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A subset of in situ breast tumor cell clusters lacks expression of proliferation and progression related markers but shows signs of stromal and vascular invasion☆

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Abstract

Background: Our previous studies in pre-invasive mammary tumors revealed that estrogen receptor negative cell clusters (ER NCC) overlying focally disrupted myoepithelial (ME) cell layers showed a significantly higher rate of genetic abnormalities and cell proliferation than adjacent cells without ME cell layer disruptions. A subset of these ER NCC, however, completely lacked expression of Ki-67, a most commonly used marker for cell proliferation. The purpose of this study was to further elucidate the immunohistochemical and morphological profiles of these ER NCC. *Methods:* Fifteen cases with such ER NCC were selected from our previous studies and assessed with a panel of commonly used biomarkers for cell proliferation, tumor progression, and normal stem cells. *Results:* Immunohistochemically, in addition to Ki-67 and ER, these ER NCC completely lacked expression of all other proliferation and progression related markers that were distinctly expressed in adjacent cells within the same duct but overlying the non-disrupted ME cell layer. These ER NCC also lacked expression of all normal stem cell-related markers tested. These cell clusters, however, showed a higher and atypical expression of c-erb-B2, compared to their adjacent counterparts. Morphologically, these ER NCC were generally arranged as triangle shaped structures penetrating into the stroma, similar to micro-invasive lesions. About 15% of these ER NCC appeared to directly spread into blood vessel-like structures. These ER NCC and their possible derivatives within the stroma and blood vessels-like structures shared the same morphologic and immunohistochemical features. No comparable ER positive cell clusters were identified in any of the cases. *Conclusions:* These findings suggest that these ER NCC and their possible derivatives are likely regulated by yet to be defined molecules and mechanisms, and they are unlikely to respond to currently available anti-mitotic agents.

Keywords: Estrogen receptor; Myoepithelial cells; Anti-mitotic agents; Stromal and vascular invasion; Stem cells; Breast tumor cells; Ductal carcinoma in situ; Morphologic features; Progression-related markers; Tumor invasion and metastasis; Vascular invasion; Immunostataining; Matrix metalloproteinase-26; c-erb-B2 protein; Cytokeratin; Basement membrane disruption; Genetic alterations

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1. Introduction

The epithelium of the normal human breast and in situ breast tumors is physically separated from the stroma by both a myoepithelial (ME) cell layer and the basement membrane [1–3]. ME cells are joined by intercellular junctions and adhesion molecules, forming a continuous layer that encircles the entire duct system, and a discontinuous layer or a basket-like structure that covers a vast majority of lobules and terminal duct-lobular units [1–3]. The basement membrane is composed of a group of fibrous proteins embedded in a hydrated polysaccharide gel, forming a continuous lining surrounding and attaching to ME cells via hemidesmosomes and focal adhesion complexes [1–3]. This architectural feature confers ME cells and the basement membrane two essential functions. First, as the epithelium is normally devoid of lymphatic and blood vessels and totally depends on the stroma for its metabolism and survival needed materials, the ME cell and basement membrane function as gatekeepers, directly regulating the communication between these two cellular compartments. Second, due to the physical interposition of the ME cell layer and basement membrane between the stroma and epithelium, ductal tumor cells must first penetrate the ME cell layer and then followed by the basement membrane, in order to reach the stroma for invasion or metastasis.

A generally accepted hypothesis for the direct cause of basement membrane disruptions and tumor invasion has been attributed primarily, if not solely, to over-production of proteolytic enzymes by tumor or stromal cells [4]. This hypothesis alone, however, may not reflect the intrinsic mechanism of tumor invasion, as results from recent worldwide clinical trials with a wide variety of corresponding enzyme inhibitors have been disappointing [5,6].

While attempting to identify early signs of ME layer disruptions and precursors of invasive lesions, we have carried out a number of studies, focusing on the correlation between the structural integrity in ME layers and the immunohistochemical and genetic profiles in adjacent epithelial cells.

In double immunostained sections from 220 patients with estrogen receptor (ER) positive, non-invasive breast tumors, we detected a total of 405 focal ME cell layer disruptions, defined as the absence of ME cells resulting in a gap equal to or greater than the combined size of three ME or epithelial cells [7]. Of these disruptions, 350 (86.4%) were overlaid by cell clusters with no or substantially reduced ER expression, in contrast to adjacent cells within the same duct, which expressed a high level of ER and overlaid a non-disrupted ME cell layer [7]. Compared to their adjacent counterparts within the same duct, most ER negative cell clusters (ER NCC) overlying focally disrupted ME cell layers displayed several unique features, including a substantially higher frequency of loss of heterozygosity at multiple chromosomal loci, a significantly higher expression of tumor progression and invasion related genes, and a significantly higher index of Ki-67 positive cells [7–10], seemingly representing a biologically more aggressive cell clone or the precursor of invasive lesions.

About 10% of these ER NCC in some cases, however, were completely devoid of Ki-67 immunoreactive cells, in a sharp contrast to both the adjacent ER positive cells within the same duct and other ER NCC in different ducts, which showed a markedly elevated number of Ki-67 positive cells [11,12]. These unusual ER NCC, however, exhibited distinct signs of growth, and stromal or vascular invasion [13–15].

As it has been well documented or suggested that ER negative tumors have a substantially worse prognosis [16–18], and deregulated cell proliferation is a direct cause of breast malignancies [19,20], this study attempted to further elucidate the immunohistochemical and morphological features of these ER NCC. The primary goal of this study was to determine whether these unusual ER NCC would have a unique immunostaining pattern for currently available proliferation, tumor progression, and other related markers that have been linked to breast tumor progression and invasion.

2. Materials and methods

Formalin-fixed, paraffin-embedded human breast tissues with ductal carcinoma in situ ($n = 15$) containing focally disrupted ME cell layers and ER NCC with no Ki-67 immunoreactive cells were selected from over 400 pre-invasive lesions from our previous studies [7–10]. Consecutive sections at 4–5 μm thickness were placed on positively charged microscopic slides, and stained with H&E for morphologic classification based on our published criteria [21]. Immunohistochemical staining was carried out, using our published protocols [22,23]. Briefly, sections were incubated at 70–80 °C for 1 h, deparaffinized with xylene, and washed with ethanol and water. Deparaffinized sections were incubated in 1× antigen retrieval solution (Biocare Medical, Foster City, CA) overnight at 60–70 °C, washed in water and phosphate-buffered saline (PBS), treated with 3% hydrogen peroxide and normal serum, and incubated with a primary antibody. After incubation with the primary antibody, sections were washed with PBS, and then sequentially incubated with the corresponding secondary antibody, avidin–biotin–peroxidase solution, and substrate diaminobenzidine (Vector, Burlingame, CA). For elucidation of a new antigen, immunostained sections were thoroughly washed with PBS, and then incubated with a new antibody. The antigen and antibody complex was detected with a corresponding secondary antibody, avidin–biotin–alkaline phosphatase detection kit, and Zymed AP-red substrate kit (Zymed Laboratories Inc., South San Francisco, CA).

To identify focal ME cell layer disruptions and to determine the size of associated ER NCC, sections 1, 11, and 21 from each case were double immunostained for ER, Ki-67, and smooth muscle actin (Vector, Burlingame, CA). ER NCC were