IN THE SPOTLIGHT

A CREB1–TGFβ2 Self-Sustaining Loop in Glioblastoma

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Summary: A subset of glioblastomas (GBM) has high levels of TGFβ signaling, and anti-TGFβ therapies are being pursued as treatments for GBM. The work presented here identifies CREB1 as a potential biomarker for TGFβ1-dependent GBM. CREB1 integrates signaling from TGFβ and the PI3K pathway and nucleates a self-sustaining signaling loop that maintains TGFβ2 expression in GBM with high CREB1 levels. Cancer Discov; 4(10): 1123–5. ©2014 AACR.

See related article by Rodón et al., p. 1230 (6).

Gliomas, which are classified into four subtypes, grades 1 to 4, are the most common primary brain tumors. Grade 4 glioma, or glioblastoma (GBM), is the most aggressive and shows minimal response to current therapies (1). Standard-of-care therapies include surgical resection and a combination of radiotherapy and chemotherapy, but tumor recurrence is rapid, and the median survival following treatment remains significantly below 2 years. The most frequent chemotherapeutic in use for GBM is temozolomide, which is a DNA alkylating agent that can inhibit DNA replication and has beneficial effects in some GBMs. However, there is significant variability in the response, and effective treatment options for GBM are still very limited (1).

One of the difficulties with treatment is that GBM appears to be driven by an interconnected network of signaling pathways that provides for functional redundancy in the protumorigenic outcomes of pathway activation (2). Genomic sequencing and expression analysis of GBM samples revealed high frequencies of alterations in three main pathways: receptor tyrosine kinase (RTK) signaling via RAS and PI3K, and the pRb and p53 pathways. Many GBMs also have alterations in genes encoding chromatin regulators that may modify the effects of the major tumor-driving pathways. Consistent with this, proteomic analysis demonstrated that predicting the tumorigenic outcomes of pathway activation (2). Genomic sequencing and expression analysis of GBM samples revealed high frequencies of alterations in three main pathways: receptor tyrosine kinase (RTK) signaling via RAS and PI3K, and the pRb and p53 pathways. Many GBMs also have alterations in genes encoding chromatin regulators that may modify the effects of the major tumor-driving pathways. Consistent with this, proteomic analysis demonstrated that predicting the biologic outcome of alterations in well-characterized signaling pathways in GBM was far from simple (2). In addition, this complexity may be further exacerbated by altered expression of noncoding RNAs and intratumor heterogeneity. Although genomic alterations affecting components of the TGFβ pathway are not common in GBM, high levels of expression of TGFβ ligands have been seen in some GBMs (3). Despite its primarily tumor-suppressive role in the early stages of many cancers, TGFβ signaling can play a protumorigenic role in more advanced cancers, including GBM, where it promotes rather than inhibits proliferation (3, 4). TGFβ signaling can also promote the self-renewal capability of glioma-initiating cells that may lead to tumor recurrence (5). This makes the TGFβ pathway a potential therapeutic target for GBM, with clinical trials either ongoing or completed, for both TGFβ receptor kinase inhibitors and antisense therapy against the ligand TGFβ2 (Clinicaltrials.gov identifiers NCT01220271 and NCT00431561). However, given the heterogeneity of GBM, the question is how to identify those patients with GBM who will respond favorably to inhibition of TGFβ signaling.

The work by Rodón and colleagues (6) addresses this problem by focusing on GBMs with high levels of TGFβ2 expression. Starting with cell lines, they identify the CREB1 transcription factor as a key mediator of TGFβ2 gene expression in GBM. CREB1 allows for the induction of TGFβ2 gene expression by TGFβ itself, and CREB1 may also integrate activating signals from RTK–PI3K signaling. To test the relevance of this pathway, they identify two patient-derived GBMs with high TGFβ2 and high CREB1 and demonstrate that they are indeed sensitive to CREB1 inhibition when grown as xenografts in nude mice. This work identifies one possible way to stratify GBMs for treatment with anti-TGFβ therapies and also provides evidence for an intriguing self-enhancing loop that may underlie activation of TGFβ signaling in some GBMs.

Rodón and colleagues (6) show that in some publicly available GBM datasets increased expression of TGFβ1 and TGFβ2 (but not TGFβ3) correlates with decreased survival. To begin to understand the mechanism, they test whether TGFβ signaling can stimulate its own expression. In several GBM lines, this appears to be true, specifically for TGFβ2 expression is increased at both the RNA and the protein level in response to TGFβ signals. Thus, in GBMs with high levels of TGFβ2 expression may be maintained, in part, by a positive feedback mechanism. Similar results are shown for breast cancer lines, suggesting that TGFβ regulation of TGFβ2 gene expression may be a more general phenomenon, although its importance in other tumors remains to be determined. Following on from the observed increase in TGFβ2 mRNA, they focus on a region of the TGFβ2 proximal promoter that contains a conserved consensus cAMP response element (CRE) that can be bound by the CRE-binding protein (CREB) family of...
transcription factors. They show that the CRE and CREB1 are required for TGFβ-mediated induction of TGFβ2 expression in cultured cells, and that this TGFβ response preferentially involves SMAD3, rather than SMAD2.

CREB transcriptional activators contain a basic-leucine zipper dimerization and DNA-binding domain, and a kinase-inducible domain (7). CREB1 phosphorylation by multiple kinases, including AKT, RSK, and PKA, results in increased interaction with the general coactivator CREB-binding protein (CBP), such that CREB1 transcriptional activity increases in response to mitogenic signals, for example, from RTK–PI3K signaling (7, 8). In the context of the TGFβ2 promoter, CREB1 and SMAD3 appear to be required for the TGFβ response, and it is possible that CREB1 coordinates signals from both TGFβ and RTKs to activate TGFβ2 expression. Indeed, Rodón and colleagues (6) show that pharmacologic inhibition of the PI3K pathway (and of RSK) reduces TGFβ2 expression and the transcriptional response to TGFβ. This sets up the possibility that CREB1 integrates two protumorigenic signals at the TGFβ2 promoter, creating a positive feedback loop that may drive tumorigenesis (Fig. 1, center). Members of the CREB–ATF family have been implicated in the regulation of other TGFβ transcriptional responses (for example, ref. 9), raising the possibility that this signal integration may also affect other downstream TGFβ target genes. It would, therefore, be interesting to know whether there are other CRE-dependent TGFβ responses in GBMs that respond to both RTK and TGFβ signaling.

Activation of RTK–PI3K signaling, which is a frequent event in GBM, would increase expression of the TGFβ2 gene. The induced TGFβ2 expression would then further activate TGFβ2 gene expression, providing a self-sustaining signal (Fig. 1, center). In addition, it is presumably possible that this autocrine induction could be kick-started by other sources of TGFβ, for example, from stroma (Fig. 1, left). A further intriguing possibility is that once TGFβ signaling has been activated, it could generate additional signals via the RTK pathway. For example, in a subset of TGFβ-responsive GBMs, expression of the PDGFB gene is induced in response to TGFβ (10), and the PDGFRA gene is frequently amplified or mutated in GBM (2), providing additional avenues by which to reinforce these signals (Fig. 1, right). Thus, tumor progression and signal amplification may advance together, proceeding from left to right as diagrammed in Fig. 1. Clearly, there are more details of these interconnections to be elucidated, and determining whether this model is applicable to other cell types and cancers will be of additional interest.

In the final set of experiments, Rodón and colleagues (6) address the importance of CREB1 for tumor growth using patient-derived xenografts. They identify two GBMs with high CREB1, and high levels of the active phosphorylated form of the protein. Both also have high TGFβ2 expression, and when CREB1 is knocked down, levels of phospho-CREB1 and TGFβ2 decrease. Importantly, when implanted orthotopically into nude mice, the patient-derived cells with reduced CREB1 formed fewer, smaller tumors, and the survival of the mice was extended. This clearly supports the importance of CREB1 in the growth of at least some GBMs, and the authors suggest that high CREB1 expression may be used as a biomarker to identify which GBMs would likely respond to anti-TGFβ therapy. A tumor with high CREB1 and TGFβ2 should certainly be a better candidate, although a tumor still might be independent of TGFβ signaling and refractory to pathway inhibition. It would, therefore, be interesting to know how many patient-derived GBMs with high CREB1 respond to anti-TGFβ therapy in a xenograft setting, and whether responses are significantly greater than those in GBMs with lower levels of CREB1. An additional possibility is that tumors that harbor this self-sustaining loop might respond well to inhibition of both TGFβ and CREB1 activation by RTK signaling. CREB1 itself could be considered a potential therapeutic target (8), although targeting transcription factors directly may be more challenging than attacking the upstream signaling pathways.

In summary, the article by Rodón and colleagues (6) provides evidence for a novel set of interconnections between TGFβ and RTK signaling in GBM, and potentially identifies a

**Figure 1.** A self-reinforcing loop in GBM. CREB1 and SMAD3 activate TGFβ2 gene expression, resulting in increased TGFβ signaling that reinforces TGFβ2 gene activation and drives GBM progression (center). Signaling may be initiated by TGFβ from nontumor cells (left), and could potentially be further enhanced by TGFβ–activated expression of PDGFB, strengthening signaling to CREB1 via the PI3K pathway (right). TβR, TGFβ receptor.
new avenue by which to better direct specific therapeutics to
the patients most likely to respond.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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