IN THE SPOTLIGHT

Targeting ETV1 in Gastrointestinal Stromal Tumors: Tripping the Circuit Breaker in GIST?

Anette Duensing[1,2]

Summary: Activating mutations in the *KIT* or *PDGFRA* receptor tyrosine kinase genes are the key oncogenic drivers in the majority of gastrointestinal stromal tumors (GIST), but novel results now show that aberrant kinase signaling is potentiated by a positive feedback circuit that involves the ETS transcription factor ETV1. Targeting ETV1 can disrupt this circuit and represents a promising new therapeutic approach for the treatment of GISTs.
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See related article by Ran and colleagues, p. 304 (5).

Gastrointestinal stromal tumors (GIST) are the most common mesenchymal tumors of the gastrointestinal tract and one of the most common subtypes of sarcomas. Because of their unique molecular properties, GISTs spearheaded the use of targeted therapies in solid tumors, a concept that eventually led to a paradigm shift in oncologic therapy. GISTs were the first solid tumor entity that could successfully be treated with small-molecule inhibitors, specifically tyrosine kinase inhibitors such as imatinib mesylate (Gleevec; ref. 1). This notion came shortly after the discovery that an oncogenic mutation in the *KIT* (75%–85%) or *PDGFRA* (platelet-derived growth factor receptor alpha; 5%–7%) gene is the tumor-initiating event in the vast majority of GISTs and leads to constitutive activation of the encoded receptor tyrosine kinase (2, 3). Major responses are seen after first-line treatment with the KIT/PDGFRA inhibitor imatinib, and approximately 85% of patients with metastatic and/or inoperable GIST benefit from this therapy. However, complete tumor remissions are rare, and about 50% of patients experience disease recurrence within 2 years of treatment. There are several reasons for this, including the emergence of secondary mutations in *KIT/PDGFRA* that confer drug resistance, entry into a state of cellular quiescence, and the potential existence of GIST stem/progenitor cells that express low levels of KIT and are intrinsically imatinib resistant. Given these reasons, it is unlikely that GISTs can be cured with imatinib alone, and additional therapeutic approaches are urgently needed.

GISTs are thought to arise from a specialized cell type in the bowel wall, the interstitial cells of Cajal (ICC), or a common progenitor. These cells are present throughout the entire digestive tract, where they serve as pacemaker cells to coordinate peristalsis. Interestingly, there are several subsets of ICCs that are located in distinct microscopical locations within the gut, and only certain types seem to give rise to GIST. In an earlier study, Chi and colleagues (4) discovered that the ETV1 transcription factor is a master regulator not only of the ICC population giving rise to GIST, but also of an ICC–GIST-specific transcriptional network. ETV1 is highly expressed in GISTs (but not in other sarcomas) and is required for the growth and proliferation of GIST cells.

A new study by the same group (5), published in this issue of *Cancer Discovery*, adds an important new spin to their previous observations. The latest data not only provide *in vivo* validation of their experimental findings; more importantly, ETV1 is identified as part of a positive feedback circuit involving KIT and MAPK signaling and hence firmly established as a key therapeutic target in GIST.

Building on their original observation that the loss of ETV1 in a mouse model leads to loss of the specific ICC subtypes that are known to give rise to GIST, Ran and colleagues now ask whether ETV1 is crucial for the development of these tumors. Indeed, when $Etv1^{-/-}$ mice are crossed with $Kit^{V558\Delta/+}$ mice (which are known to develop GIST-like tumors in the cecum as well as ICC hyperplasia throughout the gastrointestinal tract; ref. 6), no tumors are detected, indicating that ETV1 is required for the development of GIST. Interestingly, however, $Etv1^{-/-};Kit^{V558\Delta/+}$ mice can develop ICC hyperplasia. This finding suggests that constitutive activation of KIT on its own does induce a proliferative stimulus in ICCs—which is likely executed via other transcription factors—but that ETV1 is necessary for tumor formation. Notably, the importance of ETV1 for GIST maintenance is confirmed in a conditional knockout mouse model ($Etv1^{flox/flox};Kit^{V558\Delta/+};Rosa26^{CreERT2/CreERT2}$). Temporary reduction of ETV1 levels decreases proliferation of the GIST-like tumors in the cecum of these animals (as well as of the ICC hyperplasia), while also inducing tumor fibrosis as a correlate of tumor regression. These results confirm earlier observations that reduction of ETV1 levels (by shRNA) in GIST cell lines and xenografts decreases cellular proliferation (4).

One main finding of the current study is that ETV1 and KIT form a positive feedback circuit to regulate the expression of target genes in GIST. The first clue to this notion came from the authors' previous experiments, which showed that inhibition of KIT led to a substantial downregulation of ETV1 protein expression levels. When now examining the

[1]Cancer Therapeutics Program, University of Pittsburgh Cancer Institute, Hillman Cancer Center, Pittsburgh, Pennsylvania. [2]Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania.

Corresponding Author: Anette Duensing, University of Pittsburgh Cancer Institute, Hillman Cancer Center, Research Pavilion, Suite 1.8, 5117 Centre Avenue, Pittsburgh, PA 15213. Phone: 412-623-5870; Fax: 412-623-7715; E-mail: aduensin@pitt.edu

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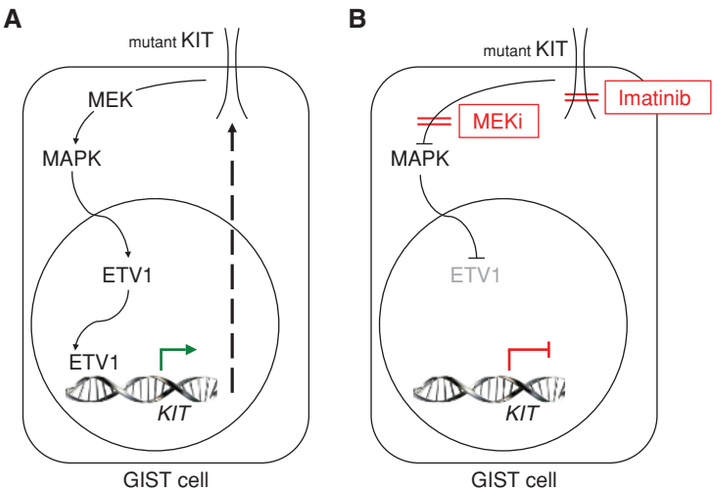

Figure 1. The KIT–ETV1-positive feedback circuit in GIST is interrupted by targeting ETV1 via inhibition of KIT and MAPK signaling. In GIST cells, constitutive activation of the oncogenically activated KIT receptor tyrosine kinase leads to target gene binding of the ETS transcription factor ETV1 via MEK–MAPK signaling (**A**). One important ETV1 target gene is *KIT* itself, thus enhancing its own expression levels via a positive feedback circuit. This loop is potently interrupted by inhibition of KIT or MAPK signaling, either alone or in combination, resulting in rapid degradation of ETV1 and reduced binding of ETV1 to target gene enhancers (**B**). ETV1 has thus been established as a key therapeutic target in GIST. MEKi, MEK inhibitor.

ETV1-regulated transcriptome in cecal tumors of *Etv1*$^{flox/flox}$;*Kit*$^{V558\Delta/+}$;*Rosa26*$^{CreERT2/CreERT2}$ mice using Gene Set Enrichment Analysis (GSEA), the authors discover that the most enriched gene set among those downregulated after ETV1 ablation is the set of genes most downregulated by imatinib treatment in the GIST-like tumors of *Kit*$^{V558\Delta/+}$ mice. The same results are obtained when looking at shRNA- or CRISPR/Cas9–mediated knockdown of ETV1 in human GIST cell line models, indicating that ETV1 and KIT regulate a common core transcriptional program.

Ran and colleagues next address the question whether ETV1—an ETS family transcription factor—could directly regulate *KIT* gene expression. Indeed, ablation of *Etv1* in *Etv1*$^{flox/flox}$;*Kit*$^{V558\Delta/+}$;*Rosa26*$^{CreERT2/CreERT2}$ mice leads not only to the downregulation of known ETV1 target genes (such as *Dusp6*, *Gpr20*, and *Edn3*) but also to downregulation of *Kit* mRNA expression. This is accompanied by reduced *Kit* protein expression in the cecal GIST-like tumors and ICC hyperplasia of the animals. Together, these results indicate that *KIT* may be a direct transcriptional target of ETV1. The fact that overexpression of ETV1 leads to upregulation of *KIT* mRNA expression and that chromatin immunoprecipitation-sequencing (ChIP-seq) analyses show multiple ETV1 binding sites in the *KIT* enhancer substantiates this hypothesis. Ultimately, ChIP–qPCR experiments establish direct binding of ETV1 to these sites, and hence show that ETV1 can directly regulate *KIT* expression levels. ETV1 therefore cooperates with mutant KIT by forming a positive feedback loop to promote GIST pathogenesis (Fig. 1A).

Establishing a positive feedback circuit involving KIT and ETV1 further underscores the role of ETV1 as a potential therapeutic target in addition to KIT inhibition in GIST. But how to inhibit the activity of a transcription factor? Although having long been desirable targets for cancer treatment, transcription factors are inherently difficult to tackle using common therapeutic strategies. The reason for this mainly lies in their large surface area for protein–DNA and protein–protein interactions in addition to their nuclear localization (7). Here, luckily, the authors had already found a clue from their previous work (4). ETV1 stability is regulated by MAPK signaling, which is one important signaling axis downstream of KIT. Thus, chemical inhibition not only of KIT but also of MEK leads to a rapid reduction in ETV1 levels.

In their current study, Ran and colleagues take these findings to the next level and investigate the efficacy as well as mechanism of action of MEK162, a MEK inhibitor that is already in late-stage clinical trials. They confirm that MEK162 (as well as imatinib) treatment destabilizes ETV1, leads to reduced binding of ETV1 at the *KIT* enhancer and, most importantly, reduces cell viability *in vitro*. Notably, combining imatinib and MEK162 has a synergistic effect on both ETV1 destabilization and cell viability, making it possible to reduce the doses of both agents (Fig. 1B).

Ran and colleagues finally go on to show that a combined treatment with imatinib and MEK162 leads to nearly complete and durable responses in two GIST xenograft models, whereas single treatment with imatinib leads only to disease stabilization (a notion that has been reported in several other studies). Similar results are obtained when treating *Kit*$^{V558\Delta/+}$ transgenic mice. When investigating the mechanism of action of the treatment, the authors confirm a dramatic loss of ETV1 expression, especially in the animals treated with the drug combination.

What is next? A clinical trial (phase Ib/II) testing the concept of ETV1 inhibition via combined imatinib/MEK162 treatment has been designed (NCT01991379) and has already started patient accrual. In addition, this study has shown once more how important it is to identify additional therapeutic options for patients with GIST that make use of these tumors' specific molecular makeup aside from *KIT*/*PDGFRA* mutations. Especially targeting the transcriptional machinery—either globally or with the aim of reducing the expression levels of specific genes (such as *KIT* and *ETV1*)—has recently emerged as an important new angle for the treatment of GIST and other oncogene-driven malignancies (8–10). For example, inhibitors of the 26S proteasome, such as bortezomib (Velcade), have been shown to inhibit ongoing gene transcription in GIST, leading to a dramatic loss of KIT protein expression and subsequent apoptosis (8). The SP1 inhibitor mithramycin A, a "classical" transcriptional inhibitor, has recently gained attention again for being the top hit in a 50,000 compound screen against the *EWS–FLI1* fusion oncogene in Ewing family sarcoma (9). It was also identified in a smaller library screen as being effective for GISTs, with its main mechanism of action lying in the reduction of KIT

expression levels (10). Specifically in GIST, targeting gene transcription and its regulators allows the bypass of secondary KIT/PDGFRA mutations that emerge during tyrosine kinase inhibitor treatment, thus paving the way to a more complete eradication of tumor cells.

Taken together, the work presented by Ran and colleagues proves once more that KIT/PDGFRA–associated regulatory networks are the Achilles heel of GIST, and that a better understanding of these intracellular signaling circuits holds the promise of more complete and long-lasting remissions for patients with GIST.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

The Pennsylvania Department of Health specifically disclaims responsibility for any analyses, interpretations, or conclusions in this work.

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