Inactivation of Stat3 in tumor cells: Releasing a brake on immune responses against cancer?

A model of immune evasion mediated by tumors expressing constitutively activated Stat3 was recently proposed in *Nature Medicine* by Wang et al., suggesting opportunities for a new therapeutic approach for cancer.

Alterations in the expression or function of critical genes that tightly regulate cell cycle checkpoints, differentiation, cell survival, and apoptosis and which can subsequently lead to cell transformation and oncogenesis has long been a model for tumor development and progression. A refinement of this model involves failure of the host immune response to recognize tumor cells due to an inherent property of the tumor cells resulting in either a switch off, or an active inhibition, of crosstalk between innate and adaptive immunity, thus allowing for tumor development and progression (Ochsenbein, 2002).

Signal transducers and activators of transcription (Stat) belong to a family of latent cytoplasmic factors that are activated by tyrosine phosphorylation by members of the Jak tyrosine kinase family in response to a variety of cytokines and growth factors. Stats dimerize and translocate to the nucleus to induce expression of critical genes essential in normal physiological cellular events.

Several investigators have reported aberrant activation of Stat3 in a variety of human cancer cell lines, in primary tumors including lymphoid/myeloid malignancies and solid tumors as well as in cell lines transformed with v-Abl and v-Src (Calo et al., 2003). Nevertheless, this constitutive activated form of Stat3 is not due to mutations in Stat3. Constitutive Stat3 activity occurs due to deregulation of protein tyrosine kinases or constitutive release of growth factors that activate Stat3. Ablation of Stat3 function in transformed or tumor cells can reverse cell transformation and/or induce apoptosis in vitro. Interestingly, tumor regression has been achieved by introduction in vivo of a dominant-negative Stat3 in tumors expressing activated Stat3 (Niu et al., 1999). Furthermore, the role of Stat3 as a negative regulator of inflammatory responses became apparent when targeted deletion of Stat3 in specific tissues was achieved (Stat3 null mice were embryonic lethal). Deletion of Stat3 in normal macrophages led to overproduction of inflammatory cytokines (Takeda et al., 1999), whereas disruption of Stat3 during hematopoeisis resulted in severe inflammatory bowel disease and lethality (Welte et al., 2003).

Recently, Wang et al. (2004) reported in *Nature Medicine* an elegant model where Stat3 signaling in tumor cells plays a pivotal role in the regulation of tumor immunity (Figure 1). Using different tumor lines expressing constitutively activated Stat3, inhibition of Stat3 by expression of a dominant-negative form of Stat3, or by introducing antisense oligonucleotides leads to tumor expression of proinflammatory cytokines and chemokines needed for the activation of the innate immune response. Stat3 signaling in tumors inhibits the expression of inflammatory cytokines and evokes the release of factors that not only impede dendritic cell maturation and activation but also the generation of antigen-specific T cells, giving rise to immune tolerance. Interruption of Stat3 signaling in tumors reverses this negative effect, resulting in enhanced ability of dendritic cells to present antigen, and consequently leads to T cell activation and a break in T cell anergy. Activated dendritic cells had increased surface expression of MHC class II and CD40 molecules that correlated with production of IL-12 and T cell production of IL-2 and IFNg. Given these findings, it was noticeable that neutrophils and macrophages, the other components of the innate immune system, also showed restored function when incubated with supernatants derived from tumor cells where Stat3 signaling was blocked. Wang et al. also demonstrated that soluble factors released from tumors expressing activated Stat3 can activate Stat3 in dendritic cells, therefore preventing their maturation. Both IL-10 and VEGF signaling through Stat3 have been reported to inhibit dendritic cell maturation, and these factors are produced in cells transformed with v-Src, and transformation by v-Src results in increased

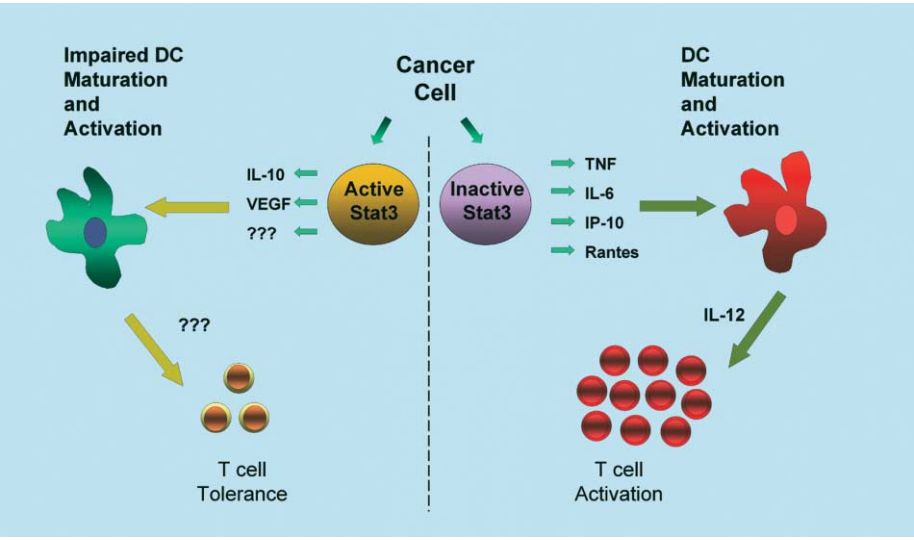

Figure 1. A model for the role of Stat3 in the regulation of tumor immunity

As shown in the left panel, activated Stat3 in cancer cells serves to constitutively inhibit T cell activation by inducing factors that impair DC maturation and activation, thus contributing to T cell tolerance. Conversely, as shown in the right panel, blocking Stat3 in tumor cells allows the production of factors that enable DC maturation and function, which contributes to T cell-mediated antitumor responses.

Stat3 activity. Although VEGF can weakly activate Stat3, the magnitude of Stat3 activation by IL-10 is higher in bone marrow progenitor cells (BMPCs). Consistent with these observations, Wang and coworkers demonstrated that activation of Stat3 in BMPCs by supernatants derived from tumor cells or by IL-10 leads to a reduction in dendritic cell maturation that was reversed with a phosphopeptide inhibitor of Stat3. In contrast, supernatant from Stat3 null BMPCs do not show any alterations in dendritic cell maturation, demonstrating a pivotal role for Stat3 in bridging innate and adaptive tumor immunity and in the reversal of systemic tolerance. It remains to be elucidated whether dendritic cells from IL-10 null mice are refractory to Stat3-mediated inhibition of dendritic cell maturation and whether there are other tumor-derived factors that need to be identified that signal through Stat3.

A recent study (Cheng et al., 2003) also presents strong evidence for pursuing Stat3 as a molecular target to enhance T cell immunity. These authors demonstrated that the crucial interaction of antigen presenting cells (APCs) with T cells resulting either in T cell activation versus T cell tolerance relies on the activation of Stat3 in those APCs. T cell tolerance can be reversed if activation of Stat3 signaling is blocked in APCs. This resulted in increased expression of MHC class II molecules and costimulatory molecules B7-1 and B7-2, expression of inflammatory cytokines, IL-12, and lack of IL-10 production by APCs.

Additional studies (Nefedova et al., 2004) demonstrate the involvement of Stat3 in abnormal differentiation of dendritic cells in cancer. Soluble factors from tumor cells expressing hyperactivated Stat3 prevented myeloid cell differentiation into dendritic cells. This was due to lack of downregulation of Stat3 activity in hematopoeitic progenitor cells and an increase in the proportion of immature myeloid cells. However, this inhibitory effect was reversible and could be prevented by expression of dominant-negative Stat3, resulting in a break in T cell tolerance.

Several mechanisms of tumor evasion by the immune system have been proposed (Ochsenbein et al., 2001). Impaired induction of antitumor cytolytic T cells (CTLs) might be due to deletion of antigen-specific CTLs by tumor cells or the inability of tumor cells to reach secondary lymphoid organs for CTL priming. Even if some tumor cells penetrate the lymphoid compartment, an immunological tolerant barrier exists, preventing T cells from recognizing the presence of tumor cells. One possible explanation for these observations is the generation of T cell tolerance mediated by tumor cells expressing activated Stat3.

Taken all together, these findings unveil Stat3 as a major player in linking innate and adaptive tumor immunity. STAT3 thus becomes a novel and important molecular target for therapeutic approaches to enhancing immune recognition and/or breaking T cell tolerance, thereby increasing the host's ability to impede cancer development and progression.

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Selected reading

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