



p53 in cancer: ready for therapeutic targeting?

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Submitted Aug 30, 2016. Accepted for publication Sep 05, 2016.

doi: 10.21037/tcr.2016.11.45

View this article at: <http://dx.doi.org/10.21037/tcr.2016.11.45>

TP53 (*p53*) is one of the oldest and best studied genes implicated in cancer formation or progression. Although originally identified as an oncogene (1,2), it has been known for several decades that wild-type (WT) *p53* functions as a tumor suppressor gene. Suppression of cancer formation involves the binding of *p53* to specific DNA response elements, followed by the induction of genes involved in one or more of the following processes, apoptosis, cell cycle arrest, senescence, DNA repair, metabolism or reactive oxygen species (ROS) modulation (3). Because of its ability to prevent cancer formation, *p53* has been referred to as the “Guardian of the Genome” (4).

In most if not all human cancer however, the “Guardian Angel” is transformed into a “Rebel Angel” (5), as *p53* becomes dysfunctional and is unable to suppress carcinogenesis. Two main mechanisms are responsible for *p53* inactivation; mutation and negative regulation of WT *p53* mediated by MDM2, MDM4 or other proteins (3). Both these processes negate the ability of WT *p53* to prevent cancer development or progression. Because inactivation of *p53* is effectively universal in human malignancy, targeting this dysfunction is currently a highly active area of research. Although a variety of approaches for targeting *p53* are undergoing investigation (Table 1), most work is focusing on blocking the degradation of WT *p53*, depleting mutant *p53* and reactivation of mutant *p53* to a WT-like form (6-8).

Blocking interaction between WT *p53* and MDM2/MDM4

In many cancers that lack *p53* mutations, the WT gene is maintained at low levels by interaction with negative

regulators such as MDM2 and MDM4 (9). MDM2 negatively regulates *p53* protein using 2 main mechanisms, i.e., by promoting its degradation and preventing it from activating its target genes. MDM4, although exhibiting strong sequence homology to MDM2, lacks ubiquitin ligase activity and is thus unable to target *p53* for proteasomal degradation. However, like MDM2, MDM4 can bind to *p53* and block its transcriptional activity. Furthermore, MDM4 can complex with MDM2 and thereby indirectly modulate levels of *p53*.

Inhibiting the binding of these proteins to *p53* would thus be expected to block its degradation and maintain WT function. However, traditionally, preventing protein-protein interaction with low molecular weight compounds has proved difficult as their binding surfaces are frequently too flat or large for efficient blockage (9). However, in the case of *p53*-MDM2 interactions, structural studies have shown that the MDM2 N-terminal domain contains a deep hydrophobic pocket into which the transactivation domain of *p53* can bind. Three amino acid residues in *p53*, i.e., Phe19, Trp23 and Leu26 appear to be primarily responsible for this protein-protein interaction (10). Based on this knowledge, several compounds (both low molecular weight organic compounds and peptides) were synthesised to inhibit the interaction between *p53* and MDM2 (11,12).

Amongst the earliest investigated were the nutlins, especially nutlin 3a (11). Nutlin 3a mimics the 3 critical residues in *p53* mentioned above that are necessary for interaction with MDM2. It thus acts as a competitive inhibitor of *p53* binding to MDM2. Although preclinical studies showed that nutlin 3a increased *p53* concentrations, enhanced apoptosis and decreased tumorigenicity in *p53* WT cancer cells (11), this compound was not pursued for

Table 1 Approaches used for targeting p53 in order to treat cancer

Mechanism of action	Drugs in clinical trials/clinical use
Inhibit WT p53-MDM2 interaction	RG7112, RG7388, MI-77301, AMG232, MK-8242, CGM097, DS-3032b, RO6839921
Reactive mutant p53	APR-246
Deplete mutant p53	Ganetespib, onalespib, luminespib
Gene replacement (gene therapy)	Gendicine (Ad-53)*
Restore zinc to zinc-deficient mutant p53	None
Promote readthrough of premature termination	None

*Gendicine is commercially available (Shenzhen SiBiono GeneTech Co.) and is currently approved for clinical use in China. WT, wild-type.

potential clinical use because of poor pharmacokinetic properties and limited efficacy. Rather, more potent p53-MDM2 inhibitors with superior pharmacokinetic properties and enhanced anticancer efficacy were developed, some of which recently entered clinical testing (*Table 1*) (13). Compared with the wide availability of p53-MDM2 inhibitors, fewer pure MDM4 or dual MDM2/MDM4 inhibitors have been described. To the author's knowledge, only one dual MDM2/MDM4 inhibitor has entered clinical trials, i.e., the stapled peptide known as ALRN-6924 (Aileron Therapeutics) (NCT 02264613).

MDM2/4 antagonists would be expected to be of most value in malignancies with WT p53 and high expression of either MDM2 or MDM4. These include sarcomas, leukemias, neuroblastomas and retinoblastomas for MDM2 inhibitors and both melanomas and retinoblastomas for MDM4 inhibitors (9). Potential side effects from the administration of MDM2/4 inhibitors include the possibility that in addition to stabilising p53 in tumor cells, these compounds might also stabilise the protein in normal cells. Levels of WT p53 could thus accumulate in normal cells and induce inappropriate apoptosis and cell death. However, the reported side effects relating to the preclinical use of MDM2 inhibitors are minimal. This limited toxicity in normal cells may be due to p53 primarily inducing cell cycle arrest rather than apoptosis in non-malignant cells (14). MDM2 inhibitors may thus exhibit selective toxicity for malignant cells over their normal counterparts. A further concern with the use of MDM2 antagonists is that if mutant p53 is present in the malignancy undergoing treatment, its stabilisation and the subsequent increased concentrations could increase the risk of cancer progression (15). Indeed, the presence of mutant p53 has been shown to confer resistance to MDM2 inhibitors (16).

Also, in the context of potential side effects from the use of MDM2/MDM4 antagonists, it should be borne in mind that in addition to p53, MDM2 can ubiquitylate other proteins (e.g., estrogen receptor, androgen receptor, p21 and Rb), resulting in their degradation (9). Furthermore, MDM2 has p53-independent activities including the ability to regulate gene expression, participate in DNA repair and modify chromatin structure (17). The impact, if any, of the available MDM2 inhibitors on these processes is unclear.

Reactivation of mutant p53

Several low molecular weight compounds (PhiKan083, MIRA-1, STIMA-1, ZMC1, PK7088, PK11000, PK11007 and PK11011, PRIMA-1 and APR-246/PRIMA-1^{MET}) as well as specific peptides (ReAcp53, pCAP-250) have been shown to reactivate mutant p53 and restore its transcription activity (6-8). Of these, the most widely studied are PRIMA-1 and APR-246 (Aprea Therapeutics). Both PRIMA-1 and APR-246 are pro-drugs that must first be converted to methylene quinuclidinone (MQ) in order to bind to p53 (18). MQ acts by attaching to specific thiol groups in mutant p53, converting it to a WT-like conformation (18). As well as binding to p53, APR-246 has also been shown to inhibit thioredoxin reductase 1 and decrease levels of GSH (7). Both of these interactions result in increased levels of ROS. Thus, PRIMA-1 and APR-246 exhibit a dual mechanism of action, i.e., reactivation of mutant p53 and generation of ROS (7).

Both PRIMA-1 and APR-246 have been shown to exhibit anticancer activity in a wide variety of preclinical models (6-8). The preclinical findings with APR-246 led to a phase I/IIa clinical trial which was carried out in patients with refractory leukemia and prostate cancer (19). In this trial,

APR-246 was administered as a 2-hour intravenous infusion once per day for 4 consecutive days. Overall, the treatment was well tolerated, the most common adverse effects being fatigue, dizziness, headache and confusion. Evidence that APR-246 acted via p53 activation included induction of cell cycle arrest, increased apoptosis and upregulation of several p53 target genes in leukemic cells recovered from the treated patients. In an extension of this trial, carried out in patients with CLL and AML, administration of APR-246 at a dose regimen of 67.5 mg/kg, given as a 6 h infusion on 4 consecutive days was found to be safe and well tolerated (20). APR-246 is currently undergoing a phase Ib/II clinical trial in patients with platinum sensitive relapsed high grade serous ovarian cancer (PISARRO trial) (21). In the phase Ib part of this study APR-246 is being administered in combination with carboplatin and pegylated liposomal doxorubicin.

Depletion of mutant p53

In order for mutant p53 protein to exert its oncogenic functions, it must be stabilized and accumulate in cancer cells (22). Stabilization is achieved by interaction with various chaperone proteins, the best known of which is HSP90. Preventing this stabilization would be expected to result in depletion of mutant p53 and thus the possibility of a reversion of the malignant phenotype. Proof of this principal emerged when it was demonstrated that inhibition of expression of HSP90 by RNAi led to accelerated MDM2 and CHIP-mediated degradation of mutant p53 (22,23).

Subsequently, several low molecular weight HSP90 inhibitors were identified (24). Most of the early compounds acted by binding to the N-terminal domain of HSP90, thus blocking ATP binding. This leads to inhibition of HSP90 which is followed by E3 ubiquitin ligase-mediated degradation of mutant p53 as well as several other HSP90 client proteins, see below. The original HSP90 inhibitors included the naturally occurring compounds, ansamycin, geldanamycin and its derivative 17-allylamino-17-demethoxygeldanamycin (17-AAG). While these compounds showed proof-of-principle that targeting HSP90 could be used to inhibit cancer cell growth, their clinical potential was limited by excessive toxicity and modest clinical efficacy (24). Some HSP90 inhibitors however, were shown to exhibit preferentially growth inhibition for malignant versus non-malignant cells (24).

One of the most widely investigated of the cancer-selective compounds is ganetespib (also known as STA-

9090 or 5-[2,4-dihydroxy-5-(1-methylethyl)phenyl]-4-1-methyl-1H-indol-5-yl-2,4-dihydro-1,2,4-triazol-3-one} (25). Indeed, in some of these experimental systems investigated, ganetespib was shown to exhibit enhanced cytotoxicity for mutant p53 versus p53-null or p53 WT cells (26). Ganetespib is currently undergoing clinical trials in a number of different cancers including non-small cell lung cancer. Other HSP90 inhibitors that have undergone or are currently undergoing clinical trials include onalespib (27) and luminespib (28).

Before concluding this section, it should be stated that HSP90 is responsible for the stabilization of several other proteins in addition to mutant p53. Amongst the other proteins stabilised by this chaperone are EGFR, HER2, AKT, CDK4 and estrogen receptor (22). Inhibition of HSP90 would thus be expected to result in the destabilization and degradation of several proteins involved in oncogenesis. HSP90 inhibitors, in contrast to other anti-cancer compounds, could thus simultaneously block multiple oncogenic pathways. However, this ability to block multiple pathways could also have detrimental effects in normal cells as HSP90 plays an essential role in normal cellular homeostasis (24). Treatment with HSP90 inhibitors might thus be expected to result in multiple toxicities, especially with long-term treatment. Whether such toxicity will emerge should soon become evident from the ongoing clinical trials (23). A further downside with HSP90 inhibitors is that although they eliminate mutant p53 (as well as other proteins) they are unable to reactivate the mutant protein.

Conclusions

Although originally discovered in the late 1970s (1,2), it has taken over 3 decades for clinical trials on targeting the p53 dysfunction in cancer to begin. The ongoing clinical trials are still at a preliminary stage. Thus, it is too early to conclude if targeting p53 will have efficacy for the treatment of cancer. If however, any of the compounds currently being evaluated in clinical trials exhibit potent anticancer activity, it is likely to usher in a new era in cancer treatment, especially as p53 dysfunction is so prevalent in human cancer.

Acknowledgments

Funding: We thank Science Foundation Ireland, Strategic Research Cluster Award (08/SRC/B1410) to Molecular

Therapeutics for Cancer Ireland (MTCI), the BREAST-PREDICT (CCRC13GAL) program of the Irish Cancer Society and the Clinical Cancer Research Trust for funding this work.

Footnote

Provenance and Peer Review: This article was commissioned by the Guest Editor (Zhi-Min Yuan) for the series “p53 Biology and Cancer” published in *Translational Cancer Research*. The article did not undergo external peer review.

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/tcr.2016.11.45>). The series “p53 Biology and Cancer” was commissioned by the editorial office without any funding or sponsorship. The authors have no other conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Cite this article as: Duffy MJ, Synnott NC, Crown J. p53 in cancer: ready for therapeutic targeting? *Transl Cancer Res* 2016;5(6):627-631. doi: 10.21037/tcr.2016.11.45