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Editorial

ΔN-p73: the enemy within

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The p73 gene belongs to the p53 gene family, which also includes p63.[1] The three genes encode proteins with extraordinary similarity at both the structural and functional level. Each member of the family functions as a sequence-specific transcription factor in response to DNA damage and activates pathways involved in the regulation of cell death.[1] Despite these similarities, *in vivo* inactivation of these genes in the mouse revealed that they are involved in completely different cellular processes. In particular, p53-deficient animals, which are normal at birth, develop a broad range of tumours while they age, thus proving p53 tumour suppressor functions.[1] In contrast, p73 null animals do not develop spontaneous tumours. Instead, they are susceptible to chronic infections and are characterized by abnormalities in the central nervous system, such as hippocampal dysgenesis and hydrocephalus.[2] Lastly, p63 is essential for the development of most epithelia, as p63 null mice lack skin and limbs and die *in utero*.[3,4] Importantly, while p53 is inactivated in 50% of human cancers, p63 and p73 are rarely inactivated or mutated. Thus, the role of p63 and p73 in cancer is not obvious.

In spite of these differences, provocative findings suggest that functional interactions can occur among family members. Such interactions are complicated by the multiple p63 and p73 isoforms expressed due to alternative splicing and the presence of two separate promoters. The upstream promoter generates full-length isoforms containing the transactivation domain (TA). By contrast, the downstream promoter produces shorter isoforms lacking the TA domain (ΔN). The ΔN isoforms act as dominant-negative proteins on the function of not only TA isoforms but also of p53.[1] The best example of such heterotypic interference was provided by Pozniak *et al*.[5] who demonstrated that ΔN-p73β is the only isoform expressed in the developing brain at day 10. In p73 null mice, which lack all the isoforms, p53-dependent apoptosis of developing neurons is greatly enhanced, thus indicating that ΔN-p73β inhibits p53 function *in vivo*. While ΔN isoforms inhibit p53 family members, evidence has been produced that TA isoforms are instead required for p53-dependent cell death and transcription.[6] Nevertheless, this has been recently challenged using a different experimental system,[7] suggesting that functional cooperation among family members varies depending on the cell type and stimuli utilized. Interestingly, a very recent paper by David Lane's group has reported the identification of p53 isoforms, which include ΔN proteins with potential dominant negative function, thus revealing unexpected degrees of complexity.[8]

The notion that ΔN-p73 isoforms inhibit p53 family members suggests that the presence of these isoforms in tumours may have an impact on clinical outcome and response to therapy. In human cancer, both TA-p73 and ΔN-p73 are expressed, thus suggesting that a balance between the two may be in place to control cell proliferation and cell death.[1] An interesting paper by Müller *et al*.[9] in this issue of 'Cell Death and Differentiation' attempts to address two fundamental questions: (i) by what mechanisms does ΔN-p73β inhibit cell death induced by chemotherapy? (ii) Does the presence of this isoform correlate with reduced survival in cancer? The authors demonstrate that TA-p73β induces apoptosis by a caspase-dependent mechanism and transcriptionally regulates several genes involved in the control of programmed cell death. In particular, transcription of genes encoding death receptors CD95, TNF-R1, TRAIL-R1, and TRAIL-R2 was induced by TA-p73β. In addition the procaspase 8-binding adapter Fas-associated death domain (FADD) was found to be upregulated. The induction of genes encoding several caspases was also observed. Lastly, the expression of a number of genes involved in the intrinsic pathway was induced. Thus, TA-p73β is able to affect both the intrinsic and the extrinsic apoptotic pathways. Interestingly, TA-p73β induces the expression of CD95 on hepatoma cells and sensitizes these cells to anti-CD95 agonistic antibodies. By contrast, ΔN-p73β inhibits TA-p73β-dependent transactivation of the CD95 gene and CD95-induced cell death, thus confirming that it can work as a dominant-negative protein (Figure 1).

Then, the authors analyze the effect of TA- vs ΔN-p73β on the response to chemotherapy in various cell lines. Expression of TA-p73β is induced by chemotherapeutic drugs such as bleomycin, mitoxantrone, and doxorubicin. Remarkably, overexpression of TA-p73β synergizes with drugs for the induction of cell death and the upregulation of CD95. In contrast, while expression of ΔN-p73β is also induced upon drug treatment, it inhibits both upregulation of CD95 and cell death. Moreover, ΔN-p73β blocked mitochondrial depolarization thus demonstrating that both extrinsic and intrinsic pathways are affected by this dominant negative isoform.

The very exciting part that follows is focussed on the potential impact of ΔN-p73β on survival of hepatocellular carcinoma (HCC) patients and its use as a prognostic marker. One of the major caveats of the field of p73 has always been the lack of reliable antibodies and in particular of isoform-specific antibodies. This has hampered the possibility to evaluate protein expression levels of different p73 isoforms in cancer. Now, the authors of this manuscript employ newly generated antibodies against either TA- or ΔN-p73 [10] and find that 37% of the HCCs displayed overexpression of ΔN-p73β. There was no correlation between ΔN-p73β expression and p53 mutation status. The authors then analysed survival of

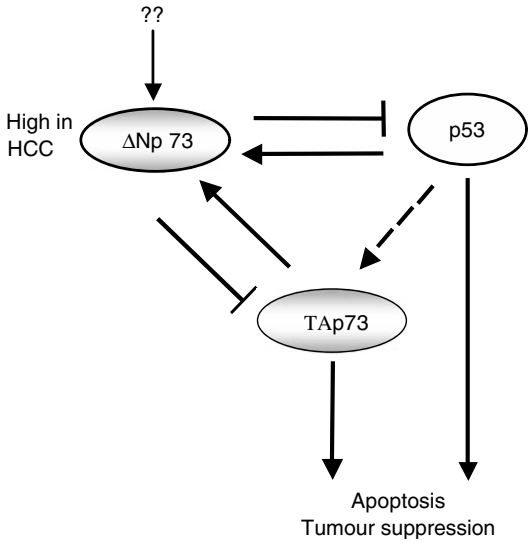

Figure 1 p53 and TA-p73 induce apoptosis. p53 proapoptotic activity is in part dependent on TA-p73. Both proteins induce the expression of ΔN-p73 acting on its promoter. ΔN-p73 acts as a dominant negative protein on the functions of p53 and TA-p73, although it is conceivable that ΔN-p73 is regulated also through alternative mechanisms. ΔN-p73 expression is high in HCC and correlates with poor survival

HCCs patients with respect to the presence of ΔN-p73β, the tumour stage and grade, and concluded that there was a strong association between ΔN-p73β expression and poor survival. These novel findings argue for an oncogenic role of ΔN-p73β in HCC.

These data require confirmation from other series of HCC and further studies are needed to determine whether overexpression of ΔN-p73β is detected in other tumours and if this also correlates with poor survival. However, the data highlight two important clinical points. Firstly, they illustrate the increasing challenges in defining chemotherapeutic resistance. Patients who are likely to respond inadequately to conventional therapy should be identified at diagnosis to allow for rational introduction of new therapeutic approaches. In many malignancies, immunohistochemical detection of TP53 expression has been the principle marker of possible resistance to chemotherapy. Similar phenotypes may be generated by other mechanisms such as ATM mutations, and as this paper suggests, expression of specific p73 isoforms. Functional assays on clinical material may be necessary to assess the global p53 status within tumours.[11] Secondly, from the point of view of therapy, there is increasing interest in the TRAIL receptors as therapeutic targets, with agonistic MAbs now entering the clinic. It may be necessary to sensitise some tumour cells for this approach to be successful.[12] Pharmacological manipulation of expression of p73 isoforms may successfully synergise with TRAIL-receptor targeted approaches through upregulation of both the receptors themselves and down-stream apoptotic effector molecules.

The existence of multiple p73 isoforms has rendered the understanding of its function difficult and challenging. At the same time, it might explain the lack of spontaneous tumour formation in p73 null animals, in which all p73 isoforms are absent. In fact, the concomitant inactivation of 'oncogenic' and 'tumour suppressive' isoforms may result in the absence of a tumour phenotype. In order to verify this working model, the field of p73 is urgently in need of isoform-specific knockout animals lacking either TA or ΔN-p73. These mouse models will be fundamental for the understanding of p73 *in vivo* functions and, in particular, of its role in cancer development. Another important question that remains to be answered is how p73 upstream and downstream promoters are regulated, and whether they are misregulated in cancer. It is known that p73 downstream promoter, which drives the expression of ΔN-p73, contains a p53 responsive element, which is regulated by both p53 and TA-p73. However, both p53 and TA-p73 expression levels are often very low in primary cells such as mouse embryo fibroblasts, where ΔN-p73 is instead expressed at quite high levels. This suggests that the p53(p73)/ΔN-p73 autoregulatory loop is not the only mechanism in place to regulate ΔN-p73 expression *in vivo*. Moreover, it is still unclear what mechanisms regulate ΔN-p73 stability and whether these are different from the ones controlling TA-p73 protein levels. Another important question is whether overexpression of ΔN-p73 can exert tumourigenic activities *in vivo* and what tissues are susceptible to its action. These are just few of the future challenges awaiting researchers involved in the understanding of the physiological functions of p63 and p73 family members and their role in cancer.

1. Melino G *et al.* 2002 Nat. Rev. Cancer 2: 605–615
2. Yang A *et al.* 2000 Nature 404: 99–103
3. Mills AA *et al.* 1999 Nature 398: 708–713
4. Yang A *et al.* 1999 Nature 398: 714–718
5. Pozniak CD *et al.* 2000 Science 289: 304–306
6. Flores ER *et al.* 2004 Nature 416: 560–564
7. Senoo M *et al.* 2004 Cancer Cell 6: 85–89
8. Bourdon JC *et al.* 2005 Genes Dev 19: 2122–2137
9. Müller *et al.* 2005
10. Sayan AE *et al.* 2005 Biochem. Biophys. Res. Commun. 330: 186–193
11. Pettitt AR *et al.* 2001 Blood 98: 814–822
12. MacFarlane M *et al.* 2005 Cell Death Differ. 12: 773–782