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Review of mutant p53 protein and the p53 targeting therapy in cancer treatment

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Abstract. Cancer in humans is a disease that has been difficult to treat due to properties it is able to obtain after being introduced to an organism and has been one of the most prominent points of research in drug development. Since cancers can take on a multitude of forms, a popular strategy employed to find therapies for it is by identifying common features among cancers. A well-known alteration in half of all human cancers is *TP53* mutations, of which there are more than 500. This literary review first discusses the additional capabilities cancer cells obtain, then a discussion of the various functions of p53 and the mutations it can take on. The central focus of this review will be an elucidation of the major approaches attempted in the development of cancer treatment through p53: viruses, targeting gain-of-function mutant p53, structural reactivation of mutant p53 to restore wild type activity, the depletion of mutant p53, and targeting mutant p53 through synthetic lethal inhibitors. Through exploring the different therapies, it is a universal goal to elicit one single treatment for mutant p53 that can impact the greatest amount of p53 mutations while retaining the ability to suppress or even prevent and inhibit cancer.

Keywords: Cancer, Mutant P53, Synthetic Lethal Inhibitor, Structural Reactivator, Gene Therapy.

1. Introduction

The search for a cure for cancer has been a lengthy journey in the field of medicine. Cancer is notoriously difficult to treat because of its ability to arise from a large family of causes, culminating in defining traits such as independence from its microenvironment, evasion of apoptosis, and sustained and rapid proliferation. The multitude of pathways that can lead to oncogene activation and tumor proliferation have, over enduring periods of brilliant research in the past, presented components of those pathways which have the potential to become targets for the treatment of cancer due to the role they play in its proliferation and survival. Due to the diverse nature of causes for cancers, it is thus also attractive to search for a target that will be effective for the largest number of cancers. One such biological agent is the p53 protein, an ancient, evolutionarily preserved signaling hub that responds to a multitude of stress signals, including its critical role as a tumor suppressor, and more specifically as an apoptotic mechanism in response to oncogene activation and tumorigenesis [43]. Mutant p53 proteins exist in more than half of all cancers, which makes it an attractive target for therapy. Furthermore, p53 protein's role as a hub for a plethora of cellular stress signals mean that it has interacts with many pathways, which presents a lot of potential for downstream or upstream components of those pathways to become involved in the inhibition or modification of the p53 protein in order to then affect the progression of cancer. Therefore,

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p53 protein's large presence in cancers, and its massive potential to be modified make it an interesting molecule to research as a target for cancer therapy.

An accumulation of past research has demonstrated that p53 has over 500 mutations, a large portion of which are missense mutations that often lead to the development of cancer [19]. The mutations can be either gain-of-function mutations that give cancers newly acquired capabilities, or loss-of-function mutations that weaken the cell and allow cancer to proliferate. Various strategies have been explored to target both gain-of-function and loss-of-function mutations in p53 proteins. Small interfering RNAs (siRNAs) was one of the first approaches to target mutant p53 that specifically exhibit a gain-of-function [48]. Other methods, like structural reactivators, are promising for its ability to target both gain-of-function and loss-of-function mutant p53 protein, since it is only specific to the structure of each mutant p53 [51-53]. While effective in one manner, the most prominent flaw of structural reactivators is that most are specific to one, or at most a small number, of p53 mutations. This presents an issue when taking into consideration the vast amount of mutations p53 can take on, furthermore implying that in order to effectively treat mutant p53 with structural reactivators, a massive and time consuming amount of screening for potential structural reactivator molecules must be conducted to either find agents for each mutation, or to find one molecule that could restore wild type function to a large number of mutant p53 protein; both are quite unrealistic. Synthetic lethality, a way to indirectly target mutant p53, is considered promising because of its ability to target mutant p53 regardless of structure [64]. More screening is required to find a synthetic lethal inhibitor that is greatly effective, since even the promising synthetic lethal inhibitor Prexasertib is more effective only when used to enhance Olaparib's antitumor effects [64]. Therefore, although attractive molecules for mutant p53 therapy have been discovered, the most promising of them – such as APR 246 and COTI-2 structural reactivators [47] as well as some ATR inhibitors [73] – have only ever managed to enter clinical trial, which brings importance to having an overview of the past and the present of various mutant p53 therapies, in order to reveal what the future of the search for effective treatments should look like.

This literary review will first discuss the six most relevant and common traits tumor cells exhibit as cancer proliferates, followed by an introduction to the p53 protein, the properties which enables its mutation, and the different types of mutations it can take on as well as a discussion of the downstream effects of prominent mutations. The purpose of the first two sections is to give context to the role that mutant p53 plays in cancer proliferation. The first two sections give background to understand the ensuing sections, which discuss important past and ongoing research that have given potential ways to treat mutant p53, citing several specific agents for treating mutant p53 that exhibit great promise and provide proof that its method of treatment is viable. The discussion will have a particular focus on the immediate upstream or downstream interactions that mutant p53 protein partakes in.

2. The common properties of cancer cell

The development of tumors in the body is a multistep process, which involves altered DNA at multiple sites in the genome, as well as the inevitable changes which bring growth advantages to tumor cells. Most cancers share six alterations in the cell that lead to malignant growth: self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis, and tissue invasion [1]. Each of these alterations works to breach an anticancer defense mechanism in cells.

Without mitosis-inducing growth signals, no normal cells can proliferate [1]. Many growth signals a cell receives come from cell-to-cell interactions, making it dependent on its environment for proliferation. Tumor cells are able to synthesize a mimicked version of growth signals, making the cell independent of its environment and also disrupting homeostasis [1].

In normal tissue, there exist anti-proliferative signals which maintain tissue homeostasis. One example of such a signal are growth inhibitors embedded in the extracellular matrix and on cell surfaces. Growth-inhibitory signals are received by transmembrane cell surface receptors working with intracellular signaling circuits, much like growth signals. From there, the cell can monitor for antigrowth signals during the G1 step of the cell cycle, and decide if the cell continues to proliferation, or is stopped

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by either forcing the developing cell out of the cell cycle and into G0, or forcing that cell into the postmitotic state, thereby removing its growth potential. The mechanism by which the cell is forced out of the cycle is via the hypophosphorylation through the pRb protein [2], which blocks proliferation of transcription factors essential for the cell to go from G1 to S phase [1]. Alternatively, normal tissue can force the cell to enter a post-mitotic state prematurely. Cancer is able to stop both of these events from occurring. The TGFβ signaling molecule is involved in a pathway for which the product is the pRb protein. Thus, with the down-regulation of TGFβ receptors, dysfunctional receptors, the elimination of the Smad4 protein (a signal transducer for the pathway) via a mutation, the up-regulation of CDK4 (which can inactivate pRb by hyperphosphorylation) by eliminating the tumor suppressor p15ink4B, and the elimination of pRb through a genetic mutation, the developing cell will not be forced into G0 [3, 4]. Since in a normal cell the C-myc oncogene in the Myc-Max growth-promoting complex is replaced by the Mad transcription factor to make the Mad-Max growth-inhibitory complex, cancers avoid the postmitotic state by overexpressing the C-myc oncogene to return the complex to Myc-Max [5].

Apoptotic mechanisms are dormant in all cells, and can be put into two components: sensors which monitor the environment for abnormalities, and effectors which receive signals from sensors and carry out apoptosis. Signals that cause apoptosis go to the mitochondria, which releases cytochrome C, a catalyst of apoptosis, in response. The p53 tumor suppressor plays a role in apoptosis by its response: the upregulation of the expression of Bax, a pro-apoptotic protein. Bax, in turn, stimulates the mitochondria to release cytochrome C [1]. Cancer cells are able to acquire resistance to apoptosis by inactivating the p53 protein, by getting rid to the FAS death signal through upregulation of a non-signaling decoy receptor for the ligand to bind to, and activating the PI3 kinase-AKT/PKB pathway (which transmits anti-apoptotic signals).

The acquired capability for cancer cells to have limitless replicative potential differs from the previous three capabilities since while the other three makes the cell independent of its environment, it does not ensure expansive tumor growth. Normal mammalian cells have a growth limit, where they reach a state called senescence, which has a control that is separate from cell cycle signaling. The p53 tumor suppressor causes massive cell death when a cell goes past senescence and into a crisis state [6, 7]. Since DNA loses 50-100 bp off of the ends of chromosomes with each replication, the telomere erodes eventually, the DNA loses its protection, and the chromosomal ends fuse, thus causing the cell to enter the crisis state [8]. Malignant cells increase replicative potential by upregulating the expression of telomerase and activating ALT, which maintains the telomere for much longer [9].

Oxygen and nutrients given by vasculature are important for cell survival. Cells with lesions initially lack the ability to make blood vessels, meaning malignant cells must acquire angiogenic abilities in order to grow [10, 11]. Tumor cells can sustain angiogenesis via an angiogenic switch. The switch is activated by changing the balance of angiogenesis inducers and counteracting inhibitors through altering gene transcription of VEGF, FGF, thrombospondin-1, and β-interferon to favor sustained angiogenesis [12].

3. The common properties of p53 protein

The p53 protein was first discovered by 4 studies that demonstrated how the T-antigen, a product of the SV40 viral oncogene, formed a complex with a protein that would come to be known as the p53 protein, and that this protein was detected at high levels in transformed cells [13-15]. At a later date in another experiment, it was proven through tests of mutant p53 cDNA clones which led to cellular transformation, and wild type cDNA which prevented transformation, that the p53 protein functioned as a tumor suppressor [16, 17]. Furthermore, p53 was found in higher concentration in areas with DNA damage [18].

There are three major ways in which the p53 protein's functions may be interrupted. Missense, frameshift, and deletion mutations can lead to a true loss-of-function in the p53 protein, with the most common being the more than 500 identified missense mutations [19]. Amplifications in the MDM-2 gene lead to the overexpression of the ubiquitin ligase, and consequently reduce the production of the

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p53 protein [20, 21]. Protein modifications may also eliminate p53 transcriptional activity. It is also important to note that although there are more than 500 identified missense mutations of the p53 protein, these mutations do not happen at the same frequency due to the mutations having different effects on the p53 protein ranging from altering amino acids that touch the template DNA to affecting the binding domain. Although the 10 most frequent mutant p53 alleles do not get transcribed, the range of mutations that can occur is a major issue for coming up with treatments for cancers containing p53 mutations [22, 23].

Missense mutations may induce both loss-of-function and gain-of-function effects in the cell. A previous experiment found that mutant p53 cDNA clones transformed a cell. The transformation is due to the contribution of a monomeric mutant p53 to a tetramer of wild type p53 proteins, negatively affecting the transcriptional activity of the whole tetramer. Another experiment demonstrated how a mutant p53 can induce a gain-of-function in a cell. In the experiment, when a mutant p53 added to a cell with no p53 was compared with a cell without p53, the cell with mutant p53 inherited enhanced transformation and tumor making capabilities [24].

Tumor regulation is only one function of the p53 protein, and it is a small part of p53's role as a regulator for genomic homeostasis, which is also only a small part of p53's general function as a stress responder. P53 enhances transcription of the MDM-2 gene, which produces ubiquitin ligase that promotes the degradation of the p53 protein, resulting in an oscillating concentration of p53 as well as a half-life of 6-20 minutes [25]. When the cell is exposed to stress, signals from the environment will allow for longer p53 protein half-life and activates it for transcription. Along with the inputs it can receive, p53 also outputs a variety of signals, making it a "hub" (Figure 1). P53's role as a cellular stress responder eliminates the need for extra connections among multiple responders as well as additional signals. This role also means that should a downstream gene that is under its regulation be deleted, tumor suppression will not be lost because p53's role in tumor suppression is to output a set of responses for environmental disturbances [23].

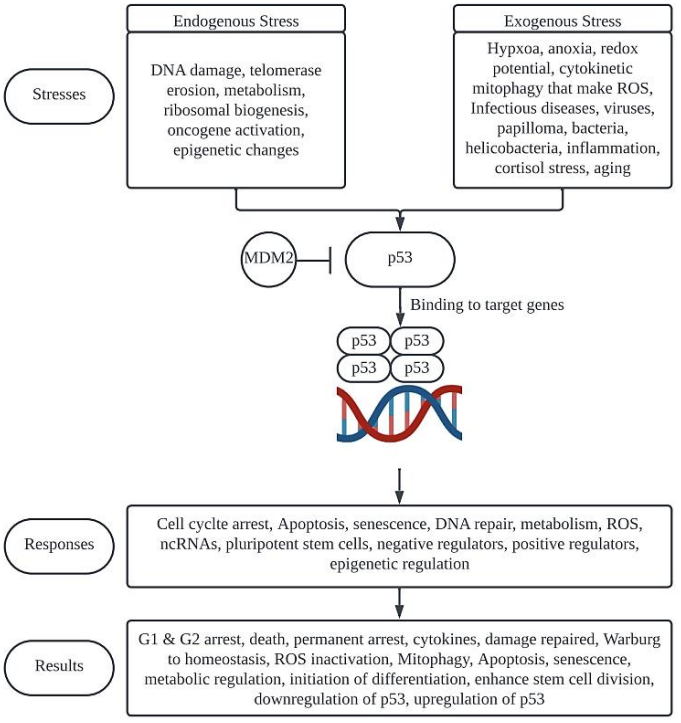

Figure 1. The main function of normal p53 protein is to maintain cellular homeostasis. P53 helps overcome cellular stresses by mediating different endogenous and exogenous responses via protein-protein interactions and transcriptional interactions depending on the type of stress.

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4. Therapies for the p53 Protein in Cancer Treatments

The p53 protein is a transcription factor that acts like a "hub" for signaling in cellular stresses. It produces and is regulated by MDM2, an ubiquitin ligase that promotes the degradation of the p53 protein. Cellular stresses such as DNA damage [18], telomere erosion [26], metabolic alterations [27], hypoxia [28], deficiencies in ribosomal biogenesis [29], mitotic spindle malfunction [30], and mutational activation of oncogenes such as *Ras, Myc, and Ets* inhibit MDM2 [31], lengthening the half-life of p53 in preparation for a response. It reacts to those stresses by inducing pathways that lead to apoptosis [32], autophagy [33], DNA repair mechanisms [34], cellular senescence [35], ferroptosis [36], differentiation [37], cell cycle arrest [38, 39], metabolic alterations [40], angiogenesis [41], and further signaling of the immune system [42]. In this paper, the focus will be the *TP53* gene and its p53 protein product, a p53 phenotype that acts as a tumor suppressor gene. The p53 protein's apoptotic and DNA repair response mechanisms are triggered by chemotherapy and irradiation, which damages both normal and cancer cells. While normal cells are able to be repaired, cancer cells cannot repair the defect, leading to apoptosis. When the *TP53* gene is mutated, however, the resulting protein, mutant p53, does not react to radiation therapy, allowing for the proliferation of cancer [43].

In order to discuss the potential therapies for cancers containing mutant p53, the mechanisms by which wild type p53 become mutant p53 must first be understood. 90% of p53 mutations are missense mutations and, in addition to poisoning p53 tetramers in cells with both wild type and mutant p53, can also lead to faulty proteins. 90% of those missense mutations happen in the DNA binding domain of the protein, which leads to a selection for faulty binding. As a result, the wild type p53 allele is selected against in cancers, and once the mutant p53 allele is established, the wild type chromosome is lost by mis-segregation [43]. In addition to the loss-of-function effect on the DNA binding domain, p53 missense mutations can also cause mutant p53 proteins to gain functions, such as increasing the rate of cell division, higher plating efficiency, and increased tumor production. In addition to the diverse number of mutations that the p53 protein can take on, the frequency of p53 mutants can vary, even in identical cancer types [43]. It was found that transcription levels in mutant p53 alleles are diverse, and correlate with the differences in the frequencies of p53 mutant alleles. In total, there are a total of 220 possible codon changes in the DNA binding domain of the p53 protein, which produce unique defects in DNA binding to the 300 genes regulated by p53, each of which may require an individual drug to target [43].

Not only is the development of treatment of p53 impeded by the wide range of mutations, there are several other factors that obstruct the path to finding a therapy for mutant p53 proteins. As mentioned in the last section, targeting loss-of-function mutations is difficult because many drugs are required for different mutant alleles, therefore the development of treatments will be slow and expensive. Not many attempts to target gain-of-function mutations have occurred, therefore success and results are limited [43]. The five most explored approaches to treat cancer via mutant p53 are using viruses, targeting gain-of-function phenotype, targeting synthetic lethal genes in p53 mutant cells, structural reactivation of a mutant p53 protein to restore wild type function, and stimulating immunological activity against the mutant p53 protein [43].

Early attempts to treat cancer cells containing mutant p53 proteins used mutant viruses to selectively kill cells with mutant p53. For example, viruses such as SV40 [14], human adenoviruses [44], and HPVs [45] encode oncoproteins (i.e. T-antigens, E1B-55 Kd, E6), which then bind and degrade p53 protein. In one case, a mutant adenovirus called ONYX-015 [46] with a deleted *E1B-55 Kd* gene replicates well in cells with mutant p53 proteins, but not in cells with wild type p53 proteins; exploration of ONYX-015 as a potential treatment is discontinued [43]. Another defective adenovirus with the *E1A* and *B* genes deleted is used to introduce a wild type p53 synthetic DNA (cDNA) into cancer cells. It was approved for neck cancers [47], where the virus is injected into the tumor accompanied by irradiation to cause DNA damage. The DNA damage causes a wild type p53-mediated apoptosis. Consequently, the tumor shrinks, but the tumors often relapse because the cDNA is not introduced into every tumor cell. Two negative effects of treating cancers with adenoviruses arise when the adenoviruses not taken up by tumor

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cells are excreted in urine and feces into the environment, and also when p53 mutations occur during the preparation of wild type p53 cDNAs [43].

Another potential angle to produce treatment for cancer with mutant p53 proteins is to target mutant p53 that exhibit a gain-of-function. Lowering mutant p53 protein levels or completely inhibiting its ability to interact with key proteins would make cancers with gain-of-function mutant p53 proteins less virulent [43]. Small interfering RNAs (siRNAs) and antisense DNA that prevent the cell from using RNA to make protein are used to create such an inhibitory effect [48]. Mutant p53 are often produced in a complex with a head shock protein - such as HSP90/HSP70 - that ensures the correct folding of the p53 protein. When the protein is not folded correctly, a stable complex of HSP90/HSP70 and mutant p53 can be detected [49]. Part of the complex is histone deacetylase 6 (HDAC6), which removes acetyl groups from the protein. Inhibiting HSP90 and HdAC6 degrades the complex, lowering mutant p53 levels [43]. Moreover, siRNAs have demonstrated to be a possible inhibitor in breast cancer. Breast cancer cells form irregular non-differentiated structures, which were restored to normality by siRNA that lowered mutant p53 protein levels [50].

Structural reactivation of mutant p53 protein by a small molecule or peptides has thoroughly been explored as potential treatment [51-53], especially since it is not limited to whether the mutation is a loss-of-function or a gain-of-function mutation. Molecules that are able to change mutant p53 protein's conformity to restore wild type conformity can be screened for by measuring conformational change using antibodies that bind to specific p53 molecules, and by selectively binding DNA to a p53-specific oligonucleotide [43]. Although yielding valuable molecules, this method has resulted in false positive compounds when the molecule bent the DNA oligonucleotide upon binding thus allowing for a mutant protein to bind to the molecule, and in cases of off-target binding [54]. There are three important successful examples of mutant p53 protein reactivators. One mutation that was targeted is p53 mutant Y220C [55, 56], the 10th most common mutation in all cancers with p53 mutations. This mutation is a loss of tyrosine that is far from p53 DNA contact sites. The loss of tyrosine lets water enter a pocket in p53 protein, which alters its conformation. The altered conformation created a temperature-sensitive mutant that doesn't bind efficiently to p53-specific DNA sites when the temperature is outside of the permissive range. Molecules that fill the pocket with hydrophobic residue were successful in restoring wild type activity in p53 mutant Y220C [56, 57]. The hydrophobic molecule has multiple advantageous properties. It binds to the Y220C mutant but not wild type proteins, it is able to reduce side effects by reactivating wild type functions in mutant proteins without activating wild type protein, it has minimized off-target side effects, and it is specific to the Y220C mutations [43]. However, a negative effect that has yet to be explored is the concern for second site mutations in the Y220C mutant allele providing resistance to the hydrophobic molecule [55, 56]. A second mutation that was explored is the p53 R175H mutant allele, which is the most common mutation of all cancers. Using computational screening, thousands of molecules were tested against the NCI-60 cell lines, and thiosemicarbazones were identified as an inhibitor of some mutant p53 alleles that exhibited lowered toxicity to other mutants and wild type proteins [43]. The IC50s of thiosemicarbazones were 10-fold lower than cells with wild type p53 or other mutant alleles. It was able to reactivate the 175 alleles, restore DNA binding to the p53 selective oligonucleotide, initiate transcription of p53-regulated genes, and kill R175H cells via apoptosis [43]. Thiosemicarbazones chelate zinc and brings it into the cell without binding to the p53 protein [58, 59], reactivating R175H mutant p53 protein as a result of increased zinc concentration, since it had been shown previously that an increased zinc concentration resulted in enhanced conformation in wild type p53 cells [60]. The zinc is cleared in ~6-10 hours because high zinc levels in the cell induce metallothionein proteins that clear the zinc [43]. A negative side effect of thiosemicarbazones is their toxicity in cases where they chelate with iron or copper. APR-246, or PRIMA-1-met, derived from PRIMA-1 (Table 1), is one of the most successful p53 structural reactivators. PRIMA-1 covalently binds to the p53 protein [61] at residues 277 and 124 [62], both residues that determine how p53 is activated. APR-246 was tested in phase I/II clinical trial and is known to be able to induce effects consistent with reactivation of p53 [63]. However, it binds both wild type and mutant p53 with little selective activation [61]. APR-246 also has not been tested rigorously for off target effects or drug resistance, but it is known

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to react with the sulfhydryl groups of cysteine of other proteins in the cell, as well as depleting the glutathione necessary for preventing damage by free radicals. APR-246 is currently being tested for reactivation of p53 mutants in a multitude of cancers, including high-grade serous ovarian, although it is unclear what the drug does by itself [43].

5. Targeting Mutant p53 for Cancer Therapy

Since cancers with p53 mutations are known to have limited response to traditional treatments such as chemotherapy and irradiation, it is attractive to explore the targeting p53 as a form of therapy. Preventing the degradation of wild type p53, suppressing mutant p53, and restoration of wild type p53 function are three approaches to p53 treatment that have been widely researched. The objective of targeting p53 as a treatment for cancer is to discover one or a small number of therapies that are able to target the vast range of p53 mutations. Examples of the three mentioned treatments are often able to effectively target certain p53 mutations that have similar structure, but one that targets a large variety of p53 mutations is rarely achieved. Furthermore, as p53 is researched more and more over time, it has become clear that TP53 is an undruggable gene, since it frequently loses its normal functions and compensates for the loss-of-function by activating a cascade of signaling pathways [64]. Synthetic lethality with p53, an emerging perspective on p53 therapy which has garnered huge amounts of attention, has the potential to overcome both the specificity of p53 mutations and also makes p53 druggable by finding synthetic lethal targets that interact with p53 [64].

Of the three treatments, the most well documented is the reactivation of p53 function. Reactivation of p53 function from mutant p53 may be more applicable to a larger scope of cancers, because while treatments preventing p53 degradation exist, it only works in the limited number of cancers that contain wild type p53. The process for restoring wild type p53 function involves first observing a mutant p53 protein that exhibits wild type activity at a certain temperature, after which a second-site suppressor mutation can adapt to delete the original mutations and restore wild-type activity. For mutant p53 that exhibit a loss-of-function in the DNA-binding domain, synthetic peptides like CDB3 (derived from the p53-binding loop) can bind to the p53 core domain and restore DNA-binding capabilities [65,66]. Large quantities of research have revealed a number of potentially qualifying compounds, notably PRIMA-1, APR-246, and COTI-2 (Table 1), and have elucidated their mechanisms of action to restore wild type p53 activity[64]. For example, APR-246 and COTI-2 are two important p53 reactivators that, despite having entered clinical trials, contain individual toxicities which hinder their progressions through the trials [47].

Another way to target mutant p53 to achieve an antitumor effect is through depleting it [67]. Observing that mutant p53 is inherently unstable, it is possible to deplete mutant p53 by using siRNA to restrict the expression of mutant p53 and promote its degradation, thereby suppressing malignant progression [68]. One method of depleting mutant p53 protein is by using histone deacetylase inhibitors (HDACis). HDACis can regulate mutant p53 by either inhibiting histone deacetylases (HDACs) or by disrupting the HDAC6/Hsp90/mutant p53 complex [69]. Inhibiting HDACs will reduce the expression level of mutant p53, and disrupting the HDAC6/Hsp90/mutant p53 complex will destabilize mutant p53 proteins, resulting in their degradation [69]. Since destabilizing mutant p53 by affecting the HDAC6/Hsp90/mutant p53 complex is viable, it would also be attractive to directly target hsp90, which is a mutant p53 protein stabilizer. Apoptosis can be induced in mutant p53 protein using its destabilization using an Hsp90 inhibitor [70]. However, these treatments do demonstrate non-specific effects that allow them to affect tumors that don't contain mutant p53 protein, further screening for agents that selectively affect mutant p53 is required.

A third potential therapy for cancers with mutant p53 is the targeting of synthetic lethal interactions that mutant p53 partakes in. The synthetic lethal method is appealing due to its flexibility with mutant p53 structure, specifically the way its efficacy is able to be less reliable on the structure of mutant p53. Therefore it is appealing as a potential therapy that could be applicable in a broad range of conditions [64], unlike other treatments like structural reactivators that are often tailored to one or a few p53 mutations.

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One potential therapy that concerns synthetic lethal interactions involving p53 uses cell cycle arrest to target mutant p53. The method was identified because intra-S and G2 arrest are heavily relied upon by cancers with mutant p53 [71]. ATR is one of the regulators of intra-S and G2, and plays a role in recognizing specific DNA sites and phosphorylating CHK1 to fulfill cell cycle regulation [72]. Thus, ATR has a synthetic lethal interaction with p53, which makes it attractive as a target to inhibit. MK6220 was the first ATR inhibitor tested in humans, and it was discovered that ATR may have the ability to enhance chemotherapy [73]. Following the same logic, it is also appealing to inhibit CHK1, since it is downstream of ATR and therefore would also be an appealing target for mutant p53 therapy by inhibition. Prexasertib, a CHK1 inhibitor that has shown antitumor activity, was discovered to have the ability to enhance olaparib's (an antitumor drug) antitumor function, allowing it to exhibit antitumor activity in models that are otherwise resistant to the drug [64]. There have been ATR inhibitors to enter clinical trials due to its strong clinical implications, though none have been successful due to its capability to induce chromosomal segregation defects [74]. Other cell cycle regulators have also been discovered as synthetic lethal partners. The p38 MAPK/MK2 pathway, a regulator of the G2/M checkpoints, is activated in response to DNA damage [75]. Synthetic lethality can be induced in this pathway using the cytarabine analogue F-Se-Ara-C, which targets MK2 in prostate cancer with p53 mutation [76]. Furthermore, induced G2 arrest was only disrupted by MK2 depletion only in p53-deficient cells, confirming that p53 does have a synthetic lethal interaction with MK2 [75].

Another way to target mutant p53 through synthetic lethality is by exploiting energy metabolism. P53 has demonstrated extensive involvement in glycolysis and oxidative phosphorylation (OXPHOS) through its ability to suppress glycolysis, limit glucose uptake, and stimulate OXPHOS [77]. Cancer reprograms energy metabolism from OXPHOS to glycolysis (known as Warburg metabolism) even under aerobic conditions [78]. Additionally, mutant p53 cannot maintain metabolic homeostasis, rather further acquiring functions to promote Warburg metabolism to assist in cancer proliferation [64]. Glucose uptake is a rate limiting step in glycolysis and wild type p53 regulates glycolysis by suppressing expression of the GLUT protein that transports glucose across cellular membranes. Contrarily, mutant p53 promotes expression of GLUT by increasing glucose uptake [79]. Therefore, it is possible to treat mutant p53 through directly inhibiting GLUT and inhibiting glucose uptake. One potential method of inhibiting glucose uptake is using the glycolysis stimulator GTPase RhoA. Although inhibiting RhoA will be effective against tumors with mutant p53, it specifically impacts mutant p53-mediated glycolysis, greatly limiting its range of effectiveness [80]. A second method of limiting glucose uptake is by inhibiting the glycolysis rate-limiting enzyme hexokinase-II (HK2). HK2 is normally downregulated by WT p53, and upregulated in the case of p53 deficiency. Inhibition of HK2 can lead to the suppression of cancer [81], although this method of treatment may not be appealing, since HK2 also maintains glycolysis in normal cells. Therefore, suppressing HK2 would not only negatively affect cancer cells, but normal cells as well. From a broader perspective, targeting OXPHOS may be risky due to its role as a major energy source [64]. Additionally, Warburg metabolism is a pathway that exists in both cancer cells with and without mutant p53 [64]. Therefore, in order to effectively locate synthetic lethal partners for p53 in energy metabolism, it is important to screen for those partners with regard to the mutations that mutant p53 takes on.

Other potential synthetic lethal pathways with mutant p53 involve autophagy, invasion and metastasis mediated by mutant p53, and ncRNAs. Autophagy inhibits tumor formation under normal cell environments, but promotes tumor growth once cancer invasion has begun. Mutant p53 gain-of-function, through the inhibition of autophagy, reduces autophagy-mediated mutant p53 degradation, thus promoting tumor proliferation [82]. The AMPK/mTOR pathway is central to the suppression of autophagy by mutant p53 [83]. Therefore, inhibition of mTOR to induce autophagy is an example of a synthetic lethal interaction with mutant p53. The potential of synthetic lethal partners for mutant p53 when through the lens of metastasis exists because mutant p53 facilitates invasion by increasing receptor expression and activating downstream signaling pathways.

A second potential lens to use to look for synthetic lethality with mutant p53 is ncRNAs (non-coding RNAs). ncRNAs can be classified into miRNAs, lncRNAs, and circRNAs; each has a different potential

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synthetic lethal interaction with mutant p53. Wild type p53 plays an important role in regulating ncRNAs, and is involved in the ncRNA network [84]; therefore mutant p53 can cause dysregulation in the network and alter ncRNA. Due to the extensive interactions that p53 has with ncRNAs, finding a lethal synthetic partner in ncRNAs is certainly possible.

One class of ncRNAs that mutant p53 has direct interactions with is microRNAs (miRNAs). miRNAs work with the degradation of mRNAs [85], therefore changing miRNA levels will lead to an altered protein concentration. Wild type p53 regulates miRNA levels [86], and mutations of the p53 protein results in a gain-of-function that upregulates miRNAs and confers oncogenic abilities such as metastasis and somatic cell programming [87]. One specific miRNA family that was explored for treatment is miR-34 [88]. miR-34a and miR-34b/c, both components of the miR-34 family, are upregulated by activated p53. Furthermore, miR-34 synergizes with the antitumor effect of p53 by demonstrating lowered expression in cancer tissues [89]. Treatment involving miR-34 was explored using MRX34, a viral vector, as a delivery system [90]. Although the study was terminated due to immune-mediated adverse effects stemming from the use of the viral vector, MRX34 served as a proof of concept for mutant p53 therapy using miRNAs [64]. Although the possibility of treating the plethora of dysregulated miRNAs resulting from loss-of-function wild type p53, it is also viable to target miRNAs mediated by mutant p53. miRNAs mediated by mutant p53 are linked with tumor progression [64], as is the case with miR-223-3p, a p53-targeting tumor inhibitor suppressed by mutant p53 in p53-mutated lung cancers [91]; miR-223-3p is a component of synthetic lethality to mutant p53. Treatments of such p53-mutated lung cancers from the perspective of miR-223-3p involves the use of a miR-223-3p agomir that mimics the function of the suppressed miR-223-3p [92]. A third example of miRNA being of synthetic lethality to mutant p53 comes from the observation that mutant p53 is capable of upregulating oncogenic miRNAs to promote tumor progression. It is possible to target and suppress miRNA such as metastasis promoter miR-30d and miR-1246, which upregulates tumor-associated macrophages, in order to suppress tumor growth [93].

A second class of ncRNA that has synthetic lethal interactions with mutant p53 is long noncoding RNAs (lncRNAs). There exist 4 types of lncRNAs that serve as signals, decoys, guides, and scaffolds [94]. lncRNAs interact with DNA, RNA, and proteins, and is specifically capable of directly regulating transcription by affecting the expression of genes adjacent to it, and has the ability to indirectly affect transcription by decoying or guiding regulatory proteins [94]. Similar to miRNAs, p53 regulates the expression of lncRNAs as a part of its signaling network [64]. The p53 pathway repressor lincRNA-p21 was the first to be identified as an lncRNA capable of being induced by p53 [95]. Mutant p53 is not normally able to regulate lincRNA-p21, but can achieve regulation under special interactions to inhibit tumor suppression [96]. Therefore, it is possible to upregulate lincRNA-p21 to achieve tumor suppression.

A third class of ncRNA that acts as a synthetic lethal partner to mutant p53 is circular RNA (circRNA). Circular RNA is an ncRNA resistant to exonuclease-mediated degradation via the connection of its 3' end to the 5' end [97]. Similar to miRNA, circRNA regulates gene expression, and is also capable of enhancing protein function. An example of synthetic lethality with circRNA involves the tumor inhibitor circ-Ccnb1, which is downregulated in breast cancer [98]. Abnormal expression of circ-Ccnb1 reduced proliferation in cancers with mutant p53. H2AX is a histone that is a part of circ-Ccnb1, and functions as a bridge to bind either wild type p53 or H2AX-dependent tumor suppressor and apoptosis inducer Bclaf1, with a higher binding affinity for wild type p53. Increased circ-Ccnb1 binding also leads to increased wild type p53 binding in cells with wild type p53, resulting in the inhibition of Bclaf1's ability to induce apoptosis. Observing that mutant p53 is unable to bind H2AX, Bclaf1 will instead bind to it, eliciting apoptosis in cells with mutant p53 [64]. Thus, Circ-Ccnb1 is a representation of a possible circRNA synthetic lethal partner of mutant p53.

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Table 1. Agents with the potential to reactivate mutant p53.

Compound name	Mechanism	Mutant p53 target
CP-31398	CP-31398 restores mutant p53's denatured DNA-binding domain to wild type p53 conformation upon binding	V173A, S241F, R249S, R273H
PRIMA-1	PRIMA-1 restores wild type DNA binding ability to non-conforming mutant p53 through an enhancement of wild type p53 stability at 37 degrees	R175H, R273H
APR-246	APR-246 restores wild type DNA binding ability to non-conforming mutant p53 through an enhancement of wild type p53 stability at 37 degrees	R175H, R273H
PK11000	PK11000 promotes conformational folding in mutant p53 by Increasing melting point temperature of DNA	Y220C
ZMC1	ZMC1 promotes conformational folding in mutant p53 by increasing Zn2+ levels in cancers with mutant p53	R175H, R172H
COTI-2	COTI-2 converts mutant p53 to wild type conformation	R175H
P5R3	P5R3 restores wild type DNA binding ability of specific sequences in several mutant p53	R175H, M237I, R273H
Chetomin	Chetomin restores wild type conformation by promoting p53 binding with Hsp40 in additional to Hsp40 expression	R175H
RITA	RITA restores wild type transcription activity in mutant p53	I254D, R175H, R213Q, Y234H, R248W/Q, R273H, R280K
WR1065	WR1065 restores wild type conformation in temperature-sensitive mutant p53	V272M

6. Conclusion

Current popular therapies to manage cancers, such as chemotherapy, are often damaging and cause severe negative counter effects to the patient's body, prompting researchers to look to mutant p53 as a therapeutic target. Although the achievement of targeting mutant p53 to degrade oncogenic processes would mean much better cancer treatments, the current reality presents many obstacles which, while currently being overcome, are still far from complete fruition. The diverse family of mutant p53 that is derived from *TP53* mutations makes designing treatments for cancers containing mutant p53 a sizable obstacle. Decades of creative and fruitful research have yielded insightful proofs of concept for many mutations of p53, though not many have been successfully integrated into medicine as treatments of

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cancer. The adenovirus with *E1A* and *E1B* deletion can be considered as an example of a therapy that was approved for use in neck cancer, even if the treatment requires being coupled with irradiation to promote anticancer effects. A structural reactivator molecule found to be effective in restoring wild type activity of mutant p53 with Y220C tyrosine deletion, is another therapy for mutant p53 that has been deemed successful. Other experimental agents such as the synthetic lethal inhibitors MRX34 and Circ-Ccnb1, the mutant p53 depletor Hsp90 inhibitor, and structural reactivator APR-246 (Table 1) have all demonstrated proof-of-concept for ways in which their respective approaches could be successful for mutant p53 treatment. All of the aforementioned proposed and accepted treatments of mutant p53 also only target individuals, or at best, a small number of p53 mutations. Therefore, further screening for potential agents, as well as dedicated research to understand the vast pathways and interactions p53 takes on as a signaling hub is necessary to find an agent that is applicable to a large quantity of p53 mutations and to consolidate viruses, structural reactivation, mutant p53 depletion, and synthetic lethal inhibitors as viable suppressors of mutant p53.

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