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Tumor-extracellular matrix interactions: Identification of tools associated with breast cancer progression

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ABSTRACT

Several evidences support the concept that cancer development and progression are not entirely cancer cell-autonomous processes, but may be influenced, and possibly driven, by cross-talk between cancer cells and the surrounding microenvironment in which, besides immune cells, stromal cells and extracellular matrix (ECM) play a major role in regulating distinct biologic processes. Stroma and ECM-related signatures proved to influence breast cancer progression, and to contribute to the identification of tumor phenotypes resistant to cytotoxic and hormonal treatments. The possible clinical implications of the interplay between tumor cells and the microenvironment, with special reference to ECM remodelling, will be discussed in this review.

1. Introduction

Although cancer has been considered as a progression of genetic mutations in an aberrant tissue mass, tumors are increasingly viewed as tissues functionally interconnected with the surrounding microenvironment [1]. Recent genome sequencing and single cell-based analyses have revealed substantial genetic heterogeneity within tumors, with subclones that differ in driver mutations. In a breast carcinoma model [2], growth was sustained by a minor cell subpopulation that facilitated the proliferation of all tumor cells. Interestingly, this subpopulation stimulated tumor growth through microenvironmental changes related to re-organization of the collagen pattern and induction of intratumoral vascularization, suggesting that progression of a tumor relies on its ability to overcome microenvironment constraints.

The tumor microenvironment consists of an insoluble extracellular matrix (ECM), a stroma composed of fibroblasts, adipocytes, endothelial and resident immune cells, and a multitude of growth factors and cytokines. The ECM itself is composed by a complex mixture of components, including proteins, glycoproteins, proteoglycans and polysaccharides [3,4]. In addition to elucidating the role of single ECM components in development and homeostasis of normal breast, many studies have revealed abnormal changes in the amount and organization of such molecules during breast carcinoma development. These changes lead to altered biochemical and physical properties of tumor-associated ECM that contribute to tumor progression and resistance to therapy. Moreover, deregulation of ECM architecture impacts on tumor surrounding stroma cells, including endothelial, immune and other stromal cells which may come to favor tumor development. Although many single ECM components, reviewed in [5], have been identified as relevant markers in breast carcinoma progression, evaluation and targeting of a single molecule appears to have limited usefulness in predicting disease outcome or improving therapeutic benefit. A possible explanation might rest in the large number of ECM components, which, even if likely redundant, collectively contribute to distinctive physical, biochemical and biomechanical properties of the tumor microenvironment.

To address the complexity of the tumor ECM and elucidate the stromal properties relevant for breast carcinoma progression and response to therapy, cancer research in the last decade has shifted to gene expression studies focused on the tumor stromal component. In this context, different experimental approaches have been

Abbreviations: ER, estrogen receptor; IDC, invasive ductal carcinoma; DCIS, ductal carcinoma *in situ*; FEC, 5-fluorouracil+doxorubicin+cyclophosphamide; NKI, Netherland Cancer Institute; ECM, extracellular matrix; SFT, solitary fibrous tumors; DTF, desmoid-type fibromatosis.

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applied, including profiling of: (a) stromal cells isolated from carcinomas or modified by cultured conditions; (b) soft tissue tumors as surrogates for different stromal responses; (c) laser-captured tumor microenvironment or whole tumor samples followed by analysis of a gene set restricted to connective tissue-related genes. The first gene expression portrait of the breast cancer microenvironment came from a study [6] in which different stromal cell types composing normal and neoplastic tissues were profiled using serial analysis of gene expression upon separation by magnetic beads armed against cell type-specific surface markers. Significant changes in gene expression profile were detected in all cell types during tumor progression, indicating that the microenvironment actively participates in cancer growth and invasion. Focusing on myoepithelial cells as constituents relevant in controlling breast cancer cell growth, the authors detected upregulated expression of several proteases (cathepsin F, K and L, MMP2 and PRSS19), protease inhibitors (thrombospondin2, SERPING1, cytostatin C and TIMP3) and different collagens (COL1A1, COL3A1, COL6A1) in DCIS myoepithelial cells [6] implying ECM remodeling during cancer development. These results were later confirmed through the gene expression portrait of fibroblasts derived from invasive and benign breast diseases [7]. While analysis of gene expression profiles of stromal tissue isolated using laser capture microdissection (LCMD) showed no significant differences between adjacent tumor and reduction mammoplasty-derived stroma [8], genes involved in ECM-receptor interaction and focal adhesion were significantly up-modulated in tumor versus normal cells microdissected from mastectomy specimens of invasive ductal or lobular carcinomas [9]. Taking advantage of LCMD, gene expression profiles of patient-matched normal stroma and tumor-associated stroma specimens showed that the highest regulated genes in the tumor-associated stroma were those encoding ECM constituents and matrix metalloproteinases, including COL10A1, COL11A1, fibronectin, collagen triple helix repeat containing 1, COL12A1, COL8A1, MMP11, and MMP2 [10]. Thus, whereas early changes involved in cancer initiation *per se* do not appear to derive from the microenvironment, the cross-talk between tumor and stromal cells mediated though the ECM is one of the first events upon mammary cell transformation. As such, there is continuing scientific interest in the role of cross-talk in neoplastic progression as well as in the promise of ECM features as biomarkers able to predict risk of progression and treatment benefit in breast carcinoma patients. Moreover, recent findings on the role of specific microRNAs regulating a network of genes involved in ECM changes by tumor microenvironment or directly targeting ECM molecules mRNAs [11,12] open new perspectives for investigating whether any of such stroma/ECM variations are associated to release/modulation of signaling molecules relevant for tumor progression which can be easily detected in body fluids for a relatively non-invasive early diagnosis/risk assessment.

Here, we provide an overview of "microenviromental signatures" proved to be relevant as markers for progression and treatment of breast cancers. Since abnormal characteristics of breast carcinoma ECM induce changes in tumor tissue architecture and rigidity, we also discuss the role of stromal stiffness on tumor behavior.

2. The role of ECM in the transition from ductal carcinoma *in situ* to invasive breast cancer

Before the introduction of mammary screening, ductal carcinoma *in situ* (DCIS) represented only 2–5% of symptomatic breast cancers while, nowadays, it accounts for more than 20% of newly diagnosed symptomatic cases and up to half of screen-detected breast cancer, but uncertainties still remain on its biological behavior and the appropriate clinical management [13–15]. Thus, the identification of biomolecular markers associated to the risk of *in situ* recurrence rather than of invasive cancer to complement standard clinical and pathological factors represents an area of intense research [16,17] for the possibility to provide patients with a more appropriate use of local and systemic treatments.

At present, the progression of DCIS to invasive breast cancer may be explained by mainly two distinct mechanisms, as the results of the accumulation of additional genetic aberrations and inherited transcriptome alterations consequent to methylation modifications or, alternatively, as a genetic-independent process. Many studies focused on the molecular and genetic alterations in neoplastic cells, but evidence is also emerging on the fact that the transition from DCIS to invasive ductal carcinoma (IDC) is strongly dependent upon alterations in the microenvironment with a particular reference to the role of myoepithelial cells and stromal-epithelial interactions.

Initial studies which focused on epithelial cells only, barely found any gene expression differences among distinct stages of progression [10,16], especially when the comparison was done between *in situ* and invasive tumors of the same grade. Indeed at the epithelial cell level, the more dramatic changes were reported in the transition from normal epithelium to DCIS, with minor changes between atypical ductal hyperplasia, DCIS and IDC. With an increasing awareness on the role of the microenvironment in tumor biology [18], studies were undertaken to molecularly dissect the contribution of single microenvironmental components, and the first systemic study [6] on pure stromal cell subpopulations, isolated from few samples of DCISs, IDCs and normal mammary glands, demonstrated alterations in all the microenvironment components across the progression to *in situ* and invasive growth, with major changes occurring in the myofibroblasts and myoepithelial cells. Such alterations involved increased secretion of chemokines (*e.g.*, CXCL14, CXCL12), which stimulate in a paracrine fashion proliferation, invasion and migration of tumor cells.

The transition from DCIS to IDC was also studied integrating genomic alterations with transcriptomic profiles and again no similarities could be observed also at the genomic level [19] suggesting that these changes are shared between DCIS and IDC. Some caution should however be used in the interpretation of data since in the absence of a common mechanism accounting for DCIS progression, individual tumor alterations could represent confounding factors in patient-matched studies of DCIS/IDC as already observed when the analysis was done at single cell level [20,21].

On the front of the non-genomic hypothesis of the transition from DCIS to IDC, Ma et al. [10] performed the molecular characterization of 14 patient-matched normal and tumor breast frozen samples in which gene expression profiles were analyzed in LCMD-isolated cells from normal breast epithelium, DCIS, IDC, normal stroma compartment, DCIS- and IDC-associated stroma. The progression from *in situ* to invasive growth proved to be associated to an extensive change in gene expression mainly in tumor-associated stroma, with an up-regulation of matrix metalloproteinases genes (MMP11, MMP2, MMP14 and MMP13) associated with invasion and with ECM remodeling. Conversely, only three typical stromal genes (*POSTN, SPARC, SPARCL1*) were up-modulated in the tumor epithelium of IDC compared to DCIS.

Later studies addressed the issue of DCIS progression in a similar way, comparing synchronous DCIS and IDC lesions, with some differences in study design (not necessarily patient-matched) and technical approaches (manually performed microdissection on FFPE samples at a distance up to 3 mm from the tumor lesion). At difference to the previous studies, Vargas et al. [22] observed major changes only in genes related to ECM remodeling in the epithelial compartment, including an increased expression of COL11A1, COL5A2 and MMP13 in IDC samples. Altogether, these

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