Review

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Hitting cancers' weak spots: vulnerabilities imposed by p53 mutation

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The tumor suppressor protein p53 plays a critical role in limiting malignant development and progression. Almost all cancers show loss of p53 function, through either mutation in the p53 gene itself or defects in the mechanisms that activate p53. While reactivation of p53 can effectively limit tumor growth, this is a difficult therapeutic goal to achieve in the many cancers that do not retain wild type p53. An alternative approach focuses on identifying vulnerabilities imposed on cancers by virtue of the loss of or alterations in p53, to identify additional pathways that can be targeted to specifically kill or inhibit the growth of p53 mutated cells. These indirect ways of exploiting mutations in p53 – which occur in more than half of all human cancers – provide numerous exciting therapeutic possibilities.

p53 and cancer therapy

p53 is a transcription factor that is activated in response to various stress signals and plays a central role in regulating key cellular processes such as apoptosis, cell cycle arrest, DNA repair, and metabolism [1,2]. Following cellular stresses, p53 mediates the transcriptional regulation of a broad network of genes that ultimately functions to contribute to tumor suppression. The critical role of p53 in tumor suppression is highlighted by the loss of p53 function – through mutations in p53 itself or disruptions in regulators of p53 activity – in most human cancers [3–5]. Furthermore, p53 has been shown in numerous models to mediate the cellular response to genotoxic therapies [6,7] and inactivation of p53 can be associated with increased resistance to chemotherapy and/or poor survival [8–11]. By contrast, several studies have indicated that retention of wild type p53 can dampen the response to chemotherapy in some tumor types, underscoring the context-dependent role of p53 in therapy [12,13]. The diverse outcomes of the p53 response are illustrated in Figure 1.

These observations, along with a plethora of evidence from mouse models demonstrating the importance of p53 as a regulator of tumor suppression and therapy *in vivo* [14,15], led to the development of various strategies to restore p53 function or inhibit aberrant p53 signaling in tumors. Adenovirus-based p53 gene therapy [16,17] has potential application in all tumors, while other approaches exploit the mutational status of p53 in tumors. Those cancers that retain wild type p53 frequently show defects in the ability to activate p53 in response to stress, which is reflected in an inability to prevent the degradation of p53 by its ubiquitin ligase MDM2. Small-molecule or peptide inhibitors of the p53–MDM2 interaction [18] protect p53 from MDM2-targeted degradation and so reactivate an endogenous p53 response. However, many tumors express mutant forms of p53, which show various degrees of conformational instability, leading to loss of wild type activity. These tumors may be targeted by drugs that bind mutant p53 proteins to induce their refolding into wild type conformation [19]. Additional strategies to target mutant p53 proteins have focused on inducing their turnover by restoring degradation [20,21] or disrupting the interaction of mutant p53 with binding partners such as the p53 family members p63 and p73 [22]. However, all of these approaches have limitations, such as on-target toxicities of systemic activation of wild type p53, dominant-negative activities of mutant p53 over any reintroduced wild type, and the large heterogeneity of mutant p53 proteins and their associated phenotypes. Although some MDM2 inhibitors for the treatment of wild type p53 cancers have shown promise in clinical trials, the only p53-based therapy currently available for clinical use is *TP53* gene therapy (Gendicine), which is approved in China but not elsewhere.

An alternative strategy for therapeutic targeting of the p53 pathway is to exploit tumor cell-specific vulnerabilities imposed by alterations in p53 signaling. In recent years, an accumulating number of studies have reported that cells that have lost functional p53 or express mutant p53 proteins exhibit specific functional dependencies on several secondary pathways that could be targeted in therapy. These synthetic lethal approaches hold the promise of significantly reducing toxicity as well as improving the response to conventional therapy. In this review, we discuss some potential approaches for the selective targeting of tumor cells with loss of or perturbations in p53 signaling.

Synthetic lethal approaches to target p53 signaling

The concept of synthetic lethality, first identified in *Drosophila* model systems [23], describes the situation where alterations in two or more separate genes or proteins, nonlethal by themselves, result in death when

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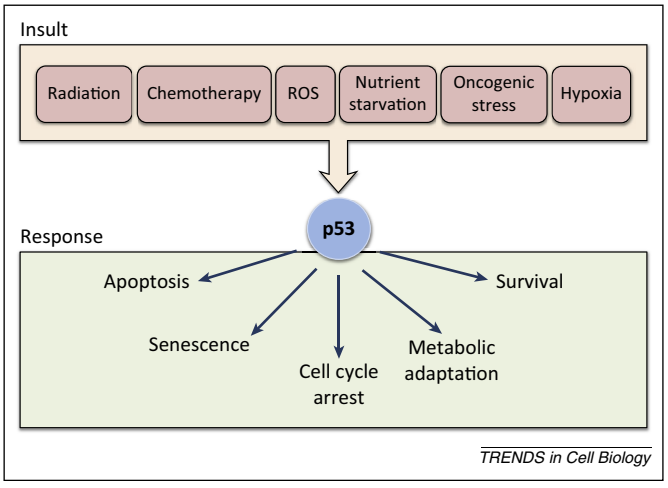

Figure 1. An overview of the p53 response. In the presence of external or internal stresses, p53 can mediate numerous cellular responses, largely through the transcriptional regulation of a diverse set of genes. The net outcome of p53 activation is dependent on the context, type, duration, and severity of the insult.

presented simultaneously within the same cell [24]. The validity of using synthetic lethality in cancer therapy is supported by multiple observations that oncogenic mutations or tumor suppressor defects may lead to the development of secondary dependencies in cancer cells [25–28]. As a key regulator of cell fate with multiple outcomes, synthetic lethal interactions within the p53 pathway can be extremely varied and context-dependent. To date, the induction of p53's death-promoting activities has been at the forefront of developments to use p53 in cancer therapy (Figure 2). However, the ability of p53 to help support the adaptation, repair, and survival of cells under some stress conditions is revealing vulnerabilities that are imposed by loss, rather than induction, of wild type p53 function (Figure 2). Additional complexity arises because most *TP53* gene alterations are missense mutations that result in the expression of a mutant p53 protein, typically at high levels [29]. While cancer-associated mutant p53 proteins generally show loss of wild type activity – meaning the cancer cells are functionally null for wild type p53 – strong evidence suggests that these mutant p53 proteins can also acquire novel oncogenic activities that further perturb cell behavior and may lead to therapy resistance by inhibiting cell death [30,31]. Therefore, to clarify the discussion on these interactions, we have broadly divided them into two categories: synthetic lethal interactions with loss of wild type p53 function in the presence or absence of genotoxic chemotherapy and synthetic lethality imposed by the oncogenic gain-of-function (GOF) activities acquired by mutant p53 proteins. The first category would most likely apply to both p53-null and mutant p53-expressing tumors while the latter interactions would apply only to mutant p53-expressing cancers and may even be specific to the particular mutation involved.

Synthetic lethality with loss of wild type p53 function

p53 and the DNA damage response

p53 is a major effector of the DNA damage response and lies downstream of ATM and ATR, the two major protein kinases responsible for detecting and repairing DNA lesions [32–34]. Depending on the type and severity of DNA damage, p53 is activated by these kinases through specific post-transcriptional modifications leading to cell cycle arrest, senescence, or apoptosis [1,35]. In response to DNA damage, normal cells undergo p53-dependent G_1 arrest, which allows time for DNA repair and is largely mediated through the transcriptional upregulation of the cyclin-dependent kinase (Cdk) inhibitor p21. The p53 response to mild stress or damage is reversible; however, if the damage is too severe the cell is instead targeted for apoptotic cell death by p53 through the activation of intrinsic and extrinsic pathways. Importantly, ATM and ATR can also sustain the intra-S and G_2 checkpoints in a p53-independent manner through the activation of their respective effector kinases Chk2 and Chk1 [36]. Cancer cells that are deficient in p53 exhibit selective loss of the G_1 checkpoint and must depend entirely on S and G_2/M checkpoints to maintain genomic integrity, resulting in synthetic lethal interactions between p53 and the ATM and ATR pathways (Figure 3). As a result, p53-deficient tumor cells are more sensitive to ionizing radiation and genotoxic agents such as cisplatin, camptothecin, and doxorubicin when treated with compounds that can suppress the G_2/S transition through the inhibition of ATR and Chk1 kinases [37–40]. This can be explained by the inappropriate entry of p53-deficient cancer cells into mitosis despite the presence of damaged DNA, resulting in failure to complete chromosomal segregation and death by mitotic catastrophe.

Synthetic lethal interactions have also been described between p53 and the ATM/Chk2 pathway. In cells and tumors that lack a functional p53 pathway, inhibition of ATM was shown to be sufficient to strongly sensitize them to genotoxic chemotherapy by topoisomerase inhibitors [41,42]. These interactions follow a model similar to ATR/Chk1 pathway inhibition whereby p53-deficient cells enter mitosis with unrepaired DNA lesions, leading to mitotic catastrophe. Interestingly, in the latter study by Jiang *et al.*, inhibition of ATM enhanced the survival of p53-proficient tumors in response to genotoxic stress – highlighting the importance of context when considering therapeutic approaches [42]. Further support for synthetic lethal interactions between p53 and ATM or ATR comes from small-molecule screens that have identified potent and selective ATR inhibitors [43–45]. Some of these agents were shown to produce synthetic lethality in p53- or ATM-deficient cells under replicative stress or treated with DNA-damaging agents. In a previous study, severe tissue degeneration was observed following ATR and p53 deletion in adult mice, indicating the co-requirement for these proteins to manage replicative stress [46]. Phase I clinical trials that showed disappointing results for the Chk1 inhibitor UCN-01 in various human cancers [47,48] initially diminished the enthusiasm for Chk1 as a good synthetic lethal target in p53 deficiency. However, a study using a more selective inhibitor of Chk1 reaffirmed the potential of targeting Chk1 alone or in combination with genotoxic chemotherapy in p53-deficient tumors. Using early-passage human-in-mouse (HIM) models of triple-negative breast cancer (TNBC), combination therapy with irinotecan (a topoisomerase I inhibitor) and the selective