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Review

Deciphering the significance of p53 mutant proteins

Alessio Butera[1] and Ivano Amelio[1,*]

Mutations in the p53 gene compromise its role as guardian of genomic integrity, yielding predominantly missense p53 mutant proteins. The gain-of-function hypothesis has long suggested that these mutant proteins acquire new oncogenic properties; however, recent studies challenge this notion, indicating that targeting these mutants may not impact the fitness of cancer cells. Mounting evidence indicates that tumorigenesis involves a cooperative interplay between driver mutations and cellular state, influenced by developmental stage, external insults, and tissue damage. Consistently, the behavior and properties of p53 mutants are altered by the context. This article aims to provide a balanced summary of the evolving evidence regarding the contribution of p53 mutants in the biology of cancer while contemplating alternative frameworks to decipher the complexity of p53 mutants within their physiological contexts.

Highlights

Every other human cancer harbors a mutation in p53, with roughly 60–80% of these mutations linked to the acquisition of gain-of-function oncogenic properties (as per the gain-of-function theory).

Before the loss of heterozygosity occurs, p53 mutant proteins can suppress the remaining wild-type p53 proteins through a dominant-negative mechanism. This mechanism, as an alternative to the gain-of-function theory, could elucidate the advantageous nature of p53 missense mutations.

While the gain-of-function theory has long accounted for the pattern of p53 mutations, conflicting observations have perpetuated its status as a postulation.

Cancer transformation is underpinned by concerted interactions between oncogenic mutations and the cell state. Notably, p53 mutant proteins consistently exhibit 'context-dependent functions'.

p53 mutations: loss-of-function, gain-of-function, and dominant-negative effects

Central to genomic integrity, p53 acts as a guardian, orchestrating cellular responses to stressors like DNA damage and oncogenic signals, thereby functioning as a key tumor suppressor [1,2]. Across various cancer types, inactivating mutations in p53 are notably common, with frequencies reaching 95% in certain cancer types and averaging about half of all cancers [3]. Unlike mutations in many other tumor suppressors, those in p53 predominantly manifest as missense mutations, observed in roughly 60–80% of cases, resulting in the expression of mutant p53 proteins within cancer cells [3] (Figure 1A–C). These mutations drive tumorigenesis by impairing the tumor-suppressive functions of p53, as most of these mutant proteins fail to retain the functional capacities of wild-type p53.

Biochemically classifying p53 missense mutants divides them into 'structural' and 'contact' mutants. Structural mutants undergo major conformational disruptions, losing the ability to form regular tetramers, while contact mutants maintain their conformation but bear mutations in DNA binding residues, preventing interaction with genomic p53 responsive elements. Consequently, in both cases, the ability of p53 mutants to regulate gene expression akin to wild-type p53 is substantially compromised, resulting in a 'loss-of-function' effect (Figure 2A,C). However, the full ramifications of the consequence of p53 mutations are still under scrutiny, raising critical questions about the selective advantage of producing mutant p53 proteins over simply inactivating p53 expression.

Several explanations have been proposed to explain the selective pressure that might promote such a peculiar pattern of missense mutations in the p53 gene. One interpretation suggests the presence of essential genes near the *TP53* gene locus on chromosome 17, countering large chromosomal deletions in these regions affecting multiple genes. An essential gene within this locus encodes the catalytic subunit of RNA polymerase II (RNAP2) [4]. Since homozygous inactivation of RNAP2 is lethal, cancer cells tend to adopt single nucleotide mutations on at least one of the two alleles, typically representing the initial genetic event at the TP53 locus

[1]Chair of Systems Toxicology, University of Konstanz, Konstanz, Germany

*Correspondence:
ivano.amelio@uni-konstanz.de (I. Amelio).

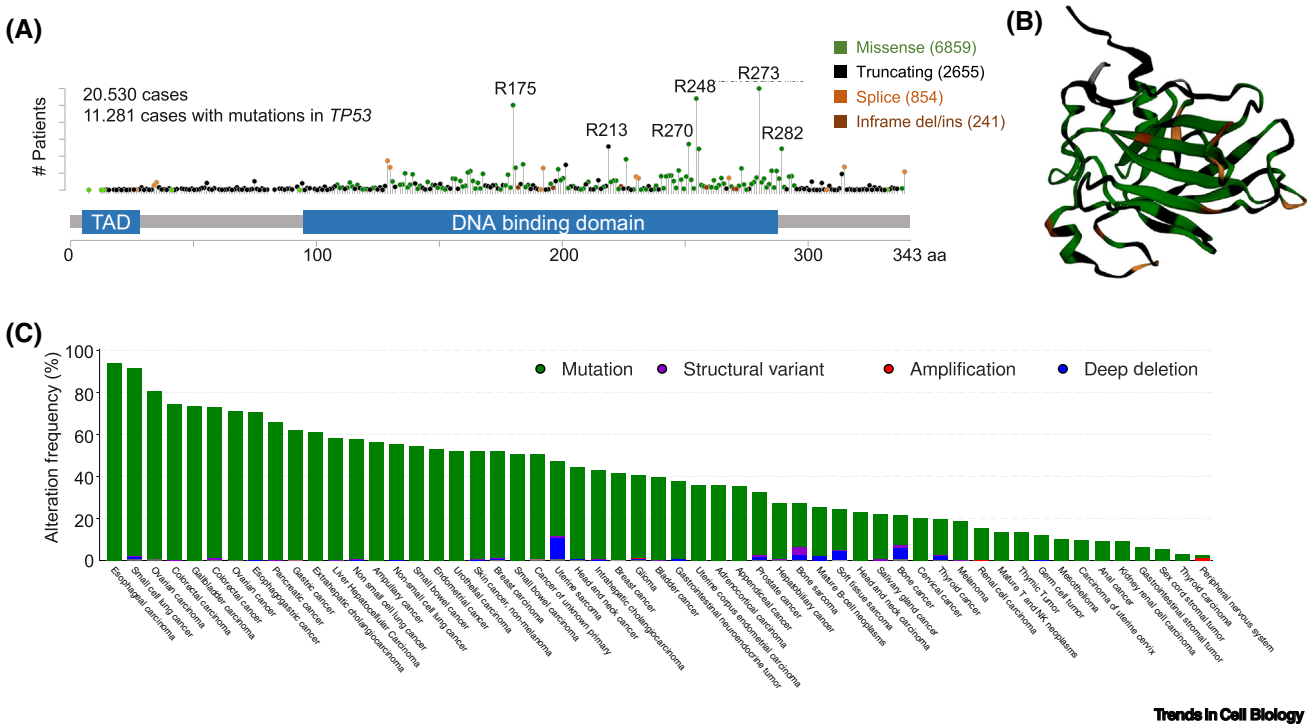

Figure 1. p53 mutations across human cancers. (A) Distribution of p53 mutations (missense, truncating, splicing site alterations, and inframe deletions/insertions) across the protein sequence in a cohort of 20 530 patients spanning diverse cancer types. Specific details are provided on the most frequently mutated residues. (B) Tridimensional rendering of the p53 DNA binding domain with color legend depicting mutation types [missense, truncating, splicing site alterations, and inframe deletions/insertions as legend in (A)], highlighting their prevalence at specific residues. (C) p53 inactivation across cancer types with frequency and modality (mutations, structural variants, amplification, and deletion). Data from the MSK-IMPACT clinical sequencing cohort [60] and China Pan-cancer [61] datasets via cBioPortal.

while preserving normal function and expression of proximal genes. However, loss of heterozygosity on the p53 locus often involves larger deletions, leading to hemizygosity for p53 proximal genes in later stages of tumorigenesis [5].

An alternative explanation posits that single nucleotide mutations initially accumulate on one allele of p53, yielding a mutant protein with dominant-negative capacity within p53 tetramers. Heterotetramers comprising mutant p53 and wild-type p53 from the intact allele exhibit impaired transcriptional capacity, resulting in abrogation of p53 tumor-suppressive signaling (Figure 2B). Nevertheless, this postulation does not readily account for high-frequency loss of heterozygosity involving p53 (>90%), which remains necessary for most cancers to progress further [3].

A prevalent postulation favors the concept of gain-of-function mutations, suggesting that missense mutations confer new oncogenic functions upon p53 mutant proteins, driving tumor progression, metastasis, and therapy resistance (Figure 2D) [6–8]. Although supported by a substantial body of literature over the past three decades, this theory is not free from conflicting observations and controversies. Recent studies have even challenged this notion, demonstrating that the ablation of multiple diverse p53 mutants in cancer cells does not affect the tumorigenic properties of these cells *in vitro* and *in vivo* [9].

Over four decades of research, p53 has remained a focal point, with its significance in cancer biology recognized early on. Clarifying the validity of gain-of-function, dominant-negative, and loss-of-function postulations holds relevance not only for experimental cancer biology but also

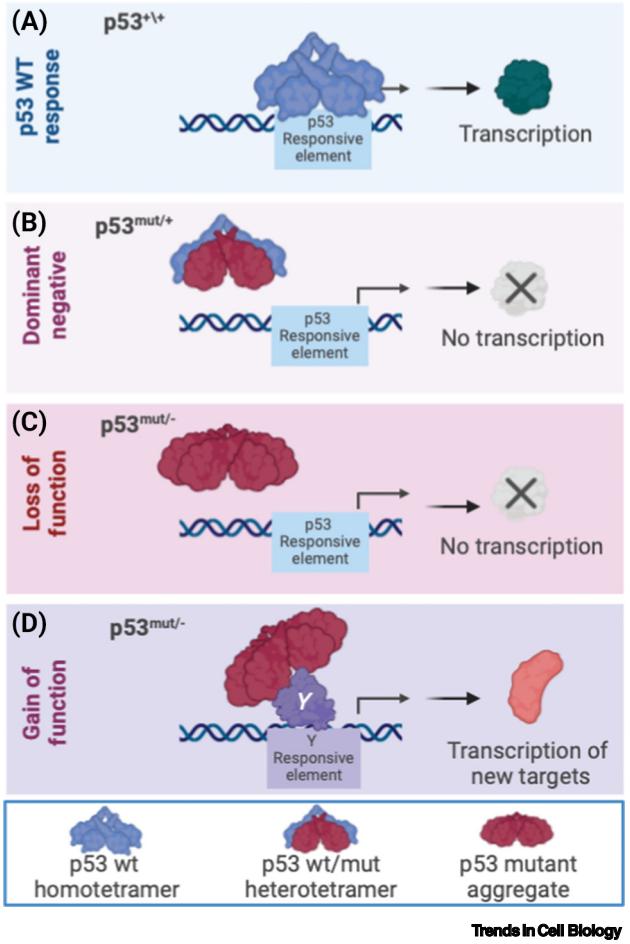

Figure 2. Pathogenic mechanisms associated with p53 mutations. (A) The wild-type response of p53 involves the formation of homotetramers, which regulate gene expression at p53 responsive elements. (B) In contrast, the dominant-negative effect of p53 mutants occurs through the formation of heterotetramers. These heterotetramers, composed of both p53 wild-type and p53 mutant monomers, lack transcriptional ability. This dominant-negative mechanism can manifest in conditions of heterozygosity, where a p53 wild-type allele coexists with a p53 mutant allele (p53mut/+). (C) Loss-of-function is characterized by the absence of p53 wild-type expression and the lack of any form of activity by the p53 mutant protein. This typically occurs when all p53 alleles are inactivated. (D) Gain-of-function involves the acquisition of neomorphic activities by p53 mutant proteins. These neomorphic activities are often described as the hijacking of additional transcriptional factors, indirectly influencing gene regulation and resulting in pro-tumorigenic phenotypes. Abbreviation: WT, wild type.

for clinical oncology. Delineating whether to target p53 mutant proteins through restoration of wild-type function or inhibition of gain-of-function necessitates distinct pharmacodynamic approaches, emphasizing the need for a clear understanding. Despite attempts to target p53 over recent decades, success has been limited, underscoring the importance of addressing this fundamental question. This review aims to provide a balanced update on the latest findings, supporting different theories while also fostering reflection to identify major knowledge gaps that could be addressed in the years to come, thus addressing this critical biomedical question.

p53 mutant proteins promote metastasis and therapy resistance: the gain-of-function theory

A wealth of evidence derived from both *in vitro* and *in vivo* investigations suggests that mutant p53 proteins confer significant growth advantages to tumor cells. A recent PubMed search conducted in April 2024 using the keywords 'p53 AND gain-of-function' yielded over 2200 publications, the majority of which provide data supporting the gain-of-function exhibited by p53 mutants.

Among the most powerful tools employed to assess the impact of p53 mutant proteins are the p53 mutant-expressing mouse models, particularly when compared with p53 knockout mice. In mice, germline biallelic inactivation of p53, whether achieved through gene deletion targeting

strategies or knock-in insertion of hotspot missense mutations, results in a remarkably high frequency of spontaneous tumors [10]. Biallelic deletion of p53 leads to cancer development in over 75% of mice within 3–6 months, with thymic T cell lymphomas and various soft-tissue sarcomas being predominant. Moreover, germline monoallelic deletion of p53 significantly predisposes mice to cancer, albeit with a delayed onset (median around 18 months) and a different tumor spectrum, primarily comprising lymphomas of B cell origin, osteosarcomas, soft-tissue sarcomas, and various carcinomas. These models exhibit a moderate rate of loss of heterozygosity of the p53 locus in tumors, with higher rates observed in certain murine genetic backgrounds, such as Balb/c.

Inactivation of p53 in mice has also been accomplished through knock-in strategies introducing hotspot mutations. The initial p53 knock-in mice harbored R172H and R270H mutations (equivalent to the human R175H and R273H mutations, respectively). Both homozygous and hemizygous models of these mutations exhibited comparable survival rates to p53 knockout mice of the corresponding genotype. However, the p53 knock-in mice demonstrated an increased propensity for developing tumors with aggressive metastatic capabilities. For instance, approximately 23% of osteosarcomas and 67% of adenocarcinomas from $p53^{R172H/+}$ mice metastasized, while in $p53^{R270H/R270H}$ mice, 29% of lung adenocarcinomas metastasized. This observation underscores the role of p53 mutant proteins in promoting metastasis through gain-of-function effects [7,8].

Building upon the observations of enhanced metastatic propensity in p53 knock-in mice, further investigation revealed a repression of p53 family members by p53 mutants. For instance, p63 suppresses metastasis through various mechanisms, including the regulation of SHARP1 expression and integrin recycling. The presence of p53 mutants disrupts the activity of p63 via direct physical protein–protein interactions [11–15]. Similarly, p73 interplay with p53 mutants promotes pancreatic cancer metastasis by regulating cell-autonomous PDGF receptor β signaling [16]. However, the body of evidence supporting the involvement of p53 mutants in a premetastatic program is steadily expanding, implicating multiple cell-autonomous and non-autonomous mechanisms [15,17].

Another significant contribution supporting the gain-of-function theory comes from an *in vivo* model of a hotspot p53 mutant capable of ablating the $p53^{R248Q}$ protein with an inducible genetic system. Ablation of $p53^{R248Q}$ strongly impacts the growth of both allotransplanted and autochthonous tumors, promoting apoptosis and tumor regression or stagnation. Consequently, mice overall displayed an approximately 40% extension of their lifespan [18]. The dependency of tumors on $p53^{R248Q}$ was extended also to $p53^{R172H}$ treating $p53^{R172H}$ knock-in mouse models with a chemical inhibitor of the protein chaperone HSP90, responsible for the general stability of p53 mutant proteins. HSP90 inhibitor significantly extended the lifespan of $p53^{R172H}$ knock-in mice [18,19]. Similarly, deletion of mutant $p53^{R172H}$ or $p53^{R245W}$ from autochthonous somatic K14-Cre-driven triple-negative breast cancer in mice blunts their tumor growth and significantly extends survival of mice [20]. Furthermore, in the delineation of the mechanisms of p53 mutant protein turnover, J-domain proteins (JDPs) have been shown to bind destabilized p53 mutants, preventing Hsp70-mediated degradation [21], providing novel potential targets to tackle p53 mutant stability. Overall, these findings pave the way for therapeutic strategies targeting p53 mutant stability.

Furthermore, beyond inducing cell death as a consequence of p53 mutant tumor ablation, gain-of-function effects have also been associated with the ability of mutant p53 to alter cell death decisions in response to standard therapeutic compounds and cell death inducers [22]. Inactivation of p53 is linked to a loss of ability to respond to DNA damage-inducing drugs, significantly influencing the responsiveness of p53 mutant tumors to chemotherapy [23]. However, there is

evidence suggesting roles of p53 mutant proteins in altering the response to cytotoxic drugs and influencing the cell death process in cancer cells. For instance, p53 mutants have been shown to alter the expression of the death receptor FAS, implicated in triggering extrinsic apoptotic pathways [24]. Multiple additional mechanisms have been described regarding the cooperation between p53 mutants and chemoresistance. These include influence of p53 mutants in the regulation of DNA repair pathways [25], or impact on detoxification of drugs [26,27], and interference effects with executions of cell death pathways.

Understanding how p53 mutants influence the decision process in cancer cells to trigger cell death and select cell death modalities is crucial. For instance, in a proteomic screen, the transcription factor BACH1 emerged as a binding partner of the DNA-binding domain of $p53^{R175H}$, but not wild-type p53. This interaction abrogated BACH1-mediated downregulation of the cystine/glutamate antiporter SLC7A11, which participates in the synthesis of the antioxidant glutathione. Enhanced expression of SLC7A11 increased cancer cell ability to prevent lipid peroxidation, thus preventing the execution of ferroptotic cell death [28]. Examination of triple-negative breast cancer models revealed that both $p53^{R172H}$ and $p53^{R245W}$ mutants possess the ability to shield cancer cells from ferroptotic cell death. This protection occurs via a regulation mediated by NRF2, influencing Mgst3 and Prdx6. These genes encode two glutathione-dependent peroxidases crucial for detoxifying lipid peroxides [29].

Gain-of-function properties of mutant p53 are not critical for cancer cell fitness

It appears there is a clear consensus favoring the importance of loss-of-function over gain-of-function following mutations in p53. When p53 is inactivated, cancer cells gain a significant advantage through the loss of the tumor-suppressive properties inherent in wild-type p53. It is not surprising, then, that the majority of missense mutations in p53 occur within its DNA binding domain, directly or indirectly impairing its ability to bind to responsive elements across the genome and regulate gene expression.

Understanding the implications of p53 mutant gain-of-function properties extends beyond mere experimental inquiry; it carries substantial clinical significance. In pursuit of this understanding, groups led by Strasser and Kelly recently undertook a rigorous assessment of targeting mutant p53 as an anticancer strategy. They conducted a comprehensive investigation into the effects of converting p53 mutants into p53-deleted cancer cells. Using CRISPR technology, they converted 12 different missense mutant p53 variants in 16 cell lines from various cancers into knockout genotypes. Surprisingly, scrutiny of cell proliferation and survival under both standard and stress conditions, including nutrient deprivation and chemotherapy treatment, revealed no discernible difference between cells expressing p53 mutants and their isogenic counterparts lacking those mutants [9].

Expanding this analysis to *in vivo* settings, implanting human breast cancer cells into mouse mammary fat pads to monitor metastasis showed no impact of mutant p53 knockout on metastasis *in vivo*. Similarly, using human colon cancer organoids cultured or transplanted in mice, as well as transplanting mouse lymphoma or breast cancer cells into syngeneic hosts with intact immune systems, revealed consistent growth, gene expression profiles, and response to drugs regardless of the presence or absence of mutant p53 [9].

To directly compare the fitness of cells expressing mutant p53 or lacking p53, competition experiments were conducted. Mutant p53-expressing cell lines and their p53 knockout derivatives were labeled with two different fluorophores, mixed at a 50:50 ratio, and monitored over time using flow cytometry. Remarkably, no competitive advantage was observed for either cell line, suggesting that loss of the p53 point mutant does not compromise cell fitness and casting doubt on a gain-of-

function effect [9]. Overall, across a wide array of settings, this study found no direct evidence supporting p53 gain-of-function activity [9].

The dominant-negative effect: mutant p53 action prior to loss of heterozygosity

A clear observation is that sporadic mutations in p53 involve a missense event on the first affected allele. This is generally followed up by a loss of heterozygosity, which more frequently involves a deletion of the p53 locus on the second allele. This mutational process leads to a stage in which the p53 mutant is present in a cancer cell concurrently with the wild type. One argument is therefore that this is the reason why the p53 locus preferentially undergoes missense mutations rather than deletion, allowing a selective pressure associated with its dominant-negative capacity.

Phenotypic and functional characterization of a panel of isogenic acute myeloid leukemia cell lines, carrying $p53^{+/+}$, $p53^{-/-}$, or different $p53^{mut/-}$ missense variants, demonstrate that different p53 mutational status does not produce any change in the oncological phenotype, nor any selective advantage. However, the presence of the mutant protein (R248Q, R172H, R270H) in heterozygosity ($p53^{mut/+}$) conferred a selective advantage to the cells when compared with $p53^{-/+}$ in response to genotoxic agents [30]. A saturation mutagenesis screen in which each amino acid in p53 was systematically mutated to all other possible amino acids and a stop codon indicated that amino acid substitutions at residues 100 to 300, corresponding to DNA binding domain, were powerfully enriched for dominant-negative activity on the transcriptional capability of the endogenous wild-type p53 to transcribe a reported gene in response to Nutlin-3a, a small chemical compound able to activate wild-type p53, preventing its MDM-2-dependent degradation [30]. Similar unbiased approaches gave conflicting results in two different studies. The findings by Kotler and colleagues underscore the varied outcomes associated with different p53 mutations. While the loss of anti-proliferative capacity typically aligns with mutations commonly observed in cancer, there seems to be a selective advantage for certain mutants, leading to their enrichment *in vivo* [31]. Another p53 saturation mutagenesis screen was conducted by Giacomelli and colleagues in an isogenic pair of p53 wild-type and null cell lines. This experiment led to the findings that dominant-negative inhibition of wild-type p53 consistently improved cellular fitness [32].

An investigation of the mechanisms behind the dominant-negative effect of p53 mutants was undertaken using a mouse model of Eμ-Myc lymphomas. p53 inactivation notably accelerated lymphomagenesis, however, discerning the mutant p53 over-expression did not impact the development of the disease on the $p53^{-/-}$ and $p53^{-/+}$ genotype. There was a substantially impact of mutant p53 over-expression on lymphoma development in an Eμ-Myc model carrying $p53^{+/+}$ tumors. A molecular analysis unveiled insights into the dominant-negative mechanisms of p53 mutants: mutant p53 does not uniformly suppress wild-type function. Instead, it disproportionately affects a specific subset of wild-type p53 target genes, disrupting pathways associated with DNA repair, proliferation, and metabolism in premalignant cells. These findings shed light on our exploration of lymphomagenesis, revealing that mutant p53 primarily drives tumorigenesis through the dominant-negative effect [33].

The significance of p53 mutant proteins: a matter of context?

'Mutant p53: it's not all one and the same' [34], 'Mutant p53: one name, many proteins' [35], 'The many faces of p53: something for everyone' [36], 'Drugging p53 in cancer: one protein, many targets' [37]. These titles of recent publications underscore the heterogeneity of p53 mutations and the vast array of biochemical and functional alterations each mutation can induce in the mutant protein. This emerging awareness has spurred a consensus within the scientific community that systematic approaches are necessary to delineate the significance of p53 mutant

proteins. Understanding how different p53 mutations function as drivers of cancer and whether tumors bearing distinct mutations exhibit unique phenotypes has therefore become a priority. This complexity is, however, compounded by the observation that specific mutants can exhibit divergent functions, resulting in seemingly contradictory findings in different 'contexts'. Here, 'context' extends beyond merely the genetic mutational background of the tumor cell, encompassing diverse cellular states defined by developmental stage and/or microenvironmental conditions.

A major example is the paradoxical role $p53^{R172H}$ and $p53^{R270H}$ display in gut tumorigenesis. While both mutants appear to exert robust tumor-suppressive effects, potentially exceeding wild-type p53 activity in the proximal gut of $CKIa^{\Delta gut}$ and $Apc^{Min/+}$ mice, their tumor-suppressive properties transition into oncogenic activities in the distal gut in the presence of the native local microbiota. In $CKIa^{\Delta gut}$ mice, p53 mutants suppress WNT-driven hyperproliferation and dysplasia and, in $Apc^{Min/+}$ mice, they inhibit tumorigenesis. Notably, these effects involve blocking WNT signaling, through mechanisms involving prevention of the binding of transcriptional factors such as Tcf4 to chromatin. However, the introduction of gallic acid, a hydroxybenzoic acid produced by gut microbiota, abolishes the tumor-suppressive effects of p53 mutants, allowing oncogenic effects to predominate in tumorigenesis processes (Figure 3A,B) [38]. These findings underscore a plasticity of p53 mutations and emphasize the critical role of the microenvironment in determining their functional outcomes.

The reciprocal interaction between p53 mutants and the microenvironment has been emerging in the past decade [39]. Firstly, the stability of p53 mutants exhibits considerable variability, particularly evident in mouse tissues [40]. Within this context, mechanical cues from the tissue microenvironment appear to play a pivotal role in governing the turnover of mutant p53 proteins. Specifically, Rho-A acts as a sensor for extracellular matrix (ECM) stiffness, initiating actin-dependent mechanotransduction in response to increased ECM rigidity. This activation triggers the mechanosensitive HDAC6 deacetylase to activate Hsp90, thereby enhancing the stability of mutant p53 proteins. Notably, mutant p53 can also modulate this signaling pathway through a positive feedback loop involving Rho-A. The regulation of GEF-H1 and RhoGDI by p53 mutants further influences Rho-A activation, reinforcing the stabilization of mutant p53 proteins. Disruption of this loop, achieved through chemical inhibition of Rho-A geranylgeranylation by geranylgeranyl transferase inhibitors such as zoledronic acid (ZA), compromises the stability of mutant p53 [41]. Furthermore, the composition of the ECM itself is subject to modulation by p53 mutants. For instance, $p53^{R273H}$ collaborates with hypoxia-inducible factor 1 (HIF-1) to form a transcriptional complex that regulates ECM components such as Laminin-γ-2 and Collagen VIIa1 in hypoxic cancer cells [42]. This regulatory mechanism is augmented by additional pathways; the p53 mutant/HIF-1 complex also controls the expression of microRNA-30d, which in turn modulates Golgi apparatus function, leading to enhanced ECM deposition and release of soluble factors. Such interplay between mutant p53 and the microenvironment produces concerted effects that likely contribute to cancer progression (Figure 3C) [43].

The presence of mutant p53 proteins has been linked to interactions with the immune-modulatory microenvironment. Mutant p53 can hinder innate immunity, facilitating evasion from immunosurveillance. It disrupts the detection of cytoplasmic DNA, a feature often observed in advanced, genetically unstable cancer cells [44]. Contrary to wild-type p53, mutant p53 physically binds to TANK-binding protein kinase 1 (TBK1), preventing the activation of STING and subsequent IRF3. When IRF3 is activated, it translocates to the nucleus to regulate the expression of interferon type I response, which supports innate immunity. However, the presence of mutant p53 appears to block this cascade of events [45]. These interactions with the immune system

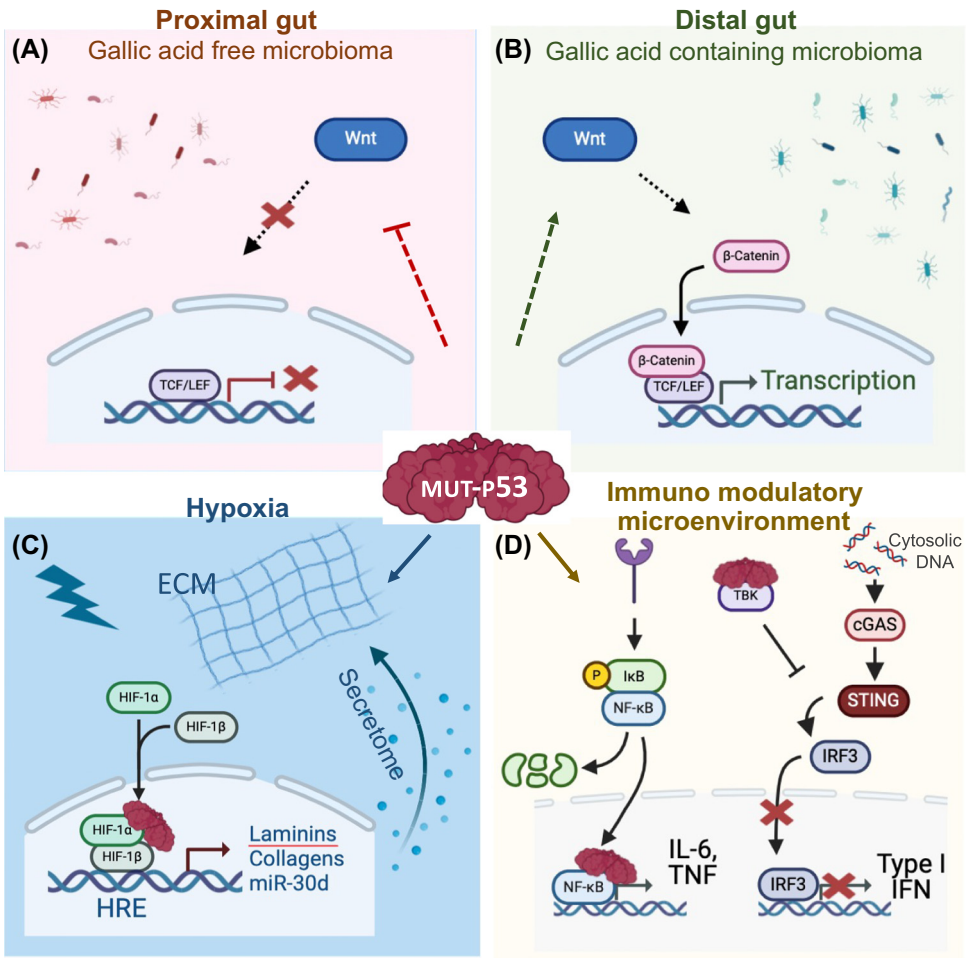

Figure 3. Context-dependent effects of p53 mutant proteins. p53 mutant proteins exhibit diverse properties upon interactions with the surrounding microenvironment. (A) Within the proximal and distal gut, variations in microbiota composition prompt a transition from the tumor-suppressive effects of p53 mutants to oncogenic. (B) Specifically, in the proximal gut, the absence of gallic acid-producing microbiota enables p53 mutants to impede Wnt-mediated activation of Tcf4 transcription, thereby exerting a tumor-suppressive influence. However, in the distal gut, the presence of gallic acid impairs this oncosuppressive capacity. (C) Under hypoxic conditions, p53 mutants form complexes with hypoxia-inducible factor, stimulating the upregulation of extracellular matrix (ECM) components such as laminins and collagens. Concurrently, the promotion of miR-30d expression facilitates the secretion of these components into the microenvironment, thereby modulating the ECM. (D) Throughout various tumorigenic stages, p53 mutants can evoke contrasting effects on the inflammatory phenotype. In models of inflammation-driven colon carcinogenesis, p53 mutants sustain the activation of NF-kB, promoting the expression of TNF and interleukins. Conversely, in genetically unstable cancers, the p53 mutant proteins interact with TBK and impede STING-mediated activation of IRF3, thereby suppressing the expression of proinflammatory type I interferon responses that support antitumor immunity. Abbreviations: cGAS, cyclic GMP-AMP synthase; ECM, extracellular matrix; HIF-1α, hypoxia-inducible factor 1-alpha; HIF-1β, hypoxia-inducible factor 1-beta; HRE, hypoxia response element; IkB, inhibitor of kappa B; IL-6, interleukin 6; IRF3, interferon regulatory factor 3; NF-KB, nuclear factor kappa-light-chain-enhancer of activated B cells; STING, stimulator of interferon genes; TBK, TANK-binding kinase; Tcf/LEF, T cell factor/lymphoid enhancer factor; TNF, tumor necrosis factor; Type I IFN, type I interferon; Wnt, Wingless-related integration site.

may also vary depending on the context. In the early stages of carcinogenesis, mutant p53 promotes the proinflammatory activation of NF-kB. In a mouse model of inflammation-driven colon cancer, germline p53 mutations exacerbate chronic inflammation and tissue damage, hastening

colon cancer development. Mechanistically, mutant p53 correlates with NF-kB activation and the transcription of proinflammatory cytokines such as TNF and interleukins (Figure 3D) [46].

The interaction between p53 mutants and cancer metabolism represents another crucial aspect. While p53 inactivation affects cellular sensitivity to metabolic stress [47,48], gain-of-function mutants also impact cancer metabolism, influencing tumorigenesis. For instance, p53R273H modulates the mevalonate pathway through transcriptional regulation of relevant metabolic genes, interfering with the function of SREBP transcriptional factors [49]. Conversely, wild-type p53 can suppress SREBP-2 activity by upregulating the cholesterol transporter gene ABCA1, thus impeding SREBP-2 maturation [50]. This suggests that the regulation of the mevalonate pathway by p53 mutations may result from a complex interplay between loss- and gain-of-function effects.

Mutant p53 proteins selectively interact with AMPKα, a key regulator of cellular metabolism and tumor suppression, impacting cellular anabolism and response to tissue conditions [51]. Similarly, akin to wild-type p53, mutant p53 sustains serine/glycine synthesis, affecting the response of cancer cells to serine/glycine deprivation [52]. These observations underscore the multifaceted influence of p53 mutations on cellular metabolism and response to environmental cues.

In light of these findings, it becomes evident that the behavior of p53 mutations can vary significantly, depending on the cellular context. Understanding this context specificity is essential for elucidating the significance of p53 mutant proteins in cancer biology and for clarifying their potential as therapeutic targets.

Concluding remarks

Cancer is often understood as a genetic disorder resulting from the gradual accumulation of numerous key genetic mutations, which empower a healthy cell to turn malignant [53]. Among these mutations, those affecting the p53 genetic locus play a significant role due to their high frequency. Surprisingly, however, mutations in p53, and in other cancer driver genes, are not confined to cancer cells [54,55]; they can also be found in normal tissue clones that never progress to cancer formation. How can this paradox be reconciled? The recently proposed 'ground state theory of cancer etiology' offers an explanation: oncogenic mutations alone are necessary but not sufficient to induce cellular transformation. These mutations require a specific cellular context, influenced by factors such as the developmental stage, external insults, and tissue damage, to fully unleash their carcinogenic potential [56,57]. Therefore, the transformation process involves a sophisticated interplay between intrinsic cellular factors and extrinsic environmental cues (cell extrinsic insults, tissue injuries). Interestingly, these environmental factors may not directly cause mutations (i.e., be mutagenic), but they can provide the necessary 'cell state' for the driver mutations to exert their full oncogenic effects [57].

While too novel to be widely acknowledged, the ground state theory of cancer etiology presents a compelling framework for comprehending numerous perplexing observations in cancer biology, also concerning p53 mutations. Indeed, p53 mutant proteins can exhibit diverse functions depending on the context [38], and hereditary p53 mutations lead to a peculiar pattern of cancers, suggesting that p53 mutations may have differing effects at different developmental stages, such as childhood versus aging [58,59]. Consequently, it is crucial to thoroughly examine the role of driver mutations within their specific physiological contexts. Accordingly, investigating the properties of p53 mutants, whether they exhibit gain-of-function, dominant-negative, or loss-of-function characteristics, requires the development of appropriate experimental models that can accurately reflect the relevant biological settings (see Outstanding questions). Evidence

Outstanding questions

Why do cancer cells frequently inactivate p53 through missense mutations? Do p53 mutant proteins exert gain-of-function or dominant-negative effects, or do other bases underlie this distinct mutation pattern?

Does the cellular state determine the significance of p53 mutant gain-of-function effects? Could the oncogenic potential of p53 mutant proteins result from cooperation with extrinsic factors present in the tissue?

How do normal tissues accommodate the presence of p53 mutations without developing cancer? What mechanisms prevent a cell from being transformed by p53 mutations? Are these cell-autonomous or cell-non-autonomous mechanisms?

Why do hereditary and sporadic p53 mutations associate with different cancer spectra? Could variations in cellular state and plasticity influence the oncogenic potential of p53 mutants across different tissues and stages of development?

Is the p53 mutant a viable therapeutic target in anticancer therapy? Should therapy aim to restore wild-type function, or is it sufficient to suppress p53 mutant expression?

suggests that different cell states (microenvironmental conditions, developmental state) confer distinct biological properties upon p53 mutations. Therefore, establishing a suitable framework for addressing these questions could significantly impact the advancement of p53-targeted therapies in cancer treatment.

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Declaration of interests

The authors declare that they have no competing interests.

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