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CURRENT TOPICS IN BREAST PATHOLOGY

The biological and clinical significance of stromal-epithelial interactions in breast cancer

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Summary

There is evidence that an aberrant tumour microenvironment (TME) facilitates cancer development, progression, and responses to treatment. While many of the mechanisms underlying the phenotype and cancer-promoting behaviour of the TME are unknown, epigenetic mechanisms in cancer cells and the TME are thought to play important roles. As a result, cancer profiling strategies for drug and biomarker development require a thorough understanding of both the epithelial tissue compartment and the TME. This review discusses recent advances in our understanding of how cancer epithelial cells interact with their microenvironment and how this knowledge can be exploited clinically.

Key words: Breast cancer; epigenetics; tumour microenvironment; cancer stem cells; epi-drugs; cancer associated fibroblasts; DNA methylation; histone.

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INTRODUCTION

Breast cancer tissues contain genetic and epigenetic changes that result in altered epithelial cell structure and function. Epigenetic regulation is defined as any heritable modifications in gene expression and chromatin structure caused by alterations that do not involve the primary nucleotide sequence.[1,2] Epigenetic changes include DNA methylation, post-translational modifications of histone proteins, nucleosomal positioning, incorporation of histone variants, and the action of non-coding RNAs [such as micro (mi)RNAs].[3] The 'classical' epigenetic effect occurs when epigenetic silencing of one allele acts in concert with an inactivating mutation in the opposite allele, resulting in total allelic loss; for example, hypermethylation and deletion of the *BRCA1* promoter in sporadic breast cancer.[4]

The tumour microenvironment (TME) also represents an important source of epigenetic regulation of the epithelial compartment in breast cancer. As well as harbouring malignant cells, the TME contains cells of mesenchymal and haematopoietic origin and non-cellular components.[5] Cells of mesenchymal origin in the TME include fibroblasts, myofibroblasts, mesenchymal stem cells (MSCs), adipocytes, and endothelial cells, while cells of haematopoietic origin include lymphoid cells [T cells, B cells, and natural killer (NK) cells] and myeloid cells [macrophages, neutrophils, and myeloid-derived suppressor cells (MDSCs)]. The non-cellular component is the extracellular matrix (ECM) formed by the basement membrane and interstitial matrix (consisting of collagens, proteoglycans, and glycoproteins) (Fig. 1).

The TME also has an important metabolic (pH, PO_2, glucose, glutamine, lactate) and chemical (e.g., nitric oxide) environment.[6] This is further discussed by Simmons *et al.* in this issue.[7] Experimental modelling has shown that epigenetic cross-talk between cells in the TME drives the efficiency of cancer formation, the rate of cancer growth, the extent of invasion, the ability of cancers to metastasise, and their response to treatments.[8]

CANCER-ASSOCIATED FIBROBLASTS IN THE TME

What are cancer-associated fibroblasts?

Fibroblasts are generally the most abundant cell type in the TME. A subpopulation of fibroblasts known as cancer-associated fibroblasts (CAFs) is thought to be of critical importance in cancer initiation, progression, survival, metastasis, and invasion via the secretion of various growth factors, cytokines, and chemokines and the degradation of ECM proteins.[9,10] The origins of CAFs in breast cancer stroma are diverse.[10] The vast majority are thought to arise from normal fibroblasts, and breast cancer cells are known to induce epigenetic changes in normal fibroblasts that transform them into CAFs. For instance, Tyan *et al.*[11] showed that breast cancer cells can induce hepatocyte growth factor (HGF) secretion by CAFs to enhance tumorigenesis and that when normal fibroblasts were cultured with the breast cancer cell line MDA-MB-231 they secreted HGF and adopted a CAF phenotype. In another example, the MCF-7 breast cancer cell line was found to reduce caveolin-1 (Cav-1) expression (a CAF biomarker) in normal fibroblasts, resulting in phenotype switching to CAFs and increased expression of CAF-associated markers.[12]

CAFs also arise when epithelial cells undergo epithelial-mesenchymal transition (EMT), from bone marrow-derived stem cells that have undergone EMT, or from trans-differentiated breast tissue cells such as pericytes, adipocytes, or smooth muscle cells (Fig. 2).[13,14] The CAF profile differs

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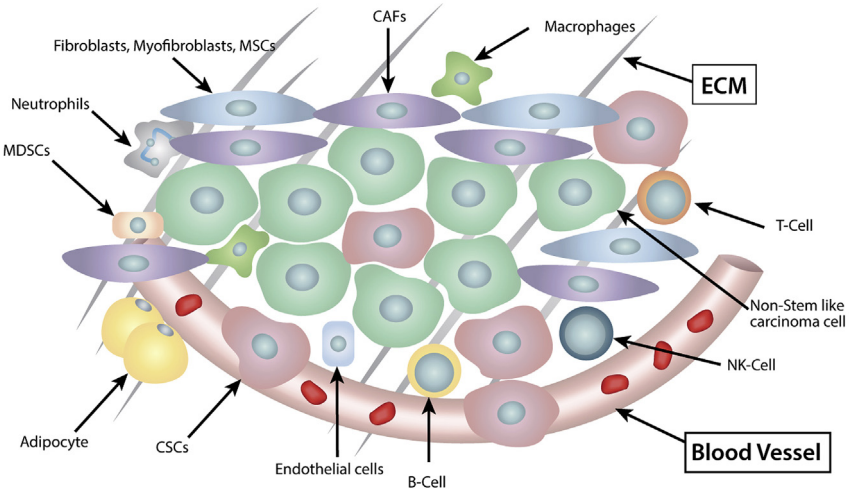

Fig. 1 Components of the tumour microenvironment (TME). The TME is a complex mixture of not only carcinoma cells but also many cells of different lineages and extra-cellular material. The cellular component includes cells of mesenchymal origin [fibroblasts, cancer-associated fibroblasts (CAFs), myofibroblasts, mesenchymal stem cells (MSCs), adipocytes, and endothelial cells] and those of haematopoietic origin: lymphoid cells [T cells, B cells, and natural killer (NK) cells] and myeloid cells (macrophages), neutrophils, and myeloid-derived suppressor cells (MDSCs). The non-cellular component is the extracellular matrix (ECM). The TME is an important epigenetic regulator of the epithelial compartment in breast cancer that ultimately influences the cancer phenotype.

depending on the TME and breast cancer subtype. In general, CAFs highly express α-SMA, p53, podoplanin, CD10, fibroblast activation protein (FAP), matrix metalloproteinases (MMPs), tenascin-C, and platelet-derived growth factor (PDGFRα/β) and lose Cav-1 expression.[10,12] Cytoskeleton- and integrin signalling-associated genes are up-regulated in HER2+ breast cancers compared to triple-negative breast cancers.[15] However, a universal CAF 'signature' has so far proven elusive.[10]

The gene expression profiles of fibroblasts from women without breast cancer have been compared to those from women with breast cancer.[16] Many genes are up-regulated in CAFs compared to normal fibroblasts including growth factors [fibroblast growth factors (FGFs), hepatocyte growth factor (HGF), transforming growth factor beta (TGF-β), and stromal cell-derived factor 1 (SDF-1)], cytokines [granulocyte macrophage colony-stimulating factor (GM-CSF), effector cell protease receptor 1 (EPR-1)], oncoproteins (K-ras), regulators of gene expression (nuclear-encoded mitochondrial elongation factor Ts, ribosomal protein S12, and spliceosome-associated protein SAP 145), and a variety of other genes associated with the cell cycle, cell-cell interactions, and cell-cell communication. Many of these gene products are pro-invasive and pro-metastatic.[10,17]

Breast cancer CAFs also show aberrations in DNA methylation, histone modifications, and dysregulated miRNAs.[18] While all DNA is coated with methyl moieties, the DNA methylation pattern is regulated by an independent enzymatic process catalysed by DNA methyltransferases (DNMTs). In terms of DNA methylation, the DNA methylation profiles of 143 human breast tumours showed significant differences in HER2 expression and DNA methylation

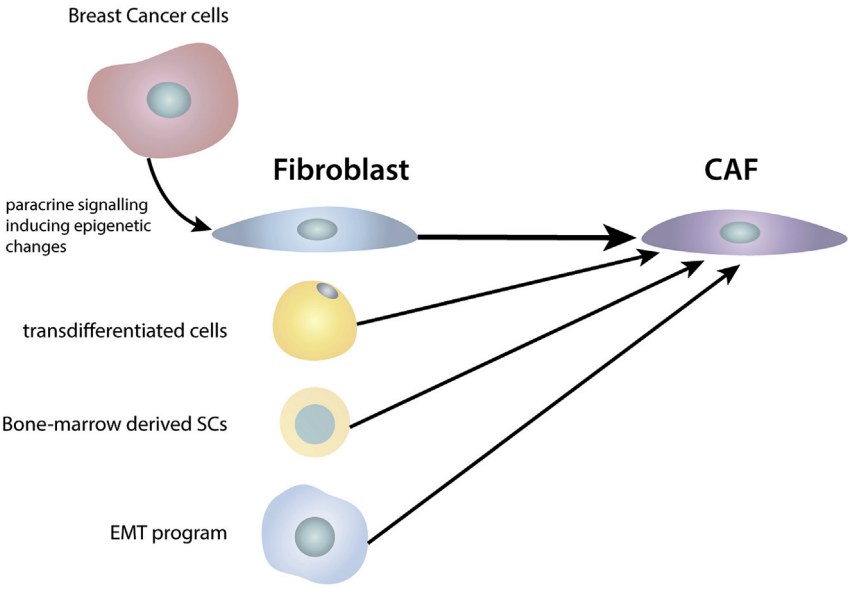

Fig. 2 Potential origins of cancer-associated fibroblasts (CAFs). There are multiple sources of CAFs, with the vast majority arising from phenotypic switching of normal fibroblasts under the influence of epigenetic signalling from breast cancer cells (non-stem-like carcinoma cells and cancer stem cells). However, CAFs can also arise from epithelial-mesenchymal transition (epithelial cells), transdifferentiated pericytes, adipocytes, or smooth muscle cells, and bone marrow-derived stem cells. These TME-influenced pathways induce epigenetic changes that promote a CAF profile.

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