PP2A fulfills its promises as tumor suppressor: Which subunits are important?

Reversible phosphorylation of proteins, catalyzed by kinases and phosphatases, is a key regulatory mechanism in the control of multiple cellular signal transduction pathways. Uncontrolled regulation by the altered phosphorylation state of the components of these pathways often leads to increased cell proliferation and cell transformation. Many viruses encode oncogenic proteins, required for their efficient viral replication, which deregulate the activity of host cell proteins. This might program cells to a malignant state, underlying the molecular mechanism of tumor formation and cancer development. Recent studies reveal a role for a specific form of protein phosphatase 2A (PP2A) in viral-induced cell transformation by interaction with the small t antigen (ST) of the DNA tumor simian virus 40 (SV40).

In an effort to determine the precise combinations of mutations that program human tumor cell growth, experimental human cell lines are developed that escape the irreversible growth arrest and subsequent apoptosis, known as replicative senescence and "crisis," respectively. By introduction of the gene encoding the catalytic subunit of human telomerase (hTERT) in combination with the SV40 early region (ER) encoding the large t (LT) and small t (ST) antigens, immortalized human cell lines were obtained. By co-expression of an oncogenic allele of the H-ras gene, these cells became tumorigenic. hTERT is necessary to compensate the difference in telomere biology between human and rodent cells that express a constitutive telomerase activity. LT sequesters and inactivates the tumor suppressors pRB and p53, whereas ST is absolutely required for the transformation and tumorigenicity of these cells. Interaction of ST with the Ser/Thr phosphatase PP2A was essential since a mutant ST, not binding PP2A, was inactive in the process (Chen et al., 2004, and references herein).

PP2A is a family of abundantly expressed Ser/Thr phosphatases, implicated in a multitude of cellular functions. The PP2A core structure consists of a catalytic subunit ($PP2A_C$) and a A/PR65 scaffold protein. This core dimer can recruit a third regulatory B subunit. There are three families of B subunits: B/B55/PR55, B′/B56/PR61, and B″/PR72. Each PP2A subunit has at least two isoforms. The differential association of all these subunits gives rise to an extensive subset of oligomeric holoenzymes. It is widely thought that PP2A exercises regulatory flexibility and differential substrate specificity through the specific association of the core dimer with one of the three regulatory B subunits. Moreover, PP2A interacts with a still growing number of cellular and viral proteins and is regulated by posttranslational modifications (see Janssens and Goris, 2001, for an extensive review on PP2A).

Building further on the knowledge of the elements in the ER of SV40, necessary for human cell transformation, Chen et al. investigated how ST deregulated PP2A and subverted the cellular machinery in order to become tumorigenic, as reported in this issue of *Cancer Cell* (Chen et al., 2004). Their observations force us to renew our thinking about the ST-PP2A interaction. Many groups contributed to the idea that the core dimer of PP2A is the only ST-interacting protein. ST might directly interact with a preexisting dimer or displace a third regulatory B subunit from a trimeric holoenzyme. It was generally believed that ST is able to replace the B/B55/PR55, but not the B′/B56/PR61 subunit. This was based on the fact that in vitro, recombinant ST could displace the B/B55/PR55 subunit from purified $PP2A_{T55}$ trimeric enzyme, but not the B′/B56/PR61 subunit from the purified $PP2A_{T61}$ trimer. Moreover, isolated B′/B56/PR61 could displace B/B55/PR55 or ST from the corresponding PP2A holoenzymes, whereas B/B55/PR55 could not (Kamibayashi and Mumby, 1995).

Now, Chen et al. (2004) reinvestigated this issue by following some functional effects of suppressing one member of the B/B55/PR55 (B55α) and one member of the B′/B56/PR61 (B56γ) subunit family and came to different conclusions. They found that suppression of the B56γ subunit expression, but not downregulation of the B55α subunit, mimics ST-induced cell proliferation and tumor formation. The authors mentioned that downregulation of B56γ as well as expression of ST reduced PP2A activity by 43%. This suggests that the PP2A-B56γ holoenzyme represents a substantial proportion of the total PP2A activity in vivo. This is surprising and might be related to the assay method that is used. Moreover, overexpression of B56γ, but not B55α, partially reverses ST-dependent, anchorage-independent growth and tumor formation. Subsequently, in an attempt to understand the molecular mechanism of these ST effects, they found that ST

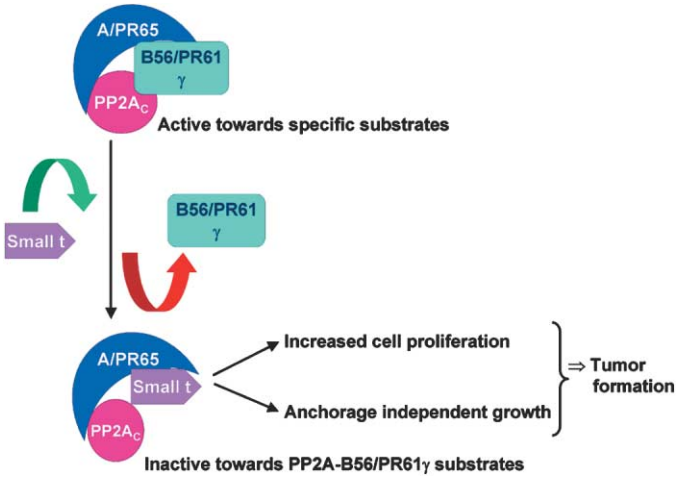

Figure 1. Schematic representation of PP2A-ST interaction

Small t interaction with PP2A displaces the B′/B56/PR61γ regulatory subunit, leading to an increased phosphorylation of specific PP2A-B′/B56/PR61γ substrates. This results in an increased cell proliferation and anchorage-independent growth, leading to tumor formation.

displaces several B′/B56/PR61 isoforms from the trimeric forms. ST also diplaces the B/B55/PR55 subunit as previously reported by others (Kamibayashi and Mumby, 1995), although this PP2A trimer does not participate in ST-induced cell transformation in these experimental cell lines. Finally, in 10 investigated lung cancer cell lines, no B′/B56/PR61γ protein could be detected, and reintroduction of B56γ, but not PR55α, partially reversed the tumorigenic phenotype.

All these results further substantiate the hypothesis that PP2A acts as a tumor suppressor and it is Chen's contribution to define the PP2A-B′/B56/PR61γ trimer as a specific target of ST-induced cell transformation. This work also focuses on the amazing specificity of the trimeric PP2As: although B′/B56/PR61α, β, δ, and ε are highly similar in primary structure and share approximately 68% amino acid identity with B′/B56/PR61γ, B56γ antisense RNA did not alter the expression of the other isoforms. Previously, Ito and coworkers (see Ito et al., 2003, and references therein) already observed that truncation of B56γ leads to an enhanced invasiveness and neoplastic progression, transforming melanoma cells from a nonmetastatic to a metastatic state. This argues again for the specific involvement of the B′/B56/PR61γ subunit in tumor formation. Other groups also contributed to the idea of PP2A being a tumor suppressor. The first strong indication came from the observation that the major cellular target of the tumor promoter okadaic acid is PP2A (Janssens and Goris, 2001). Subsequently, mutations in the PR65/A scaffold protein were detected in some cancers (Wang et al., 1998), and others found that these mutations interfered with the binding of specific third regulatory B subunits (Ruediger et al., 2001). A completely different angle came from the group of Damuni (Li et al., 1996), who identified SET, a protein that is fused to CAN in acute nonlymphocytic myeloid leukemia, as one of the heat-stable specific PP2A protein inhibitors. Therefore, disturbing the normal functioning of PP2A might contribute to leukemogenesis. It is known that the B′/B56/PR61 subunit binds to the adenomatous polyposis coli (APC) tumor suppressor, which is a component of the Wnt signaling pathway (Seeling et al., 1999), and to cyclin G, a regulator of mdm2 and p53 activity (Okamoto et al., 2002). Interestingly, both loss-of-function mutations of APC and overexpression of cyclin G are involved in colon cancer. Hence, the PP2A B′/B56/PR61 subunit might contribute to cancer development by direct interaction to APC and cyclin G. PP2A has been implicated in the regulation of cell cycle progression, DNA replication, transcription, and protein translation, and could therefore also be a causative factor in genetic instability.

Surprisingly, the authors did not observe any functional effect of downregulation of the B/B55/PR55 subunit. Does this mean that PR55 is not involved in the action of ST? Certainly not. Also, Chen and coworkers (Chen et al., 2004) found that ST displaced PR55 from the trimeric holoenzyme, but it seems not to contribute to transformation in their cellular model. In a simpler cell system, where only ST is expressed, PP2A-B/B55/PR55 is redistributed, resulting in an alteration in the actin cytoskeleton and deregulation of the tight junctions, known to play a role in cell adhesion and cancer metastasis (Nunbhakdi-Craig et al., 2003).

In conclusion, it can be said that Chen et al. (2004) succeeded in creating a human cell line that recapitulates most of the cellular changes that can lead to cancerous growth and pinpointed the B′/B56/PR61γ subunit of PP2A as a crucial player in transformation and tumorigenesis. However, the actual regulation of the PP2A-B′/B56/PR61γ activity toward specific target proteins still remains to be elucidated. The action of the oncogenic viral protein SV40 ST implicitly indicates that "third" subunit shuffling is possible, suggesting that such mechanisms also operate in normal cellular conditions. Also, partners in these mechanisms might be targets for therapeutic intervention.

A model for the involvement of a specific PP2A holoenzyme in ST-induced cell transformation is given in Figure 1. However, the molecular mechanism and particular signaling pathways participating in cell transformation and affected by ST-PP2A interaction remain to be elucidated. Probably, cell transformation results from an altered phosphorylation state of PP2A-specific substrates. Alternatively, ST changes the subcellular localization of particular PP2A holoenzymes or affects the interaction of PP2A with cellular proteins such as APC or cyclin G. One of the potential PP2A-ST targets causing cell transformation might be Akt, since the phosphatidylinositol 3-kinase (PI3-K)/Akt signaling cascade is activated by ST (Zhao et al., 2003). PP2A is a major kinase phosphatase and several groups mentioned Akt as a PP2A substrate. Identification of specific PP2A-B′/B56/PR61γ3 substrates involved in ST-induced cell transformation will be of major interest to solve this problem.

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Selected reading

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