Good guy in bad company; how STRNs convert PP2A to an oncoprotein

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Certain Protein Phosphatase 2A (PP2A) complexes are human tumor suppressors. In contrast, a paper in this issue of CANCER CELL, and two other recent studies, demonstrate that PP2A-STRN3/4 complexes inactivate Hippo tumor suppressor pathway, resulting in YAP activation and tumorigenesis. Furthermore, this new oncogenic phosphatase mechanism may be druggable.

Transformation of normal cells requires simultaneous inactivation of multiple tumor suppressors. While the prototypic tumor suppressor *TP53* is genetically inactivated in most human cancers, identification of tumor suppressors that are inhibited by non-genetic mechanisms could provide opportunities for tumor suppressor re-activation therapies.

Transcriptional regulators YAP and TAZ exemplify oncoproteins that are tightly regulated in normal cells by upstream tumor suppressors, in this case kinases MST1/2 and MAP4K4, and LATS1/2 (Fig. 1A). YAP is widely activated in human cancers, but genetic mechanisms seem to explain only a minority of the cases (Zanconato et al., 2016). However, non-mutational mechanisms driving YAP/TAZ activity in cancer have remained largely elusive. In this issue of Cancer Cell, Tang et al provide compelling evidence that the PP2A-STRN3 phosphatase complex is an upstream activator of YAP *via* MST1/2 dephosphorylation and subsequent inhibition of the tumor suppressor Hippo pathway (Tang et al., 2020)(Fig. 1A).

Protein Phosphatase 2A (PP2A) is a family of heterotrimeric phosphatase complexes (Fig. 1A). Each heterotrimer contains either of two A subunits (Aa or Ab) that acts as a scaffold for one of two catalytic C subunits (Ca or Cb). AC dimers can associate with fifteen distinct B subunits (ABC), which mediate interactions with substrates, thereby conferring specificity to PP2A holoenzymes (Fig. 1A)(Westermarck and Hahn, 2008). B subunits comprise four families with marked differences in substrate profiles and cellular

function. Out of these numerous PP2A complexes, the trimers containing B55 and B56-family of B-subunits are most often linked to tumor suppression, and their non-genetic inhibition drives cell transformation and promotes activities of many oncoproteins such as MYC (Fig.1A)(Westermarck and Hahn, 2008). Striatins (STRN, STRN3, STRN4), known to associate with a large STRIPAK protein complex, are one of the least understood B-subunit families, particularly in cancer.

Following a hypothesis that phosphatases could regulate the activity of core Hippo pathway kinases MTS1/2, Tang et al., identified PP2A B-subunit STRN3 as a suppressor of MST1/2 activity in gastric cancer cells. STRN3 knockdown restored MST1/2 phosphorylation, activation of the Hippo pathway, and phosphorylation and inhibition of YAP. They observed a significant overexpression of STRN3 in human gastric tumors compared to the adjacent normal tissue, and found high STRN3 expression was an independent prognostic marker for poor overall survival. The authors also observed a significant correlation between STRN3 and YAP target gene expression in the tumor tissue. However, the mechanisms by which STRN3 is overexpressed in gastric cancer remain uncharacterized.

In line with their observations in patients, in an MNNG-induced mouse model of gastric cancer the expression of both STRN3 and YAP increased along with tumorigenesis, and this correlated with decrease in MST1/2 activity. Furthermore, tumorigenesis was drastically impaired by conditional knock-out of either STRN3 or YAP/TAZ in the gastric mucosa. Convincingly, STRN3 knock-out could be rescued by a mutant form of YAP lacking key regulatory phosphorylation sites that render YAP insensitive to upstream regulation by the Hippo pathway.

The precise STRN3-PP2A binding interface was identified by determining the crystal structure of PP2A-A subunit in complex with STRN3 coiled-coil domain. Disrupting this interface by mutating PP2A-A or STRN3 decreased YAP activity and impaired proliferation and colony formation of gastric cancer cells, suggesting that disruption of the PP2A/STRN3 complex can be used to restore tumor suppressor activity of MST1/2-Hippo pathway. Importantly, comparison of the PP2A-STRN3 complex to the published structures of other B-subunits in complex with PP2A-A, revealed that STRN3 binds to PP2A-A in a manner distinct from the other B-subunits, thus opening a possibility for the development of a PP2A-STRN3 -specific inhibitor.

The authors then developed a peptide antagonist that mimics the core residues in STRN3 responsible for the interaction with PP2A-A. Sophisticated rational optimization yielded a STRN3-derived Hippo-activating peptide (SHAP) which potently disrupted the STRN3-PP2A-A interaction, and restored MST1/2 phosphorylation. Consistently, SHAP

suppressed YAP target gene expression both *in vitro* and *in vivo*. In an extensive panel of both established and patient-derived cell lines, SHAP potency significantly correlated with the expression of STRN3, suggesting that STRN3 expression could serve as a potential biomarker for SHAP activity. Intravenously administered SHAP also demonstrated impressive in vivo activity by suppressing tumor growth in the MNNG-induced gastric cancer mouse model and patient-derived xenografts while being seemingly well tolerated in mice. Intriguingly, therapeutic effect of SHAP was completely absent in xenografts of cells with low STRN3 expression, further supporting the role of STRN3 as the actual target, but also as a biomarker for SHAP therapies.

Although the functional role of STRN proteins as human oncoproteins was poorly understood, these discoveries did not come as a total surprise, as negative regulation of the Hippo pathway by the STRIPAK protein complex has been previously described both in Drosophila (Ribeiro et al., 2010) and in mammalian cells (Couzens et al., 2013). Furthermore, Chen and colleagues found that the STRIPAK complex is an integral part of the upstream signaling cascade regulating Hippo pathway activity by controlling, in addition MST1/2, also the phosphorylation of MAP4K4, another core Hippo regulator (Chen et al., 2019)(Fig. 1A). Very recently Hahn laboratory also connected STRN4-PP2A trimer to cellular transformation via YAP (Kim et al., 2020). They demonstrated that the SV40 small-t antigen, known as viral PP2A inhibitor protein involved in human cell transformation (Westermarck and Hahn, 2008), promotes complex formation between PP2A-STRN4 and MAP4K4. The subsequent activation of YAP in response to PP2A-STRN4-mediated MAP4K4 dephosphorylation was shown to be necessary for oncogenic transformation of human cells (Kim et al., 2020).

Collectively these studies reveal significant novel insights to non-genomic regulation of Hippo pathway activity by phosphatase-mediated dephosphorylation. The results elegantly highlight functional connections between tumor suppressors PP2A, MST1/2 and LATS1/2, and show how negative regulation of tumor suppressors can be oncogenic (“inhibitor of an inhibitor is an activator”). Demonstration that PP2A-STRN complexes are oncogenic drastically challenges the current view of PP2A as solely a human tumor suppressor phosphatase, and further emphasizes the importance of understanding the molecular functions of individual PP2A trimer complexes in different physiological and pathological contexts. The results also raise an intriguing question whether requirement of PP2A-B56 inhibition (Westermarck and Hahn, 2008), and YAP hyperactivity (Zanconato et al., 2016) for human cellular transformation, at least partly represent the two faces of the same phenomenon?

Therapeutically the results provide yet another example of inhibition of a phosphatase without affecting its catalytic activity (Westermarck and Neel, 2020). Very recently, small

molecule compounds (SMAPs and iHAPs) that selectively promote assembly of tumor suppressive PP2A-B55 and PP2A-B56 complexes, and at least in the case of iHAP, at the cost of PP2A-STRN complexes, were identified (Westermarck and Neel, 2020)(Fig. 1B). While the mechanistic basis by which the iHAP promote disassembly of PP2A-STRN complexes is different from the SHAP peptide, iHAP also showed remarkable *in vivo* antitumor activity without notable side-effects. Together these studies indicate that PP2A-STRN complexes do not have such physiological role that would jeopardize further clinical development of STRN inhibitors. Theoretically, disruption of PP2A-STRN complexes by SHAP would be expected to increase availability of PP2A core dimers for SMAP -or iHAP-induced assembly of PP2A-B56/B55 complexes (Fig. 1B). Thereby these two types of PP2A modulators used in combination could result in even stronger bias towards assembly of tumor suppressive PP2A complexes. Lastly, either inhibition of YAP (Kurppa et al., 2020), or reactivation of tumor suppressive PP2A complexes (Kauko et al., 2018), results in robust sensitization to cancer therapies. Therefore, future studies should be directed towards identification of combination therapies involving PP2A modulators (SHAP, iHAP and SMAP) to characterize drug combinations with maximal cancer cell killing activity.

Declaration of Interests

The authors declare no competing interests

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Figure legend:

Fig. 1 Two faces of PP2A complexes involved in tumor suppression and oncogenesis

A) Protein phosphatase 2A (PP2A) trimers are composed of scaffolding A-subunit (red), catalytic C-subunit (orange) and substrate recognizing B-subunits (blue). When complexed with B55 or B56 subunits, PP2A trimers recognize and dephosphorylate oncoproteins such as MYC and function as tumor suppressors. PP2A-B56 instead is inhibited in human cancer by cellular oncoprotein CIP2A. The studies discussed in this preview reveal that PP2A-STRN trimers recognize and dephosphorylate tumor suppressive MST1/2 and MAP4K4 kinases that drive Hippo pathway to suppress oncogenic activity of YAP. Notably, either inhibition of tumor suppressive PP2A complexes, or increase in PP2A-STRN complexes results in highly similar oncogenic effects. The question mark denotes the need to characterize mechanisms behind STRN expression and activity in cancer.

B) The SHAP molecules developed by Tang and collaborators inhibit PP2A-STRN3 interaction thus reactivating the Hippo tumor suppressor pathway. The other class of PP2A modulating compounds, SMAPs and iHAPs, instead increase the affinity between B55 and B56 B-subunits and PP2A AC dimer and thus also shift the balance of PP2A trimers towards tumor suppression.