REVIEW

Mutant p53: evolving perspectives

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The discovery of the p53 tumor suppressor protein raised fundamental questions about cell cycle regulation that have spanned several decades. *TP53* mutations are found in most human cancers, most frequently as missense alterations in the DNA-binding domain (DBD). As a master regulator of both cell-intrinsic and cell-extrinsic functions, mutant p53 contributes to pro-oncogenic activities through gain-of-function (GOF) properties in addition to loss-of-function (LOF) and dominant-negative effects (DNEs). New technologies and improved fidelity of model systems are uncovering the functional consequences caused by p53 mutations at the molecular, cellular, and tissue levels. In a new era of precision medicine, with the context of recent success in targeting genetic mutations, ongoing and future understanding of fundamental mutant p53 biology is of paramount importance.

Mammalian cells rely on p53 as a crucial regulator of DNA fidelity in cell division. The wild-type p53 protein is a transcription factor that binds DNA in a sequence-specific manner and modulates transcription of target genes that mediate tumor suppression (Boutelle and Attardi 2021). Its structure is consistent with its role as a transcription factor, with domains that are responsible for transcriptional activation, sequence-specific DNA binding, and oligomerization as a tetramer (Boutelle and Attardi 2021). Although roles for transcription-independent effects of p53 appear likely, regulation of gene expression is nonetheless critical for many p53-mediated responses (Boutelle and Attardi 2021; Indeglia and Murphy 2024). Transcriptional regulation is intimately associated with the tumor suppressor activity of p53 (Indeglia and Murphy 2024). Wild-type p53 mediates cellular responses to DNA damage and other forms of stress (Manfredi 2010). The levels of wild-type p53 are low in cells by virtue of binding to MDM2, a negative regulator of p53 (Karni-Schmidt et al. 2016). Stress-induced posttranslational modifications of both p53 and MDM2 disrupt their binding to each other, leading to an increase in p53 levels (Dai and Gu 2010). The p53 protein then regulates gene expression, leading to a variety of cellular responses, including cell cycle arrest, senescence, cell death, genomic stability, and metabolic programming, among other consequences (Fig. 1A; Boutelle and Attardi 2021; Wang et al. 2023; Indeglia and Murphy 2024). Despite extensive information about the normal functions of wild-type p53, we still lack a decisive understanding of the selective advantages produced by p53 mutations that drive cancer.

Mutation of the *TP53* tumor suppressor gene is the most frequent genetic event in human cancer (Freed-Pastor and Prives 2012; Kastenhuber and Lowe 2017). The proportion of tumors with genetic alterations in *TP53* varies by tissue of origin, being highest in cancers of the uterus, esophagus, and lung but rarely detected in testicular cancer and uveal melanoma (Fig. 2; Bouaoun et al. 2016). Interestingly, other genetic alterations may serve to inactivate wild-type p53 function in tumors with a low frequency of *TP53* mutations. For example, in soft tissue sarcomas, *MDM2* gene amplification is common, with mutual exclusivity between p53 mutations and MDM2 overexpression (Manfredi 2010). A similar role for gene amplification of the closely related *MDM4* gene is also found (Sun et al. 2018). In human tumors that are associated with human papillomavirus infection, the E6 protein has been shown to target p53 for degradation (Mantovani and Banks 2001). Cervical cancers that are HPV-positive have wild-type p53, while those that are negative for the virus present with *TP53* mutation (Fontan et al. 2022). One framework posits that each component of the p53 pathway can be altered in human cancer in a mutually

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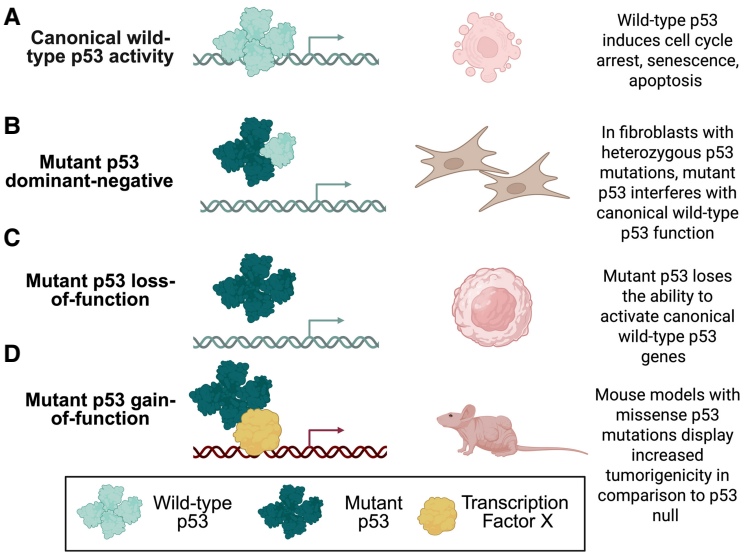

Figure 1. Wild-type p53- and mutant p53-mediated mechanisms of action. (*A*) After DNA damage, wild-type p53 acts as a tumor suppressor by activating genes that halt the cell cycle, induce senescence, and cause apoptosis. (*B*) Mutant p53 displays a dominant-negative effect wherein mutant p53 overrides wild-type p53 activity in mixed homotetramers. (*C*) Mutant p53 does not transactivate the same tumor-suppressive genes as wild-type p53 and often activates genes with opposing functions. (*D*) Beyond the loss of tumor suppression and the dominant-negative mutational effects, mutant p53 activates protumorigenic and prometastatic genes in combination with a suite of transcription factors.

exclusive manner. While this is an appealing premise, it has not been rigorously demonstrated in all cancer types. This leaves us with the notion that *TP53* mutation is selected for in some tissues but not in others.

In this review, we encompass an updated perspective on mutant p53 biology with a focus on missense mutations within the DNA-binding domain (DBD) of p53, recognizing that mutations can be found in other domains and have their importance. We discuss the diversity of p53 mutations and their underlying mechanistic basis and functional consequences and offer perspectives on advancing studies in mutant p53 biology. The p53 field is dynamic and hence perspectives evolve. As a separate consideration, the reader is referred to other reviews that cover p53 therapeutics, which are not discussed in this review (Hassin and Oren 2023; Nishikawa and Iwakuma 2023; Peuget et al. 2024).

TP53 missense mutations in the DNA-binding domain

The *TP53* gene typically harbors missense mutations in human tumors, and this is often accompanied by chromosome 17p loss of heterozygosity (Liu et al. 2016; Kastenhuber and Lowe 2017). This is in contrast to other tumor suppressors in which genetic alteration leads to loss of expression due to either deletion, frameshift mutation, or transcriptional silencing (Robles et al. 2002). For example, the *adenomatous polyposis coli* (*APC*) gene is commonly subjected to frameshift mutation that results in a truncated protein that can no longer interact with β-catenin (Robles et al. 2002). *TP53* missense mutations are clustered for the most part within the exons of the core sequence-specific DBD (Freed-Pastor and Prives 2012). Almost all p53 mutations show defects in sequence-specific DNA binding and fail to properly activate a wild-type p53 transcriptional program, which can be due to loss of either activated or basal gene expression driven by wild-type p53 (Fig. 3A; Freed-Pastor and Prives 2012; Muller and Vousden 2013). The mutational spectrum of p53 has six notable "hotspots" (sites that are mutated with extraordinarily high frequency compared with other mutations within the p53 coding sequence) that likely confer evolutionary advantage (Muller and Vousden 2014). Structural analyses of wild-type p53 bound to its cognate DNA site reveals that these mutants can be placed in two distinct classes: "Contact p53 mutants," such as those at residues p53-R248H and p53-R273H, alter sites of direct interaction with DNA (Pavletich et al. 1993), and "structural p53 mutants" (also referred to as "conformational p53 mutants"), such as those at residues p53-R175H and p53-R282H, alter regions that play key roles in establishing a domain structure that is critical to present the "contact" residues to the DNA (Freed-Pastor and Prives 2012).

It has been shown that some p53 mutations disable wild-type p53 functions, thereby evading p53-mediated tumor suppression and promoting tumor progression (Fig. 1A; Kastenhuber and Lowe 2017). Our recent work (Efe et al. 2023; Guzman et al. 2025) and other studies underscore that mutant *TP53* alleles may have neomorphic gain-of-function (GOF) properties that contribute to oncogenic functions beyond mutant p53's classic role via loss of wild-type p53 (Fig. 1D; Dittmer et al. 1993; Olive et al. 2004; Suh et al. 2011). Notably, all mutant p53 proteins do not behave the same, with some acting in a manner like the p53-null state, others only selectively losing some wild-type p53 functions, and the most aggressive variants promoting tumor invasion and metastasis (Fig. 3A). There have been studies linking different somatic *TP53* mutations to distinct clinical outcomes (Kennedy and Lowe 2022). Similarly, Li–Fraumeni patients present with varying ages of onset and tumorigenic features depending on the specific mutation (Xu et al. 2014).

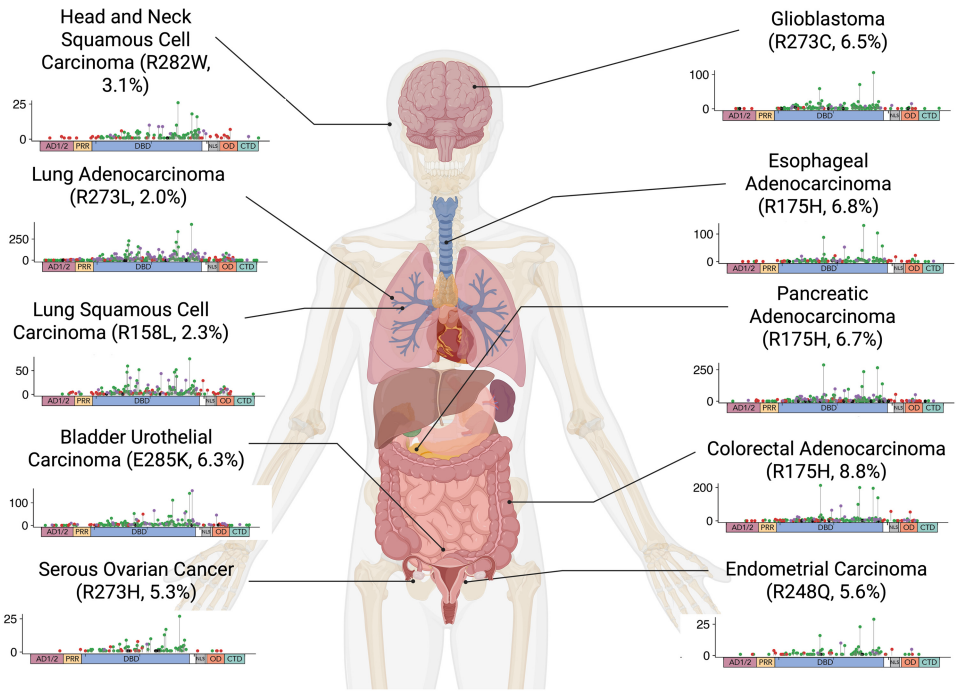

Figure 2. The distribution of p53 mutations across select cancers is depicted. The most frequent "hotspot" mutations are displayed, along with the percentage of this mutation among total cases in that cancer type with p53 mutations (e.g., in head and neck squamous cell carcinoma, the DNA-binding domain hotspot mutation R282W is the most frequent, with 3.1% of cases possessing that mutation). (Green) Missense mutations, (red) nonsense mutations, (purple) splice site mutations . Data are from The Cancer Genome Atlas (TCGA) (Weinstein et al. 2013), accessed at https://www.cbioportal.org.

p53 mutations outside of the DNA-binding domain

Besides the sequence-specific DBD, which serves as the focus of this review, the p53 protein comprises an acidic N-terminal transactivation domain (TAD; subdomains TAD1 and TAD2), proline-rich domain (PRD), oligomerization domain (OD), and basic C-terminal regulatory domain (CTD) that are important in mediating its functions as a transcriptional regulator (Laptenko and Prives 2006; Freed-Pastor and Prives 2012). Mutagenesis studies and mouse models have revealed that TAD1 is involved in acute DNA damage responses, and both TAD1 and TAD2 contribute to tumor suppression, mediated through interactions with negative regulators, other transcription factors, and chromatin modifiers (Johnson et al. 2005; Brady et al. 2011). Additionally, phosphorylation of the TADs affects p53 activity (Raj and Attardi 2017). Multiple mutations in the TAD region are needed to halt transactivation, reducing the likelihood of widespread effects after one point mutation as seen in the DBD (Lin et al. 1994).

The most well-studied and prevalent Li–Fraumeni syndrome OD mutation, p53-R337H, is overrepresented in Brazil but is associated with variable tumor penetrance (Pinto and Zambetti 2020; Jeffers et al. 2021). A small subset of patients with Li–Fraumeni syndrome harbor the OD mutation p53-A347D, which forms exclusively dimers and not the tetrameric form of the protein (Choe et al. 2023; Gencel-Augusto et al. 2023). This dimeric configuration was associated with upregulation of the PPAR pathway, metabolic reprogramming, and aberrant mitochondrial morphology, indicating novel functions of the p53 dimer (Gencel-Augusto et al. 2023). Another Li–Fraumeni-associated mutation in the OD, p53-R342P, was found to form monomeric proteins but lacked novel functions (Gencel-Augusto et al. 2023). Additionally, mutations in the CTD (p53-R379H) and PRD (frameshift and termination mutations) have been reported (Bougeard et al. 2008; Yurgelun et al. 2015; Dobbins et al. 2016). Both the CTD and PRD regulate tumor suppression: Deletion of the CTD is associated with reduced activation of apoptosis and cell cycle arrest and failure to bind p21 and PUMA gene promoters, while deletion of the PRD leads to weak transactivation of p21, and the resulting protein can induce apoptosis but not cell cycle arrest (Venot et al. 1998; Toledo et al. 2006; Hamard et al. 2012). Overall, non-hotspot mutations can affect a variety of p53 properties, such as protein stability, protein folding, or interactions with other proteins (Raj and Attardi 2017). Given the predominance of hotspot mutations and the wealth of studies focused on them, we focus here on p53 mutations in the DBD.

Diversity in mutant p53 binding partners and transcriptional regulation

Heterogeneity among different p53 mutations can be achieved through interactions with other proteins, which

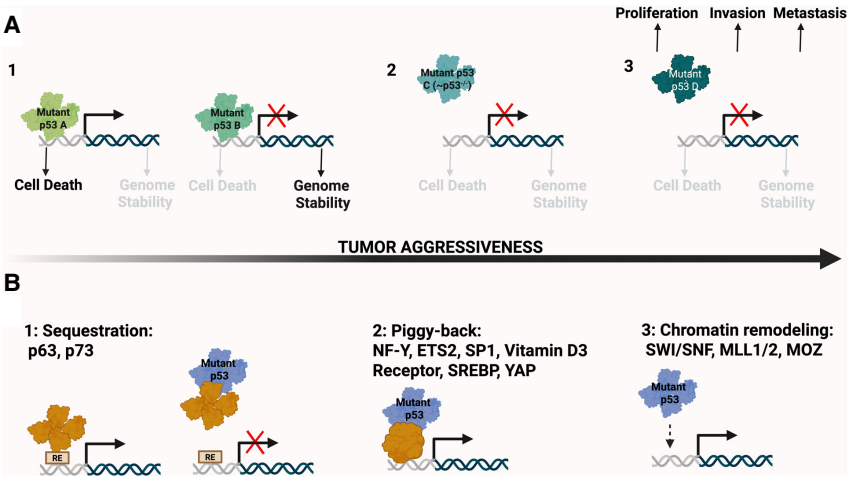

Figure 3. Tumor-derived p53 mutants have distinct alterations and modes of regulation that are associated with increasing tumor aggressiveness. (*A*) From *left* to *right*, various functions of p53 mutants increase tumor aggressiveness: (*1*) Some p53 mutants retain wild-type p53 activity. (*2*) Other p53 mutants lose the ability to bind to DNA in a sequence-specific manner, phenocopying the loss of p53. (*3*) Other mutants not only lose the wild-type functions but gain neomorphic oncogenic activities and lead to increased tumor aggressiveness. (*B*) Proposed mechanisms of mutant p53-regulated gene expression: (*1*) Mutant p53 sequesters transcription factors like p63 and p73. (*2*) Mutant p53 engages in protein–protein interactions with activators and enhances their transcriptional output. (*3*) Mutant p53 can alter chromatin remodeling.

are mostly transcription factors or chromatin-regulating proteins that lead to changes in gene expression (Fig. 3B). The selection and affinity by which distinct p53 mutations bind to these proteins may result in a diverse range of target gene transcription and, as a consequence, changes in tumorigenic phenotypes (Kim and Lozano 2018). Different models have been put forward to explain the ability of mutant p53 to affect transcriptional regulation. First, although gene occupancy can be detected for some mutant p53 proteins, these sites are often distinct from those bound by wild-type p53 and likely involve recruitment of the mutant p53 protein by other sequence-specific DNA-binding factors (Di Agostino et al. 2006; Pfister et al. 2015). Second, mutant p53 has been shown to bind to specific factors and inhibit their transcriptional regulation capacity (Di Agostino et al. 2006; Pfister et al. 2015). Finally, experimental data indicate that mutant p53 can alter chromatin remodeling through a variety of mechanisms, resulting in epigenetic changes and influencing gene expression (Di Agostino et al. 2006; Pfister et al. 2015).

Distinct mutant p53 proteins bind to transcription factors differentially to either activate or repress target genes (Kim and Lozano 2018). The most widely studied mutant p53-interacting partners in this category are the p53 family members; namely, p63 and p73 (Fig. 3B; Freed-Pastor and Prives 2012). These transcription factors are homologous at the amino acid level in the transactivation, DNA-binding, and oligomerization domains (Li and Prives 2007). Mutations in p63 or p73 are rarely detected in sporadic human cancers; however, loss of p63 can be found in metastatic tumors (Melino 2011). Multiple conformational and DNA contact tumor-derived p53 mutants (the latter less effectively) can physically interact with p63 or p73. This results in some mutant p53-mediated protumorigenic properties through the inhibition of p63 and p73 (Gaiddon et al. 2001; Strano et al. 2002; Li and Prives 2007). p53 mutations at R175, R273, Y220, and R248 interact with both p63 and p73 to disrupt the transactivation of target genes in multiple mammalian cell lines (Marin et al. 2000; Gaiddon et al. 2001; Strano et al. 2002).

The interactions of specific p53 mutations with p63/p73 have been linked to its GOF properties in tumor cell invasion and metastasis. p53 mutations at R110P, E258V, R175H, and R282W lead to coaggregation of p63 and p73, thereby preventing transcription of target genes involved in cell growth control and apoptosis (Xu et al. 2011). Notably, the common p53 polymorphism p53-R72 led to increased binding of p73 in comparison with p53-P72 in the context of p53 mutants (Marin et al. 2000). p53-R175H and p53-R273H can interact with p63 to promote α5β1 integrin and epidermal growth factor receptor (EGFR) trafficking that contributes to tumor invasion (Muller et al. 2009). Another study demonstrated that SMADs can form a platform for the p53-R175H/p63 interaction that reduces the gene expression of p63 targets and metastasis suppressors such as *Cyclin-G2* and *Sharp-1* (Adorno et al. 2009). Mutant p53 regulates tumor cell invasion in 3D environments via the mevalonate pathway-dependent Rho/ROCK signaling (Guzman et al. 2025). The mutant p53-R273H/p63 complex can also regulate microRNA expression to foster invasion and metastasis (Subramanian et al. 2015). The tumor suppressor microRNA let-7i is downregulated by mutant p53 through p63, and this promotes migration, invasion, and metastasis by inducing a network of oncogenes, including *E2F5*, *LIN28B*, *MYC*, and *NRAS* (Subramanian et al. 2015). In mouse models of pancreatic cancer, p53-R172H (mouse homolog of R175H) induces PDGFRβ through the inhibition of the p73/NF-Y complex that normally represses PDGFRβ and contributes to invasion and lung metastasis (Weissmueller et al. 2014). Furthermore, the transcription factor NF-Y interacts with p53-R175H and p53-R273C—which Liu et al. (2011) found to be mediated by TopBP1—to induce the transactivation of cell cycle genes (Fig. 4A; Di Agostino et al. 2006; Cai et al. 2025).

Studies have shown direct interaction between mutant p53 and other transcription factors, including Ets2 (Do et al. 2012; Xiong et al. 2014), Sp1 (Bargonetti et al. 1997; Chicas et al. 2000; Stieg et al. 2024), vitamin D3 receptor (Stambolsky et al. 2010), NF-Y (Di Agostino et al.

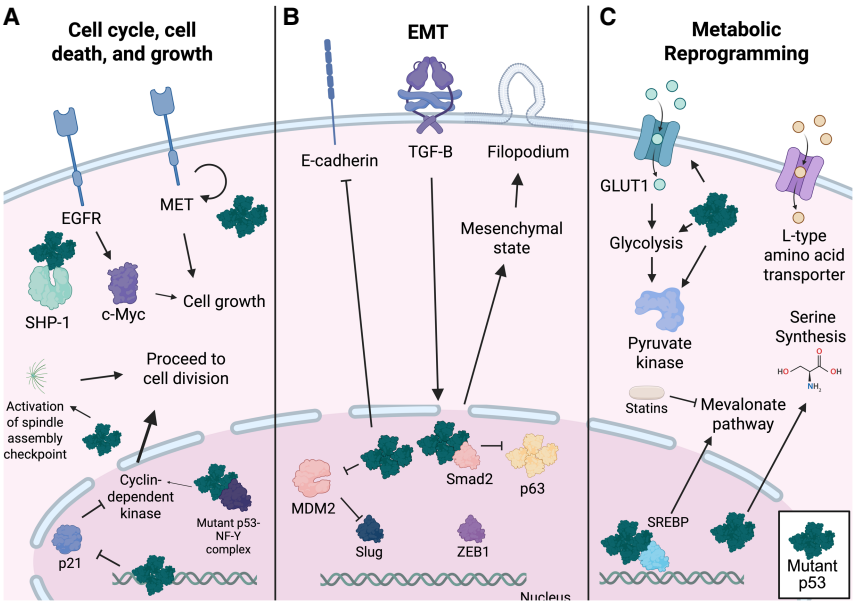

Figure 4. Mutant p53 gain-of-function activity regulates tumor cell-intrinsic mechanisms. (*A*) Mutant p53 promotes cell growth and cell division via epidermal growth factor receptor (EGFR) signaling by upregulating c-Myc, recycling the MET receptor, and activating the spindle assembly checkpoint. Additionally, mutant p53 activates cyclin-dependent kinases (CDKs) by formation of a complex with the nuclear transcription factor Y (NF-Y) and downregulation of the CDK inhibitor p21. (*B*) Mutant p53 induces the cellular plasticity program epithelial-to-mesenchymal transition (EMT) by downregulating MDM2, which reduces degradation of Slug and reduces E-cadherin expression. Using TGF-B signaling, mutant p53 forms a complex with Smad2 and p63, opposing p63's regulatory activities and causing a mesenchymal state and filopodia formation. (*C*) In cancer, mutant p53 reprograms metabolism: Mutant p53 upregulates the glucose transporter GLUT1, induces pyruvate kinase 2, and upregulates glycolysis. Mutant p53 and sterol SREBPs express genes from the mevalonate pathway, targeted by statin treatment. Mutant p53 also facilitates serine synthesis and upregulation of amino acid transporters.

2006), SREBP (Freed-Pastor et al. 2012; Ingallina et al. 2018; Parrales et al. 2018), NF-κB (Cooks et al. 2013), and YAP (Tang et al. 2021) among many others (Fig. 3B). For example, p53-R273H, R248W, C176S, R280K, and R273H proteins form a direct interaction with Sp1 on the *ENTPD5* core promoter to induce its expression, which promotes the folding of N-glycosylated membrane proteins and mediates invasion and lung colonization in pancreatic cancer models (Vogiatzi et al. 2016). p53-R172H also interacts with small nucleolar RNAs (snoRNAs), and this drives metastasis in mouse models of osteosarcoma (Pourebrahim et al. 2017).

In addition to transcriptional factors, mutant p53 proteins interact with chromatin regulators that can modify genome-wide chromatin architecture, causing changes in chromosome accessibility by transcription factors. p53-R273H, p53-R280K, and p53-R175H recruit the SWI/SNF chromatin remodeling complex to transactivate vascular endothelial growth factor receptor 2 (VEGFR2) and mutant p53-mediated oncogenic activity in breast cancer cell lines (Fig. 3B; Pfister et al. 2015). p53-R273H, p53-R249S, and p53-R175H bind to and increase transcription of methyltransferases MLL1 (KMT2A) and MLL2 (KMT2D) and acetyltransferase MOZ (KAT6A), leading to genome-wide alterations in histone methylation and acetylation with facilitation of tumor cell proliferation and growth (Zhu et al. 2015). Such findings also underscore potential combinatorial chromatin-based therapeutic strategies for cancer patients with specific p53 mutations.

Gain-of-function activity of p53 mutations

One hallmark of tumors harboring *TP53* missense mutations is the increased half-life and marked overexpression of mutant p53 (Finlay et al. 1988). This may be related to its inability to transcriptionally upregulate Mdm2 to facilitate p53 degradation that was strongly supported in an elegant mouse model (Terzian et al. 2008). While all the factors that are needed for increased stabilization have not been identified, there is strong evidence for the role of heat shock proteins in maintaining mutant p53 stability (Cordani et al. 2024). The persistence of mutant p53 in human cancers has prompted the hypothesis that there may be selective pressure to retain its expression that results in the acquisition of oncogenic properties independent of its effects on wild-type p53 (Baugh et al. 2018). Using ectopic p53 expression in cell culture studies, the first experimental evidence emerged to support the notion that tumor-derived mutant p53 gained oncogenic function(s) in comparison with p53 loss, termed "gain of function" (GOF) as noted previously (Fig. 1D; Hinds et al. 1990; Dittmer et al. 1993). In a separate study, expression of mutant p53 in cells in a p53-null background acquired protumorigenic activity, including increased transcription of the multidrug resistance gene, enhanced tumor growth in immunodeficient mice, and improved efficacy in soft agar assays (Dittmer et al. 1993).

Genetically engineered mouse models expressing either no p53 or mutant p53 have been instrumental in distinguishing between dominant-negative effects (DNEs; discussed in greater detail below) and enabling mutant protein-specific features. Multiple studies demonstrated that mice with germline knock-in of hotspot p53 mutants lead to tumor phenotypes different from those with p53-null mice (Lang et al. 2004; Olive et al. 2004; Hanel et al. 2013). Tumors from mice with R172H or R270H mutations (mouse homologs of human R175H and R273H) metastasize at high frequencies (Lang et al. 2004; Olive

et al. 2004). Notably, mice with these mutations are predisposed to unique tumor spectra—epithelial- and endothelial-derived tumors—in comparison with p53-null mice (Olive et al. 2004). In contrast, some studies did not report metastasis from mice with p53 loss (Donehower et al. 1992; Jacks et al. 1994). Since these initial results, there have been numerous examples of mutant p53 GOF activity in various tumor models (Pfister et al. 2015; Loizou et al. 2019; Efe et al. 2023). Taken together, tumor-derived GOF p53 mutants exhibit a spectra of oncogenic activities in mediating virtually all "hallmarks of cancer" (Hanahan 2022) through tumor cell-intrinsic and tumor cell-extrinsic mechanisms.

Notably, even the same p53 mutation can regulate distinct mechanisms depending on the context and tissue type. For example, the microbiome can change the protumorigenic functions facilitated by mutant p53 depending on the location in the gut of the mouse (Kadosh et al. 2020). It is our hope that further studies on the molecular and cellular bases for this apparent intra-allelic-, intertissue-, and context-dependent heterogeneity will provide better understanding of oncogenic mutant p53 functions and pave the way for novel therapeutic strategies.

Mutant p53 tumor cell-intrinsic GOF mechanisms affect cell cycle, growth, and prosurvival signaling

It is interesting that, in many cases, there is a reciprocal relationship between genes that are regulated by wild-type and mutant forms of p53. Mutant p53 often represses genes that are canonical transactivation targets of wild-type p53, such as in the case of the gene encoding the p21-dependent kinase inhibitor. For example, six missense p53 mutants (p53-R175H, p53-G245S, p53-R248W, p53-R249S, p53-R273H, and p53-R282W) were shown to downregulate p53 target genes, including p21 (Fig. 4A; Vikhanskaya et al. 2007). Conversely, degradation of mutant p53 (p53-R280K, p53-P274L/V223F, and p53-R175H) leads to increased p21 expression (Blagosklonny et al. 2005; Walerych et al. 2012; Na et al. 2019). Furthermore, in a study of pancreatic ductal adenocarcinoma, mice with the p53-R175H mutation form tumors, while mice with the p21-activating mutation p53-R175P did not (Morton et al. 2010). Another important effect of altered regulation of genes is related to cell division and genomic instability. Human mitotic arrest deficiency protein 1 (hsMAD1), part of the spindle assembly checkpoint, is activated by p53-281G (Iwanaga and Jeang 2002). Mutant p53 disrupts control of the spindle checkpoint, contributing to genomic instability (Fig. 4A; Gualberto et al. 1998).

Mutant p53 is associated with decreased apoptosis and cell death via several different mechanisms. p63 and p73, members of the p53 family, contribute to growth arrest and apoptosis (Strano et al. 2002). Specifically, p73 induces apoptosis in the setting of drug treatment by inducing p21, BAX, and Killer/DR5 (Di Agostino et al. 2008). In cells with the p53-R175H mutation, treatment with a short interfering peptide that disrupted binding between p53 and p73 restored apoptosis gene transcripts and chemical sensitivity (Di Agostino et al. 2008). However, p63 and p73 are not the only mediators of apoptosis. Indeed, cells with mutant p53 underwent apoptosis after RNA inhibition irrespective of p63 or p73 knockdown (Lim et al. 2009). Cells with p53-R175H upregulate the antiapoptotic genes BCL2L1 and Seladin 1 and the prosurvival gene MAP2K3 (Bossi et al. 2008), all in contrast to cells with a p53-null status. Cells with p53-R248Q or R273H reduce apoptosis in the setting of several drug treatments (Matas et al. 2001). In these studies, c-Myc levels were found to be higher in cells with mutant p53 than in cells with wild-type p53 (Matas et al. 2001). Through association with the ETS-binding site motif, mutant p53 upregulates TDP2, a DNA phosphodiesterase, to resist DNA damage and death by etoposide (Do et al. 2012). Similarly, mutant p53 upregulates the multidrug resistance gene MDR1 (Sampath et al. 2001).

Wild-type p53 influences ferroptosis (an iron-dependent type of nonapoptotic cell death) in a context-dependent fashion (Corazzari and Collavin 2023). For example, wild-type p53 can induce the expression of proferroptotic genes, such as spermidine/spermine N1-acetyltransferase (SAT1) and glutaminase 2 (GLS2), and represses ferroptosis-inhibiting genes, such as solute carrier family 7 member 11 (SLC7A11) (Gnanapradeepan et al. 2018). That said, under certain circumstances, wild-type 53 can inhibit or delay ferroptosis through control of DPP4 and p21 (Zhan et al. 2023). Crucially, mutant p53 can foster ferroptosis resistance. In lung cancer cells, the p53-R248W mutation inhibits ferroptosis (Peng et al. 2023). Deletion of both p53-R172H and p53-R245W mutants in triple-negative breast cancer mouse models was found to trigger ferroptosis (Dibra et al. 2024). As mentioned previously, TP53 mutations activate the Wnt/β-catenin pathway, which upregulates ΔNp63 and was found to enhance the ferroptosis inhibitor GPX4 (Cai et al. 2025). p53-R172H was found to repress ferroptosis by interacting with BACH1, which interfered with BACH-1-mediated repression of SLC7A11 (Su et al. 2023).

Mutant p53 GOF is also evident through the activation of certain receptor tyrosine kinases (RTKs), which promote cellular survival and prometastatic effects (Pfister and Prives 2017). For example, mutant p53 enhances signaling by epidermal growth factor receptor (EGFR) through blocking binding with the phosphatase SHP1, upregulating c-Myc and Cyclin D1 (Fig. 4A; Ho et al. 2023). MET, which serves as the receptor of hepatocyte growth factor, experiences increased phosphorylation and recycling in the setting of p53-R273H and p53-R175H (Fig. 4A; Grugan et al. 2013; Muller et al. 2013). In lung cancer, various p53 mutations in the DBD were found to upregulate the tyrosine kinase AXL, which increased cell growth and motility (Vaughan et al. 2012). Additionally, the insulin-like growth factor I receptor (IGFIR) and insulin receptor (INSR) are transcriptionally upregulated by p53-V143A, p53-R248W, and p53-R273H and contribute to cell growth (Werner et al. 1996; Sarfstein et al. 2020).

Mutant p53 GOF and epithelial-to-mesenchymal transition

Given its GOF roles in promoting metastasis in mouse models, it is highly relevant that mutant p53 regulates the epithelial-to-mesenchymal transition (EMT), a phenotypic spectrum in which cells lose epithelial characteristics, such as membranous keratin expression and cellular polarity, and gain mesenchymal characteristics (Gu et al. 2023). In cancer cells, EMT is associated with increased invasion and metastasis; cells in a metastatic site may utilize the reverse program of mesenchymal-to-epithelial transition (MET), which may be viewed in totality as epithelial–mesenchymal plasticity (Bakir et al. 2020; Gu et al. 2023). Analysis of liposarcoma patient tumor tissues with mutant p53 revealed high levels of EMT markers and lower survival in comparison with patients with wild-type p53 (Cho et al. 2017). Similarly, a doxycycline-regulated mouse model of p53-R270H (mouse homolog of p53-R273H) in pancreatic ductal adenocarcinoma found increased evidence of EMT when mutant p53 was induced (Schofield et al. 2018). Studies of colon and breast cancer cell lines with hotspot p53 mutations revealed that knockdown of mutant p53 increased E-cadherin expression, which was dependent on SLUG and ZEB1 transcription factors (Fig. 4B; Roger et al. 2010). SLUG degradation was found to be inhibited by mutant p53's repression of MDM2, thereby facilitating invasiveness (Wang et al. 2009).

TGF-β signaling is a potent regulator of EMT in cells with mutant p53 (Adorno et al. 2009; Tang et al. 2020). TGF-β treatment of cells with p53-R175H causes loss of the epithelial state and the emergence of a mesenchymal state with filopodia and increased migration (Fig. 4B; Adorno et al. 2009). This effect was not observed in cells with wild-type p53. Mechanistically, p63, mutant p53, and Smad2 form a complex, enabling the prometastatic phenotype (Fig. 4B; Adorno et al. 2009). Additionally, mutant p53 was shown to repress transcription of the tissue inhibitor of metalloproteinases 3 (TIMP-3), further amplifying the action of these prometastatic proteins (Thomas and Reisman 2006).

Mutant p53-regulated noncoding RNAs play a critical role in activating the EMT pathway. Mutant p53 downregulates the small noncoding RNA miR-200c in breast cancer, which increases ZEB1 and ZEB2 expression (Kim et al. 2011). Downregulation of miR-200c increases mammosphere formation, a measure of cellular stemness (Chao et al. 2021). Mutant p53 also represses the miR-130b promoter. miR-130b inhibits ZEB1, suggesting that mutant p53 induces EMT by enabling activation of ZEB1 through several strategies (Dong et al. 2013). Different p53 mutations influence noncoding RNA expression. Following the observation that cells that ectopically expressed p53-R273H exhibited more cellular stemness qualities than cells expressing p53-R175H and p53-R248W, two long noncoding RNAs (lnc273-31 and lnc273-34) regulated by p53-R273H were identified, resulting in increased stemness, EMT, survival, and chemoresistance (Dong et al. 2013).

Both wild-type and mutant p53 can regulate cellular stemness: While wild-type p53's tumor-suppressive effects limit cancer stem cell (CSC) growth, mutant p53 enhances the CSC phenotype (Park et al. 2015; Chen et al. 2022). Notably, it was demonstrated that several missense mutants of p53 associate with the promoters of CSC markers CD44, LGR5, and aldehyde dehydrogenase ALDH1A1 (Solomon et al. 2018). Similarly, mutant p53-mediated upregulation of ALDH1 expression was reported to have higher rates of chemoresistance in patients with colorectal cancer (Solomon et al. 2018). Additional mediators such as WASP-interacting protein (WIP) promote stemness through increasing expression of CD133, CD44, and YAP/TAZ (Escoll et al. 2017).

Mutant p53 GOF and metabolic reprogramming

Given a need for rapid and sustained growth, cancer cells alter metabolism to fuel their energy and anabolic needs. Not surprisingly, mutant p53 can exhibit GOF activity in rewiring cellular metabolism to promote increased malignant potential (Freed-Pastor and Prives 2012; Basu et al. 2018). However, mutant p53's effect on metabolism is highly context-dependent. In cervical cancer, cells with p53-R248Q overexpression were found to downregulate oxidative phosphorylation and upregulate glycolysis under normoxic conditions in comparison with cells with wild-type p53 (Hernández-Reséndiz et al. 2019). Cells that harbor mutant p53 translocated glucose transporter 1 (GLUT1) to the membrane and activated the small GTPase RhoA to promote increased survival in vivo (Fig. 4C; Zhang et al. 2013). Mutant p53 also induces the M2 isoform of pyruvate kinase (PKM2), the last step of glycolysis (Dando et al. 2016). Via KLF5, mutant p53 (p53-R248W and p53-R273H) was associated with upregulation of the phospholipase PLA3G16, leading to enhanced glycolysis, tumor growth, and progression (Xia et al. 2020). Additionally, in times of energy stress, mutant p53 is capable of directly inhibiting AMP-activated protein kinase (AMPK) (Zhou et al. 2014).

Mutant p53 does not exclusively prefer glycolytic pathways. In Li–Fraumeni syndrome, patients have increased oxidative phosphorylation in skeletal muscles (Wang et al. 2013). This was also observed in mice with the homologous Li–Fraumeni-causing mutation p53-R172H, which had increased levels of mitochondrial oxygen consumption in skeletal muscle in comparison with wild-type mice (Wang et al. 2013). Treatment of these mice with metformin inhibited mitochondrial biogenesis and delayed tumor growth, thereby indicating that oxidative phosphorylation may contribute to tumorigenesis (Wang et al. 2017). In a population of mesenchymal stem cells, cells with p53-R172H had increased metabolism, mitochondrial mass, and oxidative metabolism in comparison with cells with wild-type p53 (Lonetto et al. 2019). Additional study of the dynamics of mutant p53, glycolysis, and oxidative phosphorylation is needed to deconvolute the advantages of different metabolic states.

Mutant p53 mediates lipid synthesis. Mice with p53-178C (modeling human homolog p53-R181C) were found to be lean due to transactivation of the β-3-adrenergic receptor (ADRB3) (Kang et al. 2020). In high-grade serous ovarian cancer, overexpression of several p53 mutants increased production of the oncogenic lipid lysophosphatidic acid (LPA), which is associated with increased migration and invasion and poor survival (Chryplewicz et al. 2019). Comparisons between cells with p53-R273H and its shRNA-mediated depletion found that p53 interacts with sterol regulatory element-binding proteins (SREBPs) to express genes from the mevalonate pathway used for de novo cholesterol synthesis (Fig. 4C; Freed-Pastor et al. 2012). Breast cancer patients with mutant p53 have higher levels of mevalonate pathway transcripts than patients with wild-type p53 (Freed-Pastor et al. 2012). Reducing mevalonate-5-phosphate with statins was found to degrade mutant p53 by disrupting its interaction with Hsp40 DNAJA1, presenting a potential novel therapeutic scheme (Fig. 4C; Parrales et al. 2016). Similarly, treatment with atorvastatin was found to disrupt interactions between mutant p53 and the long noncoding RNA LINC00857, reducing metastasis in prostate cancer (Zhang et al. 2023). The sterol O-acyltransferase 1 (SOAT1) esterifies cholesterol to reduce negative feedback from cholesterol (Oni et al. 2020). In PDAC cells with p53-R172H/−, SOAT1 depletion markedly impaired proliferation when compared with cells with wild-type or mutant heterozygous p53 (Oni et al. 2020).

Mutant p53 also rewires amino acid metabolism and efficiency. One function of wild-type p53 is the ability to adapt to serine starvation (Humpton et al. 2018). In a serine depletion state, p53-R248W cells retain the ability to adapt by redirecting serine usage, while p53-R175H cells lose this ability (Humpton et al. 2018). Additionally, when cells with wild-type p53 are cultured in low-amino-acid conditions, proliferation decreases, while cells with p53-R280K and p53-R175H continue to grow (Tombari et al. 2023). Further exploration found that mutant p53-R175H or p53-R280K induces a transcriptional program to synthesize serine and upregulate the L-type amino acid transporter LAT1 (Fig. 4C; Tombari et al. 2023).

Mutant p53 tumor cell-extrinsic GOF mechanisms

While many studies have investigated the cell-autonomous GOF activity of mutant p53, accumulating evidence unravels how p53 mutations may function through distinct non-cell-autonomous mechanisms to change the tumor microenvironment (TME) and confer GOF properties (Efe et al. 2024). It was suggested that GOF mutations in p53 have a critical impact on promoting a proinflammatory TME and cancer progression through altering transcription of cytokines and chemokines, modulating intracellular mechanisms involved in inflammation (including NF-κB and STING signaling) and regulating the infiltration of macrophages, neutrophils, and T cells within the TME (Fig. 5A; Efe et al. 2024). p53 GOF mutations can regulate NF-κB activation by orchestrating tumor necrosis factor-α (TNF-α) signaling (Fig. 5A; Cooks et al. 2013). Notably, mutant p53-mediated NF-κB activation is persistent over time, generating inflammation-associated colon cancer in mice with a germline p53 mutation in comparison with p53-null mice, both of which are exposed to dextran sulfate sodium (Weisz et al. 2007; Cooks et al. 2013). In mouse intestinal organoids, p53-R270H mutant cells underwent Wnt activation followed by COX-2/prostaglandin E2 activation, which in turn upregulated Wnt in adjacent p53-null cells in the microenvironment (Nakayama et al. 2025). p53 mutations can also exhibit GOF activity by suppressing anti-inflammatory cytokines. Mutant p53, but not wild-type p53, was reported to bind to the promoter of anti-inflammatory secreted interleukin-1 receptor antagonist (sIL-1Ra) along with the transcriptional corepressor MAFF (v-MAF musculoaponeurotic fibrosarcoma oncogene family, protein F) to establish a chronic proinflammatory TME that mediates tumor progression (Ubertini et al. 2015).

Mutant p53 may reprogram the cellular and noncellular components of the TME to support tumor cells. p53-R175H, p53-R273H, and p53-R280K can bind and inhibit disabled homolog 2-interacting protein (DAB2IP) function in the cytoplasm, which promotes the NF-κB-mediated secretome and leads to tumor growth, invasion, and metastasis (Di Minin et al. 2014). As a consequence of this mechanism, CXCL10, CX3CL1, and LTB chemokines are secreted from breast cancer cells that serve as chemoattractants to cytotoxic T cells and natural killer (NK) cells and facilitate inflammation (Fig. 5A; Di Minin et al. 2014).

Mutant p53 can recruit and reprogram myeloid cells to facilitate tumor cell immune evasion. For example, p53-R273H can mediate infiltration of immunosuppressive myeloid cells through activation of NF-κB-mediated cytokine signatures (Cooks et al. 2013). Mutant p53 can also disrupt the STING–IRF3–TBK1 complex by binding to TANK-binding kinase 1 (TBK1), which is required for the transcriptional activity of IRF3 (Fig. 5A; Ghosh et al. 2021). This mechanism is sufficient for the polarization of $F4/80^{+}CD206^{+}$ immunosuppressive tumor-associated macrophages (TAMs) (Fig. 5A; Ghosh et al. 2021). Mutant p53 also regulates exosomal microRNAs; colon cancer cells shed exosomes enriched with miR-1246 that are taken up by adjacent macrophages, which reprogram them to an immunosuppressive and protumorigenic state with increased secretion of IL-10, TNF-α, and CCL2 (Fig. 5A; Zhang et al. 2016; Cooks et al. 2018). Additionally, mutant p53 regulates intratumoral neutrophil infiltration by inducing the secretion of CXCL2, as demonstrated using orthotopic mouse models of pancreatic cancer with $Kras^{G12D/+};Trp53^{R172H}$ (Fig. 5A; Siolas et al. 2021). Notably, p53-R249S and p53-R175H-mediated neutrophil and TAM accumulation were shown to lead to immunotherapy resistance (Asl et al. 2023).

p53 mutations are reported to play a critical role in cross-talk between tumor cells and their surrounding fibroblasts. Using a coculture system with lung cancer cells and CAFs, Madar et al. (2013) identified tumor cell

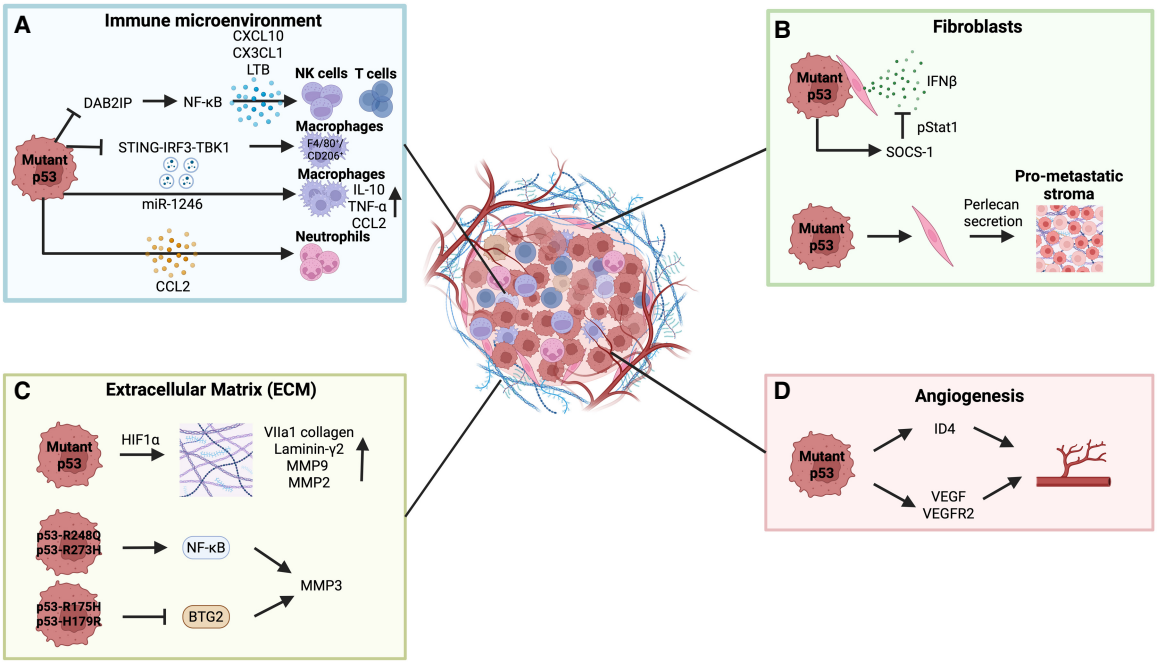

Figure 5. Mutant p53 gain-of-function activity regulates the tumor microenvironment (TME). (*A*) Mutant p53 inhibits DAB2IP function and promotes the NF-κB-mediated secretome to facilitate infiltration by T cells and NK cells, polarizes macrophages into an immunosuppressive state through disruption of the STING–IRF3–TBK1 complex and secretion of exomes with miR-1246, and regulates neutrophil infiltration by inducing CXCL2 secretion. (*B*) p53 mutations re-educate cancer-associated fibroblasts (CAFs) by diminishing IFNβ activation through SOCS1-dependent inhibition of STAT1 phosphorylation and alter CAF subpopulations to support stromal deposition of perlecan. (*C*) p53 mutations interacting with HIF1α leads to upregulation of VIIa1 collagen, laminin-γ2, MMP9, and MMP2. p53-R248Q and p53-R273H activate NF-κB, whereas p53-R175H and p53-H179R inhibit BTG2 to activate MMP3 and remodel the ECM. (*D*) Mutant p53 promotes neoangiogenesis through activation of ID4, inducing IL8 and GRO-α, and upregulates VEGF and VEGFR2 to favor tumor growth.

mutant p53-specific signatures in the CAFs in comparison with p53-null cells. They found that when tumor cells are in contact with CAFs, mutant p53 attenuates the interferon β (IFNβ) activation elicited by the CAFs via SOCS1-mediated inhibition of STAT1 phosphorylation (Fig. 5B; Madar et al. 2013). Additionally, GOF p53 mutations educate the surrounding CAF population and modify stromal deposition of perlecan to generate a protumorigenic and prometastatic microenvironment in the mouse models of pancreatic cancer (Fig. 5B; Vennin et al. 2019). Similarly, increased production of exosomes from mutant p53-expressing tumor cells was reported to moderate integrin trafficking in fibroblasts, increase their mobility, and promote deposition of proinvasive extracellular matrix (ECM) (Novo et al. 2018).

Indeed, ECM proteins are altered in the presence of p53 mutations. For example, in non-small-cell lung cancer (NSCLC), the interaction of p53-R273H with HIF1α leads to the upregulation of ECM protein VIIa1 collagen, laminin-γ2, MMP9, and MMP2, which are associated with tumor progression and adverse clinical outcomes (Fig. 5C; Rahnamoun et al. 2017; Amelio et al. 2018). Interestingly, the mechanisms by which the microenvironment is altered can be distinct depending on the type of p53 mutation. While DNA contact mutations such as p53-R248Q and p53-R273H promote cancer-related gene signature (CGS) including ECM remodeler MMP3 via NF-κB activation, conformational mutants such as R175H and H179R induce CGS via blockade of BTG2, which attenuates the inhibition on mutant H-Ras (Fig. 5C; Solomon et al. 2012). Notably, RhoA- and actin-dependent transduction of mechanical inputs such as matrix stiffness and ECM alterations can generate a feedback effect and promote sustained stabilization of mutant p53 (Ingallina et al. 2018).

Mutant p53 can also promote tumor neoangiogenesis via multiple mechanisms to support tumor progression. In NSCLC, mutant p53 activates ID4, which induces the expression of proangiogenic factors IL8 and GRO-α (Fig. 5D; Fontemaggi et al. 2009). Additionally, depletion of mutant p53 protein was shown to impair ID4 expression (Fig. 5D; Fontemaggi et al. 2009). Multiple studies have shown that mutant p53 upregulates VEGF and VEGFR2 to provide a favorable environment for cell growth (Narendran et al. 2003; Fontemaggi et al. 2009). These findings collectively underscore the multifaceted role of mutant p53 GOF activity in modulating the TME to accommodate tumor progression and suggest potential therapeutic opportunities in targeting mutant p53 or its downstream effects within the TME.

Mutant p53 loss-of-function and dominant-negative effects

The early history of p53 biology was framed by initial studies that suggested that the isolated clones of the purported wild-type *TP53* gene exerted oncogenic activity in cell culture assays either alone or with cooperating oncogenes such as *RAS* or adenoviral *E1A* (Levine and Oren 2009; Oren and Prives 2024). It was subsequently revealed that these original clones contained missense mutations, consistent with their being obtained from cell lines derived from tumors (Levine and Oren 2009; Oren and Prives 2024). One such mutant p53 clone of murine origin was actually temperature-sensitive for wild-type activity (Michael-Michalovitz et al. 1991). The temperature sensitivity of missense p53 mutants was eventually shown for many of the human "hotspot" mutants as well (Zhang et al. 1994; Friedlander et al. 1996; Shiraishi et al. 2004). Unlike wild-type p53, mutant p53 proteins were also shown to be associated with heat shock proteins (Finlay et al. 1988). These latter findings gave further support to the idea that missense mutant p53 proteins have a misfolded DBD (Alexandrova et al. 2015; Tang et al. 2022). This also provided the attractive possibility that therapeutic approaches could be developed that facilitate refolding and result in restoration of wild-type p53 function (Alexandrova et al. 2015; Tang et al. 2022). The temperature sensitivity of these mutant p53 proteins was the first means to identify wild-type p53 target genes, as these were only expressed at the permissive temperature (Buckbinder et al. 1994). This is consistent with refolding of the missense mutated core domain, loss of heat shock protein binding, and restoration of wild-type DNA binding and transcriptional activity.

Mutant p53 loss of function is due to an inability to bind DNA

Biochemical studies in vitro clearly demonstrated that missense mutation of p53 abolishes its ability to interact with wild-type p53 binding sites (Fig. 1C; Bargonetti et al. 1991; Kern et al. 1991; el-Deiry et al. 1992). Structural studies of purified p53 proteins confirm the thermodynamic instability of the mutants and confirm their inability to bind to consensus p53 motifs (Bullock et al. 1997; Bullock et al. 2000; Ang et al. 2006; Joerger and Fersht 2007). Although those studied all share the loss of DNA binding, the underlying structural basis for this appears to vary among specific missense mutants. Although to date there is no rigorous evidence for a mutant p53-specific consensus binding sequence, some mutants and not others retain varying abilities to still interact with wild-type p53 sites (Joerger et al. 2006; Eldar et al. 2013). The most striking difference is seen with the Y220C mutant protein, which has acquired a unique pocket (Joerger et al. 2006). This has allowed development of compounds that bind to this site and restore wild-type activity but only in p53 proteins that have sustained this specific Y220C mutation (Guiley and Shokat 2023).

Mutant p53 and dominant-negative effects

Early cell culture studies in which mutant p53 was ectopically expressed in the presence of an endogenous wild-type p53 demonstrated that the mutant was capable of inactivating wild-type function (Fig. 1B; Eliyahu et al. 1988; Halevy et al. 1989; Milner and Medcalf 1991; Srivastava et al. 1993; Gencel-Augusto and Lozano 2020). This supported the notion that missense mutant p53 proteins exerted a DNE over wild-type p53. Concerns were raised for these initial studies, as the mutant protein was artificially overexpressed in comparison with wild-type p53 (Gencel-Augusto and Lozano 2020). In cell culture, use of an appropriate bidirectional expression vector intriguingly showed that the DNE was not only target gene-selective but more potent on some cell fate outcomes (cell cycle arrest) than on others (apoptosis) (Fig. 3A; Aurelio et al. 2000). It was subsequently shown that the inhibitory effect of mutant p53 was dependent on the oligomerization domain (Gencel-Augusto and Lozano 2020). This supports the notion that mixed oligomers of mutant and wild-type proteins were the basis for the DNE (Gencel-Augusto and Lozano 2020). Biochemical studies demonstrated that such mixed tetramers vary in their ratio of mutant to wild-type proteins, and this results in varying extents of poisoning of wild-type activity (Chan et al. 2004; Natan et al. 2009; Gencel-Augusto and Lozano 2020). Intriguingly, it was suggested that cotranslation with mutant p53 actually caused the wild-type protein to adopt a conformation that exposed an epitope within the folded core domain (for a review, see Gencel-Augusto and Lozano 2020).

Recent studies have supported the notion that mutant p53 proteins do indeed exert a DNE over wild-type p53. When comparing *Trp53*$^{-/-}$ and *Trp53*$^{+/-}$ hematopoietic stem cells (HSCs) that were transduced with expression vectors encoding several mutant p53 proteins, no acceleration in the development of lymphoma in vivo with either genotype was observed (Aubrey et al. 2018). In contrast, the mutants produced more aggressive disease with HSCs derived from Eμ-Myc, *Trp53*$^{+/+}$ mice (Aubrey et al. 2018).

One challenge with the study of missense mutations of p53 is that different mutants often behave in manners distinct from others (Kennedy and Lowe 2022). To attempt to address this complex issue, a study was performed with *TP53* saturation mutagenesis in an isogenic set of wild-type and p53-null A549 cancer cells (Giacomelli et al. 2018). This demonstrated little difference between loss of and mutant p53-suppressing wild-type activity, leading to the conclusion that in this model system, a DNE mechanism predominates regardless of the *TP53* missense mutation (Giacomelli et al. 2018). This latter study was restricted to a single cancer cell line. More recently, a study was performed in which 15 different human cancer cell lines containing 12 different *TP53* mutations were subjected to knockout of the endogenous mutant p53 using a CRISPR/Cas9 approach. Little effect was seen using a variety of cell biological assays as well as tumorigenesis and metastasis in immune-compromised mice in vivo

(Wang et al. 2024), with the caveat of the absence of an immune-competent setting and the lack of comparison of the various p53 mutations. Using a similar approach with human acute myeloid leukemia cells, one study came to the same conclusion in favor of a role of a DNE of mutant p53 (Boettcher et al. 2019), though another study argued in favor of mutant oncogenic activity in leukemia (Loizou et al. 2019). It should be noted that all these studies in favor of mutant p53 LOF were done by either genetically engineering the endogenous *TP53* locus in established cancer cell lines or ectopically expressing mutant p53 proteins in genetically defined HSCs rather than studying the effect of p53 mutation arising during tumor development itself.

Taken together, studies both in cell culture and in vivo clearly show that dominant-negative action of mutant over wild type can be readily demonstrated experimentally (Brachmann et al. 1996; Aurelio et al. 2000; Hegi et al. 2000; de Vries et al. 2002; Wijnhoven et al. 2007; Lee et al. 2012; Boettcher et al. 2019). Nevertheless, it seems unlikely that DNE of p53 mutations can explain the role of p53 mutations in human cancer, because most tumors display loss of heterozygosity at the 17p chromosomal locus that eliminates the wild-type *TP53* gene (Liu et al. 2016). As mentioned, mouse models reveal, even in the presence of a wild-type allele, that mutant p53 can show a distinct oncogenic effect that cannot merely be explained by inactivation of wild-type function (Lang et al. 2004; Olive et al. 2004). A parsimonious explanation to accommodate these and other published findings is to propose a model in which DNEs of mutant p53 over wild-type p53 drive early disease progression. As tumors achieve full primary tumor and metastatic potential, the remaining allele is lost or mutated, leading to the full GOF phenotype. There remains a need for in-depth and systematic approaches to identify high-level principles by which such mutants can act.

Conclusions and future directions

Despite the overwhelming body of research on mutant p53, fundamental questions about how p53 mutations alter wild-type p53 function and whether they produce pro-oncogenic GOF activities remain unanswered. While there is general agreement that all p53 mutations attenuate wild-type p53 function and that many can have DNEs at least when overexpressed, the extent to which p53 dominant-negative activities are relevant for tumor progression and metastasis is poorly understood. Enigmatically, different p53 mutants have different GOF mechanisms, and even the same mutant allele appears to drive GOF effects through distinct mechanisms between biological systems. Such apparent inconsistencies have produced some concern about whether p53 GOF effects exist, and recent reports have produced results that provide evidence to the contrary (Aubrey et al. 2018; Giacomelli et al. 2018; Boettcher et al. 2019; Redman-Rivera et al. 2021). Still, these studies have their own limitations. For example, a recent study that concluded that mutant p53 does not affect mammary tumor growth and metastasis (Wang et al. 2024) is directly contradicted by other work (Dibra et al. 2023).

It is also possible that biological or technical deficiencies have produced a false impression that p53 mutations can have GOF effects. For example, it is possible that studies in mice may not apply to humans, or possibly studies employing ectopic p53 overexpression may produce nonphysiological results. Still, it is hard to refute the plethora of technically sound studies that identify a GOF role for mutant p53. Because most studies to date necessarily focus on one or a few p53 mutants, it has been extraordinarily difficult to sort out whether any shared mechanisms exist between different mutants in the same setting or the same mutant in different settings. For hotspot mutants such as R175H, understanding "private" functions is critically important and, given their high frequency, may suggest therapeutic opportunities relevant to many cancer patients. Still, it remains to be determined whether there are any common rules or principles that govern p53 mutant GOF activities and whether this might produce fundamental breakthroughs in targeting p53 mutant cancers and/or understanding metastasis.

There remain fundamental unanswered questions pertaining to the biological impact and molecular mechanisms of how p53 mutations promote cancer.

- Are all p53 mutations equivalent and, if not, are there biologically meaningful differences that impact tumor behavior?
- To what extent is there allele-specific variability in the DNE and GOF effects, and can this be related to specific transcriptional programs?
- How does variation in p53 mutant transcriptional output influence metastatic phenotypes and/or produce dependencies on ongoing activity of the p53 mutant (i.e., tumor maintenance)?
- If there is variability in p53 mutant action (which seems likely), are there common principles by which subsets of p53 alleles (hotspot, conformation/DNA contact, or truncation) influence cancer phenotypes?
- How does tissue context influence the detailed mechanisms by which individual p53 mutant alleles (e.g., p53-R175H) act?
- Is it possible to develop strategies to target mutant p53 to prevent or treat metastatic disease?

Given the range of models proposed, there has emerged some skepticism as to whether *TP53* mutations can promote cancer phenotypes beyond simply inactivating p53. Still, most studies to date have been limited to one or a few mutants in a particular cancer type or line and have examined only one or a limited number of p53 readouts. Clearly, substantial breakthroughs in understanding mutant p53 action will require systematic approaches that study a range of *TP53* mutant alleles in different tissue contexts. Building on recent studies (Funk et al. 2025), a systematic delineation of the functional and

mechanistic heterogeneity among *TP53* mutant alleles is warranted. Comprehensive in vitro and in vivo characterization will be critical to integrate information across mutant alleles across different species and between different p53 mutant alleles and tissues.

The p53 field might benefit from an iterative strategy to functionally and mechanistically distinguish between different types of mutant *TP53* alleles. This strategy relies on integration of orthogonal technologies—including precision genome editing (PGE) to generate endogenous mutant alleles—and massively parallel functional genomics (Table 1). These technologies are modular and easily reprogrammable by simply switching the sequence of a short RNA molecule, making it possible to quickly and systematically deploy them in high-throughput screening (Table 1). They can also be further combined with molecular characterization of mutant *TP53* alleles to get an idea of what cellular processes may be perturbed by a given mutant (Table 1).

The PGE technologies enable highly sophisticated models of endogenous p53 mutations and have already been used to model p53 mutations in organoids as well as in KRAS and other oncogenes (Geurts et al. 2021; Jang et al. 2023). Recent work has shown that these technologies can be used to engineer and interrogate thousands of cancer-associated p53 mutations in mouse and human cells (Sánchez-Rivera et al. 2022; Gould et al. 2025). These studies suggest that the way in which mutant *TP53* alleles are studied can influence experimental outcomes and conclusions. For instance, mutant *TP53* cDNA-based experiments have suggested that mutations in the p53 OD have little to no effect on wild-type *TP53* activity and tumor-suppressive phenotypes (Giacomelli et al. 2018; Gould et al. 2025). In contrast, cells engineered with prime editing to express endogenous *TP53* OD mutations exhibit potent disruption of wild-type p53-dependent tumor suppression (Gould et al. 2025). Interestingly, some studies have provided

Table 1. *Next-generation technologies for understanding the molecular-, cellular-, and tissue-level effects of different p53 mutations*

Technology category	Specific technology	Description	Key references
Genome editing	Base editing (BE)	This technology enables precise introduction of specific point mutations (transitions and some transversions) into the endogenous TP53 gene. This allows for the generation of cell lines and animal models carrying precise patient-derived p53 mutations.	Komor et al. 2016; Gaudelli et al. 2017
Single-cell genomics	Prime editing (PE)	This technology expands the repertoire of editable mutations beyond BE by enabling all 12 possible base-to-base conversions, as well as targeted insertions and deletions. It allows for the study of a broader spectrum of p53 mutations, including those that cannot be engineered using BE.	Anzalone et al. 2019
In vivo modeling	CRISPR/Cas9	While primarily known for gene knockout, CRISPR/Cas9 can also be used to introduce specific mutations via homology-directed repair (HDR) with a donor template. This technology provides an alternative approach for generating specific p53 mutations, although it can be less efficient than BE or PE.	Jinek et al. 2013
High-throughput screening	BE/PE sensor libraries	Couple BE or PE guide RNAs with synthetic target sites, allowing for the simultaneous assessment of the editing efficiency and precision of thousands of guide RNAs. This facilitates the rapid identification of highly effective and precise guide RNAs for introducing specific p53 mutations.	Sanchez-Rivera et al. 2022; Cirincione et al. 2025; Gould et al. 2025)
In vivo modeling	Barcoded cDNA libraries	These libraries enable the expression of a large pool of mutant p53 proteins in cells. Each mutant cDNA carries a unique barcode, allowing for the identification and quantification of each mutant in a mixed population. This approach can be used to assess the functional impact of different p53 mutations on cell proliferation, survival, and other phenotypes.	Kotler et al. 2018

Continued

Table 1. *Continued*

Technology category	Specific technology	Description	Key references
Single-cell genomics	Single-cell RNA sequencing (scRNA-seq)	This technology profiles the transcriptome of individual cells harboring specific p53 mutations. This allows for the identification of subtle, cell type-specific differences in gene expression driven by different p53 mutants. Future work should combine BE with scRNA-seq in the style of Perturb-seq to directly link specific p53 mutations to their transcriptional phenotypes at the single-cell resolution, providing a powerful approach for understanding the functional heterogeneity of mutant p53 and identifying cell type-specific effects.	Klein et al. 2015; Dixit et al. 2016; Norman et al. 2019
Other technologies	Single-cell ATAC sequencing (scATAC-seq)	This technique assesses chromatin accessibility in individual cells. It can reveal how different p53 mutations alter chromatin landscapes and regulate gene expression at the single-cell level.	Cusanovich et al. 2015; Alonso-Curbelo et al. 2021
In vivo modeling	Genetically engineered mouse models (GEMMs)	These allow for the study of mutant p53 in a physiologically relevant context. Specific p53 mutations can be introduced into the mouse genome using various techniques, including BE and traditional gene targeting.	Katti et al. 2024
Other technologies	Patient-derived xenografts (PDXs)	These involve the transplantation of human tumor tissue into immunodeficient mice. These models retain the genetic and phenotypic heterogeneity of the original tumor, allowing for the study of mutant p53 in a human tumor microenvironment.	Hulton et al. 2020
Other technologies	Somatic tissue engineering	This technology involves the direct introduction of genetic modifications into specific tissues in vivo. This can be achieved using techniques like pancreas electroporation, which allows for the delivery of BE or PE reagents directly to the pancreas, enabling the generation of PDAC models carrying specific p53 mutations.	Zafra et al. 2018; Leibold et al. 2020; Paffenholz et al. 2022; Leibold et al. 2024
Other technologies	Immunohistochemistry (IHC)/ immunofluorescence (IF)	These enable the visualization of protein expression and localization in tissue sections. This can be used to assess the impact of p53 mutations on various cellular processes, such as proliferation, apoptosis, and invasion.	Hassin et al. 2022; Leibold et al. 2024; Gould et al. 2025
Other technologies	Flow cytometry	This technique allows for the quantification and characterization of different cell populations based on their surface markers and intracellular protein expression. This can be used to assess the impact of p53 mutations on immune cell infiltration and other cellular phenotypes.	Dong et al. 2024
Other technologies	Proteomics	This technology can be used to study the global changes in protein expression and posttranslational modifications associated with different p53 mutations. This information can provide insights into the downstream signaling pathways and cellular processes affected by mutant p53 proteins.	Schaaij-Visser et al. 2009; Gao et al. 2018; Vadakekolathu et al. 2022

Continued

Table 1. *Continued*

Technology category	Specific technology	Description	Key references
Other technologies	Bioinformatics and computational analysis	These are crucial for analyzing and integrating the vast amount of data generated by high-throughput technologies. This includes the development of algorithms and software tools for analyzing sequencing data, identifying differentially expressed genes, and performing pathway enrichment analysis.	Hassin et al. 2022; Solares and Kelly 2022; Gould et al. 2025

mechanistic evidence that may explain the biochemical basis behind this phenomenon (Choe et al. 2023; Gencel-Augusto et al. 2023). Specifically, these mutant p53 alleles encode proteins that are obligate homodimers; that is, the mutant p53 OD protein pool does not interact with the wild-type p53 protein pool (Gencel-Augusto and Lozano 2020). This result suggests that these mutant *TP53* OD alleles may not act through a mixed tetramer dominant-negative mechanism; instead, these mutant alleles exhibit a biochemical phenotype that may be consistent with a GOF activity.

While the above examples were mostly discussed in the context of cell culture, the same types of approaches can be easily deployed in vivo. This includes all types of CRISPR-mediated genome editing and PGE technologies like base and prime editing for single-gene/variant and multiplexed (high-throughput) engineering and characterization of cancer-associated mutations in *TP53* and other genes (Winters et al. 2018; Sánchez Rivera and Dow 2024). Traditional mouse models based on constitutive or inducible knockout or knock-in alleles have been powerful in establishing concepts of p53-mediated tumor suppression and testing hypotheses related to mutant p53 allele-specific mechanisms of action (Lang et al. 2004; Olive et al. 2004). Notably, somatic tissue engineering, where guide RNAs are injected as plasmids or electroporated into a live Cas9 mouse, has emerged as a dynamic modeling technique, mimicking the temporal and tissue specificity of most human cancers (Liu et al. 2021).

The fact that wild-type p53 regulates transcription and that most putative GOF p53 mutant proteins work through binding to other transcriptional regulators (Pfister and Prives 2017) implies that transcriptional dysregulation is likely proximal to relevant *TP53* mutant phenotypes. By comparing the effects of different *TP53* mutations on transcriptional output in different tissue contexts, it is expected that higher-order principles by which they act can be established. If we can understand how *TP53* mutant allelic variation impacts cancer, we might be able to target cancers with more precision and efficacy by exploiting the unique biology produced by the precise *TP53* genetic configuration of the tumor. Thus, the goal will be to produce clinically relevant breakthroughs into understanding *TP53* gene mutations and their resulting mutant proteins that contribute to over half of all human cancers.

Competing interest statement

The authors declare no competing interests.

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