HISTOLOGICAL GRADING IN DUCTAL CARCINOMA *IN SITU* OF THE BREAST

XU Wei-ling 徐薇苓, ZHU Xiong-zeng 朱雄增, ZHANG Ting-qiu 张廷璆,
TU Xiao-yu 涂小予, SHI da-ren 施达仁

Department of Pathology, Cancer Hospital, Shanghai Medical University, Shanghai 200032

ABSTRACT

Objective: To study the significance of histological grading as a prognostic factor in ductal carcinoma *in situ* of the breast. Methods: According to the Van Nuy's classification, 32 cases of ductal carcinoma *in situ* (DCIS) of the breast were divided into three groups. Results: Low grade (well differentiated, low grade DCIS) 12 patients (37.5%); Intermediate grade, 9 patients (28.1%); High grade (poorly differentiated DCIS) 11 patients (34.4%). Among the high grade DCIS, the histologic subtypes were comedo (9 patients), micropapillary (1 patient) and solid (1 patient). The positive expression of c-*erb*B-2, p53 and MIB-1 in high grade DCIS was higher than that in intermediate and low grade DCIS. The difference between high grade and low grade DCIS was significant ($P<0.05$). The expression of ER in high grade DCIS was lower than that in intermediate and low grade DCIS. Conclusions: Histological grading of breast ductal carcinoma *in situ* may be a good prognostic factor.

Key words: Breast ductal carcinoma *in situ*, Histology c-*erb*B-2 p53, MIB-1, Estrogen receptor

With the early discovery and diagnoses of breast carcinoma, the diagnosis of ductal carcinomas *in situ* (DCIS) is much more frequent. It recently has been emphasized that DCIS doesn't represent a single entity. The purpose of the present study was to investigate the relationship among histologic grading, subtype and the expression of c-*erb*B-2, p53, MIB-1 and Estrogen Receptor (ER) so as to provide reliable parameters of prognosis and potential malignancy for the treatment of these patients.

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Correspondence to: ZHU Xiong-zeng, Department of Pathology Cancer Hospital, 399 Ling-Ling Road, Shanghai 200032, China; Fax: 021-62686672, E-mail: wlingxu@yahoo.com

MATERIALS AND METHODS

Thirty-two cases with pure DCIS were surgically obtained in Cancer Hospital, Shanghai Medical University, from 1987 to 1998. All the patients were female and their average age were 46 years (range 28–67 years). The patients were performed radical mastectomy or modified mastectomy except 2 cases (1 case performed quadratec-tomy and 1 case performed lumpectomy). Among all, 27 cases had the follow-up information (84.4%). The average length of follow-up were 4.2 years. All patients were all survival and no recurrence. The samples were fixed in 10% formalin, embedded in paraffin, cut and stained with hematoxylin and eosin (HE). The slides were reviewed by two pathologists according to the World Health Organization (WHO) classification. The histologic subtype was classified according to the predominant architectural pattern. Among all, there were 15 cases of cribriform type, 10 of comedo type, 4 of micropapillary type and 3 of solid type.

According to the Van Nuy's[1] histological grading classification, 32 cases of ductal carcinoma *in situ* of the breast were divided into three groups. I (low grade, well differentiated): nuclear grade 1 or 2, without comedo type necrosis. II (intermediate grade): nuclear grade 1 or 2, with comedo type necrosis. III (high grade, poorly differentiated): nuclear grade 3. Nuclear grade 1 were defined as nuclei 1–1.5 red blood cells in diameter with diffuse chromatin and inapparent nucleoli. Nuclear grade 2 were defined as nuclei 1–2 red blood cells in diameter with coarse chromatin and infrequent nucleoli. Nuclear grade 3 were defined as nuclei with a diameter greater

than two red blood cells, with vesicular chromatin, and one or more nucleoli.

Citric-Acid-Microwave-ABC immunohistochemical methods were performed using the antibodies c-*erb*B-2 (DAKO, 1:100), p53 (DAKO, 1:50), MIB-1 (Immunotech Biotech, 1:50) and ER (DAKO, working solution). c-*erb*B-2 protein expression was indicated by cell membrane and/or cytoplasm immunoreactivity. p53, MIB-1 and ER protein expressions were indicated by nuclear staining. Tumors with more than 5% of tumor cell showing ER staining were scored as ER positive. More than 10% of tumor cell expressing c-*erb*B-2, p53 or MIB-1 were interpreted as positive of c-*erb*B-2, p53 or MIB-1.

Statistical analysis was performed using "exact test".

RESULTS

Histopathology

Among 32 cases of DCIS, the most common subtype was cribriform (46.9%, 15/32), followed by comedo (31.3%, 10/32), micropapillary (12.5%, 4/32) and solid (9.4%, 3/32). Nuclear grade 3 were seen in 11 patients including 9 with comedo pattern, 1 with micropapillary pattern and 1 with solid pattern. Necrosis was present in 24 of 32, including all with the comedo subtype, 3 micropapillaries, 6 cribriform and 1 solid. The three groups of histological grading were as follows: Grade I (Figure 1): 12 patients (37.5%), including 9 cribriform, 2 solids, 1 micropapillary; Grade II (Figure 2): 9 patients (28.1%), including 6 cribriform, 2 micropapillary, 1 comedo; Grade III (Figure 3): 11 patients (34.4%), including 9 comedos, 1 micropapillary and 1 solid.

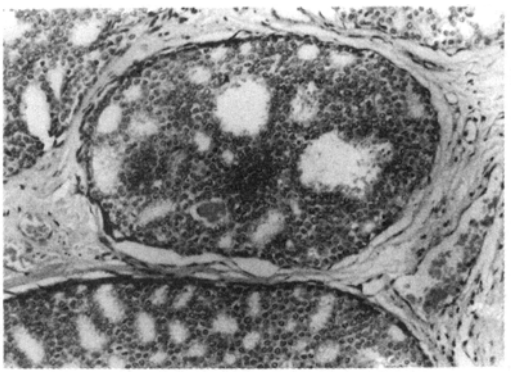

Fig. 1. DCIS Grade I HE×100

Relationship between Histological Grading, Histological Subtype and the Expression of c-*erb*B-2, p53, MIB-1 and ER

The expression of c-*erb*B-2, p53 and MIB-1 were significantly higher in comedo subtype than in non-comedo subtype ($P<0.05$), and the positive rate of ER was higher in non-comedo subtype than in comedo subtype ($P>0.05$) (Table 1). The expression of c-*erb*B-2, p53 and MIB-1 in high grade DCIS was higher than that in intermediate and low grade DCIS. The difference between high grade and low grade DCIS was significant ($P<0.05$). The expression of ER in high grade DCIS was lower than that in intermediate and low grade DCIS ($P>0.05$) (Table 2).

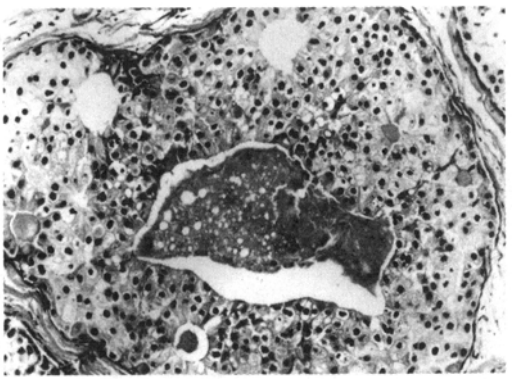

Fig. 2. DCIS Grade II HE×100

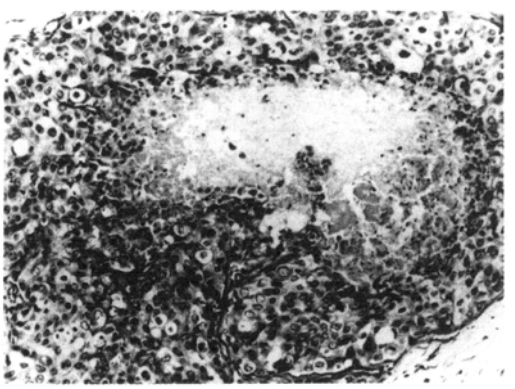

Fig. 3. DCIS Grade III HE×100

DISCUSSION

Although majority of the patients with DCIS had better prognosis, DCIS do not represent a single entity. The clinical finding, histological feature, biological character, recurrence and prognosis of these patients are heterogeneity. Till now, the best therapeutic option was unknown.[2,3]

The classification in comedo and non-comedo types is operative. In our study, the expression of c-*erb*B-2, p53 and MIB-1 were significantly higher in comedo subtype than in non-comedo subtype ($P<0.05$), and the positive rate of ER was higher in non-comedo subtype than in

comedo subtype ($P>0.05$), as reported by other authors.[2,4,5] However, some non-comedo cases had high grade malignancy and poorly prognosis, which should be recognized with other criteria.[1,2] In our group, 11 patients were divided into III (high grade, poorly differentiated), including 9 comede, 1 micropapillary and 1 solid. Micropapillary and solid subtype were belong to non-comedo and usually predict better prognosis, but these two cases were divided into III because of the presence of high nuclear grade (nuclear grade 3). 1 case of comedo pattern was referred to II because of the present of nuclear grade 1 or 2. All the cribriform subtype were divided into I or II and predicted better prognosis. There is discordance among pathologists in subtyping DCIS, but there is good agreement on the nuclear grade and the presence or absence of comedo-type necrosis for their easy to grasp. Cells with nuclear grade 3 must be large and pleomorphic, lack polarity, have prominent nucleoli and coarse chromatin, and generally show mitoses.

Table 1 Relationship between histological subtype and the expression of c-erbB-2, p53, MIB-1 and ER

Histological subtype	n	Positive rate (%)			
		c-erbB-2	p53	MIB-1	ER
Comedo	10	8 (80.0)	7 (70.0)	7 (70.0)	5 (50.0)
Non-comedo	22	5 (22.7)	6 (27.3)	3 (13.6)	14 (63.6)
Micropapillary	4	1	1	1	1
Cribriform	15	3	3	1	10
Solid	3	1	2	1	3
Total	32	13 (40.6)	13 (40.6)	10 (31.3)	19 (59.4)

Table 2 Relationship between histological grading and the expression of c-erbB-2, p53, MIB-1 and ER

Histological grading	n	Positive rate (%)			
		c-*erb*B-2	p53	MIB-1	ER
I	12	2 (16.7)	2 (16.7)	2 (16.7)	8 (66.7)
II	9	2 (22.2)	3 (33.3)	1 (11.1)	6 (66.7)
III	11	9 (81.8)	8 (72.7)	7 (63.6)	5 (45.5)
Total	32	13 (40.6)	13 (40.6)	10 (31.3)	19 (59.4)

Silverstein et al.[1] reported the Van Nuys classification to predict prognosis for DCIS patients. In their report, there are 31 local recurrences in 238 patients after breast-conservation surgery, 3.8% in low grade DCIS, 11.1% in intermediate grade and 26.5% in high grade. The 8-year actuarial disease-free survivals were 93%, 84% and 61%, respectively (all $P\leq0.05$). Van Nuys classification would be as a reliable and easily recognized prognostic factor. In invasive breast carcinoma, immunoreactivity of c-*erb*B-2 or p53 was the marker of adverse prognosis, while MIB-1 staining reflected the proliferating rate of the cell and ER positivity predicted the better prognosis.[6-10] In our group, the expression of c-*erb*B-2, p53 and MIB-1 in high grade DCIS was higher than that in intermediate and low grade DCIS. The difference between high grade and low grade DCIS was significant ($P<0.05$). The expression of ER in high grade DCIS was lower than that in intermediate and low grade DCIS ($P>0.05$).

The diagnosis of DCIS must be distinguished from intraductal epithelial proliferation (intraductal hyperplasia) and atypital proliferation (intraductal hyperplasia with atypism). Bridges of proliferating epithelium are more often in epithelial proliferation, while solid growth pattern is more common in carcinoma. In intraductal hyperplasia with or without atypism, the ductal lumens formed by cellular proliferation tend to be variable in size, shape and has a scalloped or uneven contour, rather than having uniform, round or oval, ectatic and smooth surface lumens seen in cribriform subtype of DCIS. Fibrovascular cores are present in intraductal hyperplasia with a micropapillary pattern but lack in micropapillary subtype of DCIS. Both DCIS and intraductal hyperplasia with atypism can be made dealing with the increased ratio of nuclear to cytoplasm, enlargement of nuclei, hyperchromasia, conspicuous nucleoli and increased mitotic activity, but they are more markedly in the former than in the latter. Adequate

samples must be obtained to distinguish DCIS from the early invasive of breast carcinoma. Early invasive of breast carcinoma is the period when DCIS carcinoma cells are found protruded though the basement membrane and detached in the periductal stroma. The area of invasion is small and local. Budding of carcinomas from the rupture into the stroma. The reticulin stain and immunohistochemical staining of basement membrane and myoepithelial cells are help to distinguish between them.

Although mastectomy for DCIS provides cure rates approaching 100%, this undoubtedly represents over treatment in a substantial number of patients. In our group, the patients were performed radical mastectomy or modified mastectomy except 2 cases (1 case performed quadratectomy and 1 case performed lumpectomy). Among them, 27 cases had the complete follow-up information (84.4%). At present, breast conservation surgery is appropriate for the treatment of DCIS. It was suggested that patients with non-high grade DCIS can be effectively treated with breast preservation, but mastectomy must be performed for high grade DCIS.[1]

Van Nuys histological grading of DCIS may be reliable diameters to predict potential malignancy and prognosis. Since many of our patients were performed radical mastectomy or modified mastectomy and had better prognosis, the significance of histological grading would be further discussed.

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