Ultrastructure of the Periductal Area of Comedo Carcinoma in situ of the Breast

Ping Tang, Saul Teichberg, Beth Roberts, and Steven I. Hajdu
Department of Pathology, North Shore University Hospital-New York University Medical School, Manhasset, New York

Abstract. We have previously shown that the different biological natures of comedo ductal carcinoma in situ (DCIS) and non-comedo DCIS may, in part, be explained by the different expression patterns of tenasin, a large extracellular matrix protein, as observed by immunohistochemical studies. In the present study, we compared 8 cases of comedo DCIS with 5 cases of non-comedo DCIS by ultrastructural analysis, focusing on the myoepithelium, basal lamina, and tenascin-positive extracellular periductal stromal matrix. Our observations show that the comedo type DCIS frequently has an altered basal lamina, a looser and more disorganized collagenous matrix, and a general increase in stromal cellularity, including fibroblasts, lymphocytes, histiocytes and small blood vessels. In addition, in comedo DCIS, the lateral intercellular spaces between large myoepithelial cells that border the basal lamina are often expanded, compared to those of non-comedo DCIS. These results identify structural characteristics of comedo DCIS that may play a role in its greater preinvasive potential. They may also provide a structural basis for the different strategies that are needed for clinical management of comedo DCIS, compared to non-comedo DCIS. (received 13 April 2001; accepted 23 April 2001)

Key words: breast carcinoma, ductal carcinoma in situ, comedo carcinoma

Introduction

Ductal carcinoma in situ (DCIS) of the breast has long been observed with occasional axillary lymph node metastasis, in the absence of an identifiable invasive component [1]. There are risks of both recurrent DCIS and progression to invasive carcinoma [2,3]. Although the natural history of DCIS is still largely unknown, it is generally agreed that DCIS is not a single disease, but a heterogeneous group of diseases, each with its inherent invasive biological potential, varying from low- to high-grade, depending mainly on the nuclear grade of the tumor cells and the presence or absence of intraductal comedo type necrosis [4-7].

In a effort to relate the pathologic findings with the clinical course, a 3-grade system was developed in which grade 3 is intraductal comedo carcinoma, grade 1 is classical, non-comedo carcinoma, and grade 2 is carcinoma with intermediate cytological features [8,9]. This classification has been validated by DNA analysis as well as oncogene and proliferation marker studies [10,11], and it has been shown to be a good predictor of the clinical behavior of DCIS [12-14]. Hence it is recognized that there is substantial histopathologic and cytopathologic variation among mammary intraductal carcinomas that may influence their clinical behavior and prognosis.

The aggressive behavior of comedo DCIS in contrast to the that of low-grade non-comedo DCIS is not well understood. We have reported that tenasin, a large extracellular matrix glycoprotein, found in all carcinomas studied so far [15], shows markedly different immunohistochemical patterns of expression in the various types of DCIS [16]. Furthermore, we have shown that there is a strong association between the tenasin expression pattern and the nuclear grade of the DCIS: that is, multiple thick tenasin bands in
the stroma that surrounds comedo DCIS, versus a single thin band around non-comedo DCIS. To extend these observations on the stromal compartment in DCIS, using ultrastructural analysis we evaluated the boundary between intraductal tumor cell nests and their surrounding stroma in comedo and non-comedo DCIS. Our attention was focused specifically on the basal lamina, the myoepithelium, extracellular collagen deposition, other matrix fibrils, and vascularity.

**Materials and Methods**

The pathological records of patients with DCIS were retrieved from the file of North Shore University
Hospital. H&E-stained slides from randomly chosen cases were reviewed by two of the authors (PT and SIH) and divided into the comedo DCIS and non-comedo DCIS categories. Tissue samples from selected areas were retrieved from formalin-fixed, paraffin-embedded tissue blocks, rehydrated, postfixed in osmium tetroxide, and embedded in Efapoxy resin. The samples were sectioned at 1 µm, stained with toluidine blue, and examined by light microscopy, followed by examination of thin sections with a transmission electron microscope (Jeol-JEM 100 CXII). The analysis included 8 cases of comedo DCIS and 5 cases of non-comedo DCIS.

Results

In non-comedo DCIS, the surrounding collagenous matrix typically showed densely packed mature collagen fibers, organized in thick bands that ran alternatively perpendicular and parallel to the intraductal tumor nests (Fig. 1). In some areas, the stroma surrounding non-comedo DCIS showed a

Fig. 2. Electron photomicrograph from a case of comedo DCIS, showing a typical loose, poorly organized, stromal matrix containing some mature collagen and other poorly defined material. Note the long aggregate of thinner filaments towards the top of the matrix (magnification 9,100x). In the insert, clustered fibrils in the extracellular matrix are seen at a higher magnification (30,300x)
looser pattern of collagen deposition: this pattern occasionally predominated.

In comedo DCIS, the extracellular matrix that surrounded the tumor cell nests was principally composed of loose collagenous matrix with a comparatively disorganized pattern of collagen fiber deposition (Fig. 2). This loose matrix was often mixed with areas of dense collagen. The thickness of individual collagen fibrils (35-40 nm) was similar in both types of DCIS. However, the total circumferential accumulation of the periductal fibers was different: 0.35 µm in cases of comedo carcinoma, versus 0.1 µm in cases of non-comedo carcinoma. In addition, scattered clusters of long, thin fibrils (7-11 nm in thickness), aggregated into long dense clusters and intermingling with (or on top of) collagen fibers, were frequently seen in comedo-type DCIS (Fig. 2, insert). This population of fibrils was not readily evident in non-comedo DCIS.

In comedo DCIS, the lateral intercellular spaces between periductal myoepithelial cells were variably expanded (Fig. 3). Occasionally, this resulted in direct access of tumor cell processes to the basal lamina, which then presented the only barrier to migration into the stroma. By contrast, in non-comedo DCIS the lateral plasma membranes of adjacent myoepithelial cells were in close apposition, providing a cellular barrier to tumor cell migration (Fig. 1). Myoepithelial cell shape varied from flattened to plump, in both comedo and non-comedo DCIS.

In comedo DCIS, the basal lamina beneath the myoepithelium surrounding the intraductal tumor cells showed focal loss of integrity. Pseudopodial processes of tumor cells were demonstrable, crossing into the extracellular matrix through a discontinuity in the basal lamina (Fig. 4). Furthermore, the basal lamina in comedo DCIS contained regions with a wavy, irregular contour as well as focal reduplication. In non-comedo DCIS, the basal lamina was generally intact with rare evidence of fragmentation or reduplication. The thickness of the basal lamina showed was similar (300-600 nm) in both comedo and non-comedo DCIS.

The cellularity and the vascularity of the extracellular matrix were directly related to the separation and looseness of fibers in the collagen packing. Consequently, matrix cellularity and vascularity were greater in comedo DCIS than in non-comedo DCIS. Also, there were numerous fibroblasts between collagen fibers, and occasional lymphocytes and plasma cells. Capillaries were in close proximity to the basal lamina that surrounded intraductal tumor nests in both comedo and non-comedo DCIS. The morphological characteristics of the endothelial cells and fibroblasts of comedo and non-comedo DCIS were indistinguishable.

**Fig. 3.** Electron photomicrograph from a case of comedo DCIS of the interface between the extracellular matrix at the bottom, a wavy curvilinear region of basal lamina, bordering myoepithelial cell cytoplasm, and tumor cells near the top of the micrograph. Note the expanded lateral intercellular space between the myoepithelial cells, providing a direct, open pathway for tumor cells to reach the basal lamina (magnification 13,600x).
Discussion

It is generally agreed that in carcinomas, there are derangements of cell-to-cell adhesion and alterations in the adjacent stroma, including degradation and decreased synthesis of basement membrane, neovascularization, inflammatory cell influx, and extensive remodeling of extracellular matrix [17]. Specifically, in breast tissue, there is a junction between the epithelium and the surrounding stroma [18], composed of the plasma membrane of epithelial and myoepithelial cells, lateral intercellular space, basal lamina, and layer of connective tissue fiber, with a cell population principally of fibroblasts. For cancer cells to escape from a primary intraductal site, become invasive, and metastasize, they must first enter or cross this barrier.

We previously reported a biologic difference between comedo and non-comedo DCIS in respect to
different expression patterns of tenascin in the periductal stroma (16), that may, in part, explain the higher incidence of periductal invasion and metastasis in comedo DCIS [5,9]. The present study provides ultrastructural evidence of differences in the stromal interface with intraductal tumor cells of DCIS, that may contribute to the more aggressive clinical behavior of comedo DCIS. The alterations include the imperfect myoepithelium and expanded lateral intercellular space that allow contact of tumor cell processes with the basal lamina. Together with evident gaps in the myoepithelium, these findings emphasize potential importance of this cellular barrier.

Our study provides evidence of carcinoma cell migration across a fragmented basal lamina into the surrounding stroma in comedo DCIS. This ultrastructural finding may help to explain why comedo DCIS is clinically more aggressive than non-comedo DCIS, with occasional deposits of neoplastic cells as metastases in axillary lymph nodes in the absence of demonstrable invasive carcinoma. Although comedo DCIS is classified by pathologists as carcinoma in situ (non-invasive carcinoma) based on routine examinations by light microscopy, comedo DCIS may, in some cases, show ultrastructural signs of early invasion.

The deposition pattern of collagenous matrix showed characteristic differences in comedo and non-comedo DCIS. In all cases of comedo DCIS, there was abundant, loose, and poorly organized collagen deposition, mixed in some cases with a denser component. On the other hand, in non-comedo DCIS, the collagen deposition was usually very dense with occasional loose areas. While the variations in the matrix deposition pattern limit the significance of these observations, the generally looser pattern that was evident in comedo DCIS appears to be a factor that allows more ready tumor migration in the stroma. The looser stromal matrix and the increased cellularity, including inflammatory cells, lymphocytes, plasma cells, together with their associated cytokines, are more likely to affect the tumor cells in comedo DCIS. The small capillaries that are external to the tumor cell nests may provide a pathway for tumor cell migration without stromal spread. However, such vessels are seen in comedo DCIS as well as non-comedo DCIS.

The nature of the thin fibers scattered through the stroma, which are seen frequently in the comedo type of DCIS, is unclear. They may be a type of extracellular matrix protein or glycoprotein, such as tenascin, that is upregulated by these high-grade tumor cells.

In summary, this study provides ultrastructural evidence that multiple defects in the myoepithelial lining, basement membrane, and periductal stroma of comedo DCIS may contribute to the higher propensity for its tumor cells to escape from their primary intraductal site, become invasive, and eventually metastasize. Pathologists, surgeons, and oncologists should be aware of these findings and consider them in the management of patients with DCIS.

References