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Review

p73 induces apoptosis by different mechanisms ☆

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Abstract

p73, like its homologue, the tumor suppressor p53, is able to induce apoptosis in several cell types. This property is important for the involvement of p73 in cancer development and therapy. However, in contrast with p53, the TAp73 gene has two distinct promoters coding for two protein isoforms with opposite effects: while the transactivation proficient TAp73 shows pro-apoptotic effects, the amino-terminal-deleted ΔNp73 has an anti-apoptotic function. Indeed, the relative expression of these two proteins is related to the prognosis of several cancers. Here we discuss recent developments in the control of p73-induced apoptosis. First, TAp73 induces ER stress via the direct transactivation of Scotin. Second, TAp73 induces the mitochondrial pathway by directly transactivating both Bax and the BH3 only protein PUMA promoters. While the first transactivation is weak, and not sufficient to trigger apoptosis (at least in the in vitro cellular models so far evaluated), the induction of PUMA is strong and lethal. Third, the promoter of the death receptor CD95 contains a p53 responsive element and preliminary experiments suggest that TAp73 also activates the death receptor pathway. In addition, TAp73 is able to transactivate its own second promoter, thus inducing the expression of the anti-apoptotic ΔNp73 isoform. Therefore, the balance between TAp73 and ΔNp73 finely regulates cellular sensitivity to death.

Keywords: p53; p63; p73; Apoptosis; Cell death; DNA damage; Scotin; PUMA; Bax; CD95; Death receptor; Cancer

The p73 protein [1] belongs to the p53 family, reviewed in [2–4]. Indirect experimental evidence for another protein similar to p53 was already in the literature [5], although p63 and p73 were not formally identified until nearly two decades after the identification of p53. All these proteins share the same architecture, with a significant identity in primary sequence, especially in the DNA-binding domain and, to a lesser extent, in the transactivation domain. As a result, p73 is able to transactivate several p53 responsive genes involved in the control of cell cycle arrest and apoptosis [6]. Fig. 1 shows a schematic synopsis of the p53 family members.

The TP73 gene [7] has at least two distinct promoters, which allow the formation of two proteins with opposite effects: while the transactivation proficient TAp73 shows pro-apoptotic effects, the transactivation deficient, amino-deleted ΔNp73 has an anti-apoptotic function [8]. Indeed, the relative expression of these isomers is correlated with the prognosis of several cancers [6]. Consequently, the evidence that p73 is involved in DNA

☆ *Abbreviations:* TA, full length transactivating isoforms; ΔN, amino-terminal-deleted isoforms, lacking the transactivation domain.

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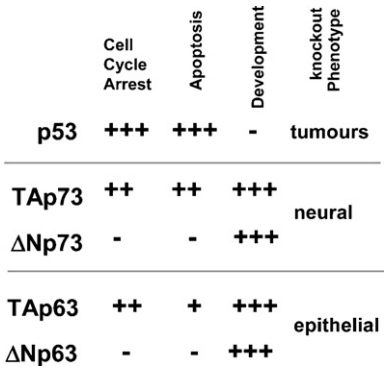

Fig. 1. Schematic outline of the functions exerted by the three p53 family members, p53, p63, and p73. Both p63 and p73 are transcribed as two distinct N-terminal isoforms, derived from distinct promoters. The dominant phenotype of the corresponding knockout mice is indicated.

damage downstream of the MLH1/c-Abl pathway [9–11] should be re-evaluated in terms of the specific isoform involved. But the balance between the two isoforms is not restricted to cancer. The p73 knockout mice show a specific neuronal phenotype [12–14], that is also evident in vitro, in neuronal cells [15,16]. Therefore, the different function of TAp73 and ΔNp73 applies also to developmental neurobiology. To make things more complicated, p73 shows at least seven distinct splicing isoforms, mostly at the 3′ end, present in the transcripts from both promoters [17,18], with a resulting complicated array of functional effects.

Despite the dramatic consequences of expression of this complex gene in the fate of cells, little is known about the mechanisms through which it induces apoptosis. Is the ability to transactivate the same promoters as p53 sufficient to explain the mechanism of death? TAp73 induces G1 cell growth arrest, activates the transcription of some endogenous p53 target genes, such as p21Waf1/Cip1, ribosomal gene cluster (RGC), Mdm2, Bax, cyclin G, GADD45, insulin-like growth factor-binding protein 3 (IGF-BP3), and 14-3-3σ, and induces apoptosis irrespective of the p53 status [4,6]. But, what are the general mechanisms controlling death at the molecular level? How is the fine regulation of cell death exerted in cancer?

Induction of ER stress via Scotin transactivation

Recently, we demonstrated that p73 is able to induce the expression of Scotin [19]. The expression of Scotin has also been shown to be driven by p53 [20]. The Scotin gene is induced after DNA damage in a p53-dependent manner in vivo. p53 responsive elements have been found in the mouse Scotin promoter (S1 p53-binding site is at circa −700 bp [20]). p53 binds specifically to this element and directly transactivates the Scotin gene. Overexpression of Scotin promotes apoptosis in a caspase-dependent manner in p53 negative tumor cells, while inhibition of endogenous Scotin strongly reduces the p53-dependent apoptosis induced by UV irradiation. Scotin is a putative transmembrane protein of 23 kDa able to localize within the ER, via its C-terminal proline-rich domain. The observation that endogenous Scotin is localized in the ER suggests that Scotin triggers apoptosis from the ER as a consequence of ER stress. We have observed that, using Tet-On Saos-2-inducible cell lines, overexpression of TAp73α induces changes in the morphology of the ER by modification of calnexin subcellular localization and by altering the intracellular concentration of free calcium. Moreover, TAp73α was also able to induce Gadd 153/CHOP, a transcription factor induced under ER stress conditions. In this system, we also found that TAp73α, but not ΔNp73α, induces Scotin expression and ER stress [19]. Thus, TAp73, like p53, can induce cell death via the ER, which is an important sensor in the cell of altered cytosolic signals (calcium depletion, accumulation of unfolded proteins, accumulation of viral proteins, and inhibition of glycosylation).

Induction of the mitochondrial pathway via PUMA and Bax

A second mechanism of p73-induced apoptosis is mediated by induction of PUMA a BH3 only protein, which in turn promotes Bax mitochondrial translocation and cytochrome *c* release [21]. In addition, overexpression of p73 promotes Bax promoter transactivation and cell death in a time-dependent manner. However, the kinetics of apoptosis do not correlate with the increase of Bax protein levels. Instead, the p73-induced and PUMA-mediated mitochondrial translocation of Bax is kinetically compatible with the induction of cell death. p73 is localized in the nucleus, and remains nuclear during the induction of cell death, indicating that the effect of p73 on Bax translocation is indirect. Therefore, TAp73 exerts two distinct effects on Bax: (i) direct transactivation of the promoter to enhance the steady state protein levels of Bax and (ii) indirect mitochondrial translocation of Bax protein from the cytosol to the mitochondrial membranes.

The ability of p73 to directly transactivate PUMA, as compared to other BH3 proteins, and the direct effect of PUMA on Bax conformation and mitochondrial relocalization, suggest a molecular link between p73 and the mitochondrial apoptotic pathway. These data, therefore, indicate that PUMA-mediated Bax mitochondrial translocation, rather than its direct transactivation, correlates with cell death. PUMA is sufficient to change the conformation of Bax protein into its active state, visualized by the 6A7 activation-specific antibody. The