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Paracrine tumor signaling induces transdifferentiation of surrounding fibroblasts

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ABSTRACT

Growth stimuli in cancer growth resemble those exhibited in wound healing. However, the process of nemosis is absent in cancer-associated fibroblasts (CAFs), which remain constitutively active. CAFs are present in almost all solid tumors but are most abundant in breast, prostate and pancreatic cancers. TGF-β1, TGF-β2, PDGF, IL-6, bFGF, reactive oxide species and protein kinase C are considered the key players in tumor-induced transdifferentiation of surrounding fibroblasts. Full-extent transdifferentiation was obtained only when the medium contained TGF-β1 or TGF-β2 (with or without other factors), whereas PDGF, bFGF or IL-6 (each alone) induced only partial transdifferentiation. Recent evidence suggests that the fibroblasts associated with primary cancers differ from those associated with metastases. The metastases-associated fibroblasts are converted by a metastasis-specific spectrum of factors. A large portion of paracrine tumor signaling is mediated by cancer cell-derived vesicles termed exosomes and microvesicles. The cancer cell-derived exosomes contain abundant and diverse proteomes and a number of signaling factors (TGF-ß1, TGF-ß2, IL-6, MMP2 and MMP9), particularly under hypoxic conditions. In contrast to the traditional view, the clonal expansion and selection of neoplastic cells should not be viewed outside the host body context. It is vital for a neoplastic cell to achieve the ability to re-program host body cells into CAFs and by this influence to modulate its microenvironment and receive positive feedback for growth and drug resistance. Neoplastic cells, which fail to develop such capacity, do not pass critical barriers in tumorigenesis and remain dormant and benign.

1. Introduction

Already in 1924, Montrose T. Burrows noticed that the growth stimuli in wound healing resemble those in cancer growth (Burrows, 1924). However, this claim is commonly attributed to

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Harold F. Dvorak, who authored the famous phrase "tumors are wounds that do not heal", which was published only in 1986. Healing processes are characterized by the activation of otherwise quiescent fibroblasts and their corruption to myofibroblasts, which are to some extent similar to cancer-associated fibroblasts (CAFs). Both myofibroblasts and CAFs express α-smooth muscle actin (αSMA) and the ED-A splice variant of fibronectin. However, wound healing of the wound results in programmed cell death (nemosis) of myofibroblasts, whereas tumors do not heal spontaneously. Thus, CAFs remain constitutively active.

2. Three-step process of CAF corruption

CAFs are found in almost all solid tumors. CAFs are highly abundant in breast, prostate and pancreatic cancers, whereas CAFs are not as prevalent usually in brain, renal and ovarian cancers (Neesse et al., 2011; Smith et al., 2013). Although CAFs form numerous subpopulations, which differ both between and within tumors, their classification is poorly understood. CAFs express several markers, including αSMA, fibroblast-specific protein-1 (FSP-1), platelet-derived growth factor (PDGF) receptors-β and fibroblast activating protein (FAP). Importantly, fibroblasts and cancer cells affect each other *via* extensive paracrine signaling, which involves dozens of secreted proteins and peptides (Table 1). CAF corruption is thought to be a three-step process. First, the distant precursor cells are recruited by the malignant or pre-malignant cells. Second, the precursors are converted from ostensibly normal cells into CAFs. Finally, the third signal is the persistence of the CAFs in the cancer microenvironment (De Wever et al., 2014).

Half a century ago, Michael G. P. Stoker demonstrated that normal quiescent fibroblasts inhibit the growth of transformed cells by direct contact between the two cell types (Stoker et al., 1966), which was later corroborated by numerous other authors. It was assumed that this negative regulatory role of normal tissue-associated fibroblasts is mediated by their ability to maintain epithelial homeostasis and proliferative quiescence (Trimboli et al., 2009), in part because of the formation of tight junctions between the normal fibroblasts or CAFs (Hinz et al., 2007). Thus, the tumor must to both recruit the fibroblasts from various sources discussed below, and re-program them using paracrine signaling to transform them into CAFs (Table 1), thereby converting them from tumor-suppressive to tumor-supportive (Öhlund et al., 2014). Following the corruption, the CAF phenotype can persist even in the absence of continued exposure to paracrine signaling from cancer cells (Orimo et al., 2005). CAF activity contributes to disease progression and is associated with more malignant phenotypes of experimentally induced tumors.

3. Central role of TGF-ß in CAFs corruption from epithelia, endothelia and other sources

Several cytokines, particularly TGF-β1, are responsible for the transdifferentiation process. TGF-ß is involved in a specialized epithelial-mesenchymal transition (EMT) process of converting epithelial cells to myofibroblasts (Petersen et al., 2003) by activating the EMT-associated pathways that involve Smad, PI3K/Akt, RhoA and p38 MAPK. This, in turn, leads to the disruption of the E-cadherin-β-catenin complex, loss of epithelial E-cadherin and gain of mesenchymal markers, including N-cadherin, vimentin, αSMA, FSP-1 and desmin, as detailed below (Bakin et al., 2000; Heldin et al., 2012; Smalley et al., 2005; Paraiso and Smalley, 2013).

Treatment of endothelial cells with TGF-β (together with bone morphogenetic protein (BMP)) can also stimulate the induction of endothelial cell-derived CAFs (Medici and Kalluri, 2012). The induction of CAFs from endothelial cells involves an incomplete EMT in which the affected cells retain low levels of endothelial markers, including VE-cadherin, CD31, TIE1 and TIE2 kinases, von Willebrand factor (vWF) and cytokeratins. The cells simultaneously upregulate mesenchymal markers, including αSMA, FSP-1, vimentin and *N*-cadherin (Medici and Kalluri, 2012). Endothelial cells may serve as major precursors of CAFs. In the murine models of B16-F10 melanoma and Rip-Tag2 spontaneous pancreatic carcinoma, over 40% of CAFs originated from endothelial cells (Zeisberg et al., 2007).

In addition to mesenchymal stem cells (MSCs), endothelia and epithelia, vessel-associated αSMA-expressing pericytes and adipocytes are considered alternative precursors of CAFs (Armulik et al., 2011; Bochet et al., 2013). Stellate cells are fibroblast-like vitamin A-storing and lipid droplet-containing cells of the liver, pancreas, kidney, intestine, lung, spleen, uterus and skin. They can activate αSMA expression upon stimulation and acquire a myofibroblast- or CAF-like phenotype. Stellate cells are responsible for most of the desmoplasias associated with pancreatic cancer, chronic pancreatitis and liver fibrosis (Bachem et al., 1998; Yin et al., 2013). Additionally, bone marrow-derived fibrocytes can be recruited to the tumor and differentiate into either myofibroblasts or CAFs (Ishii et al., 2003; Direkze et al., 2004; Mishra et al., 2008; Kidd et al., 2012).

The induction of fibroblast proliferation (desmoplasia) by TGF-β1 is partially indirect and mediated by the upregulated expression of extracellular matrix proteins (including collagen type I) and growth factors (connective tissue growth factor, PDGF, VEGF, or IL-6) in the cancer cells. Corroborating the existence of indirect TGF-β1 effects, application of PDGF-α or PDGF-β also mediates desmoplasia. In a model of breast carcinoma, breast carcinoma-secreted PDGF is a major initiator of tumor desmoplasia, and it supports the fibroblast expression of the characteristic markers of desmoplasia, stromelysin-3, IGF-II and TIMP-1. 17-β estradiol facilitates tumor growth but abolishes desmoplasia in this model.

There are already several clinically tested or already approved drugs that target TGF-β or the TGF-β-associated signaling pathways. In Europe, perfenidone, which is currently approved to treat idiopathic pulmonary fibrosis, targets the transcriptional regulation of TGF-ß, and leads to reduced fibroblast and inflammatory cell infiltration (Paraiso and Smalley, 2013; Iyer et al., 1999). The Alk5 inhibitors SB-431542 and SB-505124 inhibits TGF-β-mediated cell proliferation and CAF-mediated angiogenesis in models of osteosarcoma and esophageal squamous cell carcinoma (Noma et al., 2008; Laping et al., 2002). However, long-term TGF-β therapy is expected to be associated with deficient wound healing, dysbalanced epithelial homeostasis and possibly has even tumor promoting effects (Paraiso and Smalley, 2013).

4. Other cancer cell-derived factors contributing to CAFs corruption

In addition to TGF-ß, bFGF induces a robust growth response and αSMA, but not collagen type I or fibronectin, expression in human kidneys. This suggests a possible contribution of bFGF to CAF transdifferentiation in renal and prostate cancers (Armelin, 1973; Strutz et al., 1995; Kwabi-Addo et al., 2004). Phospholipid derivatives, such as lysophosphatidic acid (LPA), are secreted by certain cancer cells to induce differentiation of human MSCs into myofibroblast-like CAFs (Jeon et al., 2008).

IL-6 plays also a role in the transdifferentiation process. This proinflammatory cytokine is produced in high levels by cancer cells of various origins and is considered a marker of tumor aggressiveness. IL-6 induces a strong increase in FAP, but not αSMA, expression by fibroblasts concurrent with the increase in extracellular matrix secretion and autocrine IL-6 production by the CAFs