and receptor downregulation (Spangler et al., 2010). Antibody combinations are now being tested in HER2-positive breast cancer (http://www.clinicaltrials.gov).

Overall, the findings reported by Tvorogov and colleagues define a novel class of anti-VEGFR-3 antibody that blocks homodimerization and heterodimerization of receptors and complements the activity of antibodies that block ligand-binding. This work provides mechanistic insights into receptor dimerization and the promise of using inhibitors of dimerization as a biologically meaningful approach for suppressing angiogenesis and lymphangiogenesis and potentially tumor growth and dissemination. Because VEGFR-3 is one of the most highly upregulated therapeutic targets in endothelial cells of tumor vessels, the receptor could also serve as a target for antibodies coupled to therapeutic cargo such as radioisotopes, liposomes, or nanoparticles loaded with cytotoxic therapeutics, or even T cells.

This novel class of inhibitors has the potential of outperforming conventional competitive inhibitors of angiogenesis because of the insensitivity to ligand concentration and the ability to inhibit heterodimerization and influence multiple downstream signaling pathways. The use of an antidimerization antibody in combination with an antiligand binding antibody could translate into clinical benefit from more potent antiangiogenic and antilymphangiogenic activities. Further validation of the efficacy of antibody combinations in preclinical models could pave the way for inhibitors that block tumor angiogenesis and lymph node metastasis in cancer patients.

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# The Ids Have It

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DOI 10.1016/j.ccr.2010.11.028

**In this issue of *Cancer Cell,* Anido et al. demonstrate that Id1 is the likely arbiter of divergent transforming growth factor-β (TGF-β) signaling in glioma-initiating cells (GICs) from different tumors. These findings hold both the promise and potential peril of therapeutic targeting of the TGF-β pathway.**

Human glioblastoma derived GICs have stem cell properties of self-renewal and differentiation with genotypes and phenotypes similar to their parental tumors and substantially different from conventional glioma cell lines (Lee et al., 2006). In addition, there is evidence suggesting that GICs can promote tumor angiogenesis and mediate radiotherapy resistance (Bao et al., 2006). With the increasing evidence that GICs are the stem cell subcomponent of malignant gliomas, there are reasons to believe that targeting GICs hold great therapeutic potential. Terminal differentiation is a powerful tumor suppressor mechanism, and thus there is keen interest in finding ways to activate cancer stem cell differentiation programs for therapeutic purposes.

As the founding member of a group of more than 40 secreted factor family members, TGF-β plays an intricate role in the regulation of almost all cell types in the body, with an emphasis on controlling homeostasis and developmental processes including stem cell differentiation. The effects of TGF-β signaling are mediated through transmembrane serine-threonine type I (TβRI) and type II (TβRII) receptors that phosphorylate Smad proteins, which then forward signals from the TGF-β receptors to the nucleus where they regulate transcription. The role of TGFβ in cancer stem cell differentiation is of significant interest given its overexpression in a number of tumors including lung, colon, and gastric carcinoma as well as in high-grade gliomas. TGF-β had been previously shown to increase the self-renewal and oncogenic potential of GICs, although the mechanism was not known (Penuelas et al.,

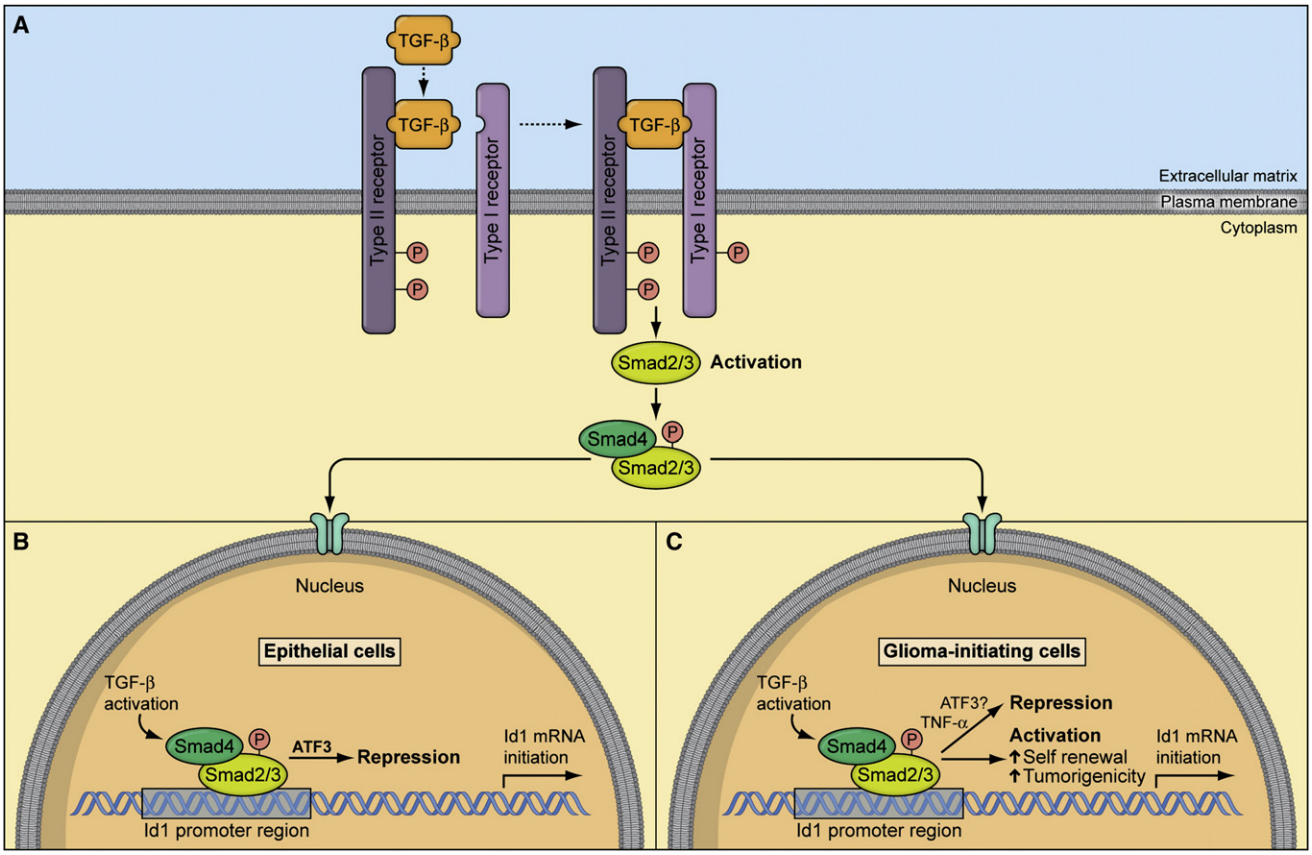


**Figure 1. The Cellular Response to TGF-β Activity Depends, in Part, on Id1 Activation or Repression**

(A) TGF-β binding to type I (TβRI) and type II (TβRII) receptors leads to Smad activation and Smad binding to TGF-β-responsive elements located at the Id1 promoter. The resulting cellular response (e.g., self-renewal, differentiation) is cell context dependent.

(B) In epithelial cells, the availability of the TNF-α associated transcriptional co-repressor, ATF3, in conjunction with the Smad complex results in transcription repression of Id1.

(C) In many, but not all, glioma initiating cells (GICs), ATF3 is not produced, leading to Smad-mediated Id1 transcriptional activation.

2009). An important clue as to how this might occur came with the demonstration that TGF-β could induce the transcription factor, inhibitor of DNA binding 1 (Id1), in tumor cells (Padua et al., 2008).

The family of Id proteins is highly expressed in embryonic tissue and in the stem/progenitor cell compartment of some adult tissues. Id proteins have a primary role in antagonizing cellular differentiation through the dominant negative inhibition of DNA-binding helix-loop-helix transcription factors. It was recently shown that Id1 expression was necessary for the self-renewal capacity of adult neural stem cells (Nam and Benezra, 2009). Along with their role in normal development, Ids are overexpressed in several different tumor types, and recently Id1 and Id3 expression was shown to be necessary for tumor initiation and metastasis in triple-negative human breast cancer (Gupta et al., 2007). Anido and colleagues now build on these findings by showing that TGF-β can induce expression of Id1 and Id3 in GICs in vitro. By contrast, blockade of TGF-β downstream signaling by a specific TβRI inhibitor significantly inhibited Id1 expression, resulting in decreased GIC neurosphere proliferation in vitro and tumorigenicity in vivo (Anido et al., 2010).

A paracrine/autocrine mechanism of glioma TGF-β overexpression leading to Id1/3 induction with subsequent GIC proliferation would seem straightforward and conducive to therapeutic interventions aimed at inhibiting TGF-β signaling. This, however, is undermined by the complexity of TGF-β signaling. Although TGFβ has long been implicated as a pro-oncogenic factor in a number of cancers, it has also been shown to have tumor suppressor affects through regulation of cell proliferation, differentiation, survival, and its effects on the tumor microenvironment. It should, therefore, not be surprising that TGF-β can not only induce Id1 expression but can also repress its expression depending on the cellular context. Thus, TGF-β can induce Id1 expression in both GICs and in breast cancer cells while repressing its expression in normal epithelia and endothelial cells (Massague, 2008; Anido et al., 2010). As a master regulator of cellular transcriptional networks, these observations suggest that Id1/3 may mediate a number of the pleotropic effects of TGF-β signaling on cellular biology.

Anido et al. provide a piece to the puzzle of how TGFβ signaling can cause such divergent effects on Id1 expression by showing that both transcriptional induction and repression is mediated through the same TGF-β responsive element in the Id1 upstream promoter. In epithelial cells, however, TGF-β signaling occurs in the setting of TNF-α activation

leading to the induction of the transcriptional repressor, ATF3, which binds to TGF-β-activated Smad proteins forming a repressor complex that then binds to the TGF-β binding element in the Id1 promoter (Kang et al., 2003). Anido et al. show, however, that ATF3 is not expressed in GICs so TGF-β-mediated activated Smads bind to the consensus element in the Id1 promoter without the ATF3 repressor protein, thus mediating transcriptional activation rather than repression (Figure 1). This suggests that the repression of Id1, and the subsequent cellular response initiated by TGF-β signaling, depends upon TNFα pathway activation in GICs.

The complexity of the TGF-β signaling pathway and its dependence on cellular context gives one pause before concluding that TGF-β inhibition will be a promising therapeutic strategy for all gliomas. In fact, Anido and coworkers reported that one of their patient-derived GIC lines responded to TGF-β inhibition with induction, rather than repression, of Id1. TGF-β inhibition could, therefore, have a pro-proliferative, pro-oncogenic affect on gliomas with inducible Id1 biology. Thus, in theory, one would optimally like to screen GICs from each patient's tumor in order to select for patients likely to benefit from therapeutic inhibition of TGF-β signaling. This is impractical, however, given the time, expense, required expertise, and inefficiency of deriving such cells from patients in real time. Thus, a more thorough molecular understanding of TGF-β signaling in GICs with divergent responses to TGF-β is necessary. For example it would be interesting to determine whether there were defects in TβRI, TβRII, or downstream components in the TGF-β signaling pathway that, like ATF3, affect Id1 induction or repression with subsequent affects on GIC tumorigenicity. Additionally, studies evaluating the posttranslational modification of TGF-β pathway components, such as the ubiquination of Smads, will probably be informative (Dupont et al., 2009).

Through such mechanistic studies of GIC lines with divergent responses to TGF-β inhibition, we will hopefully identify clinically useful biomarkers that will be predictive of tumor sensitivity to therapeutic TGF-β inhibition. Unfortunately, the complexity of translating such studies to the clinic does not end there for it is known that TGF-β has significant effects on the host immune system and on the tumor mircoenvironment. Thus, even if biomarkers predictive of GIC sensitivity to TGF-β inhibition in vitro can be identified, systemic inhibition of the TGF-β signaling pathway will have unknown and likely heterogeneous effects on different patients that will undoubtedly impact on the clinical efficacy of this therapeutic strategy.

The attraction of targeting tumor stem cell and developmental pathways is tempting and there are growing preclinical data, such as those by Anido et al., to suggest the potential benefits of doing so. The enthusiasm for such a strategy, however, must be tempered by the appreciation of the complexity and cellular context-dependent nature of these gene regulatory networks that are susceptible to both cell intrinsic and cell extrinsic (environmental) perturbations. The complexity of these stem cell networks is further confounded by affects from the diversity of genomic and epigenomic aberrations found within the landscape of the cancer genome. This diversity will undoubtedly contribute to divergent responses to therapeutic interventions aimed at targets within these networks. Nevertheless, with our increasing ability to interrogate individual tumor genomes at the sequence, expression and epigenomic level, combined with improved model systems, the understanding of the structure and function of these stem cell and developmental networks now seems to be within reach. Studies such as the one presented here by Anido et al. will help to elucidate vital nodes within these cancer-dependent developmental networks and hopefully help pave the way for patient-specific targeted therapy.

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