Cell Death and Differentiation (2013) 20, 972–973

www.nature.com/cdd

Editorial

p73 – constitutively open for business

MA Seeliger[1] and UM Moll*,[2]

Cell Death and Differentiation (2013) **20,** 972–973; doi:10.1038/cdd.2013.56

In this issue of *Cell Death & Differentiation*, Luh *et al.*[1] determine the oligomerization state and activity of TAp73α and compare it with that of closely related family members p53 and TAp63α, see Figure 1. TAp63α and TAp73α share the same domain organization, and their amino-acid sequences are the most highly conserved among the three members. The authors' finding is therefore surprising as TAp73α forms constitutively open active homotetramers, whereas p63 forms closed inactive homodimers.[1] This interesting result provides a new mechanistic model for a previously unsuspected differential regulation of activity between TAp63α and TAp73α.

p53, mutated in over half of human cancers and in the other half often indirectly inactivated via its regulators, maintains genomic integrity by triggering cellular senescence and apoptosis of damaged cells. Hence, p53 is a critically important tumor suppressor. Similarly to p53, TAp73 and TAp63 can induce cell-cycle arrest and apoptosis by transcriptional activation of many antiproliferative and pro-apoptotic p53 target genes, and by sets of unique genes. p73 also facilitates the maintenance of genomic integrity and euploidy in the absence of p53 in primary cells.[2] In addition, the p53/p63/p73 family can exert their functions by transcriptional regulation of microRNA genes, a class of non-coding regulatory RNAs that suppress stability and/or translation of target mRNAs. In contrast to p53, the p73 and p63 homologs have critical roles in development of the CNS[3,4] and skin/limbs,[5] respectively. Moreover, dependent on context, they can exert tumor suppressor activities that cooperate with p53. Unlike p53, however, p73 and p63 are rarely mutated in cancers.[6] Instead, upregulation of the anti-apoptotic dominant-negative ΔNp73 and ΔNp63 isoforms via alternative splicing or an alternative internal promoter is the most frequent abnormality in solid cancers. In hematological malignancies the most frequent p73 defect is promoter methylation and loss of expression, associated with unfavorable clinical outcomes. This suggests an essential tumor suppressor role of p73 in blood cells, also supported by genetic mouse models. Many therapeutic approaches aiming to restore p73 activity are currently being investigated. In contrast to p73, there is no firm genetic evidence in human cancers that TAp63 is a tumor suppressor. Instead, the most common alteration is upregulation of anti-apoptotic ΔNp63 in for example squamous cell and urothelial cancers, suggesting an oncogenic role.[7]

TAp63α – but not p53 – is the main guardian of the female germ line.[8] Importantly, undamaged healthy oocytes express high levels of the protein. This indicates that activity of TAp63α is tightly controlled by an inhibitory mechanism until needed to eliminate damaged oocytes.

The human p53 protein family shares a common domain architecture.[9] The aminoterminal transactivation domain is natively unfolded but promiscuously binds to translational coactivators such as p300 and negative regulators such as MDM2 and Mdmx. This domain is followed by a Zn^{2+}-complexed DNA binding core domain.[10] About 90% of cancer-associated mutations in p53 locate to the DNA binding domain. The C-terminal oligomerization or tetramerization domain in the p53 family is composed of a dimer of dimers. p63 and p73 (but not p53) can exchange dimers to form symmetrical p63:p73 heterotetramers with 2:2 stochiometry *in vitro*.[11] Helix H2 in the tetramerization domain of p63/p73 wraps around and is crucial to stabilize the active tetramer. This helix is missing in p53. In addition to these three domains, p63 and p73 have a sterile alpha (SAM) domain that mediates protein–protein interactions and stabilizes the protein.

Previously, the Dötsch lab had shown for TAp63α that the C-terminal transactivation inhibitory domain (TI, transinhibition) and the aminoterminal transactivation domain (TA) both interact with and block the oligomerization domain (OD), inducing a closed inactive dimer conformation and preventing further assembly into active tetramers. This mechanism keeps the molecule inactive. For induced activation, DNA

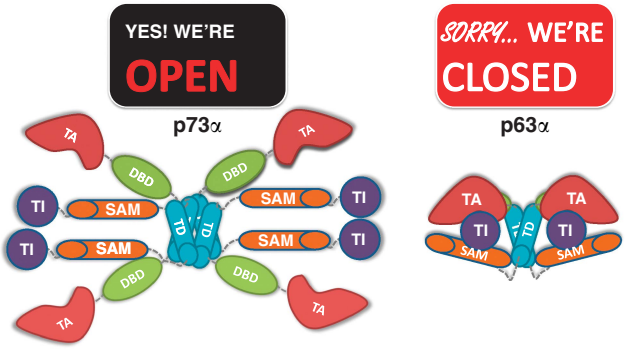

Figure 1 Proposed activation mechanisms of the TA isoforms of p73 and p63 by Luh *et al.*[1]

[1] Department of Pharmacological Sciences, Stony Brook University School of Medicine, Stony Brook, NY, USA and [2] Department of Pathology, Stony Brook University School of Medicine, Stony Brook, NY, USA
*Corresponding author: Dr UM Moll, Department of Pathology, Stony Brook University School of Medicine, Stony Brook, NY 11794, USA. Tel: +631 444 2459; Fax: +631 444 3424; E-mail: utemarthamoll@gmail.com

damage in oocytes triggers phosphorylation of the TAp63α dimers, which releases the inhibitory interactions of the OD domains. The dimers then open up and associate to form open and active tetramers.[12]

On the basis of the overall domain conservation between p63 and p73, and the high level of sequence homology, one could have expected a similar natively autoinhibited state of TAp73α in healthy cells. Surprisingly, however, in this study the Dötsch lab now shows that TAp73α is different and does not take on an autoinhibited closed dimeric conformation. A key finding was that TAp73α expressed in mammalian cells forms a higher-order species in solution that is similar in size to the open activated tetramer of TAp63α. Moreover, in p63, removal of the interaction between the transinhibition and the transactivation domain promotes formation of an open tetramer. In contrast, removal of these interactions in p73 did not change the apparent molecular size of the complex, leading the authors to conclude that the complex preexists in the open active conformation. This open conformation of (p63 and) p73 was accessible for binding to exogenous transactivation and transinhibition domains. Most importantly, in this conformation both p63 and p73 are active in transcriptional activation assays.[1]

This data immediately raises the question how TAp73α is regulated and how this could relate to its function. Apparently, while inactive p63 is constitutively expressed at very high levels and is waiting to be activated by DNA damage via phosphorylation, p73 and p53 are already active but constitutively expressed at very low levels. Thus, this suggests that during evolution, p73 might have lost the autoinhibitory interaction network, still present in p63, which is thought to be the common ancestor from which p73 and p53 evolved via gene duplication. Therefore, p73 activity regulation resembles that of p53 more closely, despite a closer domain architecture, structure and sequence between p73 and p63. Interestingly, this regulatory p53/p73 situation is reflected by their respective roles in tumor suppression.

A number of DNA damage-induced posttranslational modifications of TAp73α, including phosphorylation, acetylation and sumoylation, increase protein stability of TAp73α and consequently its net concentration in the cell.[13] Protein–protein interactions at the transactivation and C-terminal regions alter the overall activity of p73, and expression of dominant-negative ΔNp73 splice variants can shift the overall p73 activity from pro-apoptotic to anti-apoptotic. Through isoform switch, posttranslational modifications and change in subcellular localization, p73 and p53 constantly integrate the outcomes of many signaling pathways. Aside from validating this model with additional evidence, it will be interesting to see what the remaining function of the preserved transinhibitory domain in TAp73α will turn out to be.

Conflict of Interest

The authors declare no conflict of interest.

1. Luh LM *et al. Cell Death Differ* 2013; **20**: 1008–1016.
2. Talos F *et al. Mol Cell* 2007; **27**: 647–659.
3. Talos F *et al. Cell Death Differ* 2010; **17**: 1816–1829.
4. Yang A *et al. Nature* 2000; **404**: 99–103.
5. Yang A *et al. Nature* 1999; **398**: 714–718.
6. Rufini A *et al. Genes Cancer* 2011; **2**: 491–502.
7. Keyes WM *et al. Cell Stem Cell* 2011; **8**: 164–176.
8. Suh EK *et al. Nature* 2006; **444**: 624–628.
9. Joerger AC, Fersht AR. *Annu Rev Biochem* 2008; **77**: 557–582.
10. Cho Y *et al. Science* 1994; **265**: 346–355.
11. Joerger AC *et al. Proc Natl Acad Sci USA* 2009; **106**: 17705–17710.
12. Deutsch GB *et al. Cell* 2011; **144**: 566–576.
13. Conforti F *et al. Cell Death Dis* 2012; **3**: e285.