Micropapillary Carcinoma of the Breast with Necrosis-like Cell Death: A Case Report


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Micropapillary Carcinoma of the Breast with Necrosis-like Cell Death: A Case Report

ABSTRACT A primary invasive micropapillary carcinoma of the breast in a 46-year-old woman is reported. Histologically, it was composed predominantly of papillary tumor cell clusters without fibrovascular cores, surrounded by a clear space. Tumor cells were positive for cytokeratin (CK) 7, estrogen receptor (ER), and progesterone receptor (PR), but negative for p53, CK 20, CD34, c-Erb-B2, CK5, epidermal growth factor receptor (EGFR), vimentin, and c-kit. MUC1 expression was found at the reversed apical membrane of neoplastic cell clusters. Accordingly, electron microscopy showed the lack of basement membrane and presence of microvilli at the basal surface of the tumor cells. Moreover, ultrastructural examination revealed single tumor cell death characterized by patchy condensations of chromatin throughout the nucleus. These nuclear alterations were associated with the occurrence of empty cytoplasmic vacuoles, conferring a necrosis-like phenotype to this cell death. Alternative programmed cell deaths are reviewed and their morphologic distinction is discussed.

KEYWORDS breast, electron microscopy, histopathology, immunohistochemistry, micropapillary carcinoma, necrosis-like cell death

Invasive micropapillary carcinoma is a rare variant of ductal carcinoma of the breast with unique morphology [1–13]. Characteristically, this variant of carcinoma shows predominant or focal papillary cell clusters devoid of a fibrovascular core, surrounded by empty lacunar spaces. Invasive ductal carcinoma with micropapillary component is known to have frequent nodal metastases and poor prognosis. It is believed that the characteristic morphology of micropapillary carcinoma is due to a polarity reversal of the neoplastic cells where the stroma-facing (basal) surface of the cells acquires apical secretory properties. This alteration in cell polarity was demonstrated by electron microscopy of breast carcinomas showing the
presence of a large number of microvilli at the surface of the cells facing the stroma [3]. Furthermore, glycoproteins such as MUC1, expressed at the apical surface (luminal surface) of the cells in normal glandular epithelium, are present exclusively on the basal surface of micropapillary clusters, as demonstrated by immunohistochemistry [9, 14].

Patterns of cell death have been divided into apoptosis, which is actively executed by specific proteases, the caspases, and accidental necrosis. However, there is now accumulating evidence indicating that cell death can occur in a programmed fashion but in complete absence and independently of caspase activation [15]. Alternative models of programmed cell death have therefore been proposed, including paraaptosis [16], autophagy [17], mitotic catastrophe [18], and the descriptive model of apoptosis-like and necrosis-like programmed cell death [19].

To date, the identification of different cell death types is based on pathognomonic ultrastructural characteristics without a clear reference to precise biochemical mechanism [18]. We describe a rare case of micropapillary breast carcinoma, with an emphasis on the previously unreported ultrastructural features of tumor cell death.

CASE REPORT

A 46-year-old woman presented in October 2006 with a mass in her left breast. Physical examination showed an induration, but no dimpling or bulging in the upper border of the left breast. Mammogram and ultrasound findings suggested that the tumor was malignant. The patient underwent mastectomy with level II lymph node resection because the indications for only sentinel lymph node in our institution are limited to T1 and N0 lesions that are less than 2 cm in maximum dimensions. Postoperatively, tamoxifen has been administered and the patient remains well without any signs of recurrence.

MATERIALS AND METHODS

For light microscopy, the specimens were fixed in 10% formalin for 24 h at room temperature and embedded in paraffin. Sections were stained with hematoxylin–eosin (H&E), Alcian blue, and periodic acid–Schiff (AB-PAS).

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Additional sections were used for the immunohistochemical stains with ABC method. When necessary, antigen retrieval was performed using microwaving in citrate buffer for 10 min. The following primary antibodies were tested: MUC1 (clone Ma695; dilution 1:3200; Novocastra, Newcastle, UK), estrogen receptor (ER) (clone 6F11; dilution 1:50; Novocastra), progesteron receptor (PR) (clone 16; dilution 1:50; Novocastra), c-Erb-B2 (polyclonal; dilution 1:800; Dako, Glostrup, Denmark), Ki-67 (clone MIB-1; dilution 1:50; Dako), epidermal growth factor receptor (EGFR) (clone 2-18C9; dilution 1:10; Dako), c-kit (polyclonal; dilution 1:50; Dako), CK 5/6 (clone D5/16B4; dilution 1:50; Dako), CK 7 (clone OV-TL 12/30, dilution 1:200; Dako), CK 20 (clone KS 20.8; dilution 1:50; Dako), CD31 (clone MO823, dilution 1:25, Dako), p53 (clone DO-7; dilution 1:400; Dako), vimentin (clone V9; dilution 1:100; Dako), active caspase-3 (polyclonal; dilution 1:10; Dako). As controls, known positive tissue sections and negative controls devoid of primary antibody were used.

ELECTRON MICROSCOPY

Tumor tissue was fixed in 3% phosphate-buffered glutaraldehyde, pH 7.4, and postfixed in 1% osmium tetroxide. Semithin araldite-embedded sections were stained with Giemsa’s reagent for selection of fields. Thin sections were double-stained with uranyl acetate and lead citrate; they were then examined and photographed in a Zeiss EM 902 electron microscope (Carl Zeiss, Oberkochen, Germany).

PATHOLOGICAL FINDINGS

Macroscopic examination showed that the tumor was 25 x 16 mm, with an ill-defined margin. Macroscopic findings showed micropapillary carcinoma nests without fibrovascular cores. These tumor nests were morula-like, surrounded by empty, clear spaces lined with delicate strands of fibrocollagenous stroma (Figure 1). AB-PAS staining demonstrated no mucin in the clear spaces. Micropapillary carcinoma component accounted for more than 50% of total tumor volume. It was associated with an ordinary invasive ductal carcinoma. Histologic grades of micropapillary
component and ordinary invasive ductal carcinoma were both grade 2. Peritumoral lymphovascular tumor emboli were seen. Geographic zones of necrosis were not identified in the tumor. A careful observation of histological and semithin sections revealed a few tumor cells with either apoptosis or apoptotic bodies. Similarly, activated caspase-3 was occasionally observed in tumor. Metastases were found in 3/16 lymph nodes and were mostly composed of micropapillary carcinoma (pT2N1Mx). The lacunar spaces in micropapillary carcinoma were negative for CD31 immunostaining. Both estrogen (ER) and progesterone receptors (PR) were positive by immunohistochemistry, and the herceptest was negative. MUC1 expression was found at the reversed apical membranes of neoplastic cell clusters (Figure 2). None of the micropapillary and ordinary invasive ductal carcinoma component was immunoreactive for basal cell markers, including CK5/6, EGFR, and c-Kit. Tumor cells were positive for CK7 and negative for CK20. No immunoreactivity for p53 was found. Ki-67 index was 10%.

Electron microscopy showed the lack of basement membrane and presence of microvilli at the basal surface of the tumor cells (Figure 3). Other tumor cells showed nuclei with marked patches or foci of dense chromatin. These nuclear alterations were often associated with the occurrence of lipidic vacuoles, myelin-like figures, empty vacuoles, loss of microvilli, as well as disappearance of polyribosomes,
mitochondria, endoplasmic reticulum, and Golgi (Figure 4).

**DISCUSSION**

The tumor described here is classified as micropapillary carcinoma for a series of morphological, histochemical, ultrastructural, and immunohistochemical features [1–14]. The characteristic histologic appearance of the tumor was focal papillary cell clusters devoid of a fibrovascular core, surrounded by empty lacunar spaces. Histochemically, AB-PAS staining showed no presence of mucin in the clear spaces. Invasive micropapillary carcinoma may also simulate extensive lymphovascular invasion by primary carcinoma cells. Detection of CD31-positive endothelial cells around the spaces is useful in avoiding this diagnostic mistake. Immunohistochemical staining for MUC1 shows a distinctive “inside-out” staining pattern that is helpful in recognizing micropapillary carcinoma [2, 20]. However, it is sometimes difficult to differentiate micropapillary carcinoma from conventional invasive ductal carcinoma showing a micropapillary-like pattern due to artifact (pseudo-micropapillary carcinoma). Electron microscopy confirmed the presence of microvilli at the basal surface of the tumor cells, and was also useful to rule out a pseudomicropapillary carcinoma.

Tumor necrosis has garnered increased attention over the last few years, in part because a number of studies have now shown that tumor necrotic tissue represents a...
significant prognostic marker with an independent influence on metastasis-free survival in patients with neoplasm [21]. In particular, recent studies suggest that surgical pathologic evaluation of tumor necrosis should routinely record its presence or absence [22].

Tumor necrosis has been most extensively investigated in breast carcinoma. In invasive carcinoma of the breast tumor necrosis has been shown to correlate with increased tumor size, high-grade disease, microvessel density, macrophage infiltrates that express vascular endothelial growth factor, negative ER status, decreased relapse-free survival, and a worse prognosis [23, 24]. In particular, tumor necrosis is associated with the basal phenotype: a distinct subgroup of breast carcinoma characterized by poor prognosis [25, 26]. However, basal-like features based on coagulative necrosis as well as CK5/CK6 positivity, EGFR and expression, and c-kit positivity were not identified in our case. Finally, c-Erb-B2 and p53 negative immunostaining, as well as ER and PR positivity, suggests an ER-positive/luminal phenotype [27].
The fact that the nuclear morphology is currently the sole criterion allowing the accurate definition of programmed cell death pathways prompted us to perform an ultrastructural study. Under electron microscopy single tumor cell death was found. It was characterized by chromatin clustering, lipidic vacuoles, myelin-like figures, and empty vacuoles. These nuclear and cytoplasmic changes do not seem to fit the typical description of apoptosis, which is morphologically characterized by chromatin condensation into dense granular caps under the nuclear membrane [18]. The ultrastructural alterations observed in present study are compatible with the morphologic criteria for paraptosis [16]. The paraptosis-like vacuoles were clearly distinct from autophagic vacuoles because they were much greater and devoid of intracellular content. There are, however, only a few reports on paraptosis, and they do not make a comparison with other types of cell death [15]. In contrast to the specific definitions of programmed cell death such as autophagy, paraptosis, mitotic catastrophe, Leist and Jäättelä [19] proposed a descriptive model that classifies cell death into four subclasses, according to their nuclear morphology. Apoptosis is defined by stage II chromatin

FIGURE 4 Tumor cell nucleus shows patchy condensations of chromatin; the cytoplasm contains multiple empty vacuoles, myelin-like figures, and lipidic vacuoles, × 10,000.
condensation into compact figures, which are often globular or crescent shaped. Slightly different is apoptosis-like programmed cell death, which is characterized by less-compact chromatin condensation, so-called stage I chromatin condensation. In contrast, in necrosis-like programmed cell death no chromatin condensation is observed, but, at best, chromatin clustering to loose speckles, whereas necrosis is characterized by cytoplasmatic swelling and cell membrane rupture. Single cell death observed in present study is very similar to necrosis-like cell death.

Initially described as a tumor antigen over-expressed in >90% of breast cancers, MUC1 is now known to be an oncogene with roles in both tumor formation and progression [28]. It can block the p53-dependent apoptotic response to DNA damage [28]. To elucidate the role of MUC1 in alternative pathways of programmed cell death, including the necrosis-like cell death observed in the present study, additional study is needed.

In conclusion, our study suggests that the combination of MUC1 immunohistochemistry and electron microscopy was very useful for the diagnosis of micropapillary carcinoma. Recognition of this distinctive and aggressive variant of luminal-type breast carcinoma is important because of its poor prognosis and high incidence of lymph node metastases.

REFERENCES