■ REVIEW ARTICLE

Targeting the p53 Tumor Suppressor Pathway: A Comparative Review of Therapeutic Strategies

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ABSTRACT: The tumor suppressor protein p53 causes cell cycle arrest, initiates DNA repair, or triggers apoptosis to stop the development of cancer. The p53 gene regulates the cell cycle and suppresses tumors. In many cancer cases, p53 is found in a mutated state, with the mutation causing the proliferation of the damaged cells, as p53 is prevented from properly functioning. Secondary data analysis will be used to evaluate and compare traditional cancer therapy methods to CRISPR-Cas9 gene editing, exploring the potential of CRISPR-Cas9 in cancer treatment methods as a less harmful alternative.

KEYWORDS: Biology, Molecular Biology, p53, Cancer Therapy, CRISPR-Cas9, DNA Repair, Apoptosis, Cell Cycle Regulation.

Introduction

Cancer diagnoses affect millions of lives each year, with many of these diagnoses stemming from genetic mutations.[1] One genetic mutation that causes an increased chance of cancer pathogenesis is one to TP53, a gene that encodes the tumor suppressor protein p53.[2] p53, often referred to as the "guardian of the genome," is responsible for many cell processes, including regulating the cell cycle, initiating DNA repair, and triggering apoptosis as a response to stress on cells.[3] When mutated, p53 loses its protective function, which allows these damaged cells to multiply, thus leading to cancer pathogenesis.[2] Various p53 mutations have different outcomes, including the loss of the mutant p53's tumor suppression function, gain of function in the form of new oncogenic properties, or dominant-negative effects where the mutant p53 interferes with the wild-type p53.[2] Different cancer therapies that target mutations to the p53 gene will be explored, including CRISPR-Cas9's potential to offer a less harmful alternative to traditional cancer therapies as well as traditional cancer therapies. CRISPR-Cas9 makes a double-strand break at the target site, where the cell's repair pathways are used to disrupt or change genes.[4] These techniques will be examined to contribute to and aid related research in curing cancer through gene editing. This topic is extremely significant, not only because precise gene editing has the potential to minimize collateral damage as opposed to chemotherapy or radiation, but also because targeting these mutations would address this major need.

Discussion

Chemotherapy and Small Molecule Strategies that Target p53:

This section surveys the various small molecules that alter the p53 pathway and how they work to counteract the mutation in the *p53* gene.[5] Then, it compares these agents to the more traditional cytotoxic chemotherapy. Multiple therapies for mutations in the *p53* gene have emerged. One refolds and reactivates the protein's DNA-binding core, while the other one stabilizes wild-type p53 by blocking Murine double minute 2 (MDM2), which allows the p53 to initiate apoptosis or pause the cell cycle for repair.[6] Compounds such as p53 reactivation and induction of massive apoptosis (PRIMA-1) on the reactivation side and Nutlin-3a on the MDM2 side underscore the promise of pharmaceuticals but also the limitations in combating p53 mutations in patients.[7] When including traditional chemotherapies as well, there are a multitude of methods and treatments that aim to counter cancer stemming from p53 mutations.[8]

PRIMA-1 is a mutant p53 "reactivator" prodrug. It binds cysteine residues in the p53 core domain and stabilizes mutant p53, restoring transcriptional activation of p53 targets.[7] PRIMA-1 is then metabolized to methylene quinuclidinone (MQ), which forms covalent adducts with cysteines within p53's core domain. This stabilizes folding and rescues DNA binding activity.[9] Moreover, restored p53 activity following treatment increased expression of effectors including p21, p53 upregulated modulator of apoptosis (PUMA), and Bcl-2-associated X protein (BAX), all of which can trigger cell cycle arrest or apoptosis in models with mutant p53.[10] In current clinical trials, there have been encouraging responses with p53 mutant Myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) cohorts in phase 2 work. On the contrary, later pivotal trials have produced mixed results, thus underscoring translational uncertainty.[11] Whereas PRIMA-1's objective is to repair the mutant protein itself, other drugs, including Nutlin-3a, attempt to tackle the p53 mutation by blocking MDM2, thus stabilizing existing wild-type p53.[12]

Nutlin-3a is a small molecule antagonist of the p53-MDM2 interaction, occupying the p53 binding pocket on MDM2 to prevent MDM2-mediated ubiquitination and degradation of wild-type p53.[13] As a result, p53 can accumulate and trigger cell cycle arrest or apoptosis in TP53-wild-type (WT) cancers.[13] Nutlin-3a binds the p53 binding cleft on MDM2 to mimic p53 residues Phe19/Trp23/Leu26, stabilizing endogenous wild-type p53 by blocking MDM2 p53 binding.[14] p53

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activation and apoptosis in models have been tested in preclinical studies, indicating that Nutlin-type agents are effective when p53 is intact.[15] However, some limitations include the near-zero activity in p53-mutated tumors on target toxicities in p53-proficient normal tissues, and the emergence of resistance or pathway rewiring that can blunt clinical durability.[16] In clinical trials, MDM2 antagonists have shown promise in cherry-picked contexts but still require biomarker-guided patient selection to manage resistance and toxicity.[14] Both PRIMA-1 and Nutlin-3a incorporate targeted strategies around p53, but now this paper will compare these to the more traditional approach of cytotoxic chemotherapy.[5]

Traditional chemotherapies, including platinum agents, anthracyclines, taxanes, and others, exert antitumor effects by inducing DNA damage or mitotic stress, effects that p53-dependent checkpoints typically mediate.[8] p53 status also influences chemosensitivity and clinical outcomes. Many chemotherapeutics cause DNA lesions that trigger p53-dependent cell cycle arrest, apoptosis, or DNA repair. However, when mutated, these tumor cells may fail to do this and instead show resistance or altered sensitivity.[8] These chemotherapies are not targeted at p53 itself, leaving it so that p53 function is not restored; rather, they rely on exploiting vulnerabilities that may or may not be present, depending heavily on the p53 genotype and the tumor's adaptive mechanisms.[8] All in all, PRIMA-1, Nutlin-3a, and traditional chemotherapies each have their own strengths and weaknesses, thus raising the question of when small-molecule approaches are sufficient and when fundamentally different approaches must be taken.[5]

PRIMA-1 demonstrates that mutant p53 molecules are druggable by covalent stabilization and has thus produced positive early clinical signals. Nutlin-3a stabilizes wild-type p53 by blocking MDM2 and is effective in TP53-WT. However, it cannot be applied to p53 mutant tumors and faces resistance. Traditional chemotherapy also remains widely used, but its p53-dependent and independent mechanisms produce varied results when mutated, providing limited benefits for some patients.[8] All in all, small molecule strategies and alternative modalities are under research for improvement, with researchers aiming to eliminate the current limitations posed.

The Role of the p53 Gene in Cell Cycle Regulation and Cancer Pathogenesis:

This section will examine the tumor suppressor function of the p53 gene, how it regulates different checkpoints in the cell cycle, and how mutations to this gene cause an increase in risk of cancer pathogenesis. By addressing this mutation with one of many possible methods and treatments, the patient's risk of cancer pathogenesis drastically decreases.[5] p53 is a tumor suppressor that acts as a checkpoint, aiding in the prevention of uncontrolled cell growth.[3] The p53 protein has 3 main functions, those being cell cycle arrest to allow damaged DNA to repair before it replicates, activating DNA repair of damaged cells by promoting DNA repair genes, and triggering apoptosis, a process that triggers cell death of damaged cells when the damage is unrepairable[17] (Figure 1). These three functions serve as a checkpoint in the cell cycle, ensuring that any damaged cells do not proliferate and are either repaired or killed before duplicating.[18] However, when mutations are present on this gene, the protein may lose its tumor-suppressing function, thus increasing a patient's likelihood of cancer pathogenesis.

p53 holds a major role at the gap 1 / synthesis (G1/S) and gap 2 / mitosis (G2/M) checkpoints, stopping the cell cycle and preventing it from progressing until the damaged cells go through DNA repair or apoptosis. For example, p53 activates transcription of the cyclin-dependent kinase (CDK) inhibitor p21. This directly stops the progression to the S phase (DNA synthesis phase) by blocking cyclin-CDK complexes.[3] Then, at the G2/M checkpoint, p53 prompts the expression of growth arrest and DNA damage-inducible 45 (GADD45) or other repair proteins, thus preventing mitosis entry until the genome becomes stabilized.[10] (Figure 1) The function of p53 as a checkpoint regulator depends on a tightly orchestrated molecular pathway, which can be mapped to better understand where mutations exert their effects.[19]

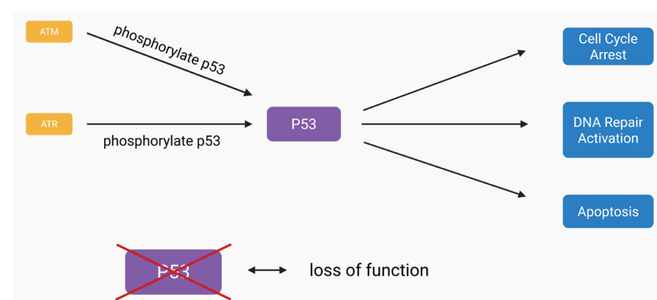

Figure 1: DNA damage triggers the ATM and ATR kinases, which phosphorylate p53, thus leading to its activation. Activated p53 can then cause cell cycle arrest by stopping cell division, repairing DNA, and triggering apoptosis. When p53 loses function, it is unable to perform these tasks, thus leading to the progression of tumors.

After sensing that the DNA is damaged, kinases such as Ataxia-telangiectasia mutated (ATM) and Ataxia-telangiectasia and Rad3- related protein (ATR) are able to activate p53. In order to do this, these proteins phosphorylate p53 and MDM2, which makes it more difficult for MDM2 to bind to p53[19] (Figure 1). Although MDM2 tags p53 for destruction, thus keeping the amount of p53 relatively low, when MDM2 isn't able to bind to p53, p53 is released and thus begins to accumulate in the nucleus.[18] Once activated, p53 transcribes target genes, which either promote DNA repair or trigger apoptosis.[3] This ensures that all damaged DNA is taken care of, whether by killing or by repairing the DNA, although mutations to the p53 gene compromise this system.

Mutations to the p53 gene heavily undermine its tumor-suppressing function, thus enabling cancers to progress and develop. These mutations typically occur in the DNA-binding domain of p53, preventing the activation of target genes that are crucial in DNA repair and apoptosis.[2] In addition to the loss of function, mutated p53 proteins exert dominant-negative effects when interfering with wild-type p53 on some occasions, speeding up tumor progression by obstructing the wild-type p53 activity.[2] Furthermore, other p53 mutations promote invasion and metastasis, in addition to the strictly defective versions of the original protein.[3] These p53 mutations

across various types of cancer make this gene a target for research into therapies, gene editing strategies included.[2]

Ultimately, the role of p53 in protecting cells from malignant transformation is extremely important, not just by regulating the cell cycle or repairing DNA, but also by initiating apoptosis when the cell damage is too severe to be repaired. Tight regulation of the pathways is typically ensured due to their complexity, but the regulation collapses following mutations, thus leading to cancer development and progression. Because of the high frequency and severe consequences of the p53 mutation, it has cemented itself as a key target of cancer therapy research, resulting in the development of many different treatments for this mutation.[5]

CRISPR-Cas9 as a Targeted Alternative for p53 Mutations:

CRISPR-Cas9 provides a solution where p53's tumor-suppressing functions can be restored, or mutations can be repaired. However, this doesn't come without drawbacks, as there is the possibility of biological risks as well as various constraints and ethical challenges, thus limiting CRISPR-Cas9's application in today's society[20] (Figure 2). In contrast to chemotherapy or small molecule approaches that aim to treat mutations in p53, CRISPR-Cas9 is an alternative solution, one that has the potential to permanently restore p53 function by correcting mutations.[4] Gene editing can restore the expression of p53 and downstream activity in cell models, with saturation mutagenesis screens mapping functionally rescuable p53 variants[21] (Figure 2). However, to transform these from concepts into authentic treatment methods, four interlinked barriers are required.[4] First, the genotoxic consequences of CRISPR-Cas9 double-strand breaks (DSBs) and off-target edits. Second, the activation of p53 responses by Cas9, which can select p53-deficient clones. Third, the low efficiency of homology-directed repair (HDR).[22] Finally, reliable and tumor-specific delivery. This section will examine therapeutic evidence for p53 editing, observed principal safety risks, and delivery or repair pathway constraints, ending with a brief overview of alternative editing strategies.

Multiple preclinical studies yield results showing that the precise editing of p53 can lead to restored expression. One example of this was in a study where CRISPR-Cas9 with repair templates was used in PC-3 prostate cancer cells. Subsequent to the gene editing, there was a reported measurable restoration of p53 sequence and downstream p53 readouts.[21] Furthermore, multiple preclinical HDR and knock-in strategies reestablish wild-type p53 sequencing, thus reducing proliferation in vitro, highlighting reproducible functional rescue within model systems.[4] These results establish feasibility, but also expose potential risks tied to how these edits are made and how cells respond to the editing process itself.[22]

Multiple safety hazards are introduced following editing associated with DNA breaks and Cas9 activity. These hazards include unintended mutations, chromosomal rearrangements, and the paradoxical selection for cells that have lost p53 surveillance.[22] Off-target cleavage from Cas9 has the possibility of creating indels at sequence-similar loci, thus raising the specter of inadvertent disruption of other tumor suppressors or activation of oncogenes[4] (Figure 2). Another study was able to provide direct evidence that CRISPR-Cas9 delivery can activate p53 pathways in TP53-WT cells, and in some experiments, the expansion of p53-inactivating clones.[20] This means that dangerous subpopulations may emerge following editing procedures.[20] In addition, genomic instability may increase as a result of variable indels or larger deletions or rearrangements being produced following double-strand break (DSB) repair proceeding by error-prone non-homologous end joining rather than the more precise HDR route.[4] The gene editing route is exploring both base/prime editors and delivery strategies to reduce DNA damage burden, as a result of the risks associated with creating DSBs and the cell's choice of repair pathway.[4]

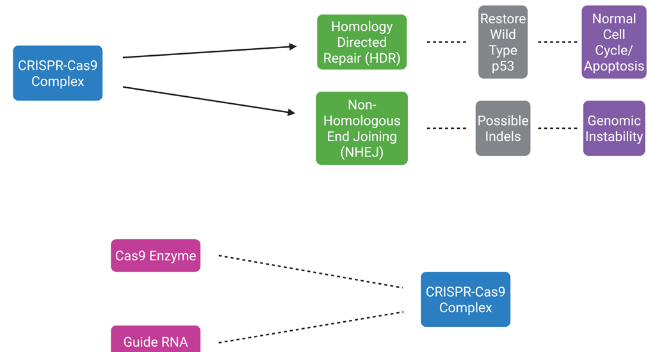

Figure 2: The CRISPR-Cas9 complex is comprised of the Cas9 enzyme and guide RNA targeting the mutation. After DNA is cut, HDR restores wild-type p53 and normal cell cycle, whereas NHEJ causes possible indels and genomic instability.

Even with safer editors, achieving meaningful gene correction requires overcoming the issue of low HDR efficiency in tumors and the problem of delivering the edited machinery to the cancer cell populations *in vivo*.[23] HDR-dependent repairs are notoriously inefficient in non-dividing tumor cells, thus limiting the fraction of cells that can be corrected by Cas9+ donor approaches.[4] Viral vectors also offer efficient transduction, but raise concerns about immunogenicity, persistence, and cargo limits.[4] On the other hand, non-viral vehicles such as lipid nanoparticles or ribonucleoprotein (RNP) delivery struggle with tumor penetration and cell type specificity, although they reduce persistence.[4] Base and prime editors can also install single-nucleotide changes or make small edits without inducing DSBs, which lowers some risks associated with Cas9 DSBs. However, they have off-target and bystander edit profiles that have to be characterized for each p53 allele and clinical context.[23] For CRISPR to achieve safe and durable clinical benefit, strategies will most likely require editor optimization, allele prioritization, and combination approaches.[4]

■ Conclusion

Mutations in the p53 gene compromise the tumor suppressive functions of p53, driving over half of human cancers.[3] This paper compared three approaches for addressing these mutations: small molecule treatments, traditional chemotherapy, and CRISPR-Cas9 gene editing. Chemotherapy remains a cornerstone of cancer treatment, but is nonspecific and oftentimes less effective when p53 is mutated.[24] Small molecules

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such as PRIMA-1 and Nutlin-3a provide targeted interventions through restoring the mutant p53 function or stabilizing the wild-type protein. However, they are limited by durability and toxicity.[5] In contrast, CRISPR-Cas9 directly edits p53 mutations, restoring wild-type function in preclinical studies,[21] but still poses risks such as off-target effects or Cas9-induced p53 pathway activation, thus preventing safe clinical use.[20] There are still multiple barriers that must be overcome before CRISPR-Cas9 can become a viable treatment method for p53-driven cancers, those being safety, precision, and delivery methods. Moreover, when discussing future research into this topic, one question that must not be overlooked is how current traditional methods of treatment can be integrated with CRISPR-based treatments to maximize treatment outcomes.

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