

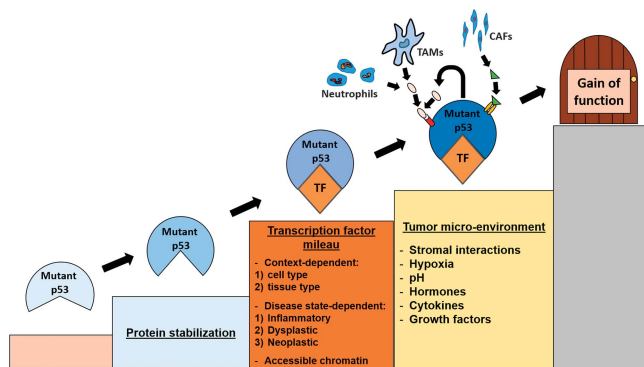
Review

Mutant p53 partners in crime

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Mutant p53 proteins impart changes in cellular behavior and function through interactions with proteins that alter gene expression. The milieu of intracellular proteins available to interact with mutant p53 is context specific and changes with disease, cell type, and environmental conditions. Varying conformations of mutant p53 largely dictate protein–protein interactions as different point mutations within protein-coding regions greatly alter the extent and array of gain-of-function (GOF) activities. Given such variables, how can knowledge regarding p53 missense mutations be translated into predicting or altering biologic activity for therapy? How may knowledge regarding mutant p53 functions within certain disease contexts be harnessed to blunt or ablate mutant p53 GOF for therapy? In this article, we review known proteins that interact with mutant p53 and result in the activation of genes that contribute to p53 GOF with particular emphasis on context dependency and an evolving appreciation of GOF mechanisms.

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Graphical Abstract

Facts

- The p53 tumor suppressor gene is a transcription factor (TF) mutated in over half of all human cancers. The majority of p53 mutations are missense mutations incurred within the DNA binding domain.
- While losing tumor suppressive function, stabilized mutant p53 proteins may simultaneously gain novel functions (gain-of-function, GOF), primarily through protein–protein interactions with other TFs. Other GOF mechanisms continue to be identified.
- Proteins that partner with mutant p53 may transactivate or subvert target gene activation with consequent changes in cellular function. Many proteins that partner with mutant p53, and associated target genes, have been identified.

Open Questions

- Does a core set of mutant p53 target genes confer GOF or does GOF result from the collective effect of many genes affected by mutant p53?

- Which proteins that partner with mutant p53 are essential for GOF and do these differ based on the site of missense mutation?
- To what degree does cellular context affect mutant p53 GOF?
- Does mutant p53 GOF evolve and change during tumor progression or in response to therapy?

The p53 tumor suppressor is a central regulator of cell proliferation and death that restricts the outgrowth of cells harboring genomic irregularities. Through several mechanisms, cells injurious to the host are discarded and cells with intact genomes remain to perform normal cellular functions. The loss of *TP53* as a signature driver of human cancers is indisputable: over half of all human cancers demonstrate alterations in p53 that attenuate or eliminate its function as a tumor suppressor. Loss of tumor suppressive activities exerted by p53 may result in unrestricted cell proliferation and the permissive accumulation of genomic infractions that culminate in tumor growth.

Alterations of the *TP53* locus occur through two genetic mechanisms: (1) deletion of the *TP53* gene and; (2) missense mutations that attenuate p53 function. According to a recent study by Scott Lowe and colleagues, ~one-third of *TP53* alterations involve deletion of one *TP53* allele with retention of the contralateral wildtype allele in examined cancers.¹ Haploinsufficiency of the *TP53* gene, leading to tumor initiation in this context, is demonstrative of the cornerstone role of *TP53* in protecting against malignancy in liquid and solid organ cancers. The remainder of *TP53* alterations (~69%) in human cancers involve loss of p53-mediated tumor suppression through genetic mutation. Most often, missense mutations occur in the DNA-binding domain and render the protein non-functional. The predilection for *TP53* mutation over the simple

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loss of wild-type *TP53* in human cancers suggests a potential selective advantage in tumor initiation and/or progression. Among cancers with *TP53* missense mutations, ~60% show concomitant deletion of the other allele, termed loss of heterozygosity (LOH),¹ whereas 40% do not undergo LOH, retaining a wild-type *TP53* allele. In cancer cells that do not undergo LOH of the wild-type *TP53* allele, a dominant negative mechanism may inhibit wild-type p53. Dominant negative effects of mutant p53 have been shown to suppress wild-type p53 expression and function. Mutant p53 proteins may heterodimerize with wild-type p53 proteins, forming complexes that attenuate the function of wild-type p53 through conformational shifts or inhibiting the DNA-binding activity of wild-type p53 on target genes.^{2–5} Through mitigation of wild-type p53 function by mutant p53 in a dominant negative fashion, a biologic advantage for cancer cells that undergo p53 missense mutation may be further inferred almost irrespective of LOH status. However, the common element underlying tumor initiation is the attenuation of p53 function below a critical threshold through either allelic deletion or mutation, irrespective of the status of the other *TP53* allele.

The prevalence of *TP53* mutations relative to deletions of *TP53* suggests that mutant p53-specific activities selectively drive tumor development. To date, mutant p53 proteins have been found to impart changes in cellular behavior and function through interactions with transcription factors (TFs) and chromatin complexes that result in altered gene expression. Such mutant p53 GOF activity is therefore dependent on the presence, accessibility, and ability of interacting proteins to partner with mutant p53 and affect target gene regulation. The milieu of intracellular proteins available to interact with mutant p53 is likely context specific; mutant GOF appears to change with disease, cell type, and even within tumors owing to heterogeneous mutant p53 stabilization.^{4,6,7} Moreover, differences in mutant p53 protein conformation, based on the site of missense mutation, should also theoretically determine protein–protein interactions and relative binding affinities to interacting proteins. Despite these factors, many studies have identified TFs or proteins capable of binding multiple mutant p53 missense proteins, suggesting that mutant p53 influence may converge on small numbers of partnering proteins. Likewise, regardless of the site of p53 missense mutation, mutant p53 GOF activity may be mediated by a core set of effector genes regulated by commonly bound TFs. Among the numerous TFs bound by mutant p53 and the multitude of transactivated genes, are only a minority essential for mutant p53 GOF activity? Alternatively, do the vast, collective effects of mutant p53 on bound TFs, proteins and downstream, transactivated genes result in mutant p53 GOF activity? Are mutant p53 GOF activities determined on a cell-by-cell basis and dependent on an array of factors such as cell identity, global protein expression, and interactions with the microenvironment? In this review, we discuss mutant p53 interactions on convergent TFs and transactivation targets with particular emphasis on context dependency and an evolving appreciation of GOF mechanisms.

Sites of p53 Missense Mutations Determine Tumor Spectrum and GOF Activity

The vast majority of missense mutations in *TP53* occur at hotspots located within the DNA-binding domain. Ineffective binding of p53 to consensus DNA sequences in target gene promoters attenuates tumor suppressive mechanisms and reduces barriers to cell proliferation. Missense mutations within the DNA binding domain of p53 may be broadly categorized as those that alter the structure (conformation mutant) of the binding domain or those that diminish the ability of mutant p53 to contact and bind DNA (contact mutant). Through either mechanism, interactions between mutant p53 and DNA are attenuated with sharply reduced transactivation of p53 target genes normally responsible for cell cycle arrest, apoptosis, or senescence activities. The relative inability of mutant p53 to directly bind DNA suggests that primary mechanisms of mutant p53 GOF are instead mediated through interactions with other proteins, many of which have been identified as TFs or chromatin-modifying proteins that alter gene expression. Importantly, numerous studies have demonstrated an intact p53 transactivation domain as requisite for specific mutant p53 GOF activities, again indicating co-dependence on other transcriptional regulators for exertion of GOF.^{8,9}

The spectrum and extent of GOF activities endowed by p53 missense mutations change with mutation type. Based on the site and nature of missense mutations – conformation *versus* structural – mutant p53 proteins likely possess different conformations that affect the spectrum of interacting proteins. Given the expanding mechanisms of mutant p53 GOF, it is conceivable that phenotypic differences observed from different p53 point mutants result from their varying ability to bind other proteins and alter gene expression. Knock-in mice that express different p53 missense mutations have provided some of the most robust evidence for alternative biologic effects of different mutant p53 proteins. Knock-in p53 alleles with point mutations at codons R172H and R270H (equivalent to R175 and R273 in humans, respectively) showed different tumor spectra: mice heterozygous for the p53R270H mutation have a higher incidence of carcinomas and B-cell lymphomas compared with mice with one null *p53* allele.¹⁰ Similarly, p53R172H mutant mice develop more osteosarcomas with higher rates of metastasis than the R270H mutant.¹⁰ Humanized p53 knock-in (HUPKI) mice express human coding sequences of the p53 DNA-binding domain and hotspot mutations at codons R248Q and G245S.¹¹ Homozygous p53R248Q mutant mice demonstrated a shortened overall survival owing to accelerated time-to-tumor formation (TTF) relative to *p53*-null mice. However, homozygous p53G245S mice had similar overall survival and tumor spectrum to *p53*-null mice, indicating altered GOF activities of mutant p53 proteins with R248Q *versus* G245S mutations. In addition, mice with mutant p53R248Q showed increased early expansion of the hematopoietic and mesenchymal stem cell pools relative to mutant p53G245S and *p53*-null mice. No significant effects, however, were observed in the ability of hematopoietic and mesenchymal stem cell to terminally differentiate, suggesting that mutant p53 GOF in this setting may be limited to the expansion of progenitor cell populations

instead of as a direct driver of tumor growth. Nonetheless, survival differences as a function of p53 missense mutation clearly indicate altered biologic function and these differences are some of the clearest evidence of mutant p53 GOF to date.

Findings of varied mutant p53 GOF phenotypes based on mutation site in mouse studies have been corroborated by observations made in patients with Li-Fraumeni syndrome (LFS), a heritable genetic disorder wherein one p53 allele is mutated from the time of conception. LFS patients with different *TP53* missense mutations have demonstrated varying times until TTF with the development of diverse tumor spectra. Specifically, patients with mutations at the R282 codon have a median onset of cancer at the age of 13 years compared with 26 years for patients with a nonsense p53 mutation.¹² In a different study, analysis of Li-Fraumeni patients with codon R248Q mutations have a TTF at a median age of 19.5 years compared to 30 years in patients with one *TP53*-null allele.¹¹ Comparison of Li-Fraumeni patients with R248Q and G245S mutations demonstrated significant differences in TTF of 19.5 and 30.5 years, respectively.¹¹ Although varying genetic backgrounds, concomitant oncogenic drivers, and LOH analyses were not considered in these human studies, the marked differences observed in TTF and the propensity for tumor initiation in LFS harboring different *TP53* missense mutations is nonetheless striking. Collectively, compelling *in vivo* data in support of mutant p53 GOF and, more precisely, GOF based on specific p53 missense codons, exist in mouse models and in human data.

Mechanistic insights into the phenotypic differences that occur as a function of p53 missense mutation is critical for the development of cancer prevention and therapy strategies. The bulk of evidence indicates that mutant p53 GOF is the product of functional partnerships between mutant p53 proteins and available interacting proteins that together mediate changes in cell phenotypes through altered gene expression (Figure 1). Mutant p53 may bind TFs and transactivate target genes or attenuate target gene expression (Figures 1a and b). Mutant p53 may also function to increase chromatin accessibility and drive the expression of genes contained within distinct regions of the genome (Figure 1c). As different p53 missense mutations generate p53 mutant proteins with varied conformations, so too might the selection and strength to which they bind enabler proteins also change, resulting in different spectra of transactivated target genes and variations in cellular phenotypes. Within cells of a particular histologic identity, different mutant p53 proteins may therefore drive diverse biologic behaviors. However, it is intriguing that many different p53 missense mutations, incurred within the p53-binding domain, nonetheless partner with identical effector proteins and transactivate identical target genes (Figure 2, Table 1). For example, the p53 missense mutants R175H, Y220C, and R248W all interact with p63 and p73 and subvert the transactivation of target genes.^{13–16} In osteosarcoma SaOS-2 cells, p63 and p73 are aggregated by the mutant p53 missense mutants R110P, E258V, R175H, and R282W, preventing their binding to target gene promoters and consequent transactivation.¹⁷ In pancreatic cancer KP¹C cells, mutant p53R175H and R273H may bind to p73 and prevent its binding to NF- κ B on the PDGFR β promoter, allowing PDGFR β expression.¹⁸ The TF NF- κ B binds the p53 missense

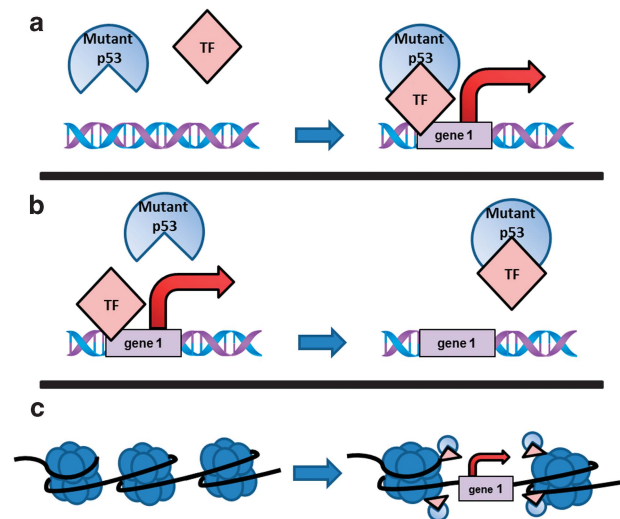


Figure 1 Multiple mechanisms of mutant p53 GOF. (a) Mutant p53 binds to transcription factors (TF) and enhances the transactivation of target genes. (b) Mutant p53 binds various transcription factors and subverts their binding to DNA motifs at the promoters of target genes, leading to reduced expression of target genes. (c) Mutant p53 (light blue circle) modifies the architecture of chromatin by binding and activation of chromatin-modifying enzymes (orange triangle), resulting in enhanced gene expression within spans of accessible chromatin. In this mechanism of mutant p53 GOF, genes occupying entire regions of chromatin may be exposed to existing transcriptional machinery, resulting in gene expression in which gene promoters are not directly bound by mutant p53. Such effects of mutant p53 would not be evident in assays that detect the occupancy of mutant p53 within gene promoters such as ChIP-seq

mutants R175H and 273C, transactivating pro-cell cycle genes.^{19,20} SP1 has been found to transactivate target genes through interactions with V134A, R175H, R249S, and R273H.^{21–24} Sp1 may also recruit mutant p53 to the ENTPD5 promoter and induce its expression, leading to increased tumor cell invasion and metastasis.²⁵ Given the multitude and biologic importance of these and other TFs bound by mutant p53 such as ETS1/2, E2F1, NF- κ B, and SMADs, a core ‘set’ of TFs may be bound by different p53 missense mutants and transactivate target genes to impart GOF activities. As summarized in Table 1, various studies have examined binding of different mutant p53 missense proteins to known TFs, many of which share significant overlap. Under certain circumstances, it appears that common GOF properties observed in different cell lines and mouse models may be largely independent of the site of p53 missense mutation and act through common TFs and core effector genes. Different mutant p53 missense mutants, acting through collateralized networks of bound TFs or proteins, may exert similar GOF through convergence on common target genes. Alternatively, specific p53 point mutants at times do produce different biologic effects, a likely effect of cell type, the milieu of bound cellular proteins, and the relative binding affinities of proteins with which mutant p53 interacts. A resounding question remains: does the sum and variety of all mutant p53 interactions across the genome result in GOF or, alternatively, can mutant p53 GOF be largely attributed to a ‘core’ set of commonly activated effector genes? Moreover, given the breadth and depth of mutant p53 interactor proteins and

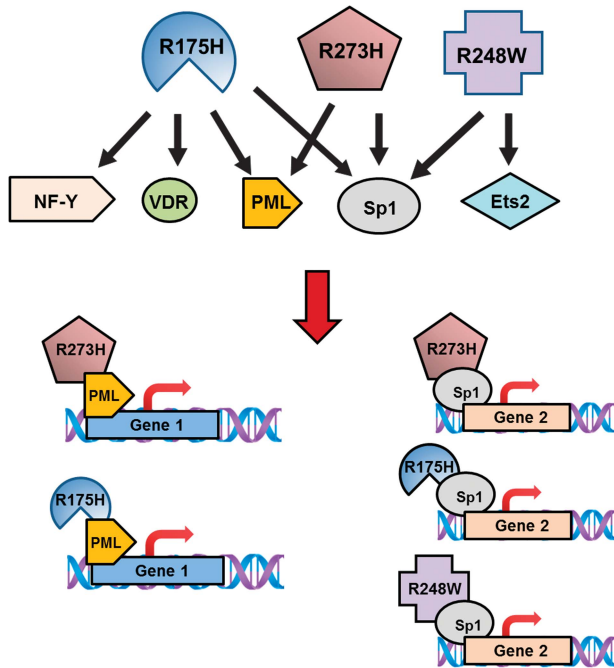


Figure 2 Different mutant p53 proteins converge on common target genes. Multiple mutant p53 proteins (R175H, R273H, R248W) bind to common transcription factors, leading to the activation of identical target genes. For example, mutant p53R175H and R273H both bind the transcription factor PML and transactivate the same target gene (Gene 1). In parallel, mutant p53R273H, R175H, and R248W may bind Sp1 and transactivate an identical target gene (Gene 2). Functionally, different mutant p53 missense mutants (p53R273H, R175H, and R248W) may act through common transcription factors to exert similar GOF through convergence on common target genes. Mutant p53 GOF may therefore depend less on the specific missense mutation site and more on the ability of mutant p53 proteins to bind to core sets of transcription factors and affect their activity

transactivation targets, it remains a challenge to determine the relative biologic impact and contribution of individual target genes or effector proteins affected by mutant p53 to specific phenotypes. Efforts to address these issues have been directed toward defining the scope and commonality of mutant p53 target genes within the genomic landscapes of numerous diseases.

Multiple Mechanisms Enable Mutant p53 GOF Activities

The preponderance of evidence indicates that mutant p53 exerts its function through protein–protein interactions with TFs that increase or reduce the transactivation of gene targets²⁶ (Table 1). Likewise, through partnerships formed with transcription factors, mutant p53 proteins increase the expression of proliferative TFs such as c-Myc and Bcl-XL that promote tumor growth.²⁷ In addition, mutant p53 proteins bind TFs like p63, p73 and Smads to subvert the activation of their respective target genes, leading to increased cell motility, invasion, and metastasis.^{13–15,28,29} The sum effect of this mechanism is potentially quite impactful: mutant p53 effects are distributed throughout vast regulatory networks managed by numerous TFs, enhancing the breadth and depth of its influence on cellular functions (Figure 3). ETS2, ETS1, SREBP, NF-Y, SP1, p63/p73, NF- κ B, YAP, and the vitamin D

receptor, to name a few, have all been found to directly interact with mutant p53 to alter gene expression.^{8,30–34} Another novel mechanism shows that mutant p53 cooperates with the TF NRF2 at proteasome gene promoters to upregulate proteasome levels, enhance the degradation tumor suppressor proteins, and confer resistance to proteasome inhibitor therapy.³⁵ Song *et al.* identified interactions between mutant p53 and Mre11 that result in impaired Ataxia-telangiectasia mutated activation, resulting in the disruption of DNA damage response elements and genetic instability.³⁶ Conceptually, as depicted in Figure 3, a single mutant p53 variant (R172H, for example) may interact with multiple TFs; TFs bound by mutant p53 may then transactivate genes normally regulated by such TFs, amplifying the number of genes affected by mutant p53 and presumably its downstream biologic effect. Collectively, partnership with other TFs has been recognized as a central mechanism through which the impact of mutant p53 GOF is mediated and amplified (Figure 3).

Recently, evidence has mounted that mutant p53 GOF may be mediated through alterations in gene expression that involve chromatin remodeling. Alterations in chromatin architecture may render portions of the genome accessible or inaccessible to available TFs, resulting in broad, genome-wide changes in gene expression (Figure 1c). Prives and colleagues showed that mutant p53 cooperates with the SWI/SNF complex to remodel the chromatin architecture of the VEGFR2 promoter, leading to increased VEGFR2 expression as a mutant p53 target gene.³⁷ Importantly, this study conceptualizes the idea that mutant p53 GOF activities are enabled by chromatin complexes through the recruitment of mutant p53 and resultant, cooperative chromatin remodeling that leads to gene expression changes that alter cellular functions. Mutant p53 also binds to and activates the methyltransferases MLL1 and MLL2, in addition to the acetyltransferase MOZ resulting in histone modifications that increase gene transcription.³⁸ Specifically, enhanced activation of MLL1, MLL2, and MOZ led to genome-wide increases in histone methylation and acetylation and knockdown of MLL1 reduced tumor cell colony formation, tumor volume, and cell proliferation. In contrast to other putative mechanisms of mutant p53 GOF, this novel mechanism produces alterations in gene expression through histone modifications, inducing chromatin accessibility to TFs without formal mutant p53 binding to these factors at specific gene promoters, as depicted in Figure 1c.

Mutant p53 Occupancy Across the Genomic Landscape

The occupancy of mutant p53 in proximity to specific gene promoters indicates active interactions with the transcriptional machinery and implies the potential for transactivation of numerous genes. The advent of ChIP and promoter microarray hybridization (ChIP-on-chip) and p53-specific immunoprecipitation with next-generation sequencing of bound DNA fragments (ChIP-seq) has enabled the identification of the genomic landscape occupied by mutant p53. Importantly, the subversion of TFs from target genes through mutant p53 binding, with resultant attenuation of target gene expression, would not be detected with these assays. Nonetheless, such studies identify genes potentially affected by mutant p53 by binding to native transcriptional complexes. Using gene

Table 1 List of proteins that partner with mutant p53 proteins to enable GOF

Missense mutant	Species	Disease	Cell line	Sp1	Ets1/ Ets2	YAP1	NF-Y	CBP	NF-kB	VDR	SREBP	E2F1	p63/p73	Smad2/ Smad3	Mre11	Nrf2	STAT3	Ref
C194D	H	Breast cancer	T47D	-	-	+	-	-	-	-	-	-	-	-	-	-	-	30
H179Y R282W	H	Keratinocytes	HACAT	-	-	-	-	-	-	-	-	-	+/-	+/-	-	-	-	25
H193L	H	Head and neck cancer	CAL27	-	-	+	-	-	-	-	-	-	+/-	+/-	-	-	-	30
M237I	H	Breast cancer	SUM149PT	-	-	-	-	-	-	-	-	-	+/-	-	+	-	-	32
R110P E258V	H	Osteosarcoma	SaOS-2	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	14
R175H R273H	H	Embryonic kidney	HEK293T	-	ND/+	-	-	-	-	-	-	-	-	-	-	-	-	35
R248Q R248W	H	Breast cancer	SKBr3	-	-	-	-	-	+	-	-	-	-	-	-	-	-	31
R175H	H	Breast cancer	SKBr3	-	-	-	+	-	-	-	-	-	-	-	-	-	-	16
R175H	H	Breast cancer	SKBr3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	37
R175H	H	Lung cancer	H1299	-	-	-	-	-	-	-	+	-	-	-	-	-	-	30
R175H	H	Lung cancer	H1299	-	-	+	-	-	-	-	-	-	-	-	-	-	-	30
R175H	H	Breast cancer	SKBr3	-	-	+	-	-	-	-	-	-	-	-	-	-	-	30
R175H	M	Fibroblasts	MEFs	-	-	-	-	-	-	-	-	-	-	-	-	-	-	25
R175H	M	Breast cancer	HCC1395	-	-	-	-	-	-	-	-	-	+/-	+/+	-	-	-	32
R175H	M	Pancreatic cancer	KP1C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15
R175H	H	Lung cancer	H1299	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	26
R248W R282W- R248L	H	Head and neck cancer	FaDu	-	-	+	-	-	-	-	-	-	-	+/-	-	-	-	30
R248W	H	LFS	MDAH087	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	27
R248W	H	Pancreatic cancer	MIA PaCa-2	-	+/-	-	-	-	+	-	-	-	-	-	-	-	-	27
R248W	H	Colon cancer	Colo320	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	33
R248W	H	Pancreatic cancer	MIA PaCa-2	-	-	-	-	-	-	-	-	-	-	-	+	-	-	22
R249S	H	Breast cancer	BT-549	-	-	-	+	-	-	-	-	-	-	-	-	+	-	32
R273H	H	Breast cancer	MDA-MB-468	-	ND/+	-	-	-	-	-	-	-	-	-	-	-	-	35
R273H	H	Breast cancer	MDA-MB-468	-	-	-	-	-	-	-	+	-	-	-	-	-	-	5
R273H	H	Glioblastoma	U373	-	-	-	+	-	-	-	-	-	-	-	-	-	-	24
R273H	H	Glioblastoma	SNB19	-	-	-	+	-	-	-	-	-	-	-	-	-	-	24
R273H	H	Colon cancer	HT-29	-	-	-	+	-	-	-	-	-	-	-	-	-	-	16
R273H	H	Lung cancer	H1299	-	-	+	-	-	-	-	-	-	-	-	-	-	-	30
R273H	H	Pancreatic cancer	PANC-1	-	-	+	-	-	-	-	-	-	-	-	-	-	-	30
R273H	H	Breast cancer	MDA-MB-468	-	-	+	-	-	-	-	-	-	-	-	-	-	-	30
R273H	H	Colon cancer	SW620	-	-	+	-	-	-	-	-	-	-	-	+	-	-	33
R273H	H	Colon cancer	SW480	-	-	-	-	-	-	-	-	-	-	-	+	-	-	33
R273H	H	Breast cancer	MDA-MB-468	-	-	-	-	-	-	-	-	-	-	-	-	-	-	32
R273H	M	Pancreatic cancer	KP1C	-	-	-	-	-	-	-	-	-	-	-	-	+	-	15
R273H, P309S	H	Colon cancer	SW480	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16
R273H, P309S	H	Colon cancer	SW480	-	-	+	-	-	-	-	-	-	-	-	-	-	-	30
R280K	H	Breast cancer	MDA-MD-231	-	-	+	-	-	-	-	-	-	-	-	-	-	-	30
R280K	H	Breast cancer	MDA-MD-231	-	-	+	-	-	-	-	-	-	-	-	-	-	-	25
R280K	H	Breast cancer	MDA-MD-231	-	-	-	-	-	-	-	-	-	+/-	+/-	-	-	-	22
R280K	H	Breast cancer	MDA-MD-231	-	-	+	-	-	-	-	-	-	-	-	-	-	-	32

All protein interactions were determined through co-immunoprecipitation assays in listed cell lines. H, human. M, mouse. ND, no data available. (-) not examined

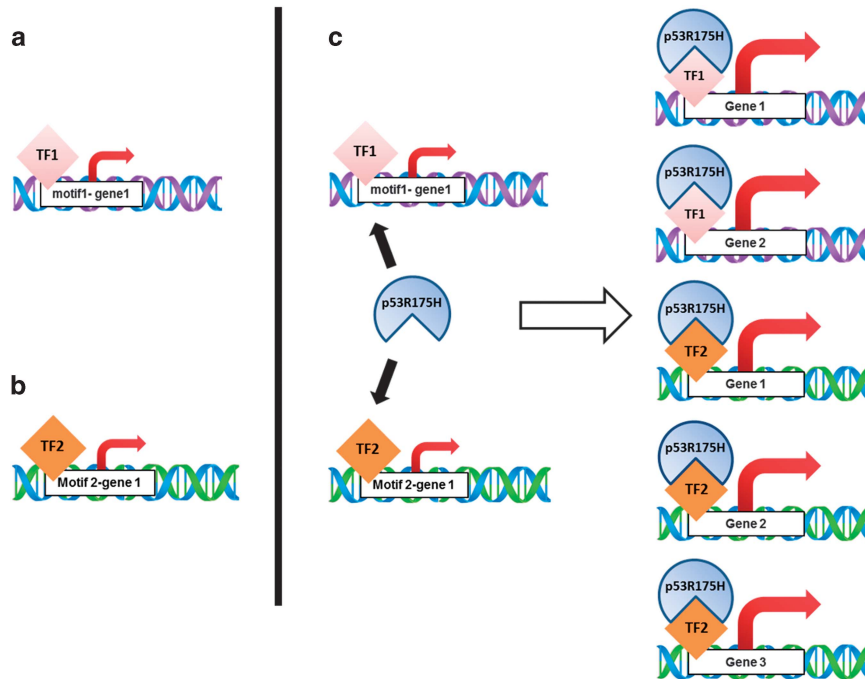


Figure 3 The scope of mutant p53 GOF is enhanced through interactions with multiple transcription factors. Transcription factor 1 (TF 1) and transcription factor 2 (TF2) normally bind to their respective motifs within promoter regions of target genes to initiate transactivation (a, b). Mutant p53R175H may bind to TF1 and TF2 and enhance the expression of target genes specific to each transcription factor. Collectively, through partnerships formed between mutant p53 missense proteins and multiple transcription factors, the influence of mutant p53 on the expression of diverse target genes is amplified. In this scenario, one mutant p53R175H protein may transactivate a total of five target genes through interactions with two different transcription factors (TF1, TF2)

expression microarrays and RNA-sequencing, comparison of increases and decreases in global gene expression in mutant p53 and p53-null samples has resulted in functional datasets identifying pertinent mutant p53 target genes. Stambolsky *et al.*³⁴ performed ChIP-on-chip analysis on the SKBr3 breast cancer cell line and identified ~ 70 gene promoters potentially bound by mutant p53R175H. Four hundred and fourteen different binding motifs were overrepresented in these 70 gene promoters. In the same study, genes potentially regulated by mutant p53 in H1299 lung cancer cells transfected with mutant p53R175H were also analyzed for overrepresented binding motifs.^{34,39} The vitamin D response element (VDRE) was found to be the most significantly overrepresented motif in both analyses. Mutant p53 was subsequently shown to bind VDRE and co-recruit the vitamin D receptor and p300, activating VDRE target genes and conferring resistance to apoptosis.³⁴

In a study conducted by Dell'Orso *et al.*,³² a similar ChIP-on-chip analysis was performed on the SKBr3 breast cancer cell line. These authors found mutant p53 occupying 40 gene promoters among 154 genes associated with or previously reported to bind mutant p53R175H.³² In addition, the authors corroborated data generated by other groups that among the 40 gene promoters bound by mutant p53, 24 promoters were also bound by either p300 or PCAF acetyltransferases.^{19,32,40} NF- κ B (p65) consensus binding sites were also enriched in gene promoters bound by mutant p53; among the 40 promoters occupied by mutant p53, NF- κ B demonstrated co-occupancy on 27 gene promoter regions.³²

Collectively, computational biology approaches to identifying gene promoters with mutant p53 occupancy and linking these data with common TF consensus sites has enabled the identification of two critical elements of mutant p53 biology: (1) the identification of numerous TFs that potentially bind to mutant p53 and; (2) transactivated target genes that affect tumor cell phenotype. Such studies have also confirmed that the activity imparted by mutant p53 on normal cellular functions is widespread and the activity of various protein partners may be either diminished or enhanced by interactions with mutant p53, potentially affecting many mutant p53 target genes in a simultaneous manner.

Subsequent studies have resulted in the identification of numerous other TFs that interact with mutant p53 to transactivate target genes. Overlapping ChIP-on chip and genome-wide ChIP-seq data for mutant p53 occupancy in the Li-Fraumeni cell line MDAH087, Do *et al.*³⁰ identified mutant p53R248W binding to 602 gene promoters. These 602 gene promoters were enriched for a motif highly similar to the consensus binding site for ETS, including ETS family members ETS1, ETS2, and SP1.³⁰ Specifically, 301/602 of gene promoters have predicted ETS binding sites in proximity to regions bound by mutant p53R248W but not to previously published sites of binding by wild-type p53.³⁰ Through a series of elegant experiments, the authors concluded that ETS2 preferentially binds to mutant p53 and that by partnering with ETS2 at the *TDP2* promoter, mutant p53 regulates expression of *TDP2*, encoding a tyrosyl-DNA phosphodiesterase, which confers resistance to etoposide therapy in the MDAH087

(R248W) cell line and the MIA-PaCa-2 (R248W) pancreatic cancer cell line.³⁰ A different study by Vaughan *et al.*³¹ localized mutant p53R273H to promoters containing ETS1 and GABPA-binding sites in H1299 lung cancer cells transfected with mutant p53R273H. Specifically, these authors showed that the probability of mutant p53R273H binding to specific promoters preferentially and exponentially increases with additional ETS1/GABPA-binding sites.³¹ In addition, these authors found that almost one-fourth of promoters bound by mutant p53R273H were bidirectional promoters enriched in inverted ETS motifs.³¹ This finding suggests that mutant p53R273H may regulate gene expression in a bidirectional manner with potential impact on cell phenotype. Collectively, through studies that localize mutant p53 to motifs bound by other TFs, it has become evident that mutant p53 exerts a broad effect through expanding downstream regulatory networks involving potentially hundreds of target genes.

Mutant p53 GOF as a Context-Dependent Phenomenon

Mutant p53 GOF hinges on interactions with other proteins present in the nucleus that may change in response to a vast array of cellular and tumor microenvironment signals. Tissue type, hypoxia, pH, inflammation, active signaling pathways, and interactions with stromal cells such as fibroblasts and immune cells may dynamically alter the nuclear proteome and the presence and volume of interactors with mutant p53. In parallel, mutant p53 is not stabilized in all tumor cells and is therefore not present at sufficiently high levels to effect changes in tumor cell function.⁷ Mutant p53 GOF activity is enabled by two main components: (1) stabilized mutant p53 and; (2) proteins and TFs that partner with mutant p53 and whose effector functions are altered. As such, mutant p53 GOF may not be a static phenomenon but rather quite dynamic with functional activity altered as conditions change within tumor cells, or as a function of the tumor microenvironment and metastasis. For example, p63 is expressed only in epithelial cells, limiting the effect of its interactions with mutant p53 to only a subset of tumors. As most of the work delineating mutant p53 GOF has been conducted in cell lines derived from tumors, confirmation of findings using *in vivo* model systems is essential to ensuring biologic significance and expression of cooperating factors. The redundancy of mutant p53 GOF, specifically the ability to bind to different TFs and transactivate wide networks of target genes, is particularly disheartening for therapies directed against individual signaling pathways or individual protein inhibitors. If mutant p53 GOF is conferred by the sum of its many effects, inhibition of only a couple proteins or signaling pathways is unlikely to produce meaningful clinical responses. Alternatively, if the majority of mutant p53 GOF function is conferred by interactions with limited, critical TFs that affect only a couple signaling pathways, therapy might prove impactful within specific disease contexts. More likely, inhibiting interactions between mutant p53 and a specific TF or individual target genes might result in the selection of tumor cells that employ alternative GOF mechanisms or TFs that transactivate identical target genes. Likewise, therapeutic efforts directed against the stabilization of mutant p53 or its ability to bind consorting GOF proteins might simply lead to the selection of tumor cells that do not stabilize mutant p53.

Collectively, owing to a variety of factors, mutant p53 GOF may be perceived as context and condition dependent with varying degrees of activity among tumor cells that incur somatic p53 mutations. Biologic selection of heterogeneous tumor cell clones may then result, depending on collective properties/abilities gained by cells that express mutant p53 and may help to explain the observation that most p53 alterations in human cancers are missense in nature rather than null.

Conflict of Interest

The authors declare no conflict of interest.

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