Paracrine Signaling between Carcinoma Cells and Mesenchymal Stem Cells Generates Cancer Stem Cell Niche via Epithelial – Mesenchymal Transition

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IN THE SPOTLIGHT

Paracrine Signaling between Carcinoma Cells and Mesenchymal Stem Cells Generates Cancer Stem Cell Niche via Epithelial–Mesenchymal Transition

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Summary: Li and colleagues present data that cancer cell–derived interleukin-1 induces prostaglandin E2 and cytokine secretion in mesenchymal stem cells (MSC) to activate β-catenin signaling in the cancer cell. This paracrine signaling between carcinoma cells and MSC leads to the creation of a cancer stem cell niche via epithelial–mesenchymal transition. Cancer Discov; 2(9):775–7. ©2012 AACR.

Commentary on Li et al., p. 840 (6).

Tumorigenesis is a multistep process, and carcinomas—cancers of epithelial origin—were long viewed as a clonal disease. However, the emergence of the tumor microenvironment’s role in cancer progression first proposed 10 years ago by Bissell and Radisky (1) brought to light the need to study each component of the stroma. The tumor stroma is the compartment that serves as a tissue framework, consisting of the vasculature (endothelial cells, smooth muscle cells, and pericytes), inflammatory and immune cells (macrophages, lymphocytes, and dendritic cells), and cancer-associated fibroblasts (CAF). Mesenchymal stem cells (MSC) are recruited from the bone marrow to the stroma, a process that is mediated mainly by inflammatory factors present in the tumor microenvironment (2). Once recruited to the tumor, MSCs can act as precursors for CAFs, which represent a distinct cell type characterized by heterogeneous expression of α-smooth muscle actin, fibroblast activation protein, and fibroblast-specific protein 1 (α-SMA, FAP, and FSP1, respectively). CAFs secrete a variety of inflammatory cytokines, growth factors, and proteinases, all of which contribute to tumor progression. These factors include interleukins (IL), chemokines C-X-C motif ligand cytokines (CXCL), vascular endothelial and hepatocyte growth factors (VEGF and HGF), and matrix metalloproteinases (MMP) that affect the surrounding components of the tumor microenvironment, including cancer cells, vascular cells, and immune cells. In addition, factors secreted by CAFs modify the extracellular matrix (ECM) by releasing collagens (COL), fibronectin (FN), and periostatin (POSTN) that further act on the cancer cells (3).

Although the existence of cancer stem cells (CSC, or alternatively called tumor-initiating cells, TIC) in carcinomas has been commonly accepted, the origin of CSCs remains under debate, and epithelial–mesenchymal transition (EMT) has been proposed as one possible mechanism (4). The hierarchical model of clonal evolution does not take into account the heterogeneity frequently observed in cancers, thus the role of the tumor microenvironment in creating dynamic subpopulations presents an alternative model (5). In this issue of Cancer Discovery, Li and colleagues (6) provide evidence that MSCs can directly promote EMT in the carcinoma cells that in turn leads to entrance into a stem cell–like state.

Using Transwell co-culture assays, the authors showed a significant increase in COX-2 expression that led to enzymatic conversion of arachidonic acid to prostaglandin E2 (PGE2) in MSCs. Concomitantly, they observed secretion of IL-1 by a panel of human colorectal and breast cancer cell lines. Similar increases in PGE2 production in MSC cells were observed when recombinant IL-1 was used. Conversely, IL-1 neutralizing antibody or a recombinant antagonist of the IL-1 receptor (IL-1ra) decreased the ability of MSCs to produce PGE2. Notably, the intracellular levels of IL-1 and IL-1ra were heterogeneous between the cell lines, and only cells that secreted IL-1 were able to induce PGE2 in MSCs. In concert with COX-2 and PGE2, levels of Gro-α (CXCL1), IL-6, and IL-8 were induced in MSCs by LoVo colorectal carcinoma cell-conditioned medium or by adding exogenous IL-1 to the MSC cultures. IL-1 short hairpin RNA–expressing LoVo cells attenuated the PGE2 production; levels of Gro-α, IL-6, and IL-8 were also decreased in MSCs. Furthermore, the COX-1/COX-2 inhibitor indomethacin and PGE2 cell surface receptor antagonists AH6809 and GW627368X (against EP2 and EP4, respectively) reduced the production of the aforementioned cytokines in both LoVo-conditioned medium and IL-1–treated MSC cells. These data confirmed the necessary role of cancer cell–derived IL-1 in driving PGE2 synthesis in the MSCs and that the cytokine production in the MSCs was dependent on the autocrine secretion of PGE2. In line with these results, a recent study by Alcolea and colleagues (7) showed that head and neck squamous cell carcinoma–derived IL-1 leads to upregulation of COX-2 and PGE2 biosynthesis in fibroblasts.

Previous work by the Weinberg laboratory has proposed a possible presence of mesenchymal-like cells that, via EMT, have gained stem cell properties in mammary carcinoma (8). In that study, EMT was induced either by TGF-β, a well-characterized inducer of EMT, or by forced expression...
of the EMT transcription factors Twist or Slug. This led to a cadherin switch from E- to N-cadherin expression and de novo synthesis of the mesenchymal proteins vimentin and fibronectin. Importantly, the majority of the mesenchymal cells acquired a CD44hi/CD24lo expression pattern that is frequently observed in mammary stem cells. In light of this, Li and colleagues (6) investigated whether MSC co-cultures led to EMT and a CSC phenotype in the carcinoma cells. Fluorescence-activated cell-sorting (FACS) analysis of colon carcinoma/MSC co-cultures revealed a significant decrease of E-cadherin expression in LoVo cells, with simultaneous upregulation of Snail, vimentin, and fibronectin, thus confirming induction of EMT in the cancer cells. Similar results were obtained when LoVo cells were treated with PGE2 together with Gro-α, IL-6, and IL-8. However, PGE2 or the cytokines alone were not able to elicit a complete induction of EMT, thus suggesting the requirement of multiple signals acting at the same time. The mesenchymal phenotype obtained via EMT significantly augmented LoVo cell migration in a Transwell assay, an effect that was reversed by the selective COX-2 inhibitor NS398 and partially rescued by addition of PGE2.

In vivo experiments conducted by Li and colleagues (6) revealed that co-injecting carcinoma cells with MSCs formed more invasive tumors and led to intravasation of LoVo cells into microvessels. Furthermore, extreme limiting dilution analysis (ELDA) uncovered that the frequency of TICs increased by several magnitudes in xenografts containing both LoVo and MSC cells, corroborating data published by Rudnick and colleagues (9) showing the expansion of the mammary CSC population by PGE2-secreting fibroblasts. What remains to be answered by these studies is whether the increased number of CSCs contributes also to metastasis, which is implied by the presence of carcinoma cells in the nearby microvessels.

Aldehyde dehydrogenase 1 (ALDH1) expression is considered to be a marker for identifying cancer stem cells. ALDH1 acts as a detoxifying enzyme in cells, thus contributing to therapeutic resistance (10). Co-culturing colorectal carcinoma cells with MSCs increased ALDH1 expression in the cancer cells and expanded the ALDHhi subpopulation, but only if the cancer cells were propagated continuously in the presence of MSC cells. To further characterize this subpopulation, the authors used a second commonly accepted CSC marker, CD133, a transmembrane glycoprotein known to be expressed in hematopoietic stem cells. As with the ALDHhi subset, the number of ALDHhi/CD133+ LoVo cells increased in MSC co-cultures. To further dissect the role of this stem cell–like population in tumor propagation, ALDHhi cells were injected subcutaneously into nude mice. ELDA calculations revealed increased TIC frequency in ALDHhi LoVo cells, an effect that was further augmented by co-injection of MSCs. The tumor-initiating capacity of ALDHhi carcinoma cells was also increased by the presence of MSC cells. This effect was mediated by the PGE2 produced in MSCs, and the EP4 antagonist was able to reduce the levels of ALDH1 in LoVo cells. In addition, ex vivo treatment of LoVo cells with PGE2 increased the number of ALDHhi cells and TIC frequency in severe combined immunodeficient (SCID) mice. This effect was abolished when PGE2 synthesis was inhibited, either with small-molecule inhibitors or with RNA interference directed against COX-2. In the xenografts, ALDHhi LoVo cells were surrounded by COX-2- and FSP-expressing MSC cells, confirming the notion that within the tumor microenvironment MSCs act as precursors for CAFs (3). These data suggest a critical role of MSC-derived PGE2 in maintaining the ALDHhi cancer cell–like subpopulation in carcinomas. Furthermore, in clinical samples, COX-2 expression correlated with IL-1 expression, with higher expression reflecting more aggressive breast and colon carcinoma phenotypes.

To further dissect the mechanism by which PGE2 elicits these functions, Li and colleagues (6) investigated the downstream signaling pathways that are known to maintain the CSC phenotype, among them β-catenin signaling. In the absence of Wnt, glycogen synthase kinase 3 (GSK-3) phosphorylates β-catenin at Ser33/Ser37/Thr41 residues, thus marking it for proteasomal degradation. PGE2 treatment of LoVo cells activated Akt at Thr473, which in turn increased β-catenin phosphorylation at the Ser552 residue, thus rendering it inaccessible for GSK-3. In addition, PGE2 increased GSK-3 Ser21 phosphorylation, thus decreasing GSK-3 activity. The EP4 receptor antagonist GW627368X was able to reverse these effects, leading to degradation of β-catenin. Similar effects were observed when PGE2-induced ALDHhi LoVo cells were treated with the phosphoinositide 3-kinase (PI3K) inhibitor LY294002, confirming the role of Akt in mediating β-catenin signaling. Downregulation of E-cadherin, orchestrated by PGE2-induced EMT, released β-catenin bound in adherens junctions and enabled its translocation into the nucleus, thus upregulating β-catenin/T-cell factor target genes, including the pluripotency regulators Nanog, Oct4, and Sox2. These data further strengthen the observation that PGE2 plays a role in creating and maintaining a cancer stem cell niche via EMT.

The cancer stem cell model has manifested new implications for cancer therapy. In addition to ALDH, many CSCs have been found to express adenosine triphosphate–binding cassette (ABC) transporters, which efficiently pump out various chemotherapeutic drugs, leading to resistance (5, 10). In addition, other components of the tumor microenvironment are known to confer drug resistance, such as leaky microvessels generated by reactivation of angiogenesis in response to targeted therapy. The key findings of Li and colleagues (6), put in context with other interactions between carcinoma cells and components of the tumor microenvironment discussed in this commentary, are summarized in Fig. 1. As is clear from the schematic illustration, a variety of factors contribute to the generation of a cancer stem cell niche, and importantly, all of these subpopulations should be targeted to achieve effective therapeutic responses. Relevantly, the data presented here by Li and colleagues (6) offer an insight into how targeting one component of the tumor could affect another component and thus lead to tumor regression. IL-1 inhibitors are used clinically to treat inflammatory conditions and are currently in early clinical trials for various solid cancers. In addition, COX-2 inhibitors are currently being investigated for the treatment of carcinomas, although severe side effects have been reported. In the future, the goal of personalized therapy should focus on targeting the various cell types present in the tumor microenvironment, thus emphasizing the need for systematic preclinical testing of combination therapies.
Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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