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Roles of Mutant *TP53* Gene in Cancer Development and Progression

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Abstract: *TP53* is a tumor suppressor gene that is mutated in most cancer types and has been extensively studied in cancer research. p53 plays a critical role in regulating the expression of target genes and is involved in key processes such as apoptosis, cell cycle regulation, and genomic stability, earning it the title "guardian of the genome." Numerous studies have demonstrated p53's influence on and regulation of autophagy, ferroptosis, the tumor microenvironment, and cell metabolism, all of which contribute to tumor suppression. Alterations in p53, specifically mutant p53 (mutp53), not only impair its tumor-suppressing functions but also enhance oncogenic characteristics. Recent data indicate that mutp53 is strongly associated with poor prognosis and advanced cancers, making it an ideal target for the development of novel cancer therapies. This review summarizes the post-translational modifications of p53, the mechanisms of mutp53 accumulation, and its gain-of-function, based on previous findings. Additionally, this review discusses its impact on metabolic homeostasis, ferroptosis, genomic instability, the tumor microenvironment, and cancer stem cells, and highlights recent advancements in mutp53 research.

Keywords: p53; Cancer; Mutant p53 (mutp53); Progression; Treatment

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1. Introduction

The "guardian of the genome" protein, known as the p53 tumor suppressor, is encoded by the *TP53* gene. Its primary biological role is the preservation of cellular DNA integrity. In addition, *TP53* is involved in cell differentiation, aging, and development. The p53 protein acts as a transcription factor, influencing numerous biological processes depending on the type of cellular stress signals it receives [1]. Oncogene activation, DNA damage, and replication stress are recognized stress signals that activate p53. In response to these stimuli, p53 undergoes post-translational modifications that promote the transcription of genes involved in specific cellular responses based on the type of stress, ultimately determining the fate of the cell [2].

Wild-type p53 (wtp53) protein binds to specific DNA response elements, leading to the expression of genes that prevent the onset and spread of cancer. Under normal conditions, the p53 signaling pathway is activated when cells encounter various stress signals. This activation allows the cells to engage in transcriptional

programs such as cell cycle arrest, DNA repair, senescence, and apoptosis, which inhibit tumor growth [3]. Inactivation of the *TP53* gene frequently results from loss-of-function mutations or negative regulation of wtp53 proteins in most, but not all, human malignancies [4]. The inactivation of *TP53* promotes cell survival, invasion, and proliferation, accelerating cancer progression. In more than 75% of *TP53* gene mutations, wtp53 functions are lost. Mutant p53 (mutp53) proteins may act as dominant negatives to wtp53 activity or acquire new tumorigenic traits that negate the tumor-suppressive effects of wtp53. p53 regulates the cell cycle, apoptosis, DNA repair, and senescence in response to hypoxia, oncogene activation, DNA damage, and nutrient deprivation [5].

The p53 transcription factor consists of six domains: the transactivation domain, the proline-rich domain, the DNA-binding domain, the nuclear localization signal domain, the oligomerization domain, and the C-terminal domain. The transactivation domain recruits transcriptional co-activators to enhance RNA transcription, while negative regulators can inhibit its activity [6]. The proline-rich domain supports transcription, and p300/CBP can bind to it to boost p53's transcriptional activity. The DNA-binding domain stabilizes the protein's structure, and single-point mutations in this domain can result in the loss of all p53 functions. The nuclear localization signal domain and oligomerization domain are essential for nuclear localization and transcriptional activity. The C-terminal domain acts as a negative autoregulatory domain by inhibiting DNA binding, which can be modulated by post-translational modifications [7].

Genomic instability is a key hallmark of human cancers and is largely driven by the gain-of-function activity of mutant p53. In breast cancer specimens, aberrant copy numbers are associated with mutant p53 and can lead to centrosome deviations, resulting in centrosome multiplication [8]. Furthermore, mutant p53 can bind to chromatin-regulated genes and promote histone methylation and acetylation, both of which contribute to genetic instability and cancer progression [9]. Mutant p53 complexes with p63 and p73 reduce their ability to control tumor growth and prevent apoptosis. Additionally, other proteins or transcription factors, such as disabled homolog 2-interacting protein (DAB2IP), poly(ADP-ribose) polymerase (PARP), nuclear transcription factor-Y (NF-Y), and sterol regulatory element binding proteins (SREBPs), collaborate with mutant p53 to promote breast cancer development via activation of the mevalonate pathway. Evidence suggests that different types of tumors have distinct *TP53* mutational spectra [10].

In a study of tissue samples from 10,000 cancer patients, *TP53* mutations were found in 42% of cases. The mutation frequency varies across cancer types, with small-cell lung cancer showing a mutation frequency of 89.02% and colorectal cancer at 72.69% [11]. However, cancers such as thyroid, cervical, and bone cancer exhibit significantly lower frequencies of *TP53* mutations. G to T transversions are commonly observed in lung and liver cancers, while CpG dinucleotide hotspot transitions are prevalent in leukemia, brain tumors, and colorectal cancer. Base pair mutations are frequently seen in esophageal cancer. Even within the same organ, different tumor subtypes can show variations in *TP53* mutation patterns. For example, an analysis of *TP53* mutations in 572 breast tumors found that truncating mutations were more common in basal breast cancers, while missense mutations, specifically A to G transitions, predominated in luminal breast cancers [12].

Additionally, there is a correlation between external risk factors and the *TP53* mutational spectrum in tumors. For instance, aggressive squamous cell carcinomas of the skin undergo CC to TT double base transitions when exposed to UV light, while smokers experience more G to T transversions in lung cancer compared to non-smokers. In primary hepatocellular carcinoma, aflatoxin B1 induces G to T transversions in codon 249 of *TP53*. Interestingly, *TP53* mutations are associated with poor prognoses in malignant tumors [13].

2. Role of microRNAs in influencing p53

It has been documented that several microRNAs (miRNAs) either directly or indirectly inhibit p53 expression and that p53, in turn, regulates the expression of miRNAs. For miRNAs to distinguish between mutant and wtp53, they must directly target the specific altered region of the mutant *p53* mRNA. So far, no miRNAs unique to mutant p53 have been shown to suppress wtp53. In specific cases, miRNAs that target important positive regulators of mutant p53 may reduce the expression of mutant p53. In line with this, artificial siRNA and shRNA oligonucleotides have been developed with the specific goal of targeting mutant p53 and reducing tumor growth *in vivo*.

Beyond its direct regulation of gene expression through interaction with the p53 response element (RE) of genes, p53 also regulates gene products by transcriptionally controlling miRNAs. It has been demonstrated that miRNAs activated by p53 play roles in the regulation of protein expression related to cell cycle progression, senescence, apoptosis, metastasis, angiogenesis, cellular stemness, and metabolic processes such as glycolysis [14,15].

p53 has been found to regulate the transcription of several miRNAs, including the *miR-34* family, *miR-145*, *miR-107*, *miR-192*, and *miR-215*. The *miR-34* family (*miR-34a-c*), which reduces the expression of proteins associated with cell cycle progression and activation of cell growth and survival, as well as immune checkpoints like Cyclin E2, cyclin-dependent kinase 4 (CDK4), CDK6, BCL-2, and programmed death-ligand 1 (PDL-1), was the first group of miRNAs identified as being stimulated by p53. Other miRNAs, such as *miR-145* and *miR-107*, which regulate the oncogene *c-Myc* and hypoxia-inducing factor-1 beta (*HIF-1β*), respectively, are involved in oncogene repression and the response to hypoxia and angiogenesis [16]. Through regulating CDK6 and RB transcriptional corepressor-like 2 (RBL2) expression, *miR-107* also plays a role in controlling the G1-S cell cycle transition.

Following genotoxic stress, p53 upregulates *miR-192* and *miR-215*, which in turn regulate the expression of molecules involved in cell cycle progression at the G1 and G2-M checkpoints, including RAD51, TOP1, MCM3, RB1, CDC7, MCM10, and MCM6 [17,18]. In addition to miRNA regulation of cell cycle genes, *miR-205* controls cell cycle progression by downregulating E2F1 and metastatic activity by targeting laminin subunit gamma-1 (LAMC1), which is associated with cell adhesion and migration. Other p53-regulated miRNAs implicated in epithelial-mesenchymal transition (EMT)-mediated metastasis include the *miR-34* family, which inhibits zinc finger E-box binding homeobox 1/2 (ZEB1/2) expression, and *miR-34*, which inhibits Snail1.

p53 also regulates its own stability and efficiency by activating miRNAs that target Mdm2 and Mdmx, such as *miR-192*, *miR-194*, *miR-215*, *miR-143*, *miR-145*, and *miR-34a* [19].

3. p53 family members

The transcription factors p73 and p63 are encoded by the *TP73* and *TP63* genes, respectively, which are homologous to *TP53*. The DNA-binding domain is the most highly conserved motif among members of the p53 family, while the oligomerization domain and transactivation (TA) domains are the least similar. Due to these similarities, p73 and p63 can bind to conventional p53 REs, oligomerize, and transactivate p53 target genes [20]. As a result, p73 and p63 also play roles in anti-tumor mechanisms, such as apoptosis and cell division regulation. However, due to their differences, p73 and p63 can be involved in distinct biological activities compared to p53. For instance, a variant of the TA domain prevents MDM2 from regulating p73 [21].

Moreover, while p53-null mice exhibit normal development but a wide range of tumor malignancies, p73- and p63-null mice survive but show developmental abnormalities. This suggests that p73 and p63 may have a stronger role in regulating cell differentiation compared to their tumor-suppressive functions, at least in the

early stages of development. Unlike p53, the tumor-suppressive properties of p73 and p63 are not lost due to mutation or allelic loss. Instead, the presence of two promoters results in the production of proteins that may either be pro-tumorigenic (ΔN, lacking the TA domain) or tumor-suppressive (TA). Indeed, the ΔN isoforms are expressed in several cancers, and alternative mRNA splicing processes produce distinct protein isoforms [22].

In *TP73*, there are 7 ΔN isoforms (ΔN-α, ΔN-β, ΔN-γ, ΔN-δ, ΔN-ε, ΔN-ξ, ΔN-η) and 7 TA isoforms (α, β, γ, δ, ε, ξ, η). In *TP63*, there are 5 ΔN isoforms (ΔN-α, ΔN-β, ΔN-γ, ΔN-δ, ΔN-ε) and 5 TA isoforms (α, β, γ, δ, ε). Although TA-α isoforms are more structurally similar to p53 and correspond with p63's transcriptional activity, TA-α is a more potent transcriptional activator and inducer of apoptosis in p73. The sterile α motif (SAM) is a critical distinction in the structures of p73, p63, and p53, as it allows p73 and p63 to interact with proteins involved in physiological processes [23].

Both p73 and p63 are activated in response to cellular stressors such as hypoxia and DNA damage. Similar to p53, their function is regulated by post-translational modifications such as ubiquitination, acetylation, and phosphorylation. Once activated, p63 and p73 can form both homo-tetramers and hetero-tetramers. Although some studies have shown that p73 can bind to p53, leading to the activation of Puma and Bax, others have found that neither p73 nor p63 forms hetero-tetramers with wtp53 [24]. This discrepancy may depend on the type of stress signal and the phosphorylation status of p53.

In certain models, p53 is required for apoptosis following DNA damage, and this process involves p73 and p63. P73 can transactivate p53 target genes such as *Puma*, *Noxa*, *RAD17*, and *p21* [25]. Similarly, p63 can upregulate p53 target genes such as *GADD45*, *PIG3*, *p21*, and *Bax*. Both p73 and p63 also regulate genes specific to their function that are not shared by p53. However, little is known about the unique gene regulation of TAp73 and TAp63 isoforms and their role in carcinogenesis. It is understood that each isoform has different functions in gene expression regulation and may be expressed differently in various tissues.

While p63 is generally expressed at low levels in both normal and malignant tissues, its high expression is primarily restricted to the female germline. Notably, the loss of the tumor-suppressive function of TA isoforms is associated with the production of ΔN isoforms. Although TA isoforms do not interact with wtp53, they can interact with mutant p53, thereby inhibiting the tumor-suppressive properties of TAp73 and TAp63 [26].

The potential cellular processes and key roles of the p53 family in cancer development, progression, and therapeutic options are summarized in **Table 1**.

Most tumor cells carry mutations in the *TP53* gene. According to genome sequencing of various human cancer cells, *TP53* mutations are present in 42% of cases. The DNA-binding domain (DBD) is the most frequently mutated region in *TP53*, and missense mutations, involving a single amino acid substitution, are the most common type of mutation. p53 mutants are classified into two major categories: structural mutations and DNA contact surface mutations. Structural mutants (e.g., R175H, R249S, G245S, and Y220C) exhibit reduced protein thermostability, leading to improper protein folding at physiological temperatures and the inability to bind DNA. Of these, R175H and C176Y mutations specifically affect the protein's affinity for zinc ions. DNA contact surface mutants (e.g., R273H/C, R248W) occur within the core DNA-binding region, where alterations prevent the protein from binding to DNA [27].

The most common mutation sites in *TP53*—R175, G245, R249, R282, R248, and R273—are collectively referred to as "hot spot" variants. These mutations not only bind to wtp53 to exert dominant-negative (DN) effects but also have the potential to acquire gain-of-function (GOF) properties, converting them into oncogenic proteins. Consequently, *TP53* differs from many "classical" oncogenes, which are typically inactivated by truncating or nonsense mutations that result in a non-functional, shortened protein [28].

Table 1. Critical roles of p53 in cancer development and treatment

Role of p53	Cancer development	Cancer progression	Treatment approaches
Tumor suppressor	p53 prevents the accumulation of DNA damage by inducing cell cycle arrest or apoptosis. Mutations in p53 lead to genomic instability and uncontrolled cell growth.	It inactivates pro-apoptotic pathways, promotes angiogenesis, and contributes to metastasis. Mutant p53 can also gain oncogenic functions.	The restoration of wild-type p53 function, inhibition of mutant p53 activity, and targeting p53-related pathways.
Cell cycle regulation	p53 pauses the cell cycle at the G1 checkpoint to allow DNA repair. Loss of p53 leads to uncontrolled cell division and tumor formation.	p53 disrupts cell cycle checkpoints, allowing for rapid cell proliferation and tumor growth.	Targeting cell cycle checkpoints and inducing apoptosis in p53-deficient cells.
Apoptosis	Induces programmed cell death to eliminate damaged cells. Loss of p53 leads to the accumulation of damaged cells and increased cancer risk.	p53 prevents apoptosis, allowing tumor cells to survive and proliferate.	Induction of apoptosis in cancer cells through various mechanisms.
DNA repair	Activates DNA repair pathways to maintain genomic stability. Loss of p53 impairs DNA repair, leading to genetic mutations and cancer development.	Contributes to genomic instability and accumulation of cancer-causing mutations.	Enhancing DNA repair mechanisms or targeting DNA damage response pathways.
Angiogenesis	p53 inhibits blood vessel formation to prevent tumor growth. Loss of p53 promotes angiogenesis, supporting tumor growth and metastasis.	p53 stimulates angiogenesis, providing nutrients and oxygen to the tumor.	Anti-angiogenic therapies to inhibit blood vessel formation.
Immune response	p53 regulates the immune response to cancer cells. Loss of p53 impairs immune surveillance, allowing tumor growth.	Suppresses immune response, creating an immunosuppressive tumor microenvironment.	Immunotherapy to enhance the immune response against cancer cells.

4. Post-translational modifications in mutant p53

Mutant p53 is also capable of undergoing post-translational modifications, although the resulting biological effects differ from those of wtp53. Interestingly, regions of wtp53 frequently modified post-translationally are also often mutated across various cancer types. This is partly due to the presence of common hotspot mutations in p53 that are not modified post-translationally. However, there are regions in all p53 domains that undergo post-translational modifications and have been identified as altered in human tumors. Post-translational modifications of mutant p53 do not affect its ability to bind to specific DNA sequences or perform tumorigenesis-related functions. While wtp53 binds to its respective RE, mutant p53 lacks a defined DNA sequence to which it binds. Instead, mutant p53 interferes with the transcriptional programs of other proteins, such as transcription factors [29].

Mutant p53 has been found to exhibit phosphorylation, ubiquitination, acetylation, and methylation. Compared to wtp53, the effects of post-translational modifications on mutant p53 are less well understood. For example, research has shown a link between the carcinogenic activity of the R175H mutant and its hyperphosphorylation at Ser392. However, this seems to be type-dependent, as hyperphosphorylation is absent in certain breast cancer cell lines. Mutant p53 interacts with cofactors and transcription factors via other phosphorylation sites, such as Ser15, which provides it with a GOF advantage. Similarly, hyperacetylation of mutant p53 at Lys373 and Lys382 promotes its localization to the nucleus.

Moreover, the effects of cellular stress signaling, such as glucose deprivation, on mutant p53 differ from those on wtp53 [30]. Under these stress conditions, mutant p53 undergoes acetylation, leading to metabolic reprogramming that enhances its survival. In contrast to wtp53, which is heavily ubiquitinated to regulate protein levels during stress response resolution, mutant p53 is rarely ubiquitinated. This is primarily due to

the lack of a negative feedback loop and the absence of *MDM2* gene transactivation. As a result, mutant p53 becomes highly stable in various tumor types. However, certain mutant p53 variants can interact with MDM2 and be targeted for degradation, in addition to other ubiquitin ligases known to regulate wtp53 stabilization [31].

5. Mutant p53 mechanisms of destabilization

The ubiquitin-proteasome system (UPS) is responsible for the degradation of mutant p53, similar to wtp53. Mutant p53 has been found to be ubiquitinated by several E3 ubiquitin ligases. MDM2 can target certain mutant p53 variants for proteasomal destruction and/or ubiquitination. However, the rate of mutant p53 degradation is not always directly correlated with its ubiquitination status. For example, hyper-ubiquitinated mutant p53 remains stable and may aggregate primarily in the cytoplasm rather than the nucleus, although this varies depending on the type of mutant p53. The primary reason for the increased stability of mutant p53 is its inability to transcriptionally regulate the target genes of wtp53, which disrupts the negative feedback loop mediated by MDM2 [32]. It is possible that additional E3 ligases may ubiquitinate mutant p53 because the interaction between mutant p53 and MDM2 depends on the MDM2 RING domain rather than its E3 ligase activity. Research has shown that while ARF-BP1 is unaffected, E3 ligases Cop1 and CHIP are involved. Mutant p53 can evade proper protein surveillance by binding to heat-shock proteins (Hsp), preventing degradation caused by its unfolded state. In this scenario, mutant p53 binds to Hsp90, protecting it from CHIP E3 ligases and MDM2. Notably, Hsp90 is overexpressed in various cancers, which may contribute to the stability and GOF properties of mutant p53 [33].

Autophagy represents a potentially unique mechanism for regulating mutant p53 protein levels. Macro-autophagy, or simply autophagy, is the process of intracellular degradation that occurs in response to cellular damage or the need to recycle components to balance energy expenditure and maintain cellular homeostasis. Vesicles are generated in an organized manner to enclose targeted cellular elements for autophagy-mediated degradation. These vesicles eventually fuse with lysosomes, where their contents are broken down [34,35]. Mutant p53 can be degraded by the autophagy machinery in response to physiological stressors such as glucose deprivation, and this process depends on the presence of deacetylated p53. Both autophagy and chaperone-mediated autophagy (CMA), which does not rely on vesicle formation, can be used to degrade mutant p53. The 70 kDa heat-shock cognate protein (Hsc70) mediates the selective autophagy mechanism known as CMA. Lysosome-associated membrane protein type 2A (Lamp-2A) directs substrate proteins associated with Hsc70 to the lysosome and facilitates their internalization into the lysosomal compartment. Under metabolic stress, hypoxia, and non-proliferative cell conditions, aggregated mutant p53 is polyubiquitinated at K63 by CHIP and interacts with Hsc70 and Lamp-2A, leading to lysosomal degradation [23].

6. Mutp53 accumulation in cancer

Elevated levels of mutp53 expression in tumor cells are necessary for its gain-of-function effects. However, the exact mechanisms of mutp53 accumulation in cancers remain unclear. Post-translational modifications are central to the regulation of p53 and are involved in numerous cellular signaling processes. Wtp53 acts as a transcriptional regulator specific to DNA sequences and becomes active in response to various stress stimuli. Its activity can be modulated by post-translational modifications, including phosphorylation, acetylation, and ubiquitination, which also affect mutp53 similarly to wtp53. Studies have shown that phosphorylation at sites such as Ser15, Thr81, and Ser392 can influence mutp53. For instance, phosphorylation of mutp53 at Ser15/Ser37 by DNA-PK enhances its stability and GOF in ovarian cancer. Conversely, in prostate cancer, nuclear

factor kappa-B (NF-κB) restriction leads to phosphorylation of mutp53 at Ser15, restoring its DNA-binding ability. Additionally, mutp53 can be modified through acetylation [36]. Transformation/transcription domain associated protein (TRRAP), a component of several histone acetyltransferase complexes, is overexpressed, increasing mutp53 levels, while TRRAP silencing reduces mutp53 accumulation in lymphomas and colon cancers. Along with phosphorylation and acetylation, ubiquitination also plays a role in modulating mutp53. Normally, MDM2 regulates wtp53 at low levels by targeting it for proteasomal degradation. However, mutp53 does not effectively activate MDM2, which impairs MDM2's negative regulatory function. Nonetheless, one study found that the p53 R172H mutant is stabilized by MDM2 deletion [37]. Mutp53 can be ubiquitinated and degraded by additional E3 ubiquitin ligases, including CHIP, COP1, and Pirh2. Co-chaperone and chaperone proteins like BAG5, Hsp90, and Hsp70 are also associated with the accumulation of mutp53 in human malignancies. BAG5 protects mutp53 from ubiquitin-mediated degradation by MDM2 and CHIP, while Hsp90 and Hsp70 help stabilize mutp53 through interactions with its DNA-binding domain [38].

7. The p53 pathway

p53 is a transcription factor that is distributed in the nucleus and cytoplasm, binds specifically to DNA, and activates a variety of genes. Under normal conditions, cellular p53 protein levels are kept very low due to strict regulation by its negative regulators, MDM2 and MDMX, which promote p53 degradation through ubiquitination. In response to internal and external stresses such as DNA damage, hypoxia, starvation, and cancer cell risk, p53 ubiquitination is inhibited. This results in a significant increase in intracellular p53 protein levels [39]. Post-translational modifications, including phosphorylation, acetylation, and methylation, enhance and maintain elevated levels of p53. Stabilized p53 binds to its target DNA, forms tetramers in the nucleus, and regulates gene transcription, thereby influencing downstream signaling pathways. As a well-studied tumor suppressor gene, p53 transcriptionally activates multiple genes involved in apoptosis and cell cycle regulation in response to cellular stress, thereby halting cellular processes and preventing the division of cells with damaged or mutated DNA. In addition to these classical functions, p53 also regulates several "non-classical" pathways, such as autophagy, metabolic balance, ferroptosis, stem cell differentiation, and the tumor microenvironment, as reported in various studies [40].

7.1. Metabolic homeostasis

For tumor cells to grow rapidly and continuously, they require substantial amounts of biological energy and raw materials. According to the Warburg effect, tumor cells use glucose differently from normal cells, characterized by increased lactate production and heightened glycolysis. By regulating the glycolytic pathway, p53 acts as a tumor suppressor by maintaining cellular metabolic balance. p53 can transcriptionally regulate genes involved in oxidative phosphorylation, such as *SCO2*, and genes that inhibit glycolysis, like *TIGAR* [41]. Additionally, to suppress the pentose phosphate pathway in tumor cells, p53 binds to G6PDH, the rate-limiting enzyme of the pathway. p53 also inhibits glucose uptake and glycolysis by reducing the production and translocation of glucose transporter proteins, including GLUT1 and GLUT4. In a dynamic sense, glycolysis and gluconeogenesis can be considered opposing mechanisms, with p53 suppressing glycolysis and promoting gluconeogenesis. Since the Warburg effect and glycolysis are crucial for tumor cell growth and metastasis, p53's suppression of glycolysis typically inhibits cancer development [42].

Cancer cells can initiate different metabolic processes depending on environmental conditions. Mutant p53 stimulates the Warburg effect and enhances tumor metabolism by promoting the translocation of GLUT1

to the plasma membrane. Mutant p53 also enhances mitochondrial efficiency and promotes cancer metastasis by binding to and activating PGC-1α, a key regulator of oxidative phosphorylation. This suggests that cancer cells with mutant p53 may exhibit greater metabolic plasticity, helping them adapt to stressful conditions and increasing their capacity for growth and metastasis. Tumor cells require lipids to proliferate and expand, and p53 promotes lipolysis, thereby inhibiting tumor growth. Cholesterol and nonsteroidal isoprenoid synthesis is regulated through the mevalonate pathway, in which SREBP2 plays a key transcriptional role. p53 prevents SREBP2 activation by transcriptionally activating the *ABCA1* cholesterol transporter gene and downregulating *USP19* and *SOAT1* to inhibit cholesterol esterification. Furthermore, p53 promotes fatty acid oxidation by upregulating *CPT1C*, *MCD*, and *PANK1* expression [43].

Ammonia is a common byproduct of cellular metabolism. Cancer cells generate large amounts of ammonia during amino acid metabolism, which can serve as a nitrogen source for tumor formation. p53 regulates ammonia levels in cancer cells through the urea cycle. By inhibiting the expression of three key enzymes in the urea cycle—*CPS1*, *OTC*, and *ARG1*—p53 controls ammonia levels and, in turn, suppresses tumor growth. In addition to other metabolic signaling pathways, p53 plays a role in regulating tumor cell metabolism. The overproduction of reactive oxygen species (ROS) in tumor cells has a dual effect: it promotes tumor growth while also triggering ROS-dependent destruction pathways that lead to tumor cell elimination. p53 regulates ROS in two ways [44]. As an upstream signal, ROS activates p53 to either promote or inhibit tumor growth, depending on the context. p53 then transcribes antioxidant genes, such as manganese superoxide dismutase and *GPX1*. Additionally, ROS can act as a downstream regulator of p53 to induce apoptosis and ferroptosis, leading to tumor cell death. p53 also influences both oxidative phosphorylation and the tricarboxylic acid cycle, mediating cancer cell death and regulating redox processes. Furthermore, p53 controls the metabolism of lipids, amino acids, and nucleotides [45].

7.2. Mutp53 exerts gain-of-function

Different p53 mutations confer GOF in various ways. First, for mutp53 to function, it must interact with transcription factors (TFs). Wtp53 binds to DNA RE and recruits TFs, RNA polymerase II (to initiate transcription at open promoters), histone acetyltransferases (HATs) like p300, and chromatin-remodeling complexes (CRCs) like SNF and SWI, which adhere to acetylated histones. However, mutp53 is unable to bind to p53 DNA RE. Instead, it exerts its GOF by alternative mechanisms, often promoting cancer. For instance, mutp53 regulates the transcription of target genes by interacting with various TFs and cofactors, including NF-Y, p73, NRF2, and Ets-1 [46]. Mutp53 binds to NF-Y in response to DNA damage, recruiting p300 to acetylate histones, which leads to the overexpression of cell cycle genes and fosters tumor growth. Mutp53 can also bind to specific DNA structures, such as matrix attachment sites, regulating transcription in specific contexts. Additionally, mutp53 interacts with other proteins, modifying or inhibiting their functions. In colorectal and pancreatic cancers, mutp53 antagonizes p63/p73-mediated tumor suppression via the Notch1 signaling pathway. Notably, the gain-of-function activity of mutp53 is also influenced by its cellular localization. While mutp53 is typically found in the nucleus, some mutations cause it to localize to the cytoplasm. For example, a study found that p53 E258K, R273H, and R273L mutants localized to the cytoplasm and inhibited autophagy in colon cancer, while p53 P151H and R282W mutants remained in the nucleus and did not exhibit this effect [47,48].

7.3. Genetic instability

Genomic instability is considered a hallmark of human cancers. As the “guardian of the genome,” wtp53 plays a critical role in maintaining genomic stability, whereas mutp53 can promote genomic instability. It has

been shown that mutp53 drives chromosomal instability and cell proliferation. For instance, in osteosarcoma, mutp53 interacts with topoisomerase I to induce gene amplification [49]. In pre-tumor thymocytes, mutp53 causes inter-chromosomal translocations. In lung cancer, mutp53 promotes the formation of DNA replication origins and stabilizes replication forks, leading to micronuclei formation and the propagation of cells with abnormal genomes. Additionally, mutp53 prevents the MRE11-RAD50-NBS1 complex from binding to DNA damage sites, thereby inactivating ATM and promoting genetic instability. In lung and breast cancer, mutp53 inhibits BRCA1 and RAD17 expression, contributing to genomic instability and impairing DNA damage repair. Intriguingly, cell-in-cell structures, a feature observed in many solid tumors, are facilitated by mutp53. In lung adenocarcinomas, mutp53 drives the formation of these structures through the engulfment of live cells, leading to abnormal mitosis, while wtp53 promotes the breakdown of these cells. Therefore, the interaction between mutp53 and genomic instability is crucial to cancer development [50,51].

7.4. Role of ferroptosis

Ferroptosis, an iron-dependent form of cell death, has been identified as a distinct mechanism for inhibiting tumor growth. Notably, the regulation of ferroptosis involves p53 in a complex but crucial manner. While most studies provide evidence supporting p53's role in promoting ferroptosis, p53 can also inhibit ferroptosis under certain conditions. In lung cancer, wtp53 induces ferroptosis by suppressing the expression of SLC7A11, which reduces cystine uptake. This reduction in cystine absorption diminishes cellular antioxidant capacity and GPX4 activity. Besides inhibiting SLC7A11, wtp53 also decreases the level of H2Bub1 by promoting the nuclear translocation of the deubiquitinase USP7 [52,53]. Additionally, wtp53-induced ALOX12 expression, via SLC7A11 reduction, triggers ALOX12-dependent ferroptosis. Mutant p53 inhibits SLC7A11 expression in lung and esophageal cancers by interacting with NRF2, a transcription factor known for its antioxidant role, which increases ROS accumulation and induces ferroptosis. For instance, researchers created acetylation-deficient p53 3KR mutant mice by replacing lysine residues at positions 117, 161, and 162 of p53 with arginine. These mice did not regulate the cell cycle or apoptosis like wtp53, but they suppressed SLC7A11 expression and induced ferroptosis. Ectopic SLC7A11 expression in tumors carrying mutp53 increases resistance to ferroptosis-inducing drugs, indicating that mutp53 suppression of SLC7A11 sensitizes cancer cells to ferroptosis [54].

The integration of ferroptosis with genomic instability could significantly accelerate senescence. In the context of *XRCC4* deletion, a gene involved in DNA double-strand break repair, p53 3KR mice exhibited senescence-like symptoms, and p53-mediated ferroptosis was markedly increased in their testes. However, the creation of p53 4KR mutant mice (K98R + 3KR) revealed that these mice not only failed to suppress tumor growth but also could not inhibit SLC7A11 expression or induce ferroptosis [55]. Tumors emerged earlier in p53 4KR mice compared to p53 3KR mice. Moreover, wtp53 interacts with SLC25A28 and translocates to mitochondria in hepatic stellate cells through its interaction with BRD7, resulting in an abnormal accumulation of redox-active iron and promoting ferroptosis. Conversely, the p53 S392A mutant reduces BRD7's binding to p53, preventing p53's mitochondrial translocation and delaying the onset of ferroptosis. In lung cancer, wtp53 regulates the level of the long noncoding RNA (lncRNA) LINC00336 by inhibiting the expression of ELAVL1, which in turn decreases the expression of cystathionine-β-synthase (CBS) and promotes ferroptosis. Wtp53 also induces ferroptosis by regulating the expression of SAT1, GLS2, and PTGS2 [56].

Remarkably, wtp53 can also prevent the initiation of ferroptosis. For example, in lung cancer, wtp53 may delay ferroptosis by promoting the expression of iPLA2β at low-stress levels, though this effect diminishes under high-stress conditions. On the other hand, p53 mutants R175H, R273H, and R248W are unable to rapidly induce iPLA2β expression. In colorectal cancer, wtp53 inhibits ferroptosis by preventing DPP4 function in a

transcription-independent manner. In fibrosarcoma, wtp53 delays ferroptosis in response to cystine deprivation by regulating CDKN1A expression. Additionally, wtp53 may inhibit ferroptosis caused by cystine depletion by upregulating Parkin expression and reducing ROS levels. These findings suggest that p53 can regulate ferroptosis, which has significant implications for cancer therapy [57].

7.5. Role of tumor microenvironment

There is increasing evidence that mutp53 may regulate the tumor microenvironment. Solid tumors are often characterized by tumor-associated macrophages (TAMs). Wtp53 promotes M1 macrophage polarization and creates an anti-tumor environment, which inhibits tumor growth. Interestingly, in colon cancer, mutp53 produces exosomes containing miR-1246 that specifically target nearby macrophages, resulting in miR-1246-dependent reprogramming into an M2 state, which supports tumor progression [58]. Mutp53 may also promote tumor neo-angiogenesis. In non-small cell lung cancer (NSCLC), mutp53 activates the ID4 protein, which encourages the expression of pro-angiogenic factors such as IL8 and GRO-α. When mutp53 is reduced, ID4 expression is also diminished. In leukemia, mutp53 stimulates the production of VEGF, fostering a microenvironment conducive to cell proliferation. Additionally, tumors are often characterized by chronic inflammation. Mutp53 exacerbates inflammation by promoting TNF-induced NF-κB activation in breast cancer. In colon adenocarcinoma, mutp53 inhibits the expression of sIL-1Ra, leading to a pro-inflammatory tumor microenvironment that can increase tumor aggressiveness [59,60].

7.6. Cancer stem cells

Mutp53 has also been found to play a role in the acquisition of cancer stem cell (CSC) phenotypes. CSCs are characterized by their ability to generate a variety of tumor cells, which is crucial for cancer development and metastasis. Wtp53 typically acts as a barrier to CSC development and suppresses the expression of CSC-associated markers. In contrast, mutp53 enhances the expression of CSC markers such as CD44, Lgr5, and ALDH, and promotes the expansion of CSC subpopulations, thereby encouraging colorectal cancer progression. In glioblastoma and breast cancer, mutp53 overexpression not only increases the expression of CSC markers but also drives CSC proliferation [61]. Additionally, in colorectal cancer, the p53 R273H mutation regulates the expression of lncRNAs, such as lnc273-31 and lnc273-34, which promote CSC self-renewal and tumor growth. Mutp53 also regulates miRNAs to enhance cancer stemness. For example, in basal-like breast cancer, mutp53 increases cancer stemness by modulating the miR-200c-PCK2 axis. In lung adenocarcinoma, mutp53 regulates the miR-324-5p-CUEDC2-NF-κB pathway to promote cancer stemness. These findings suggest that mutp53 plays a critical role in regulating cancer stemness, offering a potential new approach to targeting tumors [62].

7.7. The effect of erastin on p53 and its outlook in cancer treatment

Ferroptosis can be induced through two primary mechanisms. The first involves the cysteine-glutamate transporter, which is affected by glutamate, erastin, and sulfasalazine. The second pathway involves RSL3 and DP17, which directly inhibit glutathione peroxidase (GPX) to trigger ferroptosis. Erastin differs from other ferroptosis inducers in that it has a multi-targeted stimulating action that is efficient, fast, and long-lasting. In other words, erastin does not act through a single pathway. One of its functions is to influence the voltage-dependent anion channel (VDAC), an ion channel located in the outer mitochondrial membrane. VDAC regulates ion and molecular exchange between the cytoplasm and the mitochondria. When medications alter VDAC's accessibility, this leads to the generation of ROS, disruptions in mitochondrial metabolism, and ultimately oxidative cell death. As a tubulin antagonist, erastin can open the VDAC channel, altering the

permeability of the outer mitochondrial membrane [63].

VDAC opening results in three biological effects: increased ROS production, reduced glycolysis, and enhanced mitochondrial metabolism. Since many cancer cells exhibit suppressed glycolysis and mitochondrial metabolism, VDAC opening caused by drugs and subsequent ROS generation can target and destroy these cells. One of erastin's advantages as a VDAC-tubulin antagonist is its selective toxicity to cancer cells, as non-proliferating cells lack the high levels of free tubulin characteristic of tumor cells. Thus, by regulating metabolism, erastin offers potential as a novel anti-cancer strategy. Importantly, erastin can activate p53, enhancing ferroptosis. Wild-type p53 induces ferroptosis by inhibiting the function of the XC system [64].

Research has shown that treating A549 lung cancer cells with erastin significantly affects p53 transcription factors and increases ROS levels. The findings suggest that p53 activation, dependent on ROS generated by erastin, triggers the downstream p53 cascade. In acetylation-deficient p533KR mutant cells, even though wtp53 no longer induces apoptosis, these cells still block SLC7A11 transcription. Human cancers commonly overexpress SLC7A11, and ROS-induced ferroptosis inhibits its function. By suppressing SLC7A11, a key antiporter in the cysteine/glutamate system, p53 blocks cysteine uptake and triggers ferroptosis in cells. The results revealed that cell death was minimal (≤ 10%) in p53-deficient cells, but highly significant (> 90%) in p533KR mutant cells treated with erastin. However, erastin treatment significantly reduced cell death (20%) in p533KR mutant cells with high SLC7A11 activity [65].

A different type of mutp53, p534KR98, loses its ability to regulate SLC7A11 transcription. When treated with erastin, this mutant model showed a dramatic decrease in tumor-suppressive activity and led to cell elimination. These findings suggest that erastin-induced p53 activation may be crucial for inhibiting tumor growth by suppressing SLC7A11 transcription and promoting ferroptosis. It is important to note that while some cells may undergo ferroptosis due to erastin-induced p53 activation, this approach could reduce the side effects of chemotherapy by selectively targeting cancer cells for destruction while sparing normal cells. This leaves a promising area for future research [66].

8. Targeting wtp53 tumors through MDM2 and MDM4 inhibitors

The most common therapeutic strategy targeting p53 in cancers that still retain wtp53 is to prevent its breakdown. The most extensively studied mechanism for p53 degradation is ubiquitylation, mediated by the E3 ubiquitin ligase MDM2, which leads to p53 degradation by proteases. MDM2 overexpression is observed in various types of cancer, particularly in tumors that retain wtp53. Since MDM2-mediated ubiquitylation and degradation rely on direct interaction with p53, researchers are developing small molecules that inhibit the MDM2-p53 interaction to stabilize p53 and restore its function [67].

Nutlins, a group of cis-imidazolines, were the first of these inhibitors discovered through a chemical library screen. In cancer cells with wtp53, nutlins activated p53, while no such activation occurred in cells with mutp53. RG7112, a derivative of nutlin, was the first MDM2 inhibitor to enter clinical trials. RG7112 activated wtp53 in patients with refractory or relapsed acute myeloid leukemia (AML) and chronic myeloid leukemia (CML). The effects included stabilization of the p53 protein and increased expression of several p53 target genes, such as *CDKN1A* (encoding p21, a cyclin-dependent kinase inhibitor) and *BBC3* (encoding PUMA, a pro-apoptotic protein). Encouragingly, most of these patients showed anti-leukemic activity. A subset of patients without *TP53* mutations also showed clinical responses, suggesting that RG7112 might have p53-independent effects, such as inhibiting hypoxia-inducible factor 1α to suppress angiogenesis. However, RG7112 required high doses for efficacy, leading to adverse effects such as gastrointestinal discomfort and reduced platelet

production. High concentrations of RG7112 were also associated with neutropenia and thrombocytopenia in liposarcoma patients. Progenitor cells in the gastrointestinal tract and bone marrow may be particularly sensitive to excessive p53 activity, possibly due to high *TP53* mRNA expression, typically coupled with rapid p53 protein turnover. Moreover, RG7112 was shown to cause thrombocytopenia by impairing the ability of megakaryocytes to produce platelets [68].

Idasanutlin (RG7388), a third-generation derivative, replaced RG7112. Several clinical trials are currently evaluating the safety and efficacy of idasanutlin in various cancers, although results remain preliminary. In a phase III trial involving patients with relapsed or refractory AML, idasanutlin combined with cytarabine did not meet the primary endpoint of improved overall survival (OS) or complete responses, although the overall response rate was higher. Similarly, in a phase I trial, idasanutlin showed promising results in patients with polycythemia vera, but in a later phase II trial, it was frequently discontinued due to hematological and gastrointestinal toxicity. The presence of wtp53 in normal tissues, where p53 overexpression is not well tolerated, presents a significant challenge to the clinical use of nutlin derivatives, despite their solid scientific rationale and promising anticancer activity in early-phase trials [69].

Other compounds that inhibit MDM2-p53 binding have been developed or are in development in addition to nutlin derivatives. For instance, in preclinical models of AML, the orally bioavailable MDM2 inhibitor APG-115 exhibited potent antitumor activity and enhanced the radiosensitivity of gastric cancer xenografts. APG-115 is currently being tested in clinical trials as a monotherapy and in combination with immune checkpoint inhibitors or chemotherapy. Another oral MDM2 inhibitor, AMG 232, has shown the ability to stabilize wtp53 and induce tumor regression in osteosarcoma cells. In head-to-head comparisons, AMG 232 demonstrated greater activity than other MDM2 inhibitors, such as idasanutlin [70]. When combined with cytotoxic chemotherapy, AMG 232 showed more pronounced antitumor activity than when used alone. Now called KRT-232, AMG 232 has been evaluated in over 10 clinical trials, including a phase III trial for myelofibrosis after JNK inhibitor withdrawal. Based on its promising results, AMG 232 received fast-track designation from the FDA and has been tested in prior phase trials for other cancers. Additional MDM2 inhibitors, such as milademetan and siremadlin, are also under investigation (NCT03634228, NCT04116541). Interestingly, both drugs showed greater efficacy when administered intermittently at high doses rather than continuously for two weeks in preclinical and phase I trials [71].

While MDM2 inhibition holds promise, it is unclear whether newer MDM2 inhibitors will cause less damage to healthy tissues. Since p53 is present in almost all normal tissues, especially in proliferative regions, it is not a cancer-specific target. Therefore, it may be more feasible to combine smaller, well-tolerated doses of an MDM2 inhibitor with a cancer-specific therapy or develop a method for selectively delivering the inhibitor to tumor cells, rather than aiming to create an MDM2 inhibitor without any adverse effects. MDM4, a protein related to MDM2, is also a critical negative regulator of p53 [72]. Unlike MDM2, MDM4 lacks inherent E3 ubiquitin ligase activity but can directly bind to p53 and inhibit its transcriptional activity while supporting MDM2's E3 ligase function. MDM4 is overexpressed in many cancers, making it an attractive therapeutic target. Notably, wtp53 is commonly retained in hematologic malignancies such as AML and myelofibrosis, often in conjunction with increased MDM2 or MDM4 expression. MDM4 is highly expressed in leukemic stem cells, unlike MDM2, making MDM4 inhibitors particularly promising for leukemia treatment [73].

Stapled peptides have emerged as a novel alternative to small-molecule drugs in recent years. Hydrocarbon stapling techniques have been used to develop a stapled peptide (SAH-p53-8) that disrupts MDM2 and MDM4 interactions with p53. However, subsequent *in vitro* studies suggested that SAH-p53-8 might be cytotoxic independently of p53, raising concerns about its therapeutic potential. Later, ALRN-6924 and other bispecific

stapled peptides targeting both MDM2 and MDM4 were developed. ALRN-6924 demonstrated potent activity in p53-mutant cells and strong efficacy against several wtp53 breast cancer cell lines [74]. Similar wtp53 selectivity was observed in AML cell lines, outperforming idasanutlin, though these effects were abolished with p53 knockdown. The initial phase I trial of ALRN-6924 showed antitumor activity in solid tumors and lymphoma, with mild side effects. Recently, crystal structure analysis of the MDM4-nutlin 3a complex revealed additional intermolecular interactions that could enhance the binding affinity of nutlin 3a for MDM4. This insight may enable the development of more potent dual MDM2/MDM4 inhibitors that could suppress the growth of lung and colorectal cancer cell lines. More dual inhibitors targeting both MDM2 and MDM4 are expected to emerge [75].

9. Challenges and perspectives

Developing drugs that target p53 presents numerous challenges. Two primary issues are the lack of binding pockets and the absence of a well-established process for protein reactivation. While the activity of many proteins can be largely inhibited by blocking their active sites with small molecules, it remains unclear how drug binding can restore a protein's function. Additionally, treating p53-related issues is complicated by factors such as *TP53* deletions, off-target effects, and potentially harmful side effects from p53 overexpression in healthy tissues. The full structures of p53 and its binding partners in association with various DNA targets are not yet fully understood. This lack of structural information limits structure-based drug development for certain p53 mutants that are difficult to produce or whose architectures are not readily available. However, recent advancements in artificial intelligence and cryo-electron microscopy offer hope. With these technological developments, there is reason to believe that p53 structural research will progress, providing a stronger foundation for the development of p53-targeting drugs.

Several additional considerations must be addressed in p53-targeted therapy. First, not all TP53 mutations are alike, and they vary widely. This diversity makes it unlikely that a single drug could be effective against all *TP53* mutations, suggesting that different p53 mutants may require different therapies. Second, cancer treatment may need more than just p53-targeted therapies. Combination treatments could offer a synthetic lethal approach, such as the simultaneous inhibition of the p53-BCL-2 and MDM2-p53 pathways. Third, new therapeutic strategies might include targeting p53 mRNA, disordered structural domains, mutant protein degradation, or even genome editing using CRISPR-Cas9. Gene editing technologies, like CRISPR-Cas9, are already being explored as cancer therapies. With further scientific progress, CRISPR-Cas9 may provide effective solutions for addressing *TP53* mutations in cancer treatment.

Despite many years of unsuccessful attempts to develop drugs targeting p53, recent progress offers renewed hope. Once considered an undruggable gene, p53 is now more accessible thanks to technological advancements, much like other previously "undruggable" targets such as KRAS. Given the high frequency of *TP53* mutations in human cancers, there is good reason to believe that drugs targeting p53 will continue to advance and may lead to a breakthrough in cancer treatment.

Author contributions

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