The Unholy Trinity: Inflammation, Cytokines, and STAT3 Shape The Cancer Microenvironment

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Tumor-associated inflammation is a consequence and a driver of tumorigenesis. Three papers in this issue of *Cancer Cell* demonstrate the importance of tumor-elicited inflammation in the development and progression of pancreatic ductal adenocarcinoma and esophageal squamous carcinoma. Disruption of tissue homeostasis culminates in activation of STAT3, generating a pro-tumorigenic inflammatory microenvironment.

One hundred and fifty years after its initial description by Virchow, tumor-associated inflammation has been recognized as an important hallmark of cancer (Hanahan and Weinberg, 2011). Inflammation contributes to almost every aspect of tumor development (Grivennikov et al., 2010). Inflammation's role in tumor promotion has been extensively studied, and recent evidence supports its function in tumor recurrence and metastasis (Tan et al., 2011). However, the role of inflammatory signals in tumor initiation has been difficult to address, in part due to the paucity of adequate experimental models.

Most inflammatory signals affect tumorigenesis by activating NF-κB and STAT3 (Grivennikov et al., 2010). Persistent STAT3 activation in malignant cells stimulates proliferation, survival, angiogenesis, invasion, and tumor-promoting inflammation. Moreover, STAT3 activation within immune cells enables suppression of antitumor immunity and promotes differentiation and recruitment of immature myeloid cells (iMCs) and tumor-associated macrophages (TAMs) (Yu et al., 2009). Although in many cancers STAT3 is not directly activated by oncogenic mutations, it exerts critical oncogenic functions in both cancer and immune cells within the microenvironment.

Two papers in this issue demonstrate that STAT3 activation is essential for initiation and progression of pancreatic ductal adenocarcinoma (PDAC) (Fukuda et al., 2011; Lesina et al., 2011). In the $Kras^{G12D}$ mouse model that develops pancreatic intraepithelial neoplasia (PanINs) with high penetrance, with some of which eventually progresses to PDAC (Hingorani et al., 2003), STAT3 in pancreatic epithelial cells promotes tumor development and progression, as its ablation results in smaller lesions, lower tumor grade, and fewer metastases. Importantly, both groups demonstrate that STAT3 ablation in pancreatic epithelial cells attenuates $Kras^{G12D}$-induced PanIN formation, concluding unequivocally that epithelial STAT3 is important for tumor initiation (Fukuda et al., 2011; Lesina et al., 2011).

Effects of STAT3 on tumor initiation or early promotion are likely related to its ability to control expression of anti-apoptotic and proliferative genes, thereby increasing the likelihood that $Kras^{G12D}$-expressing cells will survive and proliferate instead of undergoing oncogene-induced senescence or apoptosis. Indeed, STAT3 in pancreatic epithelial cells controls expression of Bcl-X_L, Mcl1, survivin, c-Myc, and cyclin D1, and STAT3 deficiency decreased proliferation and increased apoptosis (Fukuda et al., 2011; Lesina et al., 2011). Equally important is STAT3's ability to promote conversion of quiescent adult pancreatic epithelial cells to cells with a progenitor-like phenotype upon pancreatitis or other insults (Fukuda et al., 2011), a process often referred to as "acinar-ductal metaplasia" (ADM). As a result of ADM, more ductal cells expressing progenitor cell markers, such as Pdx1 or Hes1, are formed, and these cells are more susceptible to KRas-mediated transformation (Murtaugh and Leach, 2007). Chronic pancreatitis carries increased risk for pancreatic cancer in humans and significantly accelerates KRas-induced tumorigenesis in mice (Guerra et al., 2007). Hence, reprogramming of normal pancreatic epithelial cells into progenitors that can assume a neoplastic fate may be a key step in PDAC initiation. Importantly, activation of STAT3 was observed during pancreatic regeneration in the context of acute pancreatitis and in $Kras^{G12D}$-induced PanIN lesions.

As for STAT3's role in pancreatic tumor promotion and progression, several mechanisms were identified. The first is the well-described ability of STAT3 to sustain cell proliferation and block apoptosis (Lesina et al., 2011). The second depends on a STAT3 target gene encoding MMP7, whose ablation in the pancreas of $Kras^{G12D}$ mice limits tumor size, progression, and metastasis but does not affect ADM and PanIN development (Fukuda et al., 2011). Other studies, nevertheless, suggest that MMP7 may also be involved in early tumorigenesis, as it is needed for pancreatitis and TGFα-induced ADM (Sawey et al., 2007). Furthermore, epithelial cell STAT3 orchestrates tumor-associated inflammation by upregulating chemokines capable of attracting immune and inflammatory cells that further propagate STAT3 activity through production of IL-6, IL-11, and other cytokines (Fukuda et al., 2011; Lesina et al., 2011; Yu et al., 2009). STAT3 ablation in the pancreas prevents macrophage recruitment during pancreatitis and tumor initiation (Lesina et al., 2011) and decreases total inflammatory infiltration and expression of proinflammatory cytokines during acute pancreatitis, which often precedes tumor development (Fukuda et al., 2011).

The ability of STAT3 to control and shape the tumor microenvironment

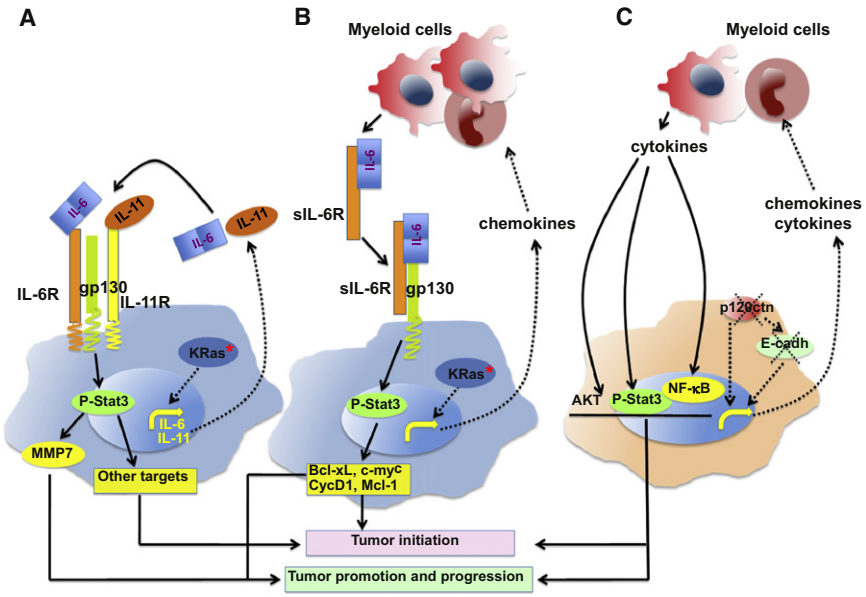

Figure 1. Mechanisms of STAT3-mediated tumorigenesis

(A) Fukuda et al. (2011) suggest that KRas activation drives epithelial cell expression of cytokines, such as IL-6 and IL-11, which in turn activate STAT3 in an autocrine fashion. STAT3-induced MMP7 is required for tumor progression but not for tumor initiation, which depends on other STAT3 targets.

(B) Lesina et al. (2011) suggest that epithelial cells harboring active KRas recruit immune cells, particularly myeloid cells, which produce IL-6 and soluble IL-6R and activate STAT3 in epithelial cells via IL-6 trans-signaling in a paracrine manner. STAT3 induces antiapoptotic and proproliferative genes, fueling tumor initiation, promotion, and progression.

(C) Stairs et al. (2011) show that loss of p120-catenin in epithelial cells alters local tissue homeostasis, generating a protumorigenic microenvironment through the recruitment of iMCs and other immune cells. Recruitment of these cells is presumably mediated by cytokines and chemokines produced by mutant epithelial cells, eventually causing activation of Akt, NF-κB, and STAT3 in neoplastic cells.

directly impacts the question of how STAT3 is activated during pancreatic tumorigenesis. Fukuda et al. (2011) found that STAT3 in pancreatic cells directly affects expression of IL-6 and IL-11, both of which are STAT3 activating cytokines. Persistent expression of IL-6 or other STAT3-activating cytokines by epithelial cells may be responsible for KRas-induced STAT3 activation. Lesina et al. (2011), however, suggest that bone marrow-derived cells, particularly TAMs, are the major source of STAT3-activating cytokines during PDAC development. They found that STAT3 is activated in cancer cells and stromal cells in the tumor microenvironment but not in isolated cancer cell lines, suggesting a non-cell-autonomous activation of STAT3. Consistent with this, two STAT3 activating cytokines, IL-6 and LIF, were found to be robustly produced upon KRas activation (Lesina et al., 2011). Ablation of IL-6 in $Kras^{G12D}$ mice demonstrated that IL-6 is one of the key STAT3 activators during PanIN initiation and progression. IL-6 uses two major modes of signaling: classical signaling through ligation of IL-6 receptor (IL-6R) and gp130 on the surface of the target cell; and trans-signaling through binding of IL-6 to a soluble form of IL-6R (sIL-6R), with subsequent binding of the IL-6-sIL-6R complex to any gp130-expressing cell. Transgenic mice that overexpress a circulating sgp130Fc fusion protein, which inhibits IL-6 trans-signaling but not classical signaling, exhibit attenuated $Kras^{G12D}$-driven PanIN progression and STAT3 activation in cancer cells, suggesting that IL-6 trans-signaling drives PanIN progression (Lesina et al., 2011). Conversely, ablation of the gp130-signaling inhibitor SOCS3 increased STAT3 activation and accelerated PanIN progression (Lesina et al., 2011). In human PDAC patients, as in many other cancers, circulating IL-6 is elevated and $STAT3^{Y705}$ phosphorylation is observed in PanIN lesions and acinar cells in tumor-adjacent tissue. An interesting and important question is whether SOCS3 expression is downregulated during PDAC development, as observed in liver and colon cancers. As in the mouse model, myeloid cells and TAMs appear to be the main sources of IL-6 in human PDAC (Lesina et al., 2011). In conclusion, both epithelial and immune cells produce protumorigenic STAT3-activating cytokines to promote PDAC development (Figure 1).

The third paper demonstrates how p120-catenin loss in the oral cavity, esophagus, and forestomach results in desmoplasia, a local inflammatory response, and leads to the development of squamous esophageal cancer (Stairs et al., 2011). p120-catenin loss results in E-cadherin downregulation and activation of NF-κB, Akt, and STAT3 pathways that contribute to local inflammation and tumorigenesis. p120-catenin-deficient cancer cells secrete GM-CSF, M-CSF, MCP-1, and TNF, which contribute to pronounced immune cell infiltration and create a tumor-promoting microenvironment. Interestingly, the main populations of immune cells that are recruited to the microenvironment upon p120-catenin deletion are $Gr1^{+}CD11b^{+}$ iMC. The authors demonstrate that iMCs are protumorigenic, due to their effects on cancer cells and cancer-associated fibroblasts (CAF). There is no evidence, however, that iMCs, frequently referred to as myeloid suppressor cells, are engaged in immune suppression. It remains largely unclear how p120-catenin loss leads to activation of NF-κB, Akt, and STAT3 and what is the relative contribution of these distinct pathways to tumorigenesis and tumor-associated inflammation in this system. Nevertheless, the recruitment of iMCs suggests that p120-catenin loss causes upregulation of chemokines and cytokines, which may account for tumor-elicited inflammation (Figure 1).

Altogether, these studies extend our understanding and appreciation of tumor-elicited inflammation in cancer development, and further underscore the notion that STAT3's role in cancer extends beyond its well-appreciated effects on cell proliferation and survival. The role of STAT3 activation in immune cells, particularly myeloid cells, during PDAC development deserves further exploration, as does the cause of STAT3 activation in nearly all human PDAC cell lines, as it has to rely on cell-autonomous mechanisms.

The paradigm that emerges from these papers and others is that disrupted tissue

homeostasis and integrity, oncogene activation, or inflammatory insults result in the induction of chemokines and cytokines that shape the local microenvironment. Recruited immune cells and CAF, as well as altered epithelial and neoplastic cells, continue to produce cytokines (such as IL-6) that activate oncogenic transcription factors (i.e., STAT3), further sustaining tumor-associated inflammation. This paradigm applies not only to pancreatic or esophageal cancers, but also to the majority of solid malignancies. This implies that drugs that disrupt tumor-associated inflammation, either by targeting STAT3 activating kinases (e.g., JAK2) or key proinflammatory cytokines, such as IL-6, should have significant therapeutic and preventive effects in a variety of cancers, regardless of their origin.

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Multiple Effects of Angiopoietin-2 Blockade on Tumors

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In this issue of *Cancer Cell*, Mazzieri, Pucci, and colleagues describe the marked effects of inhibiting the proangiogenic cytokine, Angiopoietin-2, on tumor angiogenesis and progression in spontaneous tumor models, as well as the proangiogenic functions of TIE2-expressing macrophages.

A number of antiangiogenic agents have now been developed to inhibit vascular endothelial growth factor (VEGF) signaling pathway in tumors. Like the majority of existing anticancer therapies, their clinical efficacy is limited by the transient nature of their inhibitory effects on advanced tumors. In several mouse tumor models, VEGF inhibition results in marked suppression of tumor angiogenesis, which often leads to reduced tumor growth and even tumor shrinkage. However, tumors usually revascularize and grow back upon prolonged treatment. This escape from VEGF blockade is thought to be due to various mechanisms, including the upregulation of alternative proangiogenic growth factors, the enhanced invasive/metastatic activity of tumor cells (to locate an alternative blood supply), and the increased recruitment of proangiogenic bone marrow-derived cells to the tumor site, where they promote tumor revascularization and growth (Bergers and Hanahan, 2008).

A major finding in the latter area has been that certain inflammatory cell types convey tumor resistance to antiangiogenic therapies. For example, Shojaei et al. (2007) showed, in murine tumor models, that tumor-infiltrating CD11b+ Gr1+ myeloid cells can induce tumor resistance to VEGF therapy via their release of Bv8 (prokineticin-1), a proangiogenic cytokine stimulated by their exposure to tumor cell-derived granulocyte colony-stimulating factor (G-CSF). In this way, certain mouse tumors treated with anti-VEGF drugs are able to circumvent their dependency on VEGF and render themselves resistant to anti-VEGF therapy.

Proangiogenic myeloid cells are also thought to be recruited in response to the elevated release of such chemoattractants as CXCL12 (stromal cell-derived factor-1, SDF1) by hypoxic areas of therapy-damaged tumors (Kioi et al., 2010). Among the most essential of these are a highly proangiogenic subset of monocytes/macrophages that express the angiopoietin receptor, TIE2, and so are termed