iASPP Inhibition: Increased Options in Targeting the p53 Family for Cancer Therapy

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Abstract

Strategies to induce p53 for cancer therapy offer appeal but many tumors harbor inactivating p53 mutations. One way to address this situation may be to activate the p53-related protein p73, which functions similarly, but unlike p53, is rarely lost or mutated in cancer. Along these lines, a recent study reports that a p53-derived peptide that targets iASPP—a common negative regulator of p53 family members—can effectively trigger tumor cell death by a p73-dependent mechanism. These findings promote further study of iASPP targeting as a therapeutic strategy to activate p73. [Cancer Res 2008;68(13):4959–62]

Background

The tumor suppressor protein p53 is activated by various forms of cellular stress, including oncogene activation, DNA damage, γ-irradiation, and numerous chemotherapeutic drugs (1). Once activated, the levels of p53 accumulate in the nucleus and transactivate a myriad of target genes. These target genes primarily bring about either growth arrest, during which cellular damage can be repaired, or programmed cell death, which serves to eradicate cells that may otherwise go on to form a tumor (2). In addition to its central role in preventing cancer development because many tumor cells are more likely to die after p53 activation than are their normal counterparts, p53 has also become considered a highly credible target for therapeutic intervention (1, 2). As a result, in addition to, as outlined above, the many standard forms of chemotherapy that activate p53, a number of strategies have been developed to more directly target p53’s activation. To date, the best characterized are a group of compounds termed Nutlins (3). These compounds disrupt the interaction between p53 and its major endogenous negative regulator, Mdm2. In unstressed cells, Mdm2 keeps p53 activity at bay by ubiquitilating the protein, thereby directing its ultimate degradation by the proteosome (4, 5). Nutlins counter this mechanism and cause elevation of p53 levels. On going studies continue to show the broad spectrum of tumor types that respond well to treatment with Nutlins both in vitro and in vivo (3).

Due to the major tumor suppressive role of p53, for successful tumor development, tumor cells must be able to circumvent the p53 pathway. This is most exemplified by the fact that over half of all human tumors contain p53 mutations, which result in defective p53 proteins that are unable to activate proapoptotic target genes (1, 2). As a result, therapeutic strategies like Nutlins that are designed to be dependent on the presence of wild-type p53 are limited in their application. To combat this and due to the fact that many mutations in p53 result in the production of a full-length mutant protein, an alternative therapeutic strategy has been to develop compounds that can "flip" mutant p53 back in to a wild-type conformation, which induces tumor cell death. The best described of these include CP-31398 and PRIMA-1, which have both been shown to significantly reduce tumor size in animal models (6, 7).

The massive diversity of molecular changes present in each tumor type, however, mean that there is still nevertheless scope for the development of strategies that activate cell death irrespective of p53 status—whether wild-type, mutant, or not present at all. One very promising candidate target in this regard is the p53-related protein, p73. p73 is in many ways a functional paralog of p53, being able to activate many of the same target genes as p53, resulting in the induction of cell cycle arrest and apoptosis (8). Additionally, p73 is relatively ubiquitously expressed and induced in response to a wide range of therapeutic agents, such that knockdown of transactivation competent isoforms of p73 (TA-p73), can significantly reduce levels of chemotherapy-induced cell death (9). p73 is notably different to p53, however, in that it is rarely lost or mutated in human cancer. As a result, p73 holds the potential, if successfully targeted, to cause cell death in multiple tumor cell types. Here, we describe one report from our own laboratory, which details the discovery of a p53-derived peptide that induces cell death in vitro and in vivo by derepression of p73 (10). The function and mechanism behind the action of this peptide and its relevance in the growing field of p53 family signaling pathways as targets for therapeutic intervention are discussed.

Key Findings

Our studies revealed that a 37 amino acid peptide derived from wild-type p53 was able to stimulate cell death in a variety of cells lines irrespective of whether or not the lines contain wild-type p53 (10). This peptide, which we termed 37AA, consists of a noncontiguous fusion of evolutionarily conserved regions II and III (residues 118–142 and 171–181) from within the DNA-binding domain of p53. Although from the DNA-binding domain, it was clear that 37AA could stimulate apoptosis without being able to directly transactivate p53 target genes. Reporter gene assays using regulatory elements from four previously described p53 targets (p21, Bax, PUMA, and PIG3) revealed that although wild-type p53 activated each reporter very effectively, 37AA was essentially inert (10).

Because 37AA seemed not to be modulating its effects through direct transactivation, we considered that it may function through a protein-protein interaction. One group of proteins that bind p53 in the region from which 37AA is derived, is the ASPP family, which regulate the ability of p53 to bind promoter elements of
The ASPP family consists of two proapoptotic family members, ASPP1 and ASPP2 (11), both of which bind to p53 and aid transcription of proapoptotic genes, and one antiapoptotic family member, iASPP, which represses the apoptotic transactivation potential of p53 (12). We considered therefore that 37AA may bind this family of proteins and as a result, positively regulate the apoptotic function of p53. We knew that 37AA could induce cell death in the absence of p53, so we extended our hypothesis to include the possibility that if iASPP also binds p73, then 37AA may, in p53-null cells, act to displace iASPP from p73, thereby derepressing the ability of p73 to transactivate proapoptotic target genes (Fig. 1). Our coimmunoprecipitation studies revealed that this was indeed the case with a strong iASPP:p73 interaction being almost completely disrupted by the presence of 37AA (10).

Subsequent experiments in mice where an expression vector containing 37AA was administered systemically in dendrimer-based nanoparticles revealed that 37AA was able to cause marked regression of human colorectal cancer xenografts. The introduction of either an shRNA targeting TA-p73 or overexpression of iASPP dramatically reduced this observed regression, indicating that the therapeutic effect we observed was mediated by the mechanism we had elucidated in vitro (10). Importantly, the nanoparticles used in the study exhibit striking tumor-selective delivery such that there were no noticeable side effects of the administration of 37AA in this way. In addition, our in vitro studies also revealed that nontransformed cells (mouse embryo fibroblasts, human foreskin fibroblasts, and retinal epithelial cells) showed no sensitivity to 37AA unless they were transduced with retroviruses expressing the oncogene, E1a. This therefore opens up the possibility that even in the absence of nanoparticle

Figure 1. Multiple strategies have been devised to activate p53 family members for cancer therapy. These may result in p53 and/or p73 activation when used alone or, as depicted, in combination to maximally activate either p53, p73, or both for therapeutic gain. 1, currently applied therapies such as chemotherapy and irradiation can be administered to increase p53 or p73 levels. 2, in tumors with mutant p53, molecules such as PRIMA-1 can be used to reconfigure the mutant protein into a wild-type confirmation. 3, nutlin compounds can also either be used to elevate p53 and p73 levels, or like the p53-derived apoptotic peptide, 37AA, can be used to ensure p53 and/or p73 are free of inhibitory influences (Mdm2 in the case of Nutlins and iASPP in the case of 37AA) to activate proapoptotic downstream signaling.

Also inhibited by overexpression of ΔNp73 (a naturally occurring dominant negative p73 isoform). In the absence of 37AA, death was also induced by knockdown of iASPP by RNAi, indicating that iASPP targeting is most likely the only way by which 37AA affects cell viability.

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technology, small molecule mimetics of 37AA may be designed to target p73 and cause selective killing of transformed cells. The mechanism behind the selective killing of oncogenically transformed cells, however, remains unknown and requires further investigation.

Implications

p73, and in particular, the transactivating isoforms (TA) of p73, have recently become compelling targets for drug-based screening programs aimed at instigating cell death in tumor cells. Because TA-p73 is rarely mutated in cancer and is able to activate a wide range of apoptotic genes, there is scope for the design of target-specific therapies within a broad spectrum of cancers. There have been a variety of studies that suggest that this may be the case. First, studies by Irwin and colleagues (9) have shown that TA-p73 is important for the effectiveness of traditional strategies currently used in the clinic, i.e., chemotherapy and radiation therapy. Second, novel screens completed by Wafik El-Diery’s group have isolated some novel compounds that mediate their proapoptotic responses through p73 in the absence of p53 (13). Finally and most recently, a study by Bowman and colleagues (14) has revealed that the above-described Nutlin compounds can effectively alleviate MDM2-mediated transcriptional inhibition of TA-p73, thus allowing the transactivation of proapoptotic target genes, NOXA, and PUMA. The findings in these and our own 37AA study therefore not only reillustrate the potential for therapeutics to be designed directly toward TA-p73 but also identify new targets, in our case iASPP, as potential entry points to achieve this goal.

In some tumors, however, the absence or mutation of p53 would not always lead to p73 being the default option for targeted therapy. As with most aspects of cancer genetics, the situation is far more complex than simply targeting and activating one gene. The p73 gene encodes for proteins involving a number of different COOH-terminal isoforms, many of which as yet still do not have completely defined functions (15). In addition, and more importantly, the two NH2-terminal isoforms, TA-p73 and ΔN-p73, seem to exhibit different expression profiles in different tumor types as well as strikingly different functionalities (15). Many cancers that express high levels of TA-p73 also exhibit high ΔN- levels; thus, it is possible that these may not respond as well to p73-targeted therapies (16). For example, any free TA-p73 made available after 37AA could be inhibited by high levels of ΔN- isoform. It is therefore likely that ΔN-expression levels will have to be established to predict the efficacy of such strategies. In this regard, it should also be noted that the selective targeting of ΔN- forms of p73 is also being considered as a therapeutic option, and if successful, it will be interesting to see if this approach would act cooperatively with agents designed to either elevate or derepress TA-forms of p73 to cause tumor cell death.

Our study on 37AA not only highlights the potential usefulness of TA-p73 as a target for drug design but also that of the iASPP protein. High levels of exogenously expressed iASPP correlate with increased resistance to cisplatin/UV-induced apoptosis (12). In addition, iASPP has been found to be overexpressed in a variety of cancers, including breast carcinomas and certain leukemias (12, 17). It is therefore possible that molecules, such as 37AA or compounds designed specifically to inhibit iASPP, may be particularly effective in these tumor types. It is also interesting to note that although original reports suggest that iASPP can act as a powerful oncogene that can significantly enhance the transforming activity of Ras and E1a oncogenes in rat embryo fibroblasts, a recent study by Laska and colleagues (18) suggests that in normal cells, iASPP can actually aid the induction of apoptosis via inhibition of nuclear factor-κB (NF-κB). The introduction of iASPP siRNA was found to reduce apoptosis when nontransformed lymphocytes, and human lung fibroblasts were exposed to etoposide and other stresses, indicating the consequences of iASPP targeting may be context specific. In these scenarios, it may be that 37AA-mediated inhibition of iASPP may also have a temporary protective effect on nontransformed cells when exposed to similar genotoxic strategies and, thus, may be very useful clinically. Whether 37AA can interfere with the interaction between iASPP and the relevant NF-κB subunit, however, still remains to be elucidated.

Most currently applied anticancer strategies such as chemotherapy and irradiation result in DNA damage, which can cause elevation of both p53 and p73. Because of this, it is thought that rationally designed therapeutics, which also serve to modulate the levels and or activities of p53 family members, such as the Nutlins or 37AA and its derivatives, could work in conjunction with standard chemotherapy to produce an additive or even synergistic effect (Fig. 1). Nutlins and 37AA would act to ensure that activated p53 and/or p73 are not degraded or inhibited from activating target genes. In fact, studies have already shown that nutlins can radiosensitize lung carcinoma cells and chemosensitize neuroblastoma cells (19, 20). Further studies are therefore now required to see if 37AA or its derivatives can do the same, and ultimately, whether each of these different strategies can be selectively used in combination to maximally activate p53 family members for therapeutic gain (Fig. 1).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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