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Reinforcing Suppression Using Regulators: A New Link between STAT3, IL-23, and Tregs in Tumor Immunosuppression

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STAT3 plays many roles in tumorigenesis. In this issue of *Cancer Cell*, Kortylewski et al. show that in the tumor microenvironment, STAT3 enhances the expression of the protumor cytokine IL-23 in macrophages but inhibits the antitumor cytokine IL-12 in dendritic cells. STAT3 also mediates IL-23's effect of activating tumor-infiltrating regulatory T cells.

The transcription factor STAT3 is overexpressed in tumor cells, stromal cells, and infiltrating hematopoietic cells of many types of tumors. When overexpressed in tumor cells, STAT3 contributes to their survival, proliferation, and dissemination. STAT3 activation favors proliferation of malignant cells by exerting an antiapoptotic effect, in part mediated by transcriptional downregulation of p53, and by inducing factors that drive angiogenesis and metastasis including VEGF and metalloproteases. Overexpression of STAT3 in tumors also has a major effect on recruiting tumor-infiltrating hematopoietic cells by controlling production of chemotactic factors and the expression of their receptors by infiltrating cells. Many factors released by the tumor and the tumor stroma, such as VEGF, interleukin-6 (IL-6), and IL-10, can play a role in STAT3 overexpression. Interestingly, several of these factors are themselves transcriptionally regulated by STAT3, thus creating a positive feedback regulation of their production (Yu et al., 2007).

Although STAT3 activation induces recruitment of hematopoietic cells, STAT3 activation in tumor-associated macrophages (TAMs) and dendritic cells (DCs) has a profound anti-inflammatory effect by preventing their complete maturation and blocking their ability to produce many proinflammatory cytokines such as IL-12 (El Kasmi et al., 2007; Yu et al., 2007). These contrasting activities are well suited for tumor growth because proinflammatory cells provide factors for stromal development and angiogenesis but strong inflammatory responses with antitumor and antiangiogenic effects are prevented. Indeed, progressing tumors in humans and experimental animals are characterized by the presence of infiltrating immature and anergic TAMs and DCs and a limited infiltration of T lymphocytes that often have the characteristic of regulatory T cells (Tregs). Through the production of IL-10 and TGF-β or direct cellular contacts, Tregs contribute to inactivation of antigen-presenting cells and suppress proliferation and antitumor activity of effector T cells, including IFN-γ-producing cells (Th1 cells) and cytotoxic T lymphocytes (CTLs) with antitumor activity (Zou, 2006).

Cytokines of the IL-12 family shape and control the outcome of inflammatory processes (Trinchieri et al., 2003). IL-12 is a heterodimeric cytokine composed of two covalently linked chains, IL-12a (p35) and IL-12b (p40). The IL-12 receptor is also composed of two chains, IL-12Rβ1 and IL-12Rβ2, which link to the kinases Tyk2 and Jak2 and signal prevalently through activation of STAT4. The main immunological functions of IL-12 are to induce IFN-γ production and to favor potent and long-lasting Th1 and CTL responses (Trinchieri et al., 2003). Because of these functions, IL-12 can have a strong antitumor effect. Under normal, unmanipulated conditions, hematopoietic cells in the tumor mass do not produce IL-12. Moreover, whereas macrophages and DCs of nonmalignant tissues are potent producers of IL-12 when stimulated through Toll-like receptors (TLRs) or TNF family receptors, TAMs and DCs are unable to produce IL-12 and other proinflammatory cytokines when exposed to the same stimuli (Vicari et al., 2002). IL-23 is composed of the same IL-12b chain covalently linked to the unique IL-23a (p19) chain (Trinchieri et al., 2003). IL-23 signals through a receptor composed of IL-12Rβ1 and IL-23R that also functionally links to Tyk2 and Jak2 but activates STAT3/STAT3, STAT3/STAT4, and in part STAT5/STAT5 dimers (Trinchieri et al., 2003). The expression of IL-12 and IL-23 is controlled at multiple levels. The genes encoding the two chains of these cytokines need to be simultaneously expressed in producing cells. The common IL-12β chain is normally produced in large excess, but the production of both heterodimers is limited not only by the amount of each chain but also by many posttranscriptional and posttranslational mechanisms.

IL-12 and IL-23 share some functions. However, it is now well established that IL-23, through its ability to induce STAT3, is involved in inducing IL-17 production and the pathogenic potential of the recently described Th17 cells (Trinchieri et al., 2003). Moreover, in contrast to the antitumor role of IL-12, production of IL-23 within the tumor has a tumor-promoting effect in both chemical carcinogenesis and transplantable tumor models, in part by preventing activation and function of CTLs (Langowski et al., 2007). At the time, the protumor potential of IL-23 was unexpected because of its role in driving Th17 cells that trigger tissue destruction and are associated with autoimmune conditions.

Kortylewski et al. (2009) now provide a novel framework with which we can revisit current knowledge of the complex molecular regulation by STAT3 and IL-23

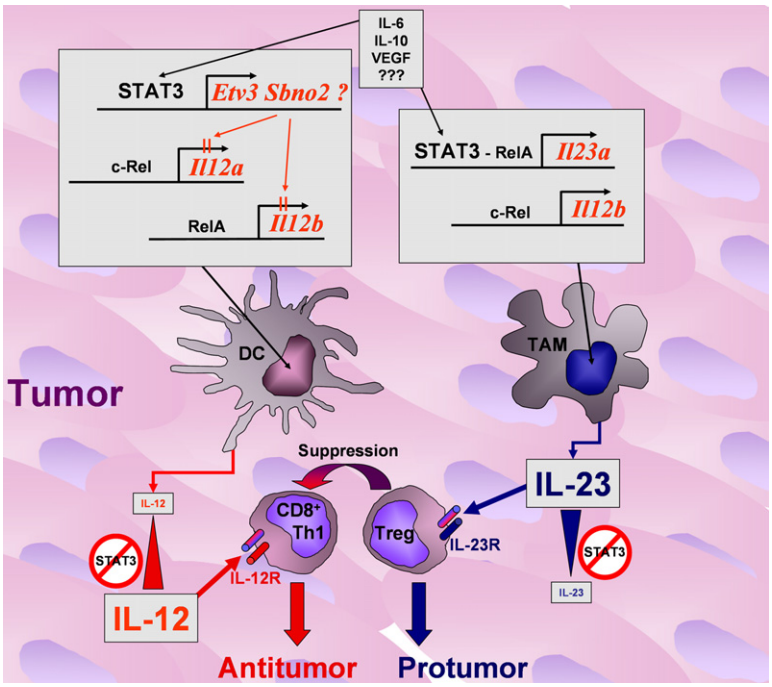

Figure 1. STAT3 Expression in Tumors Regulates IL-23 and IL-12 Production as Well as Treg Functions

STAT3 is overexpressed in many tumor types, favoring tumor growth and dissemination but also recruiting hematopoietic cells and inhibiting the maturation and function of tumor-associated macrophages (TAMs) and dendritic cells (DCs). In particular, the transcription of several proinflammatory genes such as the two genes encoding IL-12 in DCs is indirectly inhibited through the STAT3-dependent induction of the transcriptional suppressor *Etv3* and *Sbno2*. Conversely, the transcription of the *Il23a* gene and the production of IL-23 are directly induced by STAT3 in TAMs. Tumors contain a large number of immunosuppressive Foxp3$^+$ regulatory T cells (Tregs) that, unlike Tregs in other organs, express IL-23R and are functionally activated by IL-23. Deletion of STAT3 in hematopoietic cells induces the antitumor cytokine IL-12 while inhibiting the protumor cytokine IL-23 and the IL-23-dependent activation of Tregs, resulting in delayed tumor growth.

(Figure 1). They report that IL-23, but not IL-12, is spontaneously produced by TAMs in several transplantable mouse tumor models. Surprisingly, inactivation of STAT3 in hematopoietic cells results in the repression of IL-23 production by TAMs but activation of IL-12 production in DCs. The authors clearly demonstrate that, together with RelA (p65, an NF-κB component), STAT3 directly binds and activates the IL-23a (p19) promoter. NF-κB, through both RelA and c-Rel, was previously known to regulate the production of all three IL-12/23 chains, but a direct role for STAT3 in their activation had not been reported. A similar mechanism might occur when prostaglandin E2, which may also act though STAT3, inhibits IL-12 production while inducing IL-23 in DCs (Khayrullina et al., 2008). Also, nonphosphorylated STAT3 has been shown to bind to RelA-p50 dimers to induce a subset of genes such as *Rantes* (Yang et al., 2007). It would therefore be interesting to determine whether this noncanonical mechanism of NF-κB- and STAT3-induced gene transcription could be operational for IL-23. The IL-23-inducing effect of STAT3 observed by Kortylewski et al. was unanticipated because IL-10 inhibits both IL-12 and IL-23 production through STAT3 signaling. Why does STAT3 signaling promote IL-23 production under these circumstances but mediate a more global anti-inflammatory effect in other contexts? The inhibition of gene transcription by IL-10-induced STAT3 has been shown to be mediated indirectly through the ETS family transcriptional suppressor ETV3 and the helicase family corepressor SBNO2 rather than direct promoter binding to suppressed genes (e.g., *Il12a* and *Il12b*) (El Kasmi et al., 2007). IL-10 induces these repressor factors, but other STAT3-inducing cytokines such as IL-6 do not. Differential responses between IL-10 and other STAT3-inducing cytokines have been attributed to suppressor of cytokine signaling 3 (SOCS3), which is induced by high levels of STAT3 and affects function of the IL-6 receptor chain gp130 but not the IL-10 receptor. It is therefore noteworthy that TAMs and DCs can be driven to produce IL-12 with an antitumor effect by inhibiting IL-10 alone, suggesting that even if various factors induce STAT3 activation and expression in the tumor microenvironment, its anti-inflammatory effect critically depends upon IL-10 (Vicari et al., 2002).

Kortylewski et al. also observed an antitumor effect of STAT3 inhibition that was in part due to decreased activity of Foxp3$^+$ Tregs. Indeed, they showed that tumor-associated Tregs, unlike spleen Tregs, express IL-23 receptor (IL-23R) and are activated in response to IL-23, resulting in higher expression of Foxp3 and IL-10 production. This is surprising because in other settings, STAT3 activation by IL-6 or IL-21 in TGF-β-exposed T cells induces downregulation of Foxp3 and upregulation of the transcription factor RORγT leading to induction of IL-17-producing Th17 cells (Wei et al., 2008). STAT3 in the context of IL-23 signaling might also be expected to drive respecialization of Tregs to a Th17 phenotype. Although very few Th17 cells were detected in the tumor models studied, their development was completely dependent on STAT3. Several explanations can be evoked to explain this Treg-biased profile in the context of IL-23: for example, SOCS3 may limit IL-6 and IL-21 signaling through STAT3 in tumor-infiltrating T cells, thus preventing Th17 conversion, while IL-23-induced STAT5 activation persists. Alternatively, other elements (e.g., excess of TGF-β, IFN-γ, and/or IL-2 or lack of IL-1) in the tumor microenvironment may affect the ability of IL-23 and/or IL-6 to induce Th17 differentiation (Wei et al., 2008).

In conclusion, the inflammatory tumor microenvironment appears quite distinct from that of autoimmune tissues, where Tregs or Th17 cells predominate. The Kortylewski et al. study sheds light on the important question of how STAT3 and IL-23 may differentially contribute to each form of inflammation.

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Identifying the Perpetrator in Medulloblastoma: Dorian Gray versus Benjamin Button

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Tumors contain a subpopulation of tumor-propagating cells (TPCs) that are critical for their growth. In this issue, Read, Wechsler-Reya, and colleagues show that in an animal model of medulloblastoma, TPCs express the surface marker CD15 and have properties distinct from neural stem cells.

The idea that stem cells or their close derivatives might underlie tumor formation has a long history but has come to the fore with the advent of modern stem cell biology. We now know that, for a variety of tumors, there is an essential subpopulation of cells that maintains the growth of neoplastic tissue and can initiate new tumors in vivo when transplanted into a receptive host. These tumor-propagating cells (TPCs) can resemble normal tissue stem cells in their marker expression and their ability to self-renew and produce differentiated progeny. For a given tumor type, defining its constituent TPCs is of paramount importance—first to identify and target them to combat tumor growth, and second to understand their etiology and find ways to prevent tumor formation. Do TPCs arise from normal stem cells that go awry and proliferate uncontrollably, disregarding regulatory mechanisms that keep them in check, or do they arise from later-stage progenitor cells that revert to acquire stem-like features? These two views of cancer formation—reminiscent of the literary characters of Dorian Gray, who didn't age, and Benjamin Button, who was born old and grew young—are an active point of debate. Recent studies indicate that mutations in the stem cell compartment more readily phenocopy colon cancer (Barker et al., 2008). However the same might not be true for cancers in other tissues.

Medulloblastomas are the most common pediatric brain tumors. They occur in the cerebellum, a brain region involved in integrating sensory perception and movement control. The cerebellum forms from two major germinal regions, the ventricular zone and the external granular layer (Figure 1). The most abundant cell in the brain, the cerebellar granule neuron, arises from granule neuron precursors (GNPs) in the external granular layer, largely during the early postnatal period.

The remarkable expansion of GNPs is governed principally by the growth factor Sonic hedgehog (Shh) (Kenney et al., 2003). The signaling cascade is initiated by Shh binding to the cell surface receptor Patched (Ptc). A key player in the cascade is a G protein-coupled receptor-like molecule called Smoothened (Smo). In the absence of Shh, Ptc inhibits the activity of Smo. Shh binding to Ptc relieves its inhibition, and Smo can signal downstream. The targets of Smo include Gli transcription factors, which then translocate to the nucleus and initiate transcription.

Approximately 25% of medulloblastomas result from inappropriate activation of Shh signaling, and a subset of human medulloblastomas harbor mutations of the *Ptc* gene (Zurawel et al., 2000). A useful model of medulloblastoma is the $Ptc^{+/-}$ mutant mouse. *Ptc* haploinsufficiency increases proliferation of neural stem cells (Galvin et al., 2007), and 15%–20% of $Ptc^{+/-}$ mice develop medulloblastomas (Goodrich et al., 1997).

Read et al. (2009) examined medulloblastomas derived from $Ptc^{+/-}$ mice. Using fluorescence-activated cell sorting (FACS) with cell surface markers, they separated subpopulations of live cells and then stereotaxically injected these into SCID/beige mouse cerebella to examine whether they could form tumors. Surprisingly, the investigators found that medulloblastomas were not propagated by cells expressing the neural stem cell (NSC) marker CD133, leading them to search for other candidate markers. A prior study had described CD15, also known as Lewis X (LeX) or stage-specific embryonic antigen-1 (SSEA-1), as a marker of forebrain NSCs and progenitor