when these changes occur, the patients should be followed up closely.

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