Differential Pattern of Perivascular Type IV Collagen Deposits in Phyllodes Tumors of the Breast

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Deposition of basement membrane extracellular matrix is influenced by adjacent tumor cells, and in some cases, the pattern of type IV collagen deposit is characteristic in malignant tumors. In this report, we analyzed the difference in type IV collagen deposition patterns between benign and malignant phyllodes tumors (PTs) of the breast. Of the 15 cases of PTs, 8 cases were benign PTs and 7 cases were malignant PTs. Three cases of other primary sarcomas of the breast (stromal sarcoma, angiosarcoma and osteosarcoma) and 2 cases of fibroadenomas were studied for comparison. The malignant PTs were distinguished from benign ones by increased mitotic figures, cellular atypism, and a higher proliferation index of stromal cells. Immunohistochemical staining against type IV collagen in malignant PTs revealed extensive to moderate deposition of type IV collagen around the small blood vessels in duplicate or multilayering pattern, while benign PTs showed minimal deposition in a single linear pattern. All of the three cases of other sarcomas revealed multilayering or meshwork pattern of type IV collagen around the blood vessels. The deposition of type IV collagen around the blood vessels may reflect the malignant behavior of the stromal tumors of the breast.

Key Words: Phyllodes tumor, Type IV collagen, Immunohistochemistry

INTRODUCTION

Tissue architecture is established by the interaction between cellular components and extracellular matrix. Among the extracellular matrix, type IV collagen is involved in the morphogenesis of epithelium as well as vascular endothelium. Type IV collagen is one of major components of basement membrane (BM), and it represents the structural framework of BM. In malignant neoplasm, tumor invasion can be defined as the extension of neoplastic cells beyond the natural borders of the tissue compartment from which they arise. It was once believed that, during the invasion, defects in the pre-existing BM occur at the border of the tumor and parenchyma by passive expansion. But it is now

confirmed that many of the malignant cells especially invasive cells excrete type IV collagenase to degrade BM (Zucker et al., 1990). Not only type IV collagenase but several other proteases appear to form a cascade of enzymes that breaks down the extracellular matrix. In contrast, some tumors show extensive collagen deposition to induce a desmoplastic reaction and the deposition of such BM material is one of the prognostic factors of malignant tumors as shown in squamous cell carcinoma of the lung (ten Valde, 1991). In this study, we analyzed the distribution pattern of type IV collagen around the small blood vessels in phyllodes tumor of the breast and found the distinguishing features between malignant and benign tumors.

MATERIALS AND METHODS

A total of 15 PTs were collected from the files of the Department of Pathology, Seoul National University Hospital. Eight were benign PTs and the remaining 7 cases were malignant PTs. Two cases of fibroadenoma and 3 cases of primary sarcomas including stromal

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sarcoma, osteosarcoma and angiosarcoma were studied for comparison.

Monoclonal antibody (DAKO, monoclonal antibody clone CIV 22, immunized by pepsin fragments of human kidney type IV collagen) recognizing the type IV collagen was applied after deparaffinization and trypsinization procedure. Subsequent procedures included avidin-biotin-peroxidase staining with a Vector Elite kit and colorization by diaminobenzidine.

RESULTS

The profiles of benign and malignant PTs included in this study are summarized in Table 1.

The vascular basement membrane was stained linearly by type IV collagen monoclonal antibody after trypsinization. One case was excluded from the analysis because that case was not stained even after trypsinization.

In benign PTs, most of the cases showed single linear pattern with or without extension (Fig.1, 2). In malignant PTs, however, the BM around capillaries were double layered or multilayered in concentric pattern (Fig. 3). Overlapping of those two patterns between benign and malignant PTs existed in 4 cases. One case of angiosarcoma showed meshwork-like BM (Fig. 4). Osteosarcoma and stromal sarcoma of the breast showed multilayering of BM. Table 2 summarizes the type IV collagen deposition pattern.

DISCUSSION

In this study, perivascular deposition of type IV collagen was more intense in malignant PTs compared to the benign counterpart. The BM material in this study cannot be interpreted as a host reaction as seen in the desmoplastic reaction of other carcinomas, because there is no recognizable fibrosis around the blood vessels in our study. Both of the cases with multilayering of BM materials showed infiltrative margins, and all of the cases with pushing margins revealed a single layer of type IV collagen. Two cases of sarcoma which had infiltrative margins and also showed the multilayering of type IV collagen support the view that type IV collagen deposition seems to be correlated with the invasive character of the tumor cells.

PTs may exhibit foci of other stromal component (WHO, 1981). In our materials, three cases of sarcoma were excluded because they showed monotonous proliferation of osteoblast, endothelial cells, or stromal cells, respectively, without any component of epithelial glands.

In our materials, clinical outcome is not well correlated with the histologic parameters such as mitosis, proliferation index, epidermal growth factor receptor and type IV collagenase pattern. It is well recognized that the histologic and cytologic features of malignancy do not give a reliable prognosis, because at least 75% of malignant PTs did not metastasize (Pietruszka and

Table 1. Summary of clinical and pathological data.

	Benign phyllodes tumor		Malignant phyllodes tumor	
No. of cases	8		7	
Age*	37.4	(24-25)	37.0	(23-57)
Tumor Size* (cm)	5.1	(1.5-10.0)	6.3	(3.5-11.0)
No. of mitosis* (per 10 HPF)	3.9	(1-7)	33.1	(10-72)
PCNA*	1.2%	(0-6)	20.2%	(4-60)
Infiltrative margin	0.0%	(0/8)	57.1%	(4/7)
Necrosis	0.0%	(0/8)	28.6%	(2/7)
Recurrence	12.5%	(1/8)	42.9%	(3/7)

*: expressed as mean (range)

Table 2. Pattern of type IV collagen in phyllodes tumor and other primary lesions of the breast.

	Single linear	Single with extension	Double	Multiple	Not available
Benign phyllodes tumor	5	2	0	0	1
Malignant phyllodes tumor	0	2	4	1	0
Sarcomas	1*	0	0	2	0
(Fibroadenoma)	1	1	0	0	0

*: One case of angiosarcoma showed a network pattern.

Barnes, 1978) and apparently benign PTs may develop recurrence and even metastasis (Zahner and Bässler, 1989). Moreover, although benign PTs rarely exhibit metastasis, recurrence rate is higher than that of malignant tumor (18% vs. 8%) (Hajda et al., 1976). Because none of our cases were accompanied by metastasis during the follow-up period, we cannot compare the immunohistologic parameters with the clinical outcome. Also disappointing is the result of flow cytometry (Layfield et al., 1989); DNA ploidy did not correlate with any factors including recurrence, metastasis or histologic criteria of malignancy.

Studies on the immunohistochemical staining results of PTs are limited. Auger et al. (1989) summarized that stromal cells of PTs were consistently positive for vimentin and negative for desmin, actin, keratin and S-100 protein regardless of benign or malignant histology. They excluded the cases which were positively stained by desmin, actin or keratin from PTs. Those staining characteristics are similar to our cases (Kim et al.,

unpublished data).

The structural changes of BM in breast lesions have been extensively studied (Albrechtsen et al., 1981; Gusterson et al., 1982), and variable loss of BM has been known as a prominent finding around the invasive tongue of carcinoma. Our results are not inconsistent with the previously believed thought that loss of basement membrane around the ductal structure by collagenase-producing malignant cells is a hallmark of invasive carcinomas, while benign tumors do not alter the BM material (Barsky et al., 1983). Before the advent of immunohistochemistry, periodic acid Schiff (PAS) staining had been used to outline the basement membrane. However, PAS stains nonspecific glycoproteins which are present in connective tissue. The type IV collagen is present exclusively in basement membrane and most of the desmoplastic tissue around the tumor lacks type IV collagen. Ultrastructurally, BM is resolved further into basal lamina and reticular fibrils. PAS staining is positive in all layers of

Fig. 1. Immunohistochemical staining against type IV collagen of the benign phyllodes tumor. The basement membrane shows linear staining pattern.

Fig. 3. The malignant phyllodes tumor shows multilayering of the type IV collagen around the blood vessels.

Fig. 2. Staining same as Fig. 1. The basement membrane pattern is interpreted as linear staining with extension.

Fig. 4. Type IV collagen staining of the angiosarcoma. The basement membrane shows a meshwork pattern.

BM, in contrast to type IV collagen, which stains only in lamina densa (Barsky et al., 1983).

The desmoplasia has been suspected as a host reaction to the invasive tumor growth (Liotta et al., 1980). According to their hypothesis, a competent reaction would result in an extensive deposition of collagen and BM material. It was also suggested that type IV collagenase was involved in the breast remodeling, and subsequently type IV collagenase immunoreactivity is confirmed in myoepithelial cells as well as cytoplasm of carcinoma cells in the breast (Monteagudo et al., 1990).

It is very interesting that perivascular deposition of type IV collagen deposit is augmented in malignant PTs, in contrast to most malignant tumors which show thinning or disruption of basement membrane around the ductal structure. Because proliferating cells of PTs are stromal cells, they do not need to disrupt the basement membrane. The duplication or multilayering of type IV collagen around the blood vessel might be associated with proliferation of endothelial cells. Certain types of malignant tumors are associated with endothelial cell proliferation; One of the examples is the glioblastoma multiforme of the brain, which shows a meshwork pattern of blood vessels in the tumors. According to Nagashima et al. (1987), the proliferative activity of the endothelial cells assessed by bromodeoxyuridine is markedly increased in the glioblastoma multiforme. Substantial evidence reveals that the factors which promote angiogenesis of the tumor also increase the proliferation of the tumor cells. One example is the well known peptide, SIKVAV, the partial sequence of laminin A chain. The peptide is known to enhance angiogenesis, increase plasminogen activator, and stimulate tumor cell proliferation (Kibbey et al., 1992). It is probable that some factors involved in the proliferation of endothelial cells are also responsible for the proliferation of stromal cells in malignant PTs.

Although we know nothing about the basic difference between the evolution of benign and malignant PTs, vascular proliferation might be involved whether it is the primary event or a secondary result.

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