The p53 Family Protein p73 Provides New Insights into Cancer Chemosensitivity and Targeting

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Abstract

The p53 tumor suppressor (1, 2) belongs to a small family of related proteins that includes two other members, p73 and p63. Interest in the p53 family members, their functions and their complex interactions and regulation, has steadily grown over recent years and does not show signs of waning. p73 is a major determinant of chemosensitivity in humans, and mutant p53 proteins carrying specific polymorphisms can induce drug resistance by inhibiting TA-p73. Cooperation between TA (transactivating, proapoptotic, antiproliferative) and ΔN (truncated, antiapoptotic, pro-proliferative) p73 isoforms and among the three family members guarantees equilibrium between proliferation, differentiation, and cell death, thus creating a harmony that is lost in several human cancers. In this article, we review our current knowledge of the role of p73 in cancer chemosensitivity and the real prospect of therapy targeting this molecule. We also draw attention to the crucial role of specific phosphorylation and acetylation events for p73-induced apoptosis and drug chemosensitivity. (Clin Cancer Res 2009;15(21):6495–6502)

Evolution within the p53 Family

Overall structure and sequence homology indicate that p63, p73, and p53 evolved from a common ancestor (8). p73 and p63 share hallmark features that identify p53 across species from Drosophila melanogaster and Caenorhabditis elegans to human: an acidic NH2-terminal transactivation (TA) domain, a highly conserved core DNA-binding domain (DBD), and a COOH-terminal oligomerization domain (Fig. 1). Both p63 and p73 have a sterile α-motif domain implicated in protein-protein interaction. The highest degree of homology is shown within the DBD, where p53 and p63 share approximately 65% amino acidic identity with the DBD of p53 and higher identity (85%) with each other. All three genes are now known to contain a second intronic promoter that controls the expression of NH2-terminally truncated ΔN proteins (ΔNp73, ΔNp63, and Δ133p53; refs. 4, 9–13). Additional ΔN isoforms are generated by alternative splicing and initiation of translation (Δ40p53, Δex2p73, Δex2/3p73, and ΔN′p73) of mRNAs transcribed from the canonical upstream promoter regions (13–15). Δ40p53 and ΔN′p73 are originated by variations of the proline-rich domain. The term ΔTAp73 comprises ΔNp73, Δex2p73, Δex2/3p73, and ΔN′p73 isoforms. Because the lack of acidic NH2-terminal TA domain hampers activation of target genes, TA full-length isoforms (FLp53, TAp73, and TAp63) are functionally distinct from ΔN isoforms, which behave as dominant-negative isoforms and have antia apoptotic and pro-proliferative functions (12, 13). Further modifications induced by alternative splicing of COOH-terminal exons develop numerous different isoforms (α, β, γ, δ, ε, θ, Δ, and η) with still incompletely known DNA-binding properties and functions (3, 13, 15–17). Interestingly, phylogenetic analysis firmly supports the concept that p53 is not the ancestor gene but a more recent product of evolution than p63 and p73, p63 being the ancestor, followed by p73 and then by p53 (8).

p73 and Chemosensitivity of Human Tumors

p73 functions are highly integrated with p53 and p63. An analysis of the phenotype of p73 knockout mice revealed...
neurologic pheromonal and inflammatory defects, but early death precluded the evaluation of tumor development (9). The investigation by Flores et al. (18) of p73 +/- mice underlined the role of p73 as a tumor suppressor. Indeed, full necropsy showed that p73+/- and p63+/- mice developed malignant lesions: 10% of p73+/- mice developed lung adenocarcinoma, whereas 12.5% developed thymic lymphoma and 12.5% hemangiosarcomas. In addition to these malignant lesions, p73+/− mice showed an increase in premalignant lesions such as squamous cell hyperplasia and multiple lung adenoma (18). Double mutant mice for p73 and p63 developed bladder carcinomas, mammary adenocarcinomas, and myelogenous leukemias (18). p73 and/or p63 loss, when accompanied by p53 mutation, generated very aggressive, metastatic phenotypes (18), possibly due to the role of TAp73 and TAp63 in anchorage-independent growth control (19, 20). Recently, Tomasini et al. (21) showed that mice specifically lacking in TAp73 exhibited genomic instability, enhanced aneuploidy, and increased incidence of spontaneous tumors, thus reinforcing the view that the isoform exerts tumor-suppressive functions. Taken together, these results showed that close cooperation between the family members exists and is needed to amplify tumor suppression functions.

Two landmark articles recently showed that p73 is an important determinant of chemosensitivity in humans, and likewise of tumor suppression, the function of which is highly integrated with that of p53 (22, 23).

Irwin et al. found that TAp73 is induced by a large variety of chemotherapeutic agents such as camptothecin, etoposide, cisplatinum, doxorubicin, and Taxol, and that the experimental blocking of the TAp73 function with a dominant-negative mutant or small interfering RNA (siRNA) leads to enhanced chemoresistance of human tumor cells and engineered transformed cells (22) in the presence of wild-type, null, or fully inactive (not gain-of-function) p53. It is noteworthy that for certain chemotherapeutic agents, p73 induction was observed at low, but not high, drug concentrations, suggesting the presence of subtle and specific mechanisms.

Translational Relevance

The scientific evidence currently available highlights the crucial role of the p53 family member p73 in chemosensitivity. One important issue that arises from these studies is the need, both in the prognostic consideration of many cancer patients and in the evaluation of therapeutic options, to go beyond the p53 status and include an assessment of the expression of p73 proteins. The development of standardized methods and reagents and the need for reliable and rapid tests to detect p73 proteins in the clinical setting are important objectives for cancer medicine. Real-time PCR, robust immunohistochemistry reagents and methods, and protein microarray techniques will help achieve this goal.

The recent screening by different laboratories of small molecules targeting the p73 pathway and the preclinical observation of successful inhibition of tumor growth by some of them greatly encourage researchers to start clinical trials, using these new compounds alone or in combination therapies.

Fig. 1. p53 family isoforms and gene structure. p53 belongs to a multigene family that also includes p63 and p73. The overall structure and sequence homology are highly conserved from D. melanogaster to human, and consist of an acidic NH2-terminal TA domain, a central highly conserved core DBD, and a COOH-terminal oligomerization domain (OD). Both p63 and p73 have a sterile α-motif (SAM) domain implicated in protein-protein interactions. The highest degree of homology is shown within the DBD, where p63 and p73 share about 65% amino acidic identity with the DBD of p53 and higher identity (85%) with each other. All three genes contain a second intronic promoter (p2) that controls the expression of NH2-terminally truncated ΔN proteins (ΔNp73, ΔNp63, and Δ133p53). Further isoforms, generated by alternative splicing events and alternative initiation of translation, are the Δ40p53 isoform and the ΔΔAp73 isoforms Δex2p73, Δex2/3p73, and ΔΔNp73. ΔΔ40p53 and ΔΔNp73 are originated by variations of the proline-rich domain (PRD). Further modifications induced by alternative splicing of COOH-terminal exons develop numerous different isoforms (α, β, γ, δ, ε, θ, ζ, and η) with still incompletely known DNA-binding properties, transcriptional activities, and biological functions.
A further observation by the authors that mutant p53 could inactivate p73 and that downregulation of mutant p53 by transfection of p53 siRNA could enhance chemosensitivity supports a model wherein mutant p53 can induce chemoresistance through neutralization of TAp73 (22). Accordingly, specific p53 mutants that inhibit p73-dependent apoptosis have been identified, and p53-dependent inhibition of p73 is correlated with clinical drug resistance (23). Moreover, patients affected with head and neck cancers that express a mutated p53 with an arginine polymorphism at position 72 (72R) have a worse response to therapy, with shorter progression-free survival, compared with patients carrying a proline at position 72 (72P). These results have established a link between a specific polymorphism in p53, p73 inhibition, and the individual response to cancer therapy, underlining the potential effect of these factors in the prediction of clinical outcomes.

In a further study on 122 ovarian cancers, the patients with p53 mutations (65.6%) that efficiently inhibited TAp73 presented a significantly shorter overall survival than those with other p53 mutations (P = 0.044), although the p53 polymorphism at codon 72 is less relevant in these ovarian cancer series (24).

Investigations of human cancers, focused on understanding the role of the antiapoptotic and pro-proliferative isoforms of p53 family proteins, have underlined the high integration of the functions.

A study including 35 human cancers (cancers of the ovary, endometrium, cervix, vulva, vagina, breast, kidney, and colon) showed that about 70% of tumors, but not normal tissues, exhibit upregulation of the ΔNp73 isoform (25). The authors have shown that ΔNp73α suppresses the apoptotic function of wild-type p53 and TAp73 by heterocomplex formation and confers drug resistance to wild-type p53–harboring tumor cells. Consequently, suppression of ΔNp73 expression by specific siRNA enhances p53- and TAp73-mediated apoptosis.

Further investigation of 100 ovarian carcinomas revealed that transdominant ΔTAp73 isoforms are frequently upregulated and have a role as epigenetic p53 inhibitors in vivo (26).

ΔNp73 is frequently overexpressed in rhabdomyosarcoma and is essential for tumor progression in vivo (27). p53 is required to induce transcription of the retinoblastoma (RB) gene, whereas p73 and p63 induce the cyclin-dependent kinase inhibitor p57 to maintain RB in an active, hypophosphorylated state. Ablation of these functions by ΔNp73 overexpression or p53 mutations blocks myogenic differentiation and enables cooperating oncogenes to transform myoblasts to tumorigenicity (27).

Moreover, in most head and neck squamous cell carcinomas, the high levels of ΔNp63α are found to represent an essential survival factor for the tumor, due to their ability to suppress TAp73-dependent apoptosis both by direct promoter binding and by physical protein-protein interaction (28). Inhibition of endogenous p63 expression by specific siRNAs leads to the TAp73-dependent and p53-independent upregulation of apoptotic target genes to induce the selective apoptosis of tumor cells that overexpress ΔNp63α, and correlates with sensitivity to radiation and/or chemotherapy (28).

Strikingly, in a subset of breast cancers that do not express estrogen and progesterone receptors, lack Her2 amplification, and make up the majority of BRCA1-associated tumors, p63 can regulate the p73-dependent pathway that controls cisplatin sensitivity in vivo (29). In these tumors, which commonly exhibit mutational inactivation of p53 and coexpress ΔNp73 and TAp73 isoforms, ΔNp63α promotes the survival of breast cancer cells by binding TAp73 and thereby inhibiting its proapoptotic activity (29). The inhibition of p63 expression by specific siRNAs leads to the TAp73-dependent induction of proapoptotic Bcl-2 family members and apoptosis. Significantly, in these cases, the sensitivity to cisplatin is uniquely dependent on TAp73: after exposure to cisplatin, but not to other chemotherapeutic agents, TAp73 dissociates from the ΔNp63α/TAp73 protein complex and transactivates the proapoptotic members of the Bcl-2 family to induce apoptosis (29).

Figure 2 reports the crucial role of ΔNp73, ΔNp63, and mutated p53 in inhibiting TAp73 transactivation of proapoptotic target genes such as Bax, p53 upregulated modulator of apoptosis (PUMA), and p53-regulated apoptosis inducing protein 1 (p53AIP1).

Several other articles have confirmed the importance of the assessment of p73 isoform expression in the prediction of tumor chemosensitivity and in cancer prognosis by studying different tumor types. Indeed, dysfunctional equilibrium between p73 isoforms has been observed in human thyroid cancer (30), hepatocellular carcinoma (31), neuroblastoma (32), low-grade glioma (33), gynecologic cancers (34), acute myelogenous leukemia (AML; refs. 35–38), colon cancer (39), breast cancer (39, 40), and lung cancer (41, 42). In many cases (23, 24, 26, 31–34, 39, 42), patients with p73 dysfunction proved to have a worse prognosis than those with normal p73 function.
Mechanisms of p73-induced chemosensitivity. The regulatory mechanisms that control p73 drug response are primarily linked to posttranslational modifications and protein-protein interactions involving both signaling molecules and transcription factors. Indeed, mechanical behavior can be complex and variable, being dependent on the specific drugs and tissues involved.

Three seminal articles have contemporaneously shown the key role of the c-Abl oncogene in the processes of p73 activation. In cells exposed to either chemotherapeutic drugs or ionizing radiations, the tyrosine kinase c-Abl phosphorylates p73 on the tyrosine residue at position 99 and potentiates p73-mediated transactivation and apoptosis (43–45). p73 half-life is prolonged and p73 protein levels are increased by cisplatin and by coexpression with c-Abl tyrosine kinase (43). Notably, the interaction between p73 and c-Abl is potentiated by p73 phosphorylation in primary breast cancer cells as well (46), and p38 mitogen-activated protein kinase can participate in mediating this process (47). Furthermore, a robust and translational confirmation of these studies is provided by the observation that c-Abl–dependent phosphorylation of TAp73 is essential for the dissociation of the ΔNp63α/TAp73 protein complex by cisplatin in BRCA1-associated breast cancer (29).

In anaplastic thyroid cancer, c-Abl is excluded by the nucleus, losing its capacity to activate TAp73 (48). This behavior fits with the observation that the Bcr-Abl fusion protein in chronic myelogenous leukemia loses its oncogenic pro-proliferative and antiapoptotic properties when driven off the cytoplasm and entrapped in the nucleus (49). These observations prompt the need for future investigations on the relation between subcellular localization and p53 family protein functions.

c-Abl is also required for TAp73 acetylation by acetyltransferase p300. Indeed, Costanzo et al. (50) have shown that doxorubicin-induced acetylation strongly potentiates the apoptotic functions of TAp73 by enhancing its ability to selectively activate the transcription of proapoptotic target genes but not that of growth arrest genes, thus suggesting that drug-induced TAp73 acetylation can shift the balance between the antiproliferative effect of antitumor drugs toward apoptosis and tumor killing.

Protein-protein interactions with two interesting partners contribute to TAp73 activation and apoptosis in response to cell stress and DNA damage. On treatment with chemotherapeutic drugs, the prolyl isomerase Pin1 interacts with p73 and potentiates p73 phosphorylation in primary breast cancer cells as well (46), and p38 mitogen-activated protein kinase can participate in mediating this process (47). Furthermore, a robust and translational confirmation of these studies is provided by the observation that c-Abl–dependent phosphorylation of TAp73 is essential for the dissociation of the ΔNp63α/TAp73 protein complex by cisplatin in BRCA1-associated breast cancer (29).

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Protein-protein interactions with two interesting partners contribute to TAp73 activation and apoptosis in response to cell stress and DNA damage. On treatment with chemotherapeutic drugs, the prolyl isomerase Pin1 interacts with p73 and potentiates p73 phosphorylation by p300 (51). Indeed, c-Abl and p300 require Pin1 to increase p73 stability and transcriptional activity. Furthermore, the WW domain Yes-associated protein (YAP) contributes to the DNA damage–induced accumulation of p73 and acts as a transcriptional coactivator to potentiate p300-mediated acetylation of p73 and apoptosis (52). Tyrosine-phosphorylated YAP1 by c-Abl displays higher affinity to p73 and selectively coactivates p73 proapoptotic target genes (53). Overall, these breakthrough demonstrations underline the role of specific phosphorylation and acetylation events for...
p73-induced apoptosis and drug chemosensitivity. Significantly, the phosphorylation of YAP2 by tumor suppressor Kpm/Lats2, which is downregulated in poor-prognosis acute lymphoblastic leukemia (54) and breast cancer (55), leads to nuclear accumulation of p73, resulting in induction of its target genes p21 and PUMA (56). Kpm/Lats2 is downregulated in adult T-cell leukemia and natural killer leukemia/lymphoma as well (56), both of which are known to be highly resistant to conventional chemotherapy.

Lunghi et al. determined that arsenic trioxide (ATO; 1 μmol/L), the drug of choice for patients with relapsed acute promyelocytic leukemia (PML) previously exposed to all-trans retinoic acid, targets the p73 proteins to suppress acute leukemia cell growth (35, 36). Indeed, ATO reduces ΔNp73 protein levels (more in K562 than in NB4 cells) and promotes p300-mediated acetylation of endogenous TAp73, thus favoring the apoptosis of the PML NB4 and the Bcr/Abl+ erythroleukemia K562 cell lines that carry inactive p53 (35). Based on the observation that mitogen-activated protein kinases confer an aggressive, apoptosis-resistant phenotype to leukemia cells and that small-molecule inhibitors of the upstream mitogen-activated protein/extracellular signal-regulated kinase (MEK), such as PD98059 (Cell Signaling Technology) or PD184352 (Pfizer), sensitize leukemia cells to drug-induced apoptosis (57–59), Lunghi et al. explored, in a preclinical setting, the potential of a combination of MEK inhibitor PD184352 (Pfizer) and ATO in the treatment of AML.

PD184352 considerably increased ATO-induced apoptosis in 21 of 25 primary AML blasts of different FAB subtypes, showing synergism in 13 of 21 and addictiveness in 8 of 21 responders (36). At the molecular level, MEK inhibition reduced the levels of dominant-negative ΔNp73 proteins and promoted the accumulation of endogenous TAp73 through its tyrosine phosphorylation and transcriptional activation (35, 36). Thus, the combined treatment of ATO and MEK inhibitor enhanced the affinity of phospho-acetylated TAp73 for the p53AIP1 promoter in vivo, leading to p53AIP1 upregulation and increased apoptosis (35). p53AIP1, a direct transcriptional target and a primary effector of wild-type p53– and TAp73-induced apoptosis (50, 60), is located in the mitochondria, and its overexpression induces massive apoptotic cell death through the dissipation of mitochondrial ΔΨm (60, 61). Anti-TAp73 siRNA strongly decreased apoptosis, whereas anti-ΔNp73 siRNA exerted the opposite effect (35).

In blasts of AML patients with active p53 (about 50% of cases), a high concentration of ATO (2 μmol/L) promoted the accumulation of TAp73 and p53 protein levels, whereas at low concentrations (1 μmol/L), ATO only induced TAp73 (36). TAp73 induction through low doses of ATO is similar to the behavior observed by Irwin et al. (22) for certain chemotherapeutic agents.

Contrary to AML blasts, Lunghi et al. (62) showed that in multiple myeloma cells, p73 cooperates in inducing apoptosis...
when p53 is active, but in the presence of mutated or inactive p53, the apoptosis of malignant cells may occur without the aid of p73. Shammas et al. (63) discovered that the treatment of INA6 multiple myeloma cells with epigallocatechin-3-gallate is associated with elevated transcript and protein levels of p73 and p63, but not p53, whereas Raab et al. (64) showed that targeting protein kinase C by small-molecule inhibitor enzastaurin accumulates β-catenin, which triggers c-Jun–dependent induction of p73 and apoptosis of multiple myeloma cells. These findings suggest that stimulus and tissue specificity regulate p53 family member reactions, and prompt the need for further investigations of primary tumor cells of various tissues in different physiopathologic conditions.

It is clear that the PML tumor suppressor gene potentiates p73 transcriptional and proapoptotic activities by regulating the ability of p300 acetyltransferase to acetylate and stabilize p73, thus inhibiting its ubiquitin-dependent degradation (52, 65). Because PML targeting by ATO has recently been found to drive leukemia-initiating stem cells of primary chronic myelogenous leukemia patients from a quiescent drug-resistant status to a drug-responsive cell cycle entry, it would be of great interest to investigate the role of p73 in these processes (66). Indeed, a proapoptotic autoregulatory feedback loop between p73-PML-YAP has recently been found to exist, and this could have relevant implications for chemosensitivity as well (67). The cisplatin regulation of this network emphasizes the translational effect of these results (67). Mechanisms of TAp73 activation are depicted in Fig. 3.

New light could be thrown on the mechanisms of p73-dependent drug sensitivity through an analysis of the relationship with microRNAs (miRNA). A crucial relationship for inducing apoptosis has recently been shown to exist between specific miRNAs and p53 (68). Recently, Sampath et al. (69) found that drug-induced deacetylase inhibition induces miRNA 106b, which regulates p73-dependent apoptotic signaling and death in quiescent B chronic lymphocytic leukemia cells. Induction of miRNA 106b is associated with a downregulation in the levels of the E3 ubiquitin ligase Iotch. Decreases in Iotch protein levels lead to an accumulation of its proapoptotic substrate TAp73. The discovery of this interesting mechanism could lead to the development of further studies on miRNA and p73 relations.

**Targeting p73: A Promising Antitumor Strategy?**

After its discovery in 1997, more than 1,200 articles have been published over the last decade on the study of p73 expression in tumors and its involvement in carcinogenesis, but the real value of this molecule as a potential antitumor target was not apparent. For this reason, the recent recognition of molecules specifically designed toward p73 proteins or their close and direct regulators that inhibit tumor cell growth by activating p73 has generated great interest. Although confirmatory clinical studies are needed, many tumors can be potential targets because the p53-related protein p73 functions similarly, but unlike p53, it is rarely lost or mutated in cancer (70). Figure 4 depicts some strategies that have recently been tested in preclinical settings for targeting p73 to induce the transcription of proapoptotic genes.

Indeed, a p53-derived small peptide, called 37AA, as it contains 37 amino acids from p53, has been described as inducing cell death in multiple tumor cell types irrespective of p53 status, but not in normal cells (71). This peptide has the capacity to bind inhibitor of apoptosis stimulating protein of p53 (iASPP), a common negative regulator of p53 family members that antagonizes positive regulators such as ASPP1 and ASPP2 (72–74). Remarkably, in p53-null cells, this peptide derepresses TAp73, causing p73-mediated gene activation and cell death (71). Moreover, systemic nanoparticle delivery of a transgene expressing this peptide is able to induce p73-dependent tumor regression in vivo. This study supports future strategies to directly and selectively activate the therapeutic potential of TAp73.

The screening by different laboratories of small molecules structurally related to or dissimilar from p53 that activate p53 response by increasing TAp73 or releasing this molecule from the blocking complex with mutated p53 has developed reagents with strong antitumor activity that could become successful drugs (75–77). In some cases, the efficacy of these compounds has been tested in vivo in p53-deficient human tumor xenografts (77).

In tumors that express high levels of ΔNp73, strategies would be aimed at interfering by means of small molecules with the expression and function of these proteins (78, 79). The development of siRNA-based therapeutic agents with improved viability and capability to target specific tissues with poor toxicity (80) would make it possible to specifically target the expression and activity of p53 family Δ isoforms that exert an antia apoptotic, pro-proliferative function (Fig. 4B). This strategy would be used in combination treatment with compounds that positively modulate TAp73 after testing the p53 family status of individual tumors during diagnosis and relapses.

Recently, interest has grown in the small molecule Nutlin-3, which can induce p53-dependent apoptosis in AML cells and B chronic lymphocytic leukemia cells that express a p53 inhibited by murine double minute-2 overexpression (about 50% of AML patients; refs. 81, 82). The demonstration of the property of Nutlin-3 to disrupt murine double minute-2-TAp73 binding in tumor cells with null or mutated p53 deserves attention for cancer treatment (83).

Besides developing small molecules active toward p73 and related molecules, we believe that all new classes of compounds that are candidate anticancer drugs (e.g., aurora kinase inhibitors) should be investigated to assess the effect on p53 family status. Dar et al. (84) investigated the role of Aurora kinase A in regulating p73-dependent apoptosis using p53-deficient cancer cell lines. Interestingly, the authors showed that Aurora kinase A hampers endogenous TAp73 protein expression and its transcriptional activity. The Aurora kinase A inhibitor MLN8054 suppresses cell viability and induces TAp73 and its downstream proapoptotic targets PUMA, BH3-only protein NOXA, and p53AIP1, promoting apoptosis. Because interest in targeting Aurora kinases for the purpose of treating cancer is constantly growing, this article could pave the way for further studies.

It might be worth mentioning that an important class of drugs, cyclooxygenase inhibitors, has recently been shown to induce apoptosis independent of p53 and differentially modulate endogenous TAp73 and ΔNp73 isoforms in fresh neuroblastoma cells (85). Cyclooxygenase downregulation of ΔNp73 is associated with decreased levels of transcription factor E2F-1. These results encourage preclinical combination studies to enhance chemosensitivity in tumors with deregulated E2F-1 and in those with wild-type or mutant p53.
The mammalian target of rapamycin (mTOR) kinase pathway, which has a potentially great drug effect (86), has recently been focused by a gene signature-based approach to be a regulator of p73 (87). Indeed, treatment of both primary cells and cancer cell lines with rapamycin resulted in an increase in p73 levels, as did RNA interference knockdown of mTOR (87). Moreover, the intriguing observation that mTOR regulates autophagy-associated genes downstream of p73 through an unknown mechanism (87–89) could draw attention to alternative, still unexplored, ways of killing cancer.

Conclusion

This review primarily stresses the important acquisition of the role of p73 in human tumor chemosensitivity—a great success of translational and clinical oncology. This scenario leads to investigating the mechanisms and relations with p73 of new classes of compounds (e.g., Aurora kinases, cyclooxygenase, and mTOR inhibitors) that are candidates for cancer therapy. These studies could help identify dysregulated molecules that are potential targets for synergistic combination therapies.

A further great challenge concerns the design of specific small molecules directly targeted to dysregulated p73 proteins or their close regulators. Recently, to overcome laborious screens of genetic or chemical libraries, innovative methods have been proposed, such as the Connectivity Map project (i.e., a means of connecting a disease with a disease-modifying gene product and a chemical modulator of that protein by a systematized and centralized process using gene expression signatures; refs. 90, 91). In this light, the need for reliable and rapid tests to detect all p53 family proteins in the clinical setting is a major objective for research and development efforts in the near future. The integration of expression profiling tools, such as dedicated microarrays or automated multiple real-time PCR reagents, reliable immunohistochemistry reagents and methods, and protein microarray techniques, will help achieve the aim of a more personalized therapy for cancer patients.

Disclosure of Potential Conflicts of Interest

The authors declare that they do not have conflicts of interest.

Acknowledgments

The authors apologize for not being able to cite several interesting articles due to the space limitations created by the need to target primarily translational topics, in keeping with the purpose of this review.

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