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Perspective

Stromal Fibroblasts in Cancer

A Novel Tumor-Promoting Cell Type

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ABSTRACT

Tumors are highly complex tissues composed of neoplastic cells and, in the case of carcinomas, stromal cell compartments containing a variety of mesenchymal cells, notably fibroblasts, myofibroblasts, endothelial cells, pericytes, and a variety of inflammatory cells associated with the immune system. Fibroblasts and myofibroblasts often represent the majority of the stromal cells within various types of human carcinomas, yet the specific contributions of these cells to tumor growth are poorly understood. Recent work has demonstrated that stromal fibroblast fractions, named carcinoma-associated fibroblasts (CAFs), that have been extracted from a number of invasive human breast carcinomas are more competent to promote the growth of mammary carcinoma cells and to enhance tumor angiogenesis than are comparable cells derived from outside of these tumor masses. CAFs include large populations of myofibroblasts that secrete elevated levels of stromal cell-derived factor 1 (SDF-1), also called CXCL12, which plays a central role in the promotion of tumor growth and angiogenesis; CAF-derived SDF-1 not only stimulates carcinoma cell growth directly through the CXCR4 receptor displayed on tumor cells but also serves to recruit endothelial progenitor cells (EPCs) into tumors, thereby furthering neoangiogenesis. In this review, we highlight the importance of this SDF-1-CXCR4 signaling pathway in the tumor microenvironment and discuss the mechanisms by which stromal fibroblasts within mammary carcinomas enhance tumor growth.

INTRODUCTION

Neoplastic epithelial cells coexist in carcinomas with a biologically complex stroma composed of various types of stromal cells as well as extracellular matrix (ECM), both of which create the complexity of the tumor microenvironment.[1,2] The significant contribution of stroma to the development of a wide variety of tumors has been supported by extensive clinical evidence; this contribution is highlighted by the higher incidence of tumor formation in tissues exhibiting a chronically inflamed stroma as well as those undergoing various types of wound healing, in which the stroma plays a central role.[3,4] Use of mouse models of tumorigenesis also reveals that stromal cells, notably inflammatory cells, vascular cells, and fibroblasts,[5-8] actively support tumor growth.

Large numbers of myofibroblasts, which are characterized by their production of α-smooth muscle actin (α-SMA), have been observed repeatedly in the stroma of the majority of invasive human breast cancers.[9] However, the specific contributions of these cells to tumor progression are poorly defined. Myofibroblasts also exist in areas of wound healing and chronic inflammation and are often portrayed as "activated fibroblasts" that play crucial roles in wound repair; myofibroblasts possess greatly increased contractile ability, promote angiogenesis, and stimulate epithelial cell growth through the production of ECM and the secretion of growth factor and cytokines.[2,10] The striking histological resemblance of tumor stroma and the stroma present in sites of wound healing, both containing large numbers of myofibroblasts,[11] raises the following questions: (i) Do myofibroblasts play essential roles in tumor angiogenesis and can they directly stimulate the growth of epithelial carcinoma cells? (ii) Are myofibroblasts present in tumor biologically indeed equivalent to those observed in wound healing? (iii) Or alternatively, do tumor-associated myofibroblasts acquire "cancer-specific alterations" that distinguish them from those present in wounds.

In 1999, the groups of Tlsty and Cunha demonstrated a striking tumor-promoting property of stromal fibroblasts extracted from human prostate carcinomas when these were compared with control normal fibroblasts isolated from the noncancerous prostate gland. They prepared stromal fibroblasts, which they termed carcinoma-associated fibroblasts

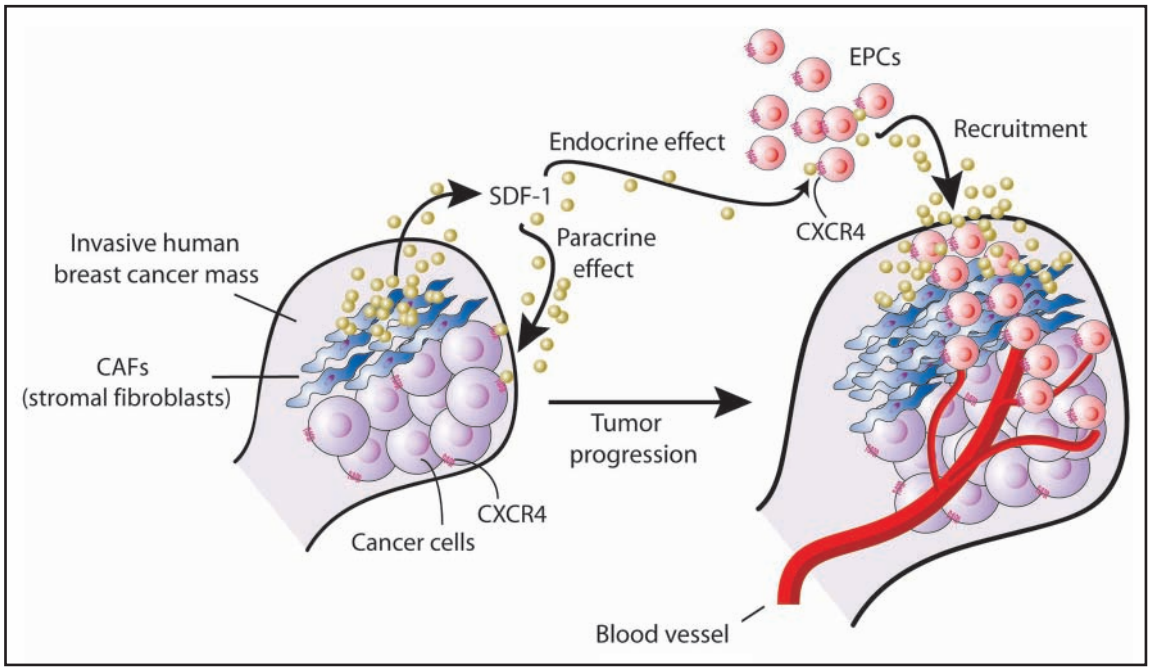

Figure 1. Schematic representation of tumor-promoting effects provoked by stromal fibroblasts within invasive human mammary carcinomas. Stromal fibroblast-derived SDF-1 enhances tumor growth not only by stimulating angiogenesis through recruiting circulating EPCs into the tumor mass but also by direct paracrine stimulation of tumor cells through the CXCR4 receptor expressed by carcinoma cells.

(CAFs), from several human prostate carcinomas. Each fibroblast preparation was then mixed with initiated, nontumorigenic human prostate epithelial cells and the mixtures were injected into immunodeficient host mice. They found that the CAFs potently stimulated the growth of tumor masses by the otherwise nontumorigenic prostate epithelial cells, while normal prostate fibroblasts failed to do so.[7] This discovery provoked a number of mechanistic questions: (i) How did CAFs succeed in promoting the observed tumor growth? (ii) Did these fibroblasts exhibit characteristics of myofibroblasts? (iii) Might the CAFs have evolved from normal prostate fibroblasts during the course of tumor progression?

We sought to elucidate the properties of stromal fibroblasts isolated from invasive human mammary carcinomas. Our work has demonstrated that fibroblasts present in the invasive human mammary carcinoma mass are biologically very different from their counterparts located outside tumor masses and from mammary stromal fibroblasts prepared from reduction mammoplasties in several important functional respects;[12]

(i) CAFs extracted from invasive human breast carcinomas are more competent than normal fibroblasts in enhancing tumor growth by comingled breast cancer cells.

(ii) CAFs include larger populations of myofibroblasts, which exhibit high levels of α-SMA expression and increased collagen contractility.

(iii) When comingled with a line of human breast cancer cells, CAFs give rise to highly vascularized tumors in contrast to the poorly vascularized tumors generated by admixed normal stromal fibroblasts.

(iv) CAFs release increased levels of SDF-1 (stromal cell-derived factor-1) which are responsible for recruiting endothelial progenitor cells (EPCs) into a tumor mass, thereby boosting tumor angiogenesis. In addition, the SDF-1 secreted from CAFs enhances tumor growth by direct paracrine stimulation via the CXCR4 receptor displayed by human breast carcinoma cells, thereby revealing a second role for stromal SDF-1 in promoting tumor progression in vivo (Fig. 1).

(v) Both the tumor-enhancing and myofibroblastic properties of CAFs are stably retained by these cells in the absence of ongoing contact with breast carcinoma cells.

SIGNIFICANT INVOLVEMENT OF THE SDF-1-CXCR4 AXIS IN FORMATION OF THE TUMOR MICROENVIRONMENT

SDF-1 is a homeostatic chemokine that signals through the CXCR4 receptor, a G-protein-coupled receptor that plays essential roles in the homing of hematopoietic progenitor cells (HPCs) and the development of B-lymphocytes[13] in the bone marrow. Furthermore, high concentrations of SDF-1 gradients generated locally by endothelial cells and fibroblasts present within ischemic injuries serve to attract circulating CXCR4-expressing progenitor cells, notably HPCs, EPCs, and possibly tissue progenitor cells into the injured sites, facilitating repair through the stimulation of angiogenesis and resulting regeneration of epithelial cell compartments.[14-17] Our findings revealed that the SDF-1-CXCR4 signaling axis is also used to recruit EPCs into tumors, thereby indicating an additional biological resemblance between tumor stroma and the stroma in sites of wound healing.

High levels of the CXCR4 expression by various types of human carcinoma cells are clinically associated with a poor prognosis.[18,19] Moreover, CXCR4 ectopically expressed in carcinoma cells enhances primary tumor growth in a mouse xenograft model,[20] and the knockdown of the CXCR4 expression in breast carcinoma cells abrogates the tumor growth.[21,22] Taken together, such findings reveal the significant roles of stromal SDF-1 in promoting tumor growth and enhancing angiogenesis through CXCR4 expressed on carcinoma cells and EPCs.

Carcinoma cells seem to recruit normal fibroblasts into tumor masses and then force the conversion of these cells into myofibroblasts

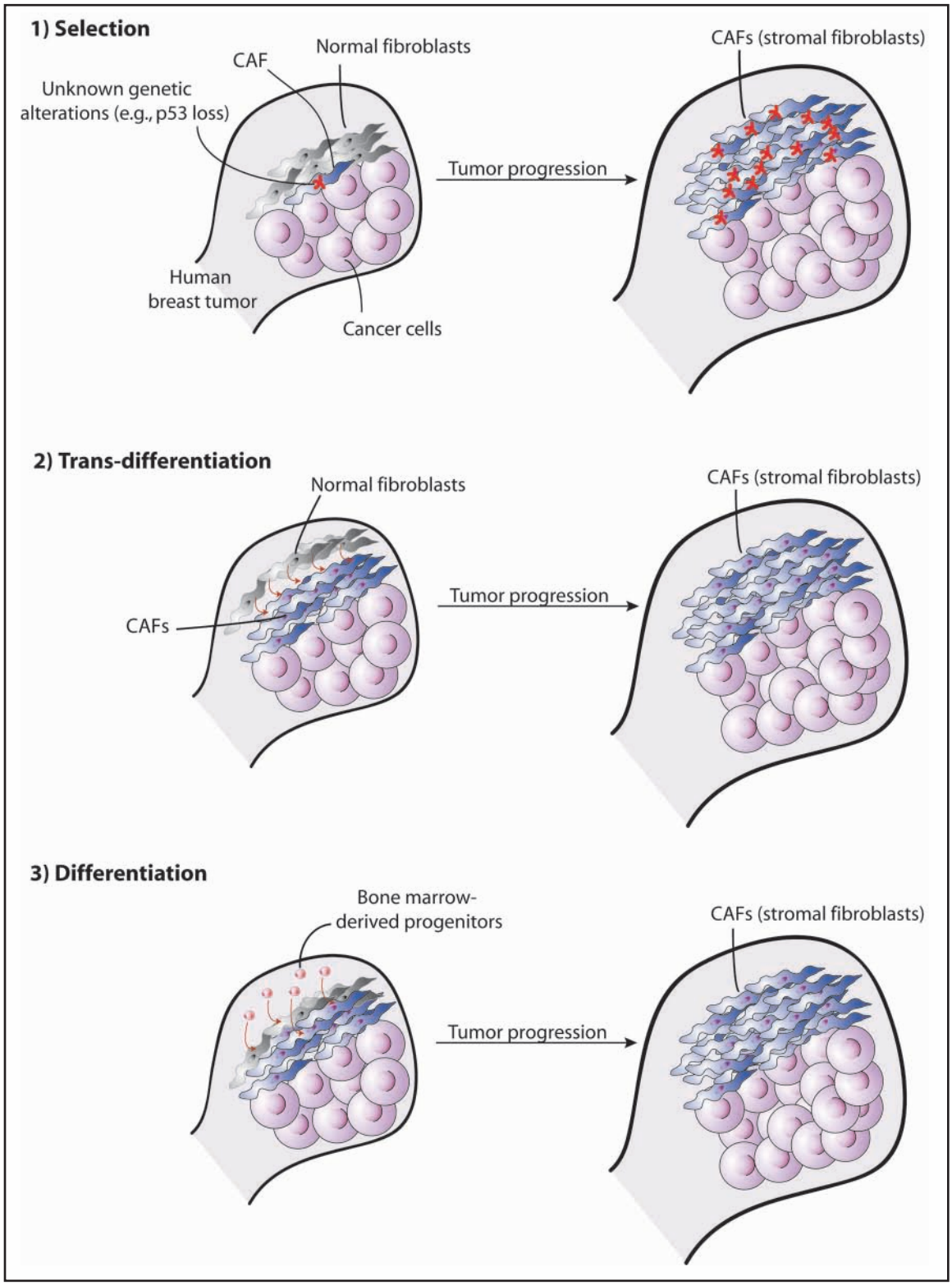

Figure 2. Tentative models for evolution of the stromal fibroblasts in human carcinomas. (1) Clonal selection from a small population of fibroblasts or progenitors that have undergone genetic alterations. (2) Trans-differentiation from, for example, pre-existing normal fibroblasts. (3) Differentiation from bone marrow-derived progenitors.

in order to promote tumor growth and progression. We note also that carcinoma cells exploit recruited immune cells, specifically macrophages and B cells; rather than displaying cytotoxic responses toward carcinoma cells, these recruited inflammatory cells facilitate their growth.[23-25]

DO STROMAL FIBROBLASTS ACQUIRE GENETIC AND/OR EPIGENETIC ALTERATIONS DURING TUMOR PROGRESSION?

CAFs retain their myofibroblastic properties and tumor-promoting phenotypes, even after they have been passaged for ten population

doublings (PDs) in vitro without ongoing contact with carcinoma cells. Accordingly, even though the CAFs appear to have initially acquired a myofibroblastic phenotype under the influence of carcinoma cells, once it is acquired, they display this trait in the absence of further signaling from the carcinoma cells. Unanswered by these observations are the following questions: (i) How do CAFs acquire and maintain their activated, tumor-enhancing phenotypes? (ii) Might CAFs harbor genetic and/or epigenetic alterations that act to confer their unique phenotypes?

Some reports indicate that stromal regions microdissected from human breast cancers exhibit a high frequency of genetic alterations, such as chromosomal regions of loss of heterozygosity (LOH) and somatic mutations.[26-28] A recent report also suggests that stromal fibroblasts that have undergone p53 loss are clonally selected during tumor progression, yielding a highly proliferative stroma.[29] However, an another report indicates that myofibroblasts isolated from human mammary breast carcinomas exhibit no detectable genetic alterations, as gauged by array CGH and SNP array analyses;[30] this suggests that any stably maintained phenotype may depend on epigenetic modifications of the genome, such as DNA methylation.[31] Alternatively, the stabilization of their phenotype may depend on some type of positive-feedback signaling of the sort created by autocrine signaling loops.

We note that our CAFs show no detectable aneuploidy as determined by karyotype analysis, no anchorage-independent growth in culture, and no tumorigenicity in vivo. Moreover, some of the CAFs begin to senesce after 15 PDs in culture, similar to the behavior of normal human stromal fibroblasts.

In Figure 2, we propose three alternative models for the evolution of the stromal fibroblasts present within invasive human carcinomas: (1) acquisition of genetic alterations (e.g., p53 loss) may allow clonal selection of a small population of fibroblasts or progenitors that have undergone such alterations; (2) populations of normal stromal fibroblasts recruited into a tumor may trans-differentiate into CAFs without acquiring any genetic alterations. Such a process would mimic that occurring during wound healing, suggesting the possibility that CAFs are essentially equivalent to the myofibroblasts present in sites of wound healing and chronic inflammation. If this were so, populations of CAFs might well be polyclonal, and examinations of their clonality may be helpful in discriminating between these alternative mechanisms; and (3) stromal myofibroblasts are recruited from specialized circulating progenitor cell types.

In the event that discrete epi/genetic alterations are indeed identified in populations of CAFs, it will be important to know whether these recur from one tumor to the next, and if so, to determine the genes and signal pathways that may be responsible for creating the CAF phenotype. In addition, it will be important to know whether such identified changes are found among various types of CAFs, such as those present in the stroma of prostate, colon, and lung carcinomas. Since we know relatively little at present about such alterations, it is even possible that each population of CAFs harbors a unique set of epi/genetic alterations that differ from all other populations, including those from the same type of tumor. Indeed, we do not even know whether CAFs from breast carcinomas are capable of promoting the proliferation of prostate carcinoma cells and vice versa.

WHAT CELLS ARE THE PRECURSORS OF CAFS?

CAFs might originally derive from several types of cells, such as pre-existing normal fibroblasts, myofibroblasts, preadipocytes, smooth muscle cells, or bone marrow-derived progenitor cells.[32,33] Importantly, CAFs are unlikely to be derived from carcinoma cells via epithelial-mesenchymal transitions (EMTs), because CAFs often fail to exhibit karyotypic alterations and are nontumorigenic.

We wished to assess whether CAFs might evolve from preexisting normal fibroblasts, presumably through interaction with the carcinoma-generated microenvironment. To do so, normal human mammary fibroblasts were comingled with human breast carcinoma cells and the mixtures were injected subcutaneously into immunodeficient mice. The admixed parental human fibroblasts were then extracted from the advanced human breast tumor xenografts. We have found that the initially present normal human stromal fibroblasts are converted into CAFs that secrete elevated levels of SDF-1, include far larger populations of myofibroblasts as gauged by α-SMA expression, and exhibit tumor-enhancing powers when mixed subsequently with weakly tumorigenic carcinoma cells. Consequently, these experimentally generated CAFs closely resemble those prepared from actual human mammary carcinomas (Orimo et al, manuscript in preparation). Taken together, these findings suggest that pre-existing normal fibroblasts have acquired such phenotypes to become CAFs during the course of tumor progression.

Almost nothing is known about the molecular determinants of this fibroblast-to-myofibroblast conversion that appears to have yielded the population of CAFs. Is the observed conversion to myofibroblasts functionally relevant to the ability of CAFs to promote tumor growth? Do myofibroblasts secrete elevated levels of growth factors or cytokines besides SDF-1 that enable them to promote carcinoma growth? Do myofibroblasts also promote angiogenesis through their production of the large amounts of ECM proteins? What signal molecules upregulate the observed SDF-1 expression in CAFs?

IMPLICATIONS FOR THERAPY AGAINST STROMAL FIBROBLASTS

Recent reviews have emphasized the advantages of therapeutic targeting of the tumor-associated stroma[34-36] because (i) the ongoing functioning of stromal cells is presumably critical to the growth of nearby neoplastic cells and (ii) the stromal cells are stable genetically in contrast to carcinoma cells, which are genetically unstable and accumulate adaptive mutations during the course of therapy in order to acquire drug resistance.

Fibroblasts and myofibroblasts are the major cell types present in tumor stroma and various types of invasive human carcinomas usually include large numbers of these cells, which help to promote tumor growth and angiogenesis. Moreover, a subset of the gene expression profiles produced by serum-stimulated activated fibroblasts, termed the "wound-response signature" of these cells, is associated with poor prognosis when displayed by the tumor-associated stromal cells of human breast cancer patients.[37] Such observations, taken together with the observations described above, suggest that stromal fibroblasts represent an attractive target for therapeutic intervention.[34,38]

At present, relatively few observations support the notion that significant numbers of stromal fibroblasts and myofibroblasts, detected through histopathology, are correlated with poor prognosis of human carcinoma patients;[39,40] in contrast, a far larger number of studies demonstrate a positive correlation of increased angiogenesis and stromal inflammation to poor clinical outcomes. Moreover, the nature of the molecules that convey heterotypic signals between the stromal and epithelial compartments are largely obscure, precluding a systematic development of such agents. Ultimately, understanding

the complex molecular networks among various types of stromal cells and tumor cells in the carcinoma mass is likely to provide highly useful information for the therapeutic targeting of human carcinomas.

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