Mechanistic Insights into Cell Death Mediated by the P53 Family

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Abstract

Development of effective anti-cancer therapies continues to be a challenge due to genetic instability that promotes intra-tumoral molecular heterogeneity and subsequent adaptation to therapy. In this review, we briefly discuss the history of the p53 family (p53/p63/p73) and how regulatory circuits affect its function in promoting apoptotic cell death. In addition, we provide perspective on how protein-protein interactions between members of the p53 family, as well as, with other regulatory proteins can dictate response to therapy. In the future, therapies that focus on targeting the p53 family to sustain pro-apoptotic pathways in combination with cancer-specific dysregulated signaling pathways is a promising approach. With further investigation at the basic science level, improvements in efficacy and quality of life for cancer patients with diverse molecular signatures may be realized.

ABBREVIATIONS

DDR: DNA Damage Response; BAX: BCL-2-Associated X protein; PUMA: p53 Upregulated Modulator of Apoptosis; BAK: BCL-2 homologous Antagonist/killer; MOMP: Mitochondrial Outer Membrane Permeabilization; TRAIL: Tumor necrosis factor-Related Apoptosis-Inducing Ligand; PTMs: Post-Translational Modifications; ASPP: Ankirin-repeat-containing, SH3-domain-containing, and Proline-rich-region containing Protein; APIID: Agile Protein Interactomes DataServer; MDM2: Mouse Double Minute protein 2; AMPK: AMP-activated Protein Kinase; NEDL2: NEDD4-like Ligase 2; NQO1: NAD(P)H Quinone oxido-reductase 1; LKB1: Liver Kinase B1; mTOR: Mammalian Target of Rapamycin; PPP: Pentose Phosphate Pathway; GLS-2: Glutaminase-2; MDMX: Murine Double Minute X; PML: Promyelocytic Leukaemia protein; IKK: I kB kinase; Plk1: Polo-like Kinase 1; Cables1: CDK5 and Abl enzyme substrate 1.

INTRODUCTION

The p53 gene was discovered in 1979 by Lionel Crawford (Imperial Cancer Research Fund, UK) [1]; Lloyd Old (Memorial Sloan-Kettering Cancer Center) [2]; as well as David P. Lane and Arnold Levine (Princeton University/University of Medicine and Dentistry of New Jersey, Cancer Institute of New Jersey) [3]. Since its discovery, p53 has undergone extensive study and is essentially positioned as a critical regulator of most aspects of cell behavior and physiology. p53 regulates cell growth, survival, differentiation, and, responds to DNA damage. The capacity of p53 to regulate and maintain genomic stability largely determines whether the cell survives or undergoes cell death, senescence, or autophagy. Additionally, numerous studies suggest a critical role of p53 in longevity [4,5], metabolism [6], epigenetic modification [7], motility [8,9], and stem cell reprogramming [10]. Furthermore, p53 activation is essential for the senescence response induced by short telomeres [11]. p53 is mutated in over half of all human tumors and the emerging role of mutant p53 and gain-of-function mutations has been thoroughly reviewed by Muller and colleagues [12]. In this review, we will focus on the role of the p53 family in cancer cell apoptosis and survival. p53 suppresses tum or cell growth through multiple mechanisms. First, following DNA damage, p53 regulates the DNA Damage Response (DDR) by activating DNA repair. Second, p53 can arrest growth by holding the cell cycle at the G1/S checkpoint following DNA damage. Third, it can initiate apoptosis (i.e., programmed cell death) if DNA damage proves to be irreparable [13-16].

After the discovery of p53 in transformed cells, two additional family members, p63 and p73, were identified by McKeon’s group
The p53 family includes three genes p53, p63, and p73. The overall domain structure of p53, p63, and p73 is conserved and consists of an amino-terminal transactivation domain (TA), followed by a proline-rich region (PR), a central DNA binding domain (DBD) and a carboxy-terminal oligomerization domain (OD). In p63 and p73, there is an additional sterile alpha motif (SAM), and transactional inhibitory domain (TID) in the carboxy-terminal. Identity shared by p63 and p73 with p53 is indicated.

(A) Simplified classification of the functions of the p53 family members.

- p53
  - TA
  - DBD
  - OD
  - SAM
  - TID
- p63
  - TA
  - DBD
  - OD
  - SAM
  - TID
- p73
  - TA
  - DBD
  - OD
  - SAM
  - TID

(B) Other characteristics

- p53
  - TA: Activation of downstream target genes
  - DBD: DNA binding
  - OD: Oligomerization
  - SAM: Sterile alpha motif
  - TID: Transactional inhibitory domain

- ΔNp53
  - NA: No activation

- ΔNp63
  - NA: No activation

- ΔNp73
  - NA: No activation

For a more detailed understanding of the functions of the p53 family members, please refer to the original document.
factor-related apoptosis-inducing ligand (TRAIL) to their respective receptors, leading to cell death through activation of caspase 8 [35].

It is generally assumed, although not necessarily proven, that because the functions of p53 and p63/p73 overlap, p63/p73 can substitute for the pro-apoptotic role of p53 in cells where p53 is deleted, mutated, or inactive. As noted below, however, complex regulatory interactions between p63/p73 and mutant p53 can exist. p63/p73 is activated in response to DNA damage and chemotherapeutic agents [36]. Once activated, p63/p73 can regulate the induction of apoptosis and cell cycle arrest similarly to p53. p63/p73-dependent apoptosis is primarily regulated by its ability to transcriptionally activate pro-apoptotic p53 target genes such as: the BCL-2 family members (BAX, PUMA, NOXA, BAD and BIK, the oxidoreductase PIG3, the tetraspan membrane protein PERP); the death receptors (CD95, TNF-R1, TRAIL-R1 and TRAIL-R2[DR5]); the mitochondrial membrane protein p53 AIP1; and the caspases (caspases-3,-6 and -8) [37,38]. Induction of apoptosis by p63/p73 in response to DNA damaging agents is intimately linked not only to its transcriptional activation, but also to post-translational modifications (PTMs) of p63/p73 and interaction with transcriptional co-activators. Several PTMs including ubiquitination, acetylation, and phosphorylation, in addition to transcriptional co-activator recruitment, have been identified as regulators of p63/p73 pro-apoptotic activity. Some of the most investigated proteins that regulate p73-dependent apoptosis are c-Abl, YAP1, p300, CBP, and Ankirin-repeat-containing, SH3-domain-containing, and proline-rich-region containing protein [ASPP] family.

REGULATION OF P53 FAMILY MEMBERS’ STABILITY AND FUNCTION

A large number of PTMs and protein-protein interactions in the non-stressed and stressed conditions control the functions of proteins of the p53 family. In the Agile Protein Interactomes DataServer [APID] database, 1082 proteins interact with human p53, 152 proteins interact with human p73, and 144 proteins interact with human p63 [39]. Many of these interacting proteins could be important in determining biological effect (survival versus death) but it is important to emphasize outcome could depend on the cellular and molecular context studied. Several possible mechanisms have been discovered that regulate the stability of these p53 family members. Furthermore, Kruse et al. provides a detail review on the regulation of p53 protein activity [40,41].

As a brief overview, the major modifications and extensively studied proteins are listed in Table 1. The key features of p53 regulation are as follows

**Regulation of transcription:** p53 mRNA is induced during S-phase, C/EBPβ and RBP-Jk are two major transcriptional factors that regulate transcription of the p53 gene.

Endogenous C/EBPβ binds to the p53 promoter and induces expression of p53 RNA [42], while RBP-Jk binds to the p53 promoter and represses transcription of the p53 gene [43]. ASP1/2 can bind to DBD domain of p53 and increases p53 transcriptional activity, while other members of ASPP family iASPP inhibit p53 activation and p53-mediated apoptosis [44,45].

**Regulation by phosphorylation:** Phosphorylation of p53 leads to stabilization and promotes p53 transcriptional activity to facilitate p53-mediated cell-cycle arrest and apoptosis. Phosphorylation of p53 is the first crucial step for its stabilization. p53 can be phosphorylated by a broad range of kinases, including ATM/ATR/DNA-PK, and Chk1/Chk2. Article by Thompson and colleagues provides further insight on p53 phosphorylation [46].

### Table 1: Regulation of p53 expression and its functional outcome.

<table>
<thead>
<tr>
<th>Protein</th>
<th>p53 region of Interaction</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ubiquitination</td>
<td>MDM2/MDMX</td>
<td>TA domain</td>
</tr>
<tr>
<td></td>
<td>Pirh2</td>
<td>DBD</td>
</tr>
<tr>
<td>Acetylation</td>
<td>P300/CBP</td>
<td>C-terminal acetylation sites</td>
</tr>
<tr>
<td></td>
<td>Sirt1</td>
<td>DBD</td>
</tr>
<tr>
<td>Transcription regulation</td>
<td>C/EBPβ</td>
<td>p53 promoter</td>
</tr>
<tr>
<td></td>
<td>RBP-Jk</td>
<td>p53 promoter</td>
</tr>
<tr>
<td>Phosphorylation</td>
<td>ATM, ATR,DNAPK</td>
<td>Phosphorylate p53 at TA domain</td>
</tr>
<tr>
<td></td>
<td>P38 kinase</td>
<td>Phosphorylate p53 at ser15 &amp; ser46</td>
</tr>
<tr>
<td></td>
<td>CHK1/CHK2</td>
<td>Phosphorylate p53 at ser20</td>
</tr>
<tr>
<td>Methylation</td>
<td>Set7/9</td>
<td>K372</td>
</tr>
<tr>
<td>ASPP family</td>
<td>ASPP1/2</td>
<td>DBD domain</td>
</tr>
<tr>
<td></td>
<td>iASPP</td>
<td>DBD domain</td>
</tr>
<tr>
<td>Redox-dependent &amp; -independent interactions</td>
<td>Ref-1/APE1</td>
<td>Not known</td>
</tr>
</tbody>
</table>
p53 is phosphorylated on Ser15 and Ser20 by ATM, ATR, DNA-PK, Chk1, and Chk2 after DNA damage and other types of stress [47-49]. The outcome of Ser15 and Ser20 phosphorylation is that p53 is stabilized since it can no longer efficiently interact with MDM2, an E3-ligase for p53. In addition, p53 can be phosphorylated by several kinases at Ser46 [50]. Smeeink and colleagues have performed genome-wide DNA-binding and expression analysis upon different chemotherapeutic treatments. They found that chromatin-associated p53 phosphorylated at Ser46 increases significantly upon apoptosis-inducing etoposide treatment, whereas, the amount of DNA-bound p53 that is phosphorylated at Ser15 remains similar following exposure to etoposide. The authors concluded that p53 phosphorylated at Ser46 is most likely involved in apoptosis induction [50].

**Regulation by ubiquitin-like modifications:** Cellular p53 levels are tightly controlled through its ubiquitin-mediated proteasomal degradation [51,52]. MDM2 is the principal endogenous E3-ligase with high specificity for p53 [53-55]. Multiple E3-ligases ubiquitinate p53 and lead to p53 degradation, emphasizing how critical it is to fine tune p53 levels. MDM2 independent pathways for p53 degradation include E3-ligases COP1 [56,57], Pirh2 [58], and Arf-BP1 [59]. Other p53 modifications, such as acetylation, methylation, and others that are reviewed in detail by Kruse et al. [40,41].

**Regulation of p53 by Ref1/APE1:** The multifunctional Ref1/APE1 protein has redox and DNA repair activities. Hanson et al. showed that Ref-1 directly regulates p53 transcription factor activity. Ref1/APRE1 functions in a redox-dependent and independent manner to activate p53. Ref-1 reduces cysteine residues in p53 and via direct interaction with p53 promotes the formation of p53 tetramers in vitro, which collectively enhance p53 binding to target DNA [60].

As previously discussed, p63 is predominantly involved in the regulation of skin and limb development [20, 21]. p63 can mediate apoptosis and the major regulators of p63 are summarized in Table 2.

**Regulation of transcription:** At the transcriptional level, RBM24 is an RNA-binding protein that binds to multiple regions in the p63 3’ untranslated region and destabilizes the p63 transcript [61]. This leads to decreased p63 mRNA and protein. In terms of p63 activation, Bernassola et al. have demonstrated that the promyelocytic leukemia protein (PML) physically interacts with p63, thereby, resulting in accumulation of p63 in the PML nuclear-bodies (PML-NBs) and increased p63 transcriptional activity [62]. The ASPP family member ASPP1 and ASPP2 bind to the DNA binding domains of TAp63, activate the transcription of TAp63, and regulate target genes such as BAX and PIG3 in p53-mutant cells [44].

**Regulation by phosphorylation:** c-Abl can phosphorylate p63 at Tyr149, Tyr171, and Tyr 289 residues to enhance the transcriptional and apoptotic activity of this protein upon DNA damage [63]. I-kappa B kinase activated by radiation or TNF-can phosphorylate TAp63 which blocks p63 ubiquitylation and degradation [64]. Polo-like kinase 1 (Plk1) phosphorylates p63 at Ser52, decreases the TAp63 protein stability, and suppresses TAp63-induced cell death [65].

**Regulation by ubiquitin-like modifications:** The NEDD4-like ubiquitin E3 ligase Itch directly binds to the PYY motif of p63 and polyubiquitinates p63 which triggers pro tease-dependent degradation [66]. WWP1, the homologue of Itch, can also bind to the PYY motif of p63 and ubiquitinate it [67]. p53-

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**Table 2:** Regulation of p63 expression and its functional outcome.

<table>
<thead>
<tr>
<th>Protein</th>
<th>p63 region of interaction</th>
<th>function</th>
</tr>
</thead>
<tbody>
<tr>
<td>WWP1</td>
<td>PPPY motif of SAM domain</td>
<td>Sequesters p63 and downregulates p63 activity</td>
</tr>
<tr>
<td>ITCH</td>
<td>PPPY motif of SAM domain</td>
<td>Polyubiquitinates p63 proteasome degradation</td>
</tr>
<tr>
<td>Pirh2</td>
<td>Not known</td>
<td>Polyubiquitinated p63 proteasome degradation</td>
</tr>
<tr>
<td>NQO1</td>
<td>Not known</td>
<td>Stabilizes p63 and enhances its activity</td>
</tr>
<tr>
<td>RBM24</td>
<td>3'UTR</td>
<td>Decreases p63 mRNA and protein</td>
</tr>
<tr>
<td>ASPP1/2</td>
<td>DBD domain</td>
<td>Upregulates p63 transcription</td>
</tr>
<tr>
<td>PML</td>
<td>Not known</td>
<td>Stabilizes p63 and enhances its activity</td>
</tr>
<tr>
<td>c-Abl</td>
<td>Phosphorylate p63 at Y149, Y171 AND Y289</td>
<td>Stabilizes p63 and upregulates p63 transcription</td>
</tr>
<tr>
<td>IKKB</td>
<td>Phosphorylate p63 at TAD</td>
<td>Stabilizes p63</td>
</tr>
<tr>
<td>Plk1</td>
<td>Phosphorylate p63 at Ser52</td>
<td>Destabilizes p63</td>
</tr>
<tr>
<td>Mutated p53</td>
<td>DBD domain</td>
<td>Downregulates p63 activity</td>
</tr>
<tr>
<td>Cables1</td>
<td>TAD and SAM domain</td>
<td>Stabilizes p63 and enhances its activity</td>
</tr>
<tr>
<td>Pin1</td>
<td>PPPY motif of SAM domain</td>
<td>Stabilizes p63 and enhances its activity</td>
</tr>
</tbody>
</table>
induced RING-H2 (Pirh2), another E3 ubiquitin ligase, physically interacts with p63 and targets p63 for polyubiquitination and subsequently proteasomal degradation [68,69]. The p63 isoform, TAp63 can be degraded by the 20S proteasomes. NAD(P)H quinone oxidoreductase 1 (NQO1) physically interacts with, stabilizes TAp63 and protects it from ubiquitin-independent degradation by the 20S proteasome. The stabilization of TAp63 by NQO1 was especially prominent under stress induced by chemotherapy [70].

Other regulators of p63: As mentioned in the p73 section below, both p63 and p73 can be sequestered by mutated p53. In addition, CDK5 and Abl enzyme substrate 1 (Cables1) can bind to both the TA and SAM domain of TAp63 and prevent TAp63 degradation [71]. Pin1 is a ubiquitously expressed peptidyl-prolyl isomerase. Li et al. demonstrated that Pin1 binds to PPPY motif of SAM domain in TAp63a and prevents it from proteasomal-mediated, degradation and thereby increases p63-dependent apoptosis [72].

Furthermore, major regulators of p73 can occur at both the transcriptional and translational levels and are summarized in Table 3.

**Regulation of transcription:** The E2F1 transcription factor directly binds to p73 P1 promoter E2F consensus site [TT (G/C) (G/C) CG (G/C)] to activate TAp73 transcription [73,74]. ASPP1 and ASPP2 bind to the DNA binding domains of TAp73 activate the transcription of TAp73 and regulate its target genes such as BAX and PIG3 in p53-mutant cells [44]. ASPP1/2 can increase both p73 and p63 transcriptional activity, while other members of ASPP family like iASPP inhibit p73 activation and p73-mediated apoptosis [45]. p73 is also regulated by the ribosomal protein RPL26 at both mRNA and protein levels [75]. Zhang et al. reported RPL26 directly binds to p73 3’ untranslated region (3’UTR) of the mRNA and regulates p73 mRNA stability. RPL26 also interacts with eIF4E and enhances the association of eIF4E with p73 mRNA, leading to increased p73 mRNA translation and p73 protein level [75].

**Regulation by phosphorylation:** c-Abl can phosphorylate p73 at Tyr99 and enhance the transcriptional and apoptotic activity of this protein upon DNA damage [76-79]. In contrast, when p73 is phosphorylated by cyclin/CDK complexes, its transcriptional activity is reduced [80]. p73 proteins have cyclin recognition motifs (CRM) located within the N-terminal portion of the DNA-binding domain. Cyclins regulate p73 by binding to the CRM and phosphorylating p73 at Thr86 [80]. This would be the case when cyclin/CDK complexes are activated and favor a period of cell cycle arrest instead of apoptosis following a genotoxic or metabolic stress. p73 is also involved in sensing metabolic stress via AMP-activated protein kinase (AMPK), a serine/threonine protein kinase that is a central energy sensor in the cell. AMPK has been found to directly phosphorylate p73 at Ser426. Following its phosphorylation by AMPK, p73 accumulates in the nucleus where it can escape Itch-mediated proteasomal degradation, thereby, inducing apoptosis [81]. Chk1

### Table 3: Regulation of p73 expression and its functional outcome.

<table>
<thead>
<tr>
<th>Protein</th>
<th>p73 region of interaction</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDM2</td>
<td>TA domain</td>
<td>Sequesters p73 and downregulates p73 activity</td>
</tr>
<tr>
<td>ITCH</td>
<td>PY motif</td>
<td>Polyubiquitinates p73 proteasome degradation</td>
</tr>
<tr>
<td>YAP1</td>
<td>PY motif</td>
<td>Stabilizes p73, competes with ITCH binding to PY motif of p73</td>
</tr>
<tr>
<td>FBXO45</td>
<td>SAM domain</td>
<td>Ubiquitination leading to p73 proteasome degradation</td>
</tr>
<tr>
<td>UFD2a</td>
<td>SAM domain</td>
<td>Ubiquitination-independent proteasome degradation</td>
</tr>
<tr>
<td>NEDL2</td>
<td>C-terminal PR domain</td>
<td>Stabilizes p73 and enhances its transcription</td>
</tr>
<tr>
<td>NQO1</td>
<td>SAM domain</td>
<td>Stabilizes p73 and enhances its transcription</td>
</tr>
<tr>
<td>E2F1</td>
<td>TAp73 promoter region</td>
<td>Upregulates p73 transcription</td>
</tr>
<tr>
<td>RPL26</td>
<td>3’UTR</td>
<td>Increases p73 mRNA and protein</td>
</tr>
<tr>
<td>ASPP1/2</td>
<td>DBD domain</td>
<td>Upregulates p73 transcription</td>
</tr>
<tr>
<td>iASPP</td>
<td>DBD domain</td>
<td>Inhibits p73 transcription</td>
</tr>
<tr>
<td>c-Abl</td>
<td>Phosphorylate p73 at Tyr99 [PY motif]</td>
<td>Stabilizes p73 and upregulate p73 transcription</td>
</tr>
<tr>
<td>Cyclin/CDK</td>
<td>Phosphorylate p73 at Thr86</td>
<td>Inhibits p73 transcription</td>
</tr>
<tr>
<td>AMPK</td>
<td>Phosphorylate p73 at Ser 426</td>
<td>Stabilizes p73</td>
</tr>
<tr>
<td>CHK1</td>
<td>Phosphorylate p73 at Ser47</td>
<td>Stabilizes p73</td>
</tr>
<tr>
<td>others</td>
<td>Mutated p53</td>
<td>Downregulates p73 activity</td>
</tr>
</tbody>
</table>
and Chk2 are the downstream effector kinases of ATM and ATR, which play a critical role in the regulation of DDR [82]. Gonzalez et al., reported that Chk1 phosphorylates p73 at Ser47 in DDR [83]. Chk1-mediated phosphorylation enhanced p73-dependent transactivation activity and its apoptotic function.

**Regulation by ubiquitin-like modifications:**

(a) The NEDD4-like ubiquitin ligase, Itch, binds to and polyubiquitinates p73 which triggers proteasome-dependent degradation [84]. The transcriptional co-activator YAP1 prevents Itch-mediated ubiquitination of p73 by competing with Itch for the PY motif of p73 [85].

(b) MDM2, the main E3 ubiquitin ligase controlling p53 stability, also binds to p73 and interferes with p300/CBP acetylation of p73. Additionally, it blocks p73-dependent transcriptional activities [86-89]. A previous report indicates that MDM2 does not ubiquitinate p73 but rather catalyzes p73 neddylation which also inhibits p73 transcriptional activity [88]. Recently Wu et al. demonstrated that MDM2 ubiquitinates p73 under specific cell-free conditions, as well as, in p53 null Saos-2 cells. In MDM2-null MEFs, Wu and colleagues observed that overexpression of MDM2 not only promoted p73 degradation but they also noticed that this effect was dependent on Itch [89]. They further found that Itch interacts with the overexpressed MDM2 in MDM2-null cells. Further studies are needed to determine whether direct ubiquitination of p73 by MDM2 is dependent on the cell type and the molecular context.

(c) F-box protein FBXO45 binds to and promotes the ubiquitination and degradation of TA- and ΔN p73 isoforms [90].

(d) The U-box-type E3 ubiquitin ligase, UFD2a, interacts with the SAM domain of TAp73, and promotes its ubiquitination-independent proteasomal degradation [91].

(e) In contrast, NEDD4-like ligase 2 (NEDL2), a HECT-type E3 ligase, regulates p73 in a different manner. Ubiquitination of p73 by NEDL2 stabilizes p73 in a NEDL2-dependent manner. Accordingly, p73 decays at faster rates in the absence of NEDL2 than in its presence. Consistent with the NEDL2-mediated stabilization of p73, NEDL2 enhances the p73-dependent transcriptional activation [92].

(f) NQO1 physically interacts with p73 in an NADH-dependent manner and protects p73 ubiquitin-independent degradation by the 20S proteasome [93].

**Mutated p53:** Di Como et al., demonstrated that mutant p53 could sequester p73 and inhibit its transcription and proapoptotic activity [94]. Surface plasmon resonance and atomic force spectroscopy revealed that a stable complex is formed between mutant p53 and p73 protein, but not between p73 and wild type p53 [95]. The mutant p53 DBD domain interacts with the DBD and oligomerization domain (OD) of p73 proteins [96]. p53 mutants, most of which have mutations in the DBD domain, exhibit an increased aggregation propensity and can induce misfolding and coaggregation of wild-type p53, p63, and p73. Inactivation of p63 and p73 by mutant p53 results in blockade of p63-and p73-mediated downstream target genes and promotes cancer cell survival [23,97]. The precise role for the association between mutant p53 and p63/p73 warrants further investigation to uncover if blocking these interactions or potential interactions with heat shock proteins such as HSP90 [98] could be a useful option for anti-cancer treatment in mutant p53 cancers.

**p53/p63/p73 and cancer cell metabolism**

Cancer cells proliferate rapidly due to metabolic changes or metabolic reprogramming. Nutrients, particularly glucose and glutamine, are used by cancer cells to produce energy through the Warburg effect which increases survival in low-oxygen environments. Oncogenic pathways (Ras, PI3K or Myc) promote glycolysis, while tumor suppressors like p53 and liver kinase B1 (LKB1) inhibit it. p53 and its family members, p63 and p73, have been implicated in many aspects of cellular metabolism, including AMPK and mTOR signaling, carbohydrate and lipid metabolism, the regulation of autophagy, and the maintenance of mitochondrial integrity and redox balance [99-102].

**p53/p63/p73 inhibit mTOR signaling**

Akeno et al. demonstrated that p53 can suppress carcinogenesis by inhibiting mammalian target of rapamycin (mTOR) signaling. They used mouse models (Rb+/− vs Rb+/−/p53−/−) to demonstrate that mTOR activity was markedly increased in p53-deficient tumors and rapamycin treatment suppressed tumor cell growth, identifying mTOR inhibition as a critical p53 tumor suppressive function. p53 transcriptionally induces expression of multiple genes that repress mTOR pathway signaling such as Sestr, Tsc2, pkl2 [102]. ThemTOR protein is an important positive regulator of cell growth and proliferation that can influence the development of diabetes, aging, and cancer [101]. mTOR forms two multimeric protein complexes, each with distinct functions [103,104]. The mTORC1 complex plays multiple roles in the regulation of protein translation and synthesis, mitochondrial biogenesis, lipid synthesis, and autophagy [99]. The mTORC2 complex has not been studied as extensively, but clearly functions in regulating cytoskeletal assembly [104] and cell survival [105]. Activation of p53 in response to nutrient stress is essential for tumor suppression. Although previous studies have emphasized the importance of p53-dependent cell cycle arrest and apoptosis for tumor suppression, recent studies have suggested that other areas of p53 regulation, such as metabolism and DNA damage repair, are also essential for p53-dependent tumor suppression. However, the intrinsic connections between p53-mediated DNA damage repair and metabolic regulation remain unclear. Franklin et al. reported a rate-limiting enzyme that promotes glycolysis called PFKFB3 [105]. The suppression of PFKFB3 increases the flux of glucose through the pentose phosphate pathway (PPP), which increases nucleotide production. Subsequently, this results in more efficient DNA damage repair and increased cell survival [105]. Interestingly, PFKFB3 suppression by p53 could increase the two major PPP products, NADPH and nucleotides, but only nucleotide production is essential to promote DNA damage repair [105]. In this study, they reported that metabolic stresses activate AMPK, which phosphorylates and inactivates murine double minute X (MDMX), a homolog of MDM2 [106]. This results in p53 stabilization and activation [106]. TAp73 transcriptionally activates the expression of glutaminase-2 (GLS-2) which favors conversion of glutamine into glutamate, thus, providing cancer cells an energy resource to help maintain cellular redox homeostasis [107].
FUTURE DIRECTIONS

A significant hurdle for treating cancer is not only the molecular heterogeneity associated with cancer, but also the development of small molecule inhibitors to pertinent signaling pathways that are delivered to the site(s) of the tumor at adequate levels as to engage the target(s) for sufficient duration. Effective target modulation that has a significant inhibitory impact on tumor growth without major debilitating toxicities will be essential to the development of anti-cancer therapies. To this end, multiple phases of combination therapy, each tailored for the molecular signature of the relapsed tumor, will most likely be required to stabilize and/or cure various cancers. Therapies that exploit the DNA damage response by sustaining p53/p63/p73-mediated apoptosis in combination with those that target multiple signaling pathways require further investigation. In wildtype p53 and in some mutated p53 cancers, combination frontline therapy with MDM2 protein-protein interaction inhibitors (PPI) that block binding of p53 [108-111] and p73 [112-114] to MDM2 and sustain p53- or p73-mediated cell death can increase tumor cell kill in vitro and in some human xenograft models. It is important to note that not all MDM2 PPIs can block binding of p73 to MDM2 in mutant p53 cells and that the molecular context of the cell type may dictate response to MDM2 PPIs in mutant p53 cells [112-114]. For example, early generation MDM2 PPIs Nutlin3a and RG7112, but not third generation RG7388, can block binding of p73 to MDM2 (Ding and Pollok, unpublished observations) [112]. In contrast to p53, p73 is rarely mutated in cancer and appropriate pharmacological manipulation of the p73 pathway for cancer therapy is underexplored.

There are three major areas of investigation that should be considered to increase our understanding of p73 mechanism of action, as well as, identifying new therapeutic targets for cancer: (1) Increase TAp73 levels, for instance, by stimulating its transcription or by inhibiting degradation of the protein; (2) Modulate the expression or function of TAp73 upstream regulators such as kinases; and (3) Release p73 from inhibitory interactions with other cellular proteins, such as ΔN p73 isoforms, mutant p53, or E3 Ligases such as MDM2.

Initial clinical trials that utilize MDM2 PPI report evidence of therapeutic responses in relapsed leukemia [115] and liposarcoma [108]. For detailed information regarding clinical trials of MDM2/MDMX inhibitors, refer to the review by Burgess et al. [116]. In summary, investigations to date have uncovered multiple mechanisms that regulate the ability of the p53 family members to effectively promote death of cancer cells. Continued studies focused on p53/p63/p73-mediated cell death will contribute to uncovering further mechanistic insights that can be exploited therapeutically and lead to improved stabilization of disease and, ultimately, curing cancers with wildtype or mutant p53 backgrounds.

REFERENCES

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