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The linchpin? Pin1 meets p73

A recent paper shows that the peptidyl-prolyl isomerase Pin1 conformationally alters p73, promoting its acetylation by p300 in a c-Abl dependent manner. Given previous findings with p53, Pin1 may represent a common mediator linking pro-apoptotic cooperative activity of the p53 family members.

When considering the p53 gene family, one is struck by their conspicuous similarities as well as their intriguing differences. Structurally, the p53 family members' kinship is obvious, as they share a common domain topology and bear significant sequence homology within their transactivation, DNA binding, and tetramerization domains (reviewed in Melino et al., 2002). At this point, however, far more is understood about p53 and its function as a pivotal node in the DNA damage checkpoint. Upon genotoxic insult, p53 becomes modified, stabilized, and thereby activated to regulate transcription of its downstream target genes important in many cellular functions, including cell cycle arrest and apoptosis. While p53's central role in apoptosis and tumor suppression is proven, the extent to which the other members of the p53 family can support or recapitulate its functions is unclear. Nevertheless, p73 can activate at least a subset of p53 target genes independently of p53 and also can mediate p53-independent apoptosis in response to a number of chemotherapeutic agents (Irwin et al., 2003). Interestingly, however, p63 and p73 are required for p53-dependent apoptosis as well as activation of some proapoptotic p53 target genes in mouse embryo fibroblasts expressing E1a in response to cytotoxic agents (Flores et al., 2002). Understanding the mechanisms by which the p53 family members individually and together regulate transcription continues to be an important challenge for revealing their respective roles in tumor suppression.

A recent elegant paper from Montovani et al. links p73 with the Pin1 peptidyl-prolyl-isomerase in apoptosis, and as such may begin to provide a mechanism for coregulation of the p53 family (Montovani et al., 2004). Pin1 mediates *cis*/*trans* isomerization of proteins at serine-proline or threonine-proline (SP or TP) motifs. The ensuing conformational change produces a variety of functional outcomes in many proteins central to tumorigenesis, including cyclin D1, β catenin, NF-κB, and even p53 itself. Substrate recognition by Pin1 requires phosphorylation of SP or TP motifs by multiple families of proline-directed kinases, including cyclin-dependent kinases (CDKs) and mitogen-activated protein kinases (MAPKs) (reviewed in Lu, 2003).

Montovani et al. demonstrate that Pin1 strongly enhances p73-dependent apoptosis in p53 null cell lines, an observation that correlates with the ability of Pin1 to augment p73's ability to induce proapoptotic target genes, including Bax, Pig3, and p53AIP1. The Pin1-p73 interaction is phosphorylation-dependent and seems to be mediated by three key phosphorylation sites within the poly-proline region of p73 that exist only in the α and β isoforms of this protein. Mutation of these 3 residues abrogates Pin1 binding as well as transcriptional regulatory activity of p73. Furthermore, the authors show by a partial proteolysis assay that Pin1 can directly induce conformational change(s) in p73 in vitro, indicating that the Pin1-p73 interaction is direct and that p73 is a bona fide substrate for Pin1 isomerization. Binding of Pin1 often enhances substrate half-life, and indeed, siRNA-mediated knockdown of Pin1 destabilizes p73 protein both in the presence and absence of DNA damage.

The signaling pathway converging on p73, activated by two chemotherapeutic agents cisplatinum (CDDP) and adriamycin (ADR), relies principally on the c-Abl tyrosine kinase (White and Prives, 1999). Direct phosphorylation of p73 by c-Abl on Y99, among other sites, can stabilize and activate p73 protein. Connecting this pathway with Pin1 is the finding that c-Abl activates proline-directed p38 MAPK to phosphorylate TP sites within p73 (Sanchez-Prieto et al., 2002). Thus, the c-Abl pathway likely creates Pin1 recognition motifs on p73 through MAPK. Indeed, Montovani et al. show that CDDP and ADR as well as overexpression of c-Abl or p38-MAPK stimulate Pin1-p73 association. This is dependent on c-Abl, as p73 mutated at Y99 is not active in these assays, and siRNA depletion of c-Abl inhibits the p73-Pin1 association. Conversely, c-Abl cannot stabilize or activate p73 in the context of Pin1 knockdown by siRNA. Taken together, these observations strongly implicate a strict functional interdependence between c-Abl and Pin1 in activating p73 after genotoxicity.

In addition to tyrosine phosphorylation, c-Abl is also required for p300-dependent acetylation of p73, and this modification enhances activation of pro-apoptotic p73 target promoters (Costanzo et al., 2002). Indeed, Montovani et al. found that DNA damage-dependent p73 acetylation is defective in Pin1 null cells, and coexpression of Pin1 enhances both the p300-p73 interaction as well as the

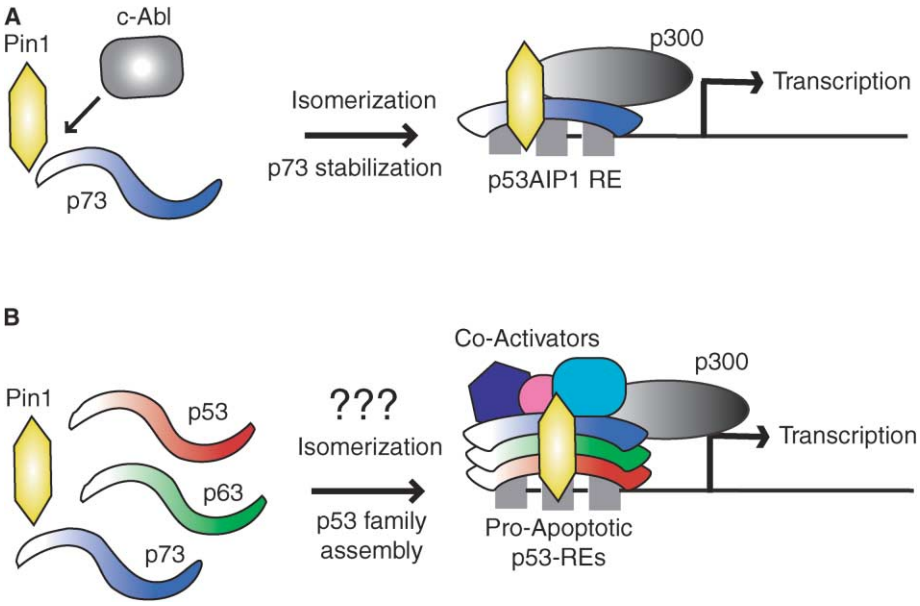

Figure 1. Pin1 and the p53 family

A: Model proposed by Montovani et al. (2004) in which c-Abl and Pin1 promote p73's stable interaction with the p53AIP1 promoter.

B: Speculative model of p53 family coregulation by Pin1, whereby Pin1 facilitates assembly of transcriptional complexes containing p53, p63, and p73, as well as other cofactors.

ensuing acetylation of p73. In a well construed experiment, they further demonstrated that purified Pin1 enhances the interaction of in vitro-translated p73 with purified p300. Thus, their data imply that the Pin1-induced p73 conformational change directly promotes association between p73 and a vital transcriptional coactivator, p300.

Supporting the in vivo relevance of their observations, Montovani et al. show that siRNA-mediated knockdown of Pin1 or p73 reduces CDDP induced apoptosis in a p53 null cell line and also reduces induction of an important p53-dependent proapoptotic target gene, PUMA. Finally, using a chromatin immunoprecipitation (ChIP) assay, the authors show that Pin1 siRNA treatment leads to a marked decrease in the association of endogenous p73 with the p53AIP1 promoter after treatment with CDDP. Whether this reflects a direct effect of Pin1 on the intrinsic ability of p73 to bind to DNA or is the indirect result of increased p73 protein levels was not determined. Whatever the mechanism, these data suggest that the intermolecular associations and posttranscriptional modifications promoted by Pin1 are required for stable association of p73 with at least some of its target promoters (Figure 1A).

p73 is not the only member of the p53 family regulated by Pin1. Indeed, it was previously demonstrated by this group and others that p53 itself is regulated by Pin1 in a manner very similar to that shown for p73 (Pulverer, 2002). Pin1 inducibly binds p53 after DNA damage and alters both its stability and transcriptional activity. Interestingly, ADR-induced association of p53 with two of its target promoters, p21 and Mdm2, is compromised in Pin1 null mouse embryo fibroblasts (Zheng et al., 2002), a similar observation to that described for p73. The fact that Pin1 can regulate both p53 and p73 may help to explain why, in some settings, the absence of p63 and p73 precludes stable association of p53 with a subset of its target promoters, and conversely that the absence of p53 similarly affects promoter binding by p63 (Flores et al., 2002). An admittedly speculative model to reconcile these mechanistically mystifying observations would posit that Pin1 (alone or in concert with other factors) acts as a molecular keystone of p53 family transcriptional activation (Figure 1B). Pin1 may function to stabilize and assemble a transcriptional complex involving p53, p63, p73, and perhaps other coactivators like p300 (Figure 1B).

Future experimentation will hopefully explore this exciting prospect and many questions can be approached. First, is the third p53 family member, p63, also a substrate for Pin1? Intriguingly, p63 has several conserved phosphorylation sites that, like p73, may be Pin1 substrates. Second, does Pin1 itself participate in complexes containing p53 family members that are associated with chromatin? It should be possible using a ChIP assay to determine Pin1's presence at p53 target promoters. Third, does the absence of Pin1 affect recruitment of additional factors to p53 responsive promoters? A recent study demonstrated that the previously shown transcriptional coregulators of p53, ASPP1 and ASPP2, potentiate the function of endogenous p63 and p73 (Bergamaschi et al., 2004). Perhaps they too are present in a complex involving p53 family members and Pin1. Further supporting the possibility that Pin1 may play a specific role in transcriptional regulation is the observation that Pin1 can directly regulate the function of RNA polymerase II (Xu et al., 2003).

In sum, the data presented in Montovani et al. not only delineates a new regulatory interaction between p73 and Pin1, but also describes how this integrates into the known p73 signaling pathway, and ultimately how Pin1 affects p73's proapoptotic function in vivo. As with all good studies, in hinting that Pin1 may be the glue holding the p53 family together, it sets the field off in new directions.

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