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Previews

How do you mend inactive tumor suppressor mutants? You glue them!

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SMAD4 mutations that disrupt its interaction with SMAD3 and attenuate tumor suppression by TGF- β are major oncogenic drivers. Tang et al. (2020) report the discovery of small molecules that restore the SMAD4:SMAD3 complex and its cytostatic activity, exemplifying the therapeutic potential of fixing tumor suppressor mutants using molecular glues.

Signaling by the transforming growth factor- β (TGF- β) is fundamental for the control of tissue growth and differentiation, and the maintenance of homeostasis in multicellular organisms. Mounting clinical evidence frames TGF- β as a tumor-derived immunosuppressor, an inducer of tumor mitogens, and an enabler of pro-metastatic cytokine secretion (Batlle and Massagué, 2019). In this issue of *Cell Chemical Biology*, Tang et al. (2020) sought to identify small molecules to restore the cytostatic activity of TGF- β as anti-cancer therapies. Upon TGF- β stimulation, SMAD4 (a common partner SMAD, or co-SMAD) forms a complex with the TGF- β receptor-phosphorylated proteins SMAD2 or SMAD3 (the receptor-specific SMADs, or R-SMADs), translocates to the nucleus, and modulates gene expression to carry out various cellular responses (Figure 1) (Massagué, 2012). A complication in working with TGF- β signaling resides in its paradoxical role during tumor progression, where it can exhibit both tumor-suppressive and tumor-promoting effects depending on the context (Figure 1) (Liu et al., 2021).

SMAD4 was originally identified as a tumor suppressor frequently mutated or deleted in pancreatic cancer, and was accordingly named *DPC4* (deleted in pancreatic carcinoma locus 4) (Hahn et al., 1996). Subsequently, inactivating mutations in SMAD4 (SMAD4^{mut}) were identified in a range of solid tumors, including colon and hepatocellular cancer, so it became clear that the significance of the tumor-sup-

pressive activity of SMAD4 extends beyond pancreatic cancer. The missense mutations in SMAD4—including D351H and R361H—cluster at the protein-protein interaction (PPI) interface with R-SMAD (SMAD2 or SMAD3), where they can prevent the formation of a stable SMAD4^{mut}:R-SMAD complex upon TGF- β stimulation and lead to the loss of the tumor-suppressive effects of TGF- β (Figure 1). Restoring a PPI between SMAD4^{mut} and SMAD3, therefore, has the potential to reactivate the tumor-suppressive TGF- β signal and block tumor progression. Unlike oncoproteins, however, tumor suppressors have been considered “undruggable” targets because of the formidable task of using small molecules to restore faulty PPIs in comparison, for example, to inhibiting the enzymatic reaction of a kinase. Undaunted, Tang and colleagues launched a high-throughput screen (HTS) of over 2,000 compounds to successfully identify two bisindolylmaleimide compounds—Ro-31-8220 and Go-6983—that increase the PPI between SMAD4^{R361H} and SMAD3 (hereafter referred to as SMAD4:SMAD3 glues) (Tang et al., 2020). Interestingly, the SMAD4:SMAD3 glues also facilitated the interaction between SMAD4^{D351H} mutant and SMAD3 (Tang et al., 2020), implying their effectiveness in cancer cells harboring a variety of SMAD4 mutations that affect the PPI with SMAD3. The SMAD4:SMAD3 glues did not seem to make SMAD4 “sticky,” as they did not induce a PPI between SMAD4 and the bone morphogenetic protein (BMP)

signaling R-SMADs—SMAD1/5/8, for example (Tang et al., 2020). Although TGF- β stimulation did not inhibit the colony formation of colon carcinoma HCT116 cells harboring SMAD4^{R361H}, the cells treated with the SMAD4:SMAD3 glue formed fewer colonies in response to TGF- β , indicating the successful restoration of the cytostatic activity of TGF- β by the SMAD4:SMAD3 glue (Tang et al., 2020). Consistently, the transcriptome profile of the SMAD4^{R361H} cells treated with TGF- β and the SMAD4:SMAD3 glue recapitulated that of the TGF- β -treated HCT116 cells containing wild-type SMAD4 (SMAD4^{WT}) (Tang et al., 2020), indicating that the SMAD4^{mut}:SMAD3 complex aided by the molecular glues can mediate the transcriptional responses of the SMAD4^{WT}:SMAD3 complex.

Targeting PPIs with small molecules is an increasingly popular therapeutic approach based on the recognition that the complex network of PPIs (known as interactome) formed under pathological conditions, such as cancer, are distinct from the physiological interactome (Li et al., 2017). Several small molecule drugs (PPI inhibitors) that disrupt a specific PPI found only in pathological interactomes have been developed despite the challenges of the size and the number of weak chemical bonds of the PPI surfaces (Scott et al., 2016). The alternative approach—inducing or stabilizing a specific PPI using small molecule inducers—has been less successful, with only a handful of drugs reported (e.g.,

Figure 1. TGF- β pathway and cancer

In normal cells with wild-type (WT) *SMAD4* (*SMAD4^{WT}* cells; left), upon TGF- β binding to the TGF- β receptor complex, an R-SMAD (SMAD2/3) (green) becomes phosphorylated by the TGF- β receptor kinase, then interacts with the co-SMAD (SMAD4) (orange), translocates to the nucleus, binds DNA in association with other cofactors (blue), and modulate gene expression as a transcription factor. TGF- β mediates both tumor-suppressive and tumor-promoting effects through the canonical, SMAD-dependent pathway. Inactivating *SMAD4* mutations is one of many ways in which cancer cells circumvent cell-cycle control to develop into malignant cells (*SMAD4^{mut}* cells; right). The PPI inducers developed by Tang et al. (SMAD4:SMAD3 glues) facilitate the PPI formation between *SMAD4^{mut}* (red) and R-SMAD (green), restoring the transcriptional activity of the co-SMAD:R-SMAD complex and the tumor-suppressive effects of TGF- β in cancer cells carrying *SMAD4^{mut}*.

Cyclosporine A and Rapamycin) to date (Schreiber, 2021). Therefore, the report by Tang et al. (2020) is a true breakthrough and a reminder that PPI inducers (or “molecular glues”) that restore the interaction between a mutant tumor suppressor and its protein partner(s) might be effective at preventing cancer progression (Figure 1).

Considering that *SMAD4* is mutated in more than 50% of pancreatic cancer and, with a lower frequency, in other solid tumors, the SMAD4:SMAD3 glue has the potential of treating a range of cancers (Liu et al., 2021). There are several issues that, once addressed, could pave the way to leveraging the SMAD4:SMAD3 glue as cancer therapeutics. A primary issue is the double-edged sword unsheathed by restoring the TGF- β pathway by the SMAD4:SMAD3 glue: it could possibly reinstate not only the tumor-suppressive effects,

but also the tumor-promoting effects of TGF- β (Figure 1) (Batlle and Massagué, 2019). Indeed, in *SMAD4^{R361H}* cells treated with the SMAD4:SMAD3 glue, TGF- β treatment induced the transcriptional signature of epithelial-mesenchymal transition—the hallmark of the tumor-promoting TGF- β effects—in addition to the tumor-suppressive transcriptional signature (Figure 1) (Tang et al., 2020). Another issue is a potential induction of the immunosuppressive effects of TGF- β by the SMAD4:SMAD3 glue. TGF- β is known to impair the antitumor potential of effector T cells, natural killer cells, and antigen-presenting cells, and to promote tumor growth, especially in late-stage cancer (Batlle and Massagué, 2019). To minimize the tumor-promoting effects, it would be critical to treat patients with the SMAD4:SMAD3 glue when cancer is in its early stage; however, this is challenging for pancreatic and

colon cancer, which are often diagnosed at an advanced stage.

In addition to disabling *SMAD4*, cancer cells can circumvent the tumor-suppressive actions of TGF- β by inactivating other mediators, such as the TGF- β receptors, R-SMADs, or other molecules involved in the cytosolic TGF- β responses, or the alternative (SMAD-independent) TGF- β pathway. Although the SMAD4:SMAD3 glue could activate the luciferase reporter gene in *SMAD4^{R361H}* cells, the transcriptome analysis suggests that the treatment with the SMAD4:SMAD3 glue alone was not sufficient to mediate the tumor-suppressive TGF- β transcriptome signature (Tang et al., 2020). Therefore, a TGF- β input would still be required to unleash the full potential of the SMAD transcription complex and recapitulate the TGF- β transcriptome signature in *SMAD4^{mut}* cells. In that case, the effectiveness of the SMAD4:SMAD3 glue in cells

harboring mutations in the TGF- β receptors or R-SMADs would be in question. The SMAD4:SMAD3 glue would probably not be effective for cancer cells driven by the activation of the alternative TGF- β pathway. Further mechanistic studies and pharmacological evaluations of SMAD4:SMAD3 glues are needed to address these challenges.

Finally, a growing number of studies have illustrated the role of the BMP signaling pathway in tumorigenesis (Gomez-Puerto et al., 2019). Juvenile polyposis syndrome (JPS), a hereditary condition with a risk of polyps and gastrointestinal cancer, is caused by inactivating mutations in the BMP receptor *BMPR1A* (*ALK3*) or *SMAD4* (Gomez-Puerto et al., 2019). One of the *SMAD4* mutations found in JPS is *SMAD4*^{R361C} (Houlston et al., 1998), which affects the same amino acid position of *SMAD4*^{R361H} in pancreatic cancer. It is plausible that the molecular glues that restore the PPI between SMAD4^{mut} and SMAD1/5/8 might prevent the formation of polyps and/or the transition toward cancer in JPS patients. Additionally, inactivating mutations in *SMAD4* are associated with vascular disorders, such as hereditary hemorrhagic telangiectasia (HHT) and pulmonary artery hypertension (PAH) (Gomez-Puerto et al., 2019). It is intriguing to speculate that the PPI inducer of the SMAD4:SMAD1/5/8 complex might also ameliorate the vascular phenotype in HHT and PAH patients with *SMAD4*^{mut}.

Future studies aimed at resolving the structure of the PPI inducer in action between SMAD4^{mut} and SMAD3 should reveal whether the PPI inducer directly glues SMAD4^{mut} to SMAD3 or whether it requires endogenous cofactor(s). Before the SMAD4:SMAD3 glues can be considered as viable therapeutics, though, they will need to be evaluated for their anti-tumor effects in patients-derived cancer cells and animals with *SMAD4*^{mut}. It would also be crucial to determine *in vivo* the potential adverse effects of SMAD4:SMAD3 glues in the context of immune suppression and the development of fibrosis, both hallmarks of excessive TGF- β signal. Despite the dual and still enigmatic role of the TGF- β pathway during cancer progression, several types of “TGF- β blockers” have shown significant clinical potential as cancer therapies. The systematic HTS of small molecules and successful discovery of SMAD4:SMAD3 glues by Tang et al. (2020) will place other tumor suppressor mutants on the cusp of becoming therapeutic targets, renewing interest in previously uncharted avenues to anti-cancer therapies.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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