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Transcriptional Regulation by Wild-Type and Cancer-Related Mutant Forms of p53

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TP53 missense mutations produce a mutant p53 protein that cannot activate the p53 tumor suppressive transcriptional response, which is the primary selective pressure for *TP53* mutation. Specific codons of *TP53,* termed hotspot mutants, are mutated at elevated frequency. Hotspot forms of mutant p53 possess oncogenic properties in addition to being deficient in tumor suppression. Such p53 mutants accumulate to high levels in the cells they inhabit, causing transcriptional alterations that produce pro-oncogenic activities, such as increased pro-growth signaling, invasiveness, and metastases. These forms of mutant p53 very likely use features of wild-type p53, such as interactions with the transcriptional machinery, to produce oncogenic effects. In this review, we discuss commonalities between wild-type and mutant p53 proteins with an emphasis on transcriptional processes.

TP53 is the most frequently mutated gene found in human cancers (Olivier et al. 2010). Wild-type p53 is a sequence-specific transcription factor that when activated by various stresses, such as DNA damage, oncogenic signaling or nutrient depletion, promotes cellular outcomes, such as cell arrest, cell death, senescence, metabolic changes, and others, depending on the extent and context of the stress (Vousden and Prives 2009). In human cancer, p53 primarily sustains missense mutations in its conserved DNA-binding domain. The small number of residues (~5 to 6) within this region that are mutated with extraordinarily high frequency are termed hotspot mutations. These mutations can be loosely divided into two categories, the contact mutants (e.g., R273H), which remain well folded but whose mutated residues fail to make specific contact with elements within the DNA-binding site and conformational mutants (e.g., R175H) that are partly unfolded leading to loss of zinc coordination and general DNA binding (Cho et al. 1994; Joerger et al. 2005, 2006). p53 hotspot mutant proteins have been reported to associate with chromatin and alter a cell's transcriptional profile, leading to oncogenic cellular changes (Di Agostino et al. 2006; Stambolsky et al. 2010; Do et al. 2012; Freed-Pastor et al. 2012; Cooks et al. 2013; Pfister et al. 2015; Zhu et al. 2015).

p53 BACKGROUND AND DISCOVERY

p53 was initially thought to be a cellular oncogene, because early experiments showed cooperation of p53 with SV40 or H-Ras to transform cells (DeLeo et al. 1979; Lane and Crawford 1979; Linzer and Levine 1979; Eliyahu et al.

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1984; Parada et al. 1984; Hinds et al. 1989). Histology supported the hypothesis that p53 is an oncogene, as high levels of p53 are observed in transformed cells, whereas low levels of p53 are observed in normal tissues (Rotter 1983; Cattoretti et al. 1988; Bartek et al. 1990a). However, it was eventually realized that transforming versions of p53 were derived from cancer cell lines, whereas p53 cloned from normal cells did not show such oncogenic properties and in fact was able to suppress transformation by other oncogenes. Coupled with the fact that the 17p13.1 chromosomal region containing the *TP53* gene is frequently lost in tumors that contain point mutations in the other allele, p53 became firmly established as a tumor suppressor (Baker et al. 1989, 1990; Nigro et al. 1989). Mutant p53 is a dominant-negative inhibitor of wild-type p53 (Bargonetti et al. 1992; Kern et al. 1992) and wild-type p53 confers G_1 checkpoint control to cells lacking functional p53 (Kuerbitz et al. 1992; Yin et al. 1992) in a dose-dependent manner, which is mediated by p21 (Waldman et al. 1995). Mechanistically, wild-type p53 binds DNA in a sequence-specific manner to mediate its functions, whereas mutant p53 fails to bind to a wild-type p53 consensus sequence (Bargonetti et al. 1991, 1993; Kern et al. 1991; el-Deiry et al. 1992; Funk et al. 1992). Critically, p53 null mice are predisposed to acquiring tumors, firmly establishing p53 as a tumor suppressor in mice (Donehower et al. 1992). These data provided a framework to understand Li–Fraumeni syndrome, in which germline mutations in *TP53* predispose affected individuals to breast cancer, sarcomas, lymphomas, and other neoplasms (Malkin et al. 1990; Srivastava et al. 1990; Varley et al. 1997; Wong et al. 2006).

TP53 MUTATIONS IN HUMAN CANCERS

On average, *TP53* is mutated in ~50% of all human tumors although frequencies vary enormously with tumor type ranging from ~1% in papillary thyroid cancer (Cancer Genome Atlas Research Network 2014) to 95% in serous ovarian cancer (Cancer Genome Atlas Research 2011 Network, cbioportal.org). Importantly, *TP53* is unusual for a tumor suppressor in that it is not frequently deleted. Instead, this gene primarily sustains various other types of mutations, the most frequent of which are missense mutations in the p53 DNA-binding domain. Different datasets may have varying frequencies for *TP53* mutation within the same cancer type. Using breast cancer as an example, published *TP53* mutation rates range from 27.2% for breast cancers unstratified by subtype (Banerji et al. 2012) to 53.8% (35/65 samples) in triple-negative breast cancer (Shah et al. 2012). *TP53* mutation is an independent prognostic indicator (correlating with worse prognosis) in breast cancer (Olivier et al. 2006; Petitjean et al. 2007), stage I non-small-cell lung cancer (Ahrendt et al. 2003), lymphomas (Levine and Vosburgh 2008; O'Shea et al. 2008; Young et al. 2008), and with certain hotspot mutations in colon cancer (Samowitz et al. 2002). *TP53* mutations occur at multiple stages in the progression of a tumor, and the stage in which *TP53* mutation occurs may affect the malignancy of the tumor (reviewed in Rivlin et al. 2011).

ESSENTIAL SELECTIVE ADVANTAGES OF *TP53* MUTATIONS IN TUMORS

With few exceptions, mutations within the *TP53* DNA-binding domain either reduce, alter, or preclude interaction of p53 with its consensus DNA-binding sequence (Bargonetti et al. 1991; Kern et al. 1991). The p53 consensus sequence (el-Deiry et al. 1992; Funk et al. 1992; Riley et al. 2008) is a *cis*-regulatory element in the promoter of canonical p53 target genes, such as *MDM2* (Barak et al. 1993), *NOXA* (Oda et al. 2000), *PUMA* (Nakano and Vousden 2001), and *p21* (*CDKN1A*) (el-Deiry et al. 1993). Abrogation of DNA binding by mutation in the p53 DNA-binding domain dysregulates the induction of p53-mediated cellular arrest (through p21) and apoptosis (through PUMA and NOXA), and leads to the accumulation of elevated levels of the mutant form of p53 in part because of the impairment of inducing p53's primary negative regulator, the E3 ubiquitin ligase MDM2 (reviewed in Freed-Pastor and Prives 2012). Loss of p53 function enables a

cell to evade cell-arrest mechanisms that would allow adequate repair of damaged DNA and allow a cell to evade apoptosis, which would have eliminated a cell that sustained DNA damage. Hence, abrogation of p53 function is a critical step in oncogenesis (reviewed in Vousden and Prives 2009). Indeed, even when *TP53* is not directly affected, its negative regulators MDM2 and MDMX are often overexpressed (reviewed in Toledo and Wahl 2006). Loss of wild-type p53 function is the primary selective advantage conferred by mutation in *TP53*.

TP53 HOTSPOT MUTATIONS

The spectrum of *TP53* missense mutations suggests an oncogenic role of *TP53* mutations in promoting tumorigenesis. Six codons have been designated as "hotspots" because of increased selection for these mutants: codons 175, 245, 248, 249, 273, and 282 (Olivier et al. 2010). Each of these codons occurs within the DNA-binding domain of p53, the location of the overwhelming majority of p53 mutations, with other known mutations less frequently occurring outside this domain (Soussi et al. 2005; Petitjean et al. 2007). p53 hotspot mutants are classified as either a DNA-contact mutant (e.g., codon R248, codon R273, codon R282) or a conformational mutant (e.g., codon R175, codon G245) based on the mechanism of alteration of the DNA-binding domain, with conformational mutants being more structurally abnormal, and each class leading to abrogation of sequence-specific DNA binding (Bartek et al. 1990b; Gannon et al. 1990; Bargonetti et al. 1993; Cho et al. 1994; Legros et al. 1994).

p53 DOMAIN STRUCTURE

Structurally, p53 is composed of a bipartite amino-terminal transactivation domain (TAD), a proline-rich domain (PRD), a central DNA-binding domain, an oligomerization domain, and a carboxy-terminal regulatory domain (CTD) (Joerger and Fersht 2008). p53 is a dimer of dimers that through hydrophobic interactions between leucines 344 and 348 in the oligomerization domain form the functional p53 tetramer (Jeffrey et al. 1995; Joerger and Fersht 2008). The p53 protein should, thus, be thought of as a tetramer, allowing for four distinct binding sites per structural element within each tetramer.

The amino-terminal TAD is subdivided into two subdomains, TAD1 within the first 40 amino acids and TAD2 within amino acids 41–61 (Chang et al. 1995; Walker and Levine 1996). TAD1 functionally requires residues 22 (leucine) and 23 (tryptophan) and TAD2 functionally requires residues 53 (tryptophan) and 54 (phenylalanine). These residues can be mutated to polar amino acids (mTAD1 into L22Q/W23S and mTAD2 into W53Q/F54S), leading to abrogation or reduction in p53 transactivation of many target genes (Lin et al. 1994, 1995; Candau et al. 1997; Zhu et al. 1998; Venot et al. 1999; Yan and Chen 2010). Mouse models of p53 transactivation mutants revealed that TAD1 is required for activation of the acute DNA damage response but is dispensable for tumor suppression, whereas simultaneous TAD1 and TAD2 mutations also impairs p53 tumor suppression (Johnson et al. 2005; Brady et al. 2011). The PRD (amino acids 64 to 92) is likely necessary for efficient Pin1 prolyl isomerase-dependent loading of WT p53 on target promoters (Mantovani et al. 2007). On the other hand, the very same domain is required for mutant p53 conformation and function (Girardini et al. 2011), and this domain can be deleted to study PRD-dependent effects (Yan and Chen 2010). The CTD (residues ~363 to 393) has been shown to be necessary for certain mutant p53 target gene activation but dispensable or even inhibitory for mutant p53 pro-proliferation phenotypes, and this domain can be deleted to study CTD-dependent effects (Frazier et al. 1998; Yan and Chen 2010).

The amino and carboxyl termini of p53 are involved in the majority of described protein–protein interactions with p53 (Boehme and Blattner 2009). The CTD and oligimerization domain (OD, residues 326–356) are required for wild-type p53 interaction with Sp1 and Sp3, as well as other transcriptional proteins (Koutsodontis et al. 2005; Kamada et al. 2011). The CTD contains numerous lysines that are

modified to regulate p53, and these residues can be mutated to investigate mutant p53 CTD interactions. A significant challenge to studying CTD interaction is that many studies co-delete the OD along with the CTD, which destabilizes the quaternary structure of p53 and changes its binding interface so it cannot be clearly determined whether an interaction requires the CTD specifically or other aspects of the p53 tetramer. Indeed, specific mutant p53 domains are either required or dispensable to mutant p53 target gene activation, and the mechanisms of this regulation are incompletely defined (Table 1). It is plausible that the charged amino- and carboxy-terminal domains of p53 retain protein–protein interactions in the DNA-binding domain mutants, even though the genomic localization of these mutants is altered. Not only the TAD and CTD but also the DNA-binding domain form interactions with cellular proteins. For example, mutant p53 interactions with members of p63 and p73 families involve DNA-binding domain interactions (Li and Prives 2007). It will be critical to investigate domain-specific p53 interactions to mechanistically define mutant p53 gene regulation.

TRANSCRIPTIONAL ACTIVATION BY WILD-TYPE p53

The following section describes the most well-understood mechanisms of how wild-type p53 initiates transcription at genes containing a p53 response element (reviewed in Beckerman and Prives 2010). It must be stated from the outset that the precise mechanisms by which p53 initiates transcription at its target genes is incompletely understood and undoubtedly more complex than discussed here (Espinosa 2008). Although somewhat of an oversimplification, the basic model for activation of a p53 target gene from promoter engagement through transcription initiation occurs in two distinct phases: (1) p53 recognition and association with its DNA response element, followed by (2) recruitment of transcriptional modifiers that culminate in the formation of the RNA polymerase II pre-initiation complex (Fig. 1A) (Laptenko and Prives 2006).

p53 mediates gene expression changes through interaction with multiple chromatin regulators (Laptenko and Prives 2006). The potential for gene regulatory mechanisms is especially complex when the tetrameric structure of p53 is considered. The functional p53 protein composed of four identical monomers allows for four distinct binding sites for binding partners within each tetramer. This allows for a high degree of complexity in the regulation of transcription, as a single tetramer could bind to multiple transcriptional modifiers at its amino or caboxyl termini (or with its DNA-binding domain).

p53 has been proposed to preferentially associate with genomic regions with a high degree of nucleosomal occupancy (Lidor Nili et al. 2010). Increased DNase I cleavage (termed hypersensitivity) is a hallmark of active genes, corresponding to an open promoter configuration in which nucleosomes are not present (reviewed in Krebs and Peterson 2000). DNase I hypersensitivity is a feature of multiple wild-type p53 target gene promoters including *p21* (*CDKN1A*) (Braastad et al. 2003). Interestingly, the p53 binding site within these promoters occurs in regions that tend to be resistant to DNase I (Braastad et al. 2003). These genomic regions where p53 is bound undergo nucleosomal displacement, which is specific to p53 recruitment as the nucleosomal displacement is reversible on p53 inactivation using a temperature-sensitive p53 mutant (Lidor Nili et al. 2010).

At the *p21* promoter, which is the best-studied p53 responsive gene, both distal and proximal p53 response elements contain high levels of nucleosomal occupancy (Laptenko et al. 2011). On p53 activation, two nucleosomes at the distal (5′) response element are rapidly displaced on p53 activation (Laptenko et al. 2011). Nucleosomal displacement is most likely to occur subsequent to p53 DNA binding as it is unlikely that the alternative—that nucleosomes and p53 compete for the same site—occurs, especially considering that p53 can bind to its response element while the response element is engaged by a nucleosome (Sahu et al. 2010; Laptenko et al. 2011). Interestingly, the ability of p53 to bend DNA within the p53–DNA com-

Table 1. Studies exploring mutant p53 domain-specific effects on gene expression

Gene	Cell line	Mutant	Method	Study
MDR1	NIH3T3, Saos-2, Caco-2, BHK	175H, 281G, requires TAD residues 14/19 for gene expression; requires ETS-1 site; Del22/23 blocks Ets1 binding, Del360CTD still has Ets binding to 281G to 143A, 175H, 248W, 273H; 213Q and 234H no effect on MDR1; 281G mTAD1 ineffective	Overexpression—CAT reporter assay, reporter assay	Chin et al. 1992; Lin et al. 1995; Strauss and Haas 1995; Candau et al. 1997; Sampath et al. 2001
dUTPase	SK-OV-3 and 10(1)	175H, 248W, 273H mTAD1	Overexpression, qPCR/ northern	Pugacheva et al. 2002
TIM50 (Ets-1, CREB ChIP)	1299, SKBR3, MDA-MB-468, Saos-2	175H, 273H, 281G, mTAD1 281G ineffective	Overexpression, siRNA, reporter assay, ChIP	Sankala et al. 2011
NF-κB2	H1299, 21PT, Saos-2	175H, 273H, 281G mTAD1-281G; 175H has increased NF-κB activity	Gene expression array with ectopic expression, qPCR, reporter assay, EMSA	Scian et al. 2005
EBAG9, *ITGA6*, *E2F5*, *MCM6*, *C-SYN*	H1299	281G but not mTAD1-281G effective in gene expression	Gene expression array with ectopic expression, qPCR,	Scian et al. 2005
VDR gene (protein), VDR promoters RNA (IGFBP3, CYP24A1)	SKBR3, H1299, SW480, MDA-MB-231	175H (VDR motif overrepresented), 273H; mTAD1-175H no effect on reporter assay	ChIP-on-chip, overexpression, Southwestern blot, reporter assay	Scian et al. 2005
EGR1	H1299, PC3, SKBR3, HeLa	175H, 248W, 273H, 281G, but not mTAD1-175H or 179E or wt p53 increase gene expression	Overexpression, gene expression array, ChIP, reporter assay, VEGF-induction by EGR1	Weisz et al. 2004
CXCL1 (GRO1)	SW480, MIA-PaCa-2, HCT116	273H/309S, 248W, 175H but not wt p53, mTAD1, mTAD2, PRD required in 245S and 248W, CTD inhibitory 248W, no effect 245S	siRNA, ectopic expression, ChIP	Yan and Chen 2009, 2010
Id2 (inhibition by mutant p53)	SW480, MIA-PaCa-2, HCT116	273H/309S, 248W, 175H but not wt p53, TAD1, TAD2, PRD required in 245S and 248W, CTD not required and perhaps inhibitory	siRNA, ectopic expression, ChIP	Yan et al. 2008; Yan and Chen 2010
c-Myc (mTAD1 and CTD required)	Cx3Ras (rat), 10(1) mouse cells, Saos-2, SK-OV-3 and 10(1)	143A, 281G, 175H, 273H, 248; mTAD1 and CTD with intermediate phenotype with 281G; 175H, 248W, 273H activated c-myc but mTAD1 does not	Overexpression, reporter assay, qPCR/northern	Frazier et al. 1998; Pugacheva et al. 2002

Continued

Table 1. *Continued*

Gene	Cell line	Mutant	Method	Study
c-Myc, apoptosis suppression	M1/2 myeloid cells	143A, mTAD1 required for c-myc and apoptosis suppression	Overexpression	Matas et al. 2001
Induction of invasion/metastasis or p63/p73 inactivation	H1299	175H, 273H; TAD not required for transactivation	Overexpression	Adorno et al. 2009; Oren and Rotter 2010
Spindle checkpoint control	Li–Fraumeni fibroblasts	281G; TAD not required for transactivation	Overexpression	Gualberto et al. 1998
Apoptosis suppression, G_2 arrest suppression	M1/2 myeloid cells	135V; CTD required for apoptosis suppression	Overexpression	Sigal et al. 2001
CXCL1	WI-38, Ras expressing	175H, 179R; TAD1 not required for gene expression	Overexpression	Solomon et al. 2012
IL-1β	WI-38, Ras expressing	175H, 179R; TAD1 not required	Overexpression	Solomon et al. 2012
MMP3	WI-38, Ras expressing	175H, 179R; TAD1 not required	Overexpression	Solomon et al. 2012
TGF-β receptor 2	H1299	175H; mTAD1 required for expression	Overexpression, reporter assay	Kalo et al. 2007
Axl	H1299, H1437, H1048	175H, 267P, 273C, 273H, 273P, 281G; utilized mTAD1 mutants	siRNA, qPCR, +/−ChIP	Vaughan et al. 2012

Summary of known studies that have investigated domain-specific effects of mutant p53 on transcription. The cell lines in which the stated gene was found to be regulated, the p53 mutants that were used, the experiment system that was used, and the associated references are listed.

TGF, Transforming growth factor; wt p53, wild-type p53.

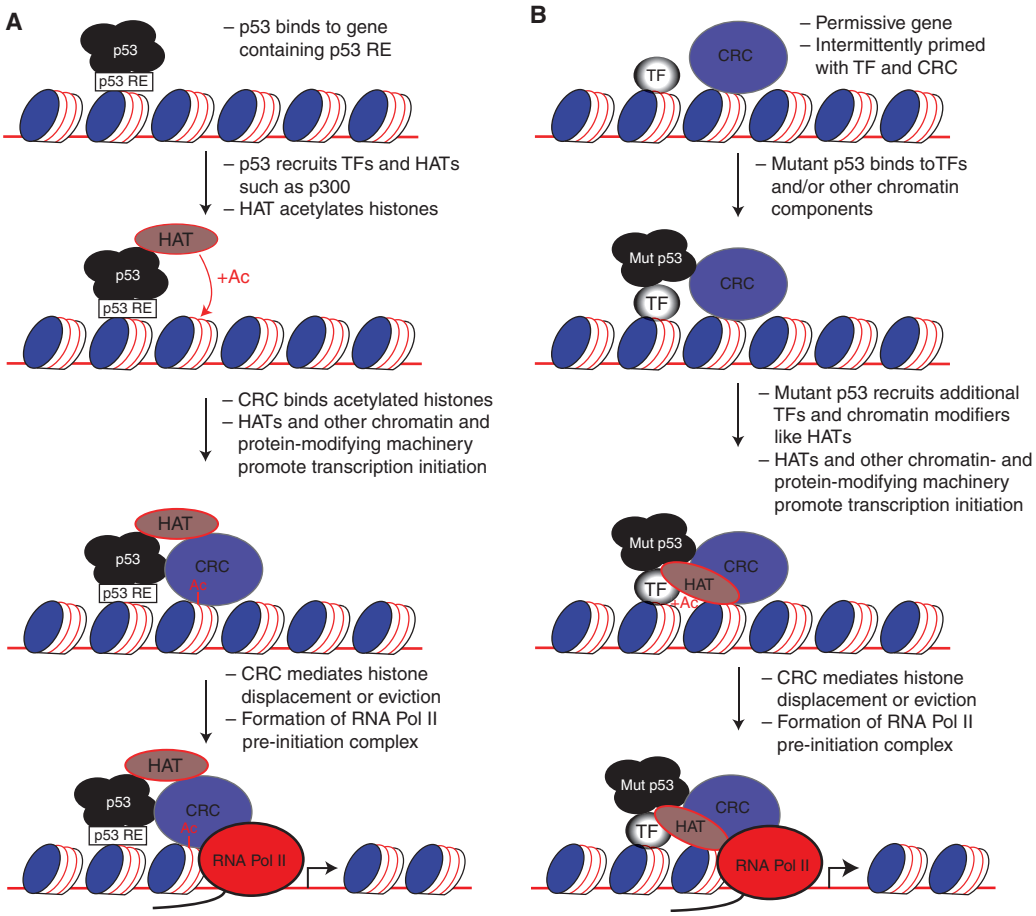

Figure 1. Transcriptional model for wild-type and mutant p53. (*A*) Transcriptional model for wild-type p53. Wild-type p53 is modified at specific amino acids in response to cellular stress signals, such as DNA damage, and accumulates at the protein level in part because of decreased degradation by MDM2. p53 has an intact DNA-binding domain and thus recognizes its cognate p53 response element (RE), which it preferentially binds. p53 then recruits other transcriptional regulatory components, such as transcription factors (TFs) and histone acetyltransferases (HATs) such as p300. HATs acetylate (+Ac) p53 as well as chromatin, promoting additional steps toward transcription, such as the stable recruitment of chromatin remodeling complexes (CRCs) such as SWI/SNF. CRCs recognize histone tails that are primed with histone modifications at specific residues, which promotes ATP-dependent histone displacement or eviction by the CRC. RNA polymerase II and other general TFs bind to the open promoter, forming the pre-initiation complex, followed by steps leading to transcriptional elongation. For a more detailed explanation, see the text. (*B*) Transcriptional model for mutant p53 (Mut p53). Mutant p53 is accumulated at the protein level because of impairment of MDM2 induction and deregulated posttranslational modifications, among other mechanisms. The mutant p53 DNA-binding domain does not recognize the p53 RE, so mutant p53 interaction with chromatin is primarily through protein–protein interactions with other transcriptional regulators such as transcription factors. Because mutant p53 is not restricted through specific binding to the p53 RE, mutant p53 is associated across the genome in a promiscuous manner. At genes that are primed for transcription, such as by being bound by a pioneering transcription factor as well as a chromatin remodeling complex (see Pfister et al. 2015), mutant p53 may promote transcription. Mutant p53 is recruited through protein–protein interactions (that may be conserved in wild-type p53) to such promoters, followed by mutant p53-dependent recruitment of additional transcriptional regulators such as TFs and HATs. Transcription then proceeds in a manner generally similar to that of wild-type p53. Therefore, a key distinction is that wild-type p53 is capable of independently facilitating CRC recruitment and transcriptional activation, while mutant p53 is dependent on the presence of specific promoter factors such as SWI/SNF. Most importantly, wild-type p53 is restricted to specific genomic sites (at p53 responsive genes) by virtue of its DNA-binding domain, whereas mutant p53 is not restricted to specific genomic sequences.

plex is directly correlated with the stability of the p53–DNA interaction (Nagaich et al. 1997). It is conceivable that the intrinsic sequence of the p53 response element (which dictates the degree of DNA bending on p53 binding) facilitates nucleosomal repositioning and impacts subsequent steps in transcriptional activation and resetting of the promoter (promoter turnover) (Nagaich et al. 1997; Laptenko and Prives 2006). As mutant p53 does not bind to DNA in a stable manner like wild-type p53, these initial steps of transcriptional activation likely differ.

Relevant to the above-mentioned findings of its association with nucleosomes in vivo, p53 has been reported to cooperate with nucleosomal remodeling complexes including the Mi-2/NuRD complex (Luo et al. 2000) and multiple members of the SWI/SNF chromatin remodeling complex (Lee et al. 2002). p53 is not yet known to function with the INO80, SWR, or ISWI families of CRC.

The genetic and physical interactions between SWI/SNF components and p53 have been well described. Multiple components of the SWI/SNF complex have been identified as wild-type p53-binding partners including BRG1 (Lee et al. 2002; Naidu et al. 2009), SNF5 (Lee et al. 2002), BAF60A and BAF155 (Oh et al. 2008), ARID1A (Guan et al. 2011), and BRD7 (Burrows et al. 2010). p53 has been described to function with the SWI/SNF complex to mediate transcription from nucleosomal promoters, and p53 interaction with the SWI/SNF complex has been proposed to be mediated, at least in part, through interaction with BAF60A (Oh et al. 2008). BRG1 depletion has been reported to activate p53 signaling (Naidu et al. 2009), whereas SNF5 depletion has been reported to lead to inhibition of p53 mRNA translation (Xu et al. 2010). A proline-rich region of BRG1 is necessary for interaction with p53 (Naidu et al. 2009). CBP (which is closely related to p300) has been reported to dissociate from BRG1 on DNA damage, correlating with decreased CBP and BRG1 levels at the p21 promoter and increased p300 and p53 levels at the p21 promoter (Naidu et al. 2009). As previously mentioned, the p21 promoter undergoes nucleosomal remodeling on p53 binding (Laptenko et al. 2011) and SWI/SNF complex components including BAF180 (Xia et al. 2008), SNF5 (Lee et al. 2002), and BRG1 (Lee et al. 2002; Xu et al. 2007) are required for p21 expression, so the relationship with p53 and SWI/SNF components is intricate.

The Mi-2/NuRD complex is interesting because a component of this complex, PID (also known as metastasis-associated protein 2, MTA2), significantly restricts a p53 response by preventing acetylation of p53. The Mi-2/NuRD complex is an atypical chromatin-remodeling complex in that it has both histone deacetylase and chromatin remodeling activities in the same complex (Denslow and Wade 2007). The Mi-2/NuRD complex is composed of the HDAC1 and HDAC2 histone deacetylases and the CHD3 (Mi-2α) and CHD4 (Mi-2β) chromatin remodeling components, among other proteins (Denslow and Wade 2007). The CHD3 and CHD4 ATPases are chromodomain-containing proteins in the SNF2 family of proteins, which includes the SWI/SNF ATPases BRG1 and BRM (Eisen et al. 1995; Woodage et al. 1997; Denslow and Wade 2007). The PID component of the Mi-2/NuRD complex binds to the p53 amino terminus between amino acids 1–80, and binding is abolished when the first TAD of p53 (amino acids 22, 23) is inactivated (Luo et al. 2000). This finding is intriguing because theoretically the Mi-2/NuRD complex could be recruited by p53 to remodel chromatin and then deacetylate p53 to terminate the p53 transcription activation cycle.

p53 is known to direct transcription through interaction with the Mediator complex (Zhang et al. 2005; Meyer et al. 2010). The Mediator complex was initially identified as a group of thyroid hormone receptor–associated proteins (TRAPs) that function as gene-specific transcriptional coactivators (Fondell et al. 1996). The pre-initiation complex is composed of RNA polymerase II, the Mediator complex, and the general TFs TFII-A, -B, -D, -E, -F, and -H (Fondell et al. 1996; Esnault et al. 2008). The human Mediator complex was suggested to interact directly with TFIID in the process of forming the pre-initiation complex (Johnson

et al. 2002). In yeast, Med11 is required to recruit TFIIH and TFIIE to the pre-initiation complex that leads to serine 5 phosphorylation of the RNA Pol II CTD (Esnault et al. 2008). Mediator has also been described to interact directly with the unmodified RNA Pol II CTD, which causes the Mediator complex to adopt a specific CTD-bound conformation (Naar et al. 2002). p53 has been reported to interact with Mediator components (Gu et al. 1999), including Med17 (TRAP80) (Gu et al. 1999) and MED1 (RB18A) (Drane et al. 1997; Meyer et al. 2010). Med17 interacts with p53 TAD1 (Gu et al. 1999) and Med1 interacts with the p53 CTD (mapped to residues 363–393) (Meyer et al. 2010). Interestingly, increasing titrations of Med1 lead to decreased p53-dependent p21 expression and increased p53-dependent Bax expression (Frade et al. 2000) and increased MDM2 expression (Frade et al. 2002). Notably, the D5 domain of Med1 has been reported to interact with mutant p53 in Raji lymphoma cells (R213Q, Y234H) (Lottin-Divoux et al. 2005).

Arginine methyltransferases PRMT1 and CARM1 have been implicated in p53 transcriptional activation (An et al. 2004). PRMT1 and CARM1 bind directly to the p53 amino terminus (in a region encompassing TAD1) and carboxyl terminus (OD and CTD), respectively, and cooperate with p300 to stimulate transcription of the p53 target gene GADD45A (An et al. 2004). Histone methylation marks may serve dual roles to recruit co-activators that recognize those sequences and by indirectly enhancing acetylation by blocking histone deacetylases (Nishioka et al. 2002) including the NuRD complex (Zegerman et al. 2002). PRMT1 has been described to modify histones in a manner that permits them to be subsequently modified to transcriptionally active modification modes (Huang et al. 2005), and PRMT1 is known to cooperate with CARM1 to mediate gene expression (Hassa et al. 2008).

The amino terminus of p53 (involving the TAD residues 22 and 23) interacts with p300 (Gu et al. 1997). p300 is a histone acetyltransferase that serves to regulate p53 through direct acetylation (Avantaggiati et al. 1997; Gu and Roeder 1997; Lill et al. 1997). Acetylation of the p53 CTD correlates with increased acetylation of histones H3/H4 and increases the interaction of p53 with the p300 homolog CBP (Barlev et al. 2001). Interestingly, at the p21 promoter it has been reported that p300 does not function in transcriptional coactivation through acetylation of p53 (Espinosa and Emerson 2001). Rather, p53 was found to recruit p300 to acetylate nucleosomal histones to mediate transcriptional activation (Espinosa and Emerson 2001; see also An et al. 2004). Acetylated histone lysine residues are known binding and activating marks for other transcriptional components, especially those that possess bromodomains, including SWI/SNF (Agalioti et al. 2002), which functions in an ATP-dependent manner to reorganize chromatin to allow the binding of TFs (Kwon et al. 1994).

It can be surmised that at the p21 locus p53 binds to its response element and recruits an acetyltransferase, such as p300, to acetylate key histone residues that promote nucleosomal remodeling complex activity to reposition nucleosomes, which facilitates the recruitment of other transcriptional components that culminate in the formation of the RNA Pol II preinitiation complex. Although this is a simplified model, these key ideas—specifically, that wild-type p53 binds to its response element, recruits chromatin-modifying proteins that modify both p53 and/or neighboring histones and chromatin-associated proteins to stimulate subsequent transcriptional processes, such as additional coactivator recruitment—will serve as a guide to understand mutant p53 gain-of-function, whereby one of the most critical actions of mutant p53 may be the recruitment of transcriptional regulators to specific promoters to impact transcription.

MUTANT p53 GAIN OF FUNCTION

It is firmly established that there is a pro-oncogenic gain-of-function role for mutant p53. Li–Fraumeni patients with missense mutations are associated with a 9-year earlier tumor onset than patients with other mechanisms of p53 haploinsufficiency (Bougeard et al. 2008). Mice

engineered with haploinsufficient mutant p53 ($p53^{+/mut}$) have accelerated tumor growth, increased tumor count, altered tumor spectrum, and increased metastases than mice with true haploinsufficiency ($p53^{+/-}$) (Dittmer et al. 1993; Liu et al. 2000; Lang et al. 2004; Olive et al. 2004; Caulin et al. 2007). Critically, $p53^{mut/-}$ mice spontaneously acquire a variety of additional carcinomas in addition to the tumors associated with $p53^{-/-}$ mice, defining an in vivo gain-of-function phenotype for mutant p53 (Olive et al. 2004).

It is generally appreciated that the majority of mutant p53 gain-of-function effects derive from the ability of mutant p53 to affect transcription of a variety of genes. Indeed, mutant p53 transcriptional effects have been shown to lead to increased cell proliferation (Preuss et al. 2000; Strano et al. 2002; Scian et al. 2004; Bossi et al. 2006, 2008; Yan et al. 2008; Haupt et al. 2009; Yan and Chen 2009; Freed-Pastor et al. 2012), resistance to apoptosis (Bossi et al. 2008; Lim et al. 2009), which can be mediated through mutant p53 interaction with Ets-2 (Do et al. 2012), increased migration (Adorno et al. 2009; Weissmueller et al. 2014), increased invasion through Matrigel (Muller et al. 2009, 2013), increased tumor inflammation (Scian et al. 2005), and increased metastases (Strano et al. 2002; Weissmueller et al. 2014).

In mouse models, mutant p53 is associated with increased rates of metastasis (Pohl et al. 1988; Hsiao et al. 1994; Heinlein et al. 2008; Adorno et al. 2009; Weissmueller et al. 2014). This effect was initially identified in 1988 (Pohl et al. 1988) and was defined to be missense mutant specific by using xenotransplants of leukemia cells expressing different missense p53 mutations (Hsiao et al. 1994). Leukemia cells (Be-13 cells) expressing p53 R175H, R248Q, and R213Q were able to disseminate to distant sites and induce further hematological disease, whereas the same cells expressing Y234H and R273C were unable to metastasize (Hsiao et al. 1994). The missense mutations that resulted in disseminated disease correlated with decreased survival, and these mice underwent rapid death following the mutant p53-mediated metastases (Hsiao et al. 1994). In a mouse model of invasive breast cancer, the mouse equivalent of p53 R273H (R270H) increases the rate of lung metastases (Heinlein et al. 2008). R248Q and G245S have been shown in mouse models to confer different abilities to hasten tumorigenesis, with the R248Q mutant having accelerated tumor onset and shorter survival compared with G245S mice (Hanel et al. 2013).

Mutant p53 has been shown in mouse models to impair p63- and p73-mediated transcription (Lang et al. 2004). Two more recent studies describe how mutant p53 can impede p63 (Adorno et al. 2009) or p73 function to promote metastasis (Weissmueller et al. 2014). Contact mutant p53 R280K (endogenously expressed in MDA-231 breast cancer cells) were studied in xenotransplants. Twenty-one out of 22 mice with unaltered levels of mutant p53 had lymph node positivity, whereas 12 out of 22 mice showed lymph node positivity when mutant p53 was depleted (Adorno et al. 2009). This correlated with the number of micrometastases to the lung, as depletion of mutant p53 resulted in 75%–90% (depending on the clone) reduction in micrometastases (Adorno et al. 2009). Furthermore, when R175H was overexpressed in the presence of depleted R280K, the number of lung micrometastases was increased back to normal levels, demonstrating that both contact (R280K) and conformational (R175H) mutants can mediate this metastatic phenotype (Adorno et al. 2009). This study further describes how Smad3 (a component of transforming growth factor [TGF]-β signaling) cooperates with mutant p53 to oppose p63 action (which impedes metastatic spread to the lung) (Adorno et al. 2009). A second study defined how a p73/NF-Y complex repressed PDGFRβ expression in a mouse model of pancreatic cancer (Weissmueller et al. 2014). Pancreatic cancer cell lines were derived from mice engineered with the mouse equivalent of p53 R175H (R172H). These cell lines have been previously defined and also express constitutively active K-Ras under tissue specific control (Hingorani et al. 2005). These cells were analyzed in the presence and absence of p53 R172H. Following orthotopic injection into the pancreas, metastasis to the lung and

liver are significantly reduced when mutant p53 is depleted (Weissmueller et al. 2014). Mutant p53 interaction with p73 was found to inhibit p73 interaction with NF-YB, allowing NF-Y to activate PDGFRβ expression that is critical to the metastatic phenotype of mutant p53 in these cells (Weissmueller et al. 2014). Hence, it is well established that mutant p53 promotes metastasis in mouse models, which is consistent with the observations that mutant p53 correlates with worse survival in human cancers (Elledge et al. 1993; Olivier et al. 2006; Langerod et al. 2007; Petitjean et al. 2007; Alsner et al. 2008).

Mutant p53 is known to promote NF-κB signaling through tumor necrosis factor (TNF)-α (Weisz et al. 2007). This observation was especially interesting because mutant p53 is documented to impact the expression of genes in the NF-κB pathway that lead to increased cell growth and survival (Scian et al. 2005). Mutant p53 has recently been shown through cytoplasmic interaction with DAB2IP to alter TNF-α downstream signaling by promoting NF-κB and dampening ASK1/JNK signaling, respectively, suggesting a role for mutant p53 in harnessing the inflammatory effects of the immune system to promote tumor development and metastases (Di Minin et al. 2014). Hotspot p53 mutants have also been reported to contribute to chronic inflammation that underlies the development of colorectal cancer by cooperation with NF-κB (Cooks et al. 2013). This suggests that early p53 mutation can promote tumor development in early stages of tumor progression (Cooks et al. 2013) as well as promote invasiveness and metastases in later stages (Di Minin et al. 2014). Inflammation has a multifaceted role in cancer (reviewed in Grivennikov et al. 2010), and it is worth considering that novel immunomodulatory agents, such as PD1, PDL1, or CTLA4 checkpoint inhibitors (reviewed in Pardoll 2012), could be effective in mutant p53-containing tumors, especially in conjunction with radiotherapy.

These studies identify a variety of mechanisms through which mutant p53 promotes oncogenesis. As each tumor is unique in its development, it should be considered at this point that mutant p53 may be a promiscuous transcription factor that is used by the tumor cell–based on its specific mutations, dominant signaling pathways, and interaction with the microenvironment—in a manner that is selectively advantageous. The next section will summarize what is known about mutant p53-mediated transcription with a focus on specific mutant p53 interacting partners and transcriptional targets.

TRANSCRIPTIONAL ACTIVATION BY MUTANT p53

It remains to be fully understood how mutant p53 mechanistically affects transcription. Mutant p53 does not directly bind to a consensus DNA sequence (Bargonetti et al. 1991, 1993; Kern et al. 1991; el-Deiry et al. 1992). Rather, it is likely that mutant p53 through its individual domains associates with other transcription factors, histone-modifying machinery, or the transcription initiation complex to promote transcription (Fig. 1B; summarized in Table 2).

Mutant p53 has been shown to interact with the following transcription factors: p53 homologs p63 (Davison et al. 1999; Gaiddon et al. 2001; Strano et al. 2002; Adorno et al. 2009) and p73 (Davison et al. 1999; Di Como et al. 1999; Marin et al. 2000; Gaiddon et al. 2001; Bensaad et al. 2003; Di Agostino et al. 2008; Oren and Rotter 2010), Sp1 (Gualberto and Baldwin 1995; Bargonetti et al. 1997; Chicas et al. 2000; Lee et al. 2000; Torgeman et al. 2001; Koutsodontis et al. 2005; Hwang et al. 2011), Smad2 (Adorno et al. 2009), Smad3 (Adorno et al. 2009; Ji et al. 2015), NF-Y (Imbriano et al. 2005; Di Agostino et al. 2006; Liu et al. 2011), E2F1 (Fontemaggi et al. 2009), Ets-1 (Sampath et al. 2001; Kim et al. 2003; Kim and Deppert 2007; Strano et al. 2007; Do et al. 2012), Ets-2 (Do et al. 2012), and the Med1 component of the Mediator complex (Drane et al. 1997; Lottin-Divoux et al. 2005; Meyer et al. 2010) (see Table 2). It is presumed that mutant p53 depends on these interactions (and others not yet identified) to mediate the transcription of numerous genes to mediate its gain-of-function effects.

Table 2. Transcription factors (TFs) that interact with mutant p53

Protein	Interaction with wild-type p53	Mutant	Study
Ets-1	Yes	V143A, D281G, CTD may be required	Sampath et al. 2001; Kim et al. 2003; Kim and Deppert 2007; Strano et al. 2007; Do et al. 2012
Ets-2	Yes		Do et al. 2012
Sp1	Yes	V134A, R175H, R249S, R273H, CTD/OD required for wt p53 interaction	Gualberto and Baldwin 1995; Bargonetti et al. 1997; Chicas et al. 2000; Lee et al. 2000; Torgeman et al. 2001; Koutsodontis et al. 2005; Hwang et al. 2011
NF-Y	Yes	R175H, R273H, R273C, CTD required for binding to wt p53	Imbriano et al. 2005; Di Agostino et al. 2006; Liu et al. 2011
VDR	Yes	R175H, interaction does not occur in 1–292 amino acid mutant	Stambolsky et al. 2010
SMADs (2/3, maybe 4)	Unclear	175H, 273H, TAD likely required	Wilkinson et al. 2008; Adorno et al. 2009; Ji et al. 2015
E2F1	Yes	E2F1 binds wt p53 carboxyl terminus, 175H and perhaps 280K recruit E2F1 to CDE consensus sequence	Fogal et al. 2005; Fontemaggi et al. 2009
TBP	Yes	mTAD1 and 2 required for wt p53	Seto et al. 1992; Ragimov et al. 1993; Chang et al. 1995; Lee et al. 2000
p63	No	R175H, Y220C, R248W, R273H (not D281G), interaction may not require TAD	Davison et al. 1999; Gaiddon et al. 2001; Strano et al. 2002; Adorno et al. 2009
p73	No	R175H, Y220C, V143A, R248W (not R273H), interaction may not require TAD	Davison et al. 1999; Di Como et al. 1999; Marin et al. 2000; Gaiddon et al. 2001; Bensaad et al. 2003; Di Agostino et al. 2008; Oren and Rotter 2010
MED1	Yes	R213Q, Y234H	Drane et al. 1997; Lottin-Divoux et al. 2005; Meyer et al. 2010
p300	Yes	R175H	Avantaggiati et al. 1997; Di Agostino et al. 2006; Ali et al. 2013

Summary of known transcription factors (TFs) or chromatin modifiers for which there is a reported interaction with mutant p53. Whether or not wild-type (wt) p53 is known to interaction with the same factor is listed. Known domain-specific TF interactions with wild-type p53 may predict domain interactions with mutant p53. Note that NF-κB may interact with mutant p53 (Solomon et al. 2012). The table is an extension of a similar table prepared by Freed-Pastor and Prives (2012).

The list of mutant p53-regulated genes, most of which have shown a functional purpose, is extensive and includes ABCB1 (MDR) (Chin et al. 1992; Lin et al. 1995; Strauss and Haas 1995; Sampath et al. 2001; Bush and Li 2002), the GRO1 chemokine (Yan and Chen 2009), PCNA (Deb et al. 1992), the ID2 transcription regulator (Yan et al. 2008), the ID4 transcription regulator (Fontemaggi et al. 2009), the hsMAD1 mitotic spindle checkpoint protein (Iwanaga and Jeang 2002), 15-lipoxygenase (Kelavkar and Badr 1999), the galectin-3 antiapoptotic protein (Lavra et al. 2009), insulin-like growth factor II (Lee et al. 2000), insulin-like growth factor receptor I (Werner et al. 1996), epidermal growth factor receptor (Ludes-Meyers et al. 1996), c-Fos (Preuss et al. 2000), NF-κB2 (Scian et al. 2005; Weisz et al. 2007; Cooks et al. 2013),

c-Myc (Frazier et al. 1998), the EGR1 transcription factor (Weisz et al. 2004), mitogen-activated protein kinase kinase 3 (Gurtner et al. 2010), asparagine synthetase (Scian et al. 2004), human telomerase reverse transcriptase (Scian et al. 2004), the stathmin microtubule-destabilizing protein (Singer et al. 2007), matrix metalloproteinase 13 (Sun et al. 2000), Pla2g16 phospholipase (Xiong et al. 2014), genes of the mevalonate pathway (Freed-Pastor et al. 2012), genes of nucleotide metabolism (Kollareddy et al. 2015), as well as numerous others (Table 3).

Mutant p53 has been reported to stimulate expression of multiple receptor tyrosine kinases and other signaling components. It is well described that receptor tyrosine kinases promote pro-proliferative signaling (Lemmon and Schlessinger 2010). Common signaling nodes can be engaged by multiple receptor tyrosine kinases, even though the output is different; for example, the same signaling pathway can shift from promoting a differentiated state (common to normal cells) to a pro-proliferative state (Marshall 1995; Lemmon and Schlessinger 2010). It is conceivable that mutant p53 may affect cell signaling pathways to promote dedifferentiation of tumor cells. Mutant p53 has been reported to stimulate multiple receptor tyrosine kinases, including EGFR (Ludes-Meyers et al. 1996), IGF1R (Werner et al. 1996), Axl (Vaughan et al. 2012), MET (Muller et al. 2013), PDGFRβ (Weissmueller et al. 2014), and VEGFR2 (Pfister et al. 2015), all of which promote pro-proliferative signaling. In the case of EGFR and MET, this activation has been shown to be dependent on Rab-coupling protein, which increases recycling of these receptor tyrosine kinases to enhance their signaling outputs (Muller et al. 2009, 2013). It is worth considering that as a tumor forms, acquisition of a hotspot mutation in *TP53* may facilitate transcriptional plasticity, whereby tumor cells increase capacity for gene expression changes and, therefore, undergo selection for the greatest pro-proliferative transcriptional program for the particular tumor context. This hypothesis explains, for instance, why such a wide array of genes and pathways has been reported to mediate mutant p53 gain of function.

Mutant p53 regulation of all of these genes varies based on the specific p53 mutant and the cell line. Notably, many of these genes were studied by overexpressing mutant p53 in p53-null cell backgrounds (indicated in the table as overexpression) and often with reporter assays (Table 3). Reporter assays are inadequate to study mutant p53-dependent transcription because they lack the full complexity of chromatin, and mutant p53 does not bind to a DNA response element. Although overexpressing mutant p53 in p53-null cell backgrounds may be adequate to study mutant p53 gain-of-function effects because there is no impact of wild-type p53, this approach is imperfect because the cell lines underwent selective changes without regard to the selective advantages conferred by mutant p53. Such selective changes—the tumor evolving to harness and depend on mutant p53 for its proproliferative or other capacities—must impact how mutant p53 functions in a particular tumor. Therefore, we suggest that cell culture studies involving mutant p53 be performed using cell lines that express single-point mutations in one allele of p53 and have undergone loss of heterozygosity (for example, see Freed-Pastor et al. 2012; Pfister et al. 2015).

Tumor-specific genetic alterations, cellular metabolism, chromatin landscape, and the availability of specific TFs affect mutant p53 function (Kim and Deppert 2003, 2004; Strano et al. 2007; Li et al. 2008; Adorno et al. 2009; Haupt et al. 2009; Dell'Orso et al. 2011; Rodriguez et al. 2012). It follows that one p53 mutant may be observed to behave differently than another p53 mutant based on changes in conformation, binding partners, cellular localization, and transactivation capability. Indeed, mutant p53 gain of function depends on cell and tumor context. Note that posttranslational modifications, nucleosome state, quarter-site orientation and spacer length of the p53 response element, and cofactors affect wild-type p53 gene regulation (Riley et al. 2008). Post-translational modifications, nucleosome state, and cofactors likely impact mutant p53 transcription as well.

These observations (in addition to the fact that mutant p53 does not bind to the p53 re-

Table 3. Known mutant p53 target genes

Gene	Cell line	Mutant	Method	Study
RhoGDI α	H1299, SKBR3, HT29	175H, 175H, 273H	Overexpression, qPCR, siRNA	Bossi et al. 2008
RANGAP1	H1299, SKBR3, HT29	175H, 175H, 273H	Overexpression, qPCR, siRNA	Bossi et al. 2008
RAB6KIFL	H1299, SKBR3, HT29	175H, 175H, 273H	Overexpression, qPCR, siRNA	Bossi et al. 2008
Seladin1 (*DHCR24*)	H1299, SKBR3, HT29, MDA-MB-468	175H, 175H, 273H	Overexpression, qPCR, siRNA	Bossi et al. 2008; Freed-Pastor et al. 2012
MAP2K3 (no TATA)	H1299, SKBR3, HT29/also MDA-MB-468, MDA-MB-231	175H, 175H, 273H/ 280K, regulated by NF-κB, NFY	Overexpression, qPCR, siRNA/ overexpression (175/273), reporter assay, siRNA, ChIP	Bossi et al. 2008; Gurtner et al. 2010
IGFR1	H1299, SKBR3, HT29, Saos-2, RD, HeLa	175H, 175H, 273H, 143A, but wild-type (wt) p53 suppresses	Overexpression, qPCR, siRNA, reporter assay	Werner et al. 1996; Bossi et al. 2008
Paxillin β	SKBR3, HT29	175H, 273H	qPCR, siRNA	Bossi et al. 2008
BCL2L1	SKBR3, HT29	175H, 273H	qPCR, siRNA	Bossi et al. 2008
MDR1	NIH3T3, Saos-2, Caco-2, BHK	175H, 281G, requires TAD residues 14/19; requires ETS-1 site; Del22/23 blocks Ets binding, Del360CTD still have Ets binding to 281G	Overexpression-CAT reporter assay, reporter assay	Chin et al. 1992; Lin et al. 1995; Strauss and Haas 1995; Candau et al. 1997; Sampath et al. 2001
PCNA	HeLa, Saos-2	V143A, R175H, R248W, R273H, D281G	Overexpression-CAT reporter assay	Deb et al. 1992
CCNA2	SKBR3, HT29, SW480, H1299	175H, 273H, 273H/309S; induction by adriamycin inhibited	WB, ChIP, reporter assay	Di Agostino et al. 2006
CCNB1	SKBR3, HT29, SW480	175H, 273H, 273H/309S; induction by adriamycin inhibited	WB, ChIP	Di Agostino et al. 2006
CCNB2	SKBR3, HT29, SW480, H1299-281G	175H, 273H, 273H/309S, 281G; induction by adriamycin inhibited	WB, ChIP, overexpression, reporter Assay	Di Agostino et al. 2006
CDK1	SKBR3, HT29, SW480	175H, 273H, 273H/309S; induction by adriamycin inhibited	WB, ChIP	Di Agostino et al. 2006
CDC25C	SKBR3, HT29, SW480, H1299-281G	175H, 273H, 273H/309S, 281G; induction by adriamycin inhibited	WB, ChIP, overexpression, reporter assay	Di Agostino et al. 2006
ID4	H1299, SKBR3	175H, 273H, Sp1 and NF-κB implicated	Overexpression, gene array, ChIP, EMSA	Fontemaggi et al. 2009
c-Myc	Cx3Ras (rat), (10)1 mouse, Saos-2	143A, 281G, 175H, 273H	Overexpression, reporter assay	Frazier et al. 1998
CXCL5, *CXCL8*, *CXCL12*	H1299, MDA-MB-231, MDA-MB-435	175H, 273H, 281G	Overexpression, siRNA	Yeudall et al. 2012

Continued

Table 3. *Continued*

Gene	Cell line	Mutant	Method	Study
ACAT2, *HMGCS1*, *HMGCR*, *PMVK*, *MVD*, *IDI1*, *FDPS*, *SQLE*, *LSS*, *CYP51A1*, *SC4MOL*, *DHCR7*	MDA-MB-468	273H	siRNA, qPCR, +/−ChIP	Freed-Pastor et al. 2012
MVK, *FDFT1*, *TM7SF2*, *NSDH*	MDA-MB-468, MDA-MB-231	273H, 280K	siRNA, qPCR, +/−ChIP	Freed-Pastor et al. 2012
TGF-β receptor 2	H1299	175H	Overexpression, reporter Assay	Kalo et al. 2007
EPB41L4B, *BUB1*, *MIS18A (C21orf45)*, *NCAPH*, *CENPA*, *FAM64A*, *DEPDC1*, *CCNE2*	MDA-MB-231, MDA-MB-468	280K, 273H	siRNA, qPCR, ChIP	Girardini et al. 2011
CPSF6, *WDR67*	MDA-MB-231	280K	siRNA, qPCR, ChIP	Girardini et al. 2011
hsMAD1	HeLa, HCT116	281G, not 143A	Overexpression, reporter assay	Iwanaga and Jeang 2002
Galectin-3	Saos-2, SW-1736, ARO	273H	Overexpression	Lavra et al. 2009
EGFR	Saos-2	V143A, R175H, R248W, R273H, D281G, and also wt p53	Overeexpression, reporter assay	Ludes-Meyers et al. 1996
Fos	Saos-2	C174Y	Reporter assay	Preuss et al. 2000
dUTPase (DUT) (TAD1 required)	SK-OV-3 and 10(1)	175H, 248W, 273H	Overexpression, qPCR/northern	Pugacheva et al. 2002
TIM50 (Ets-1, CREB target)	H1299, SKBR3, MDA-MB-468, Saos-2	175H, 273H, 281G	Overexpression, siRNA, reporter assay, ChIP	Sankala et al. 2011
Asparagine synthetase	H1299, 10(3), Saos-2	143A, 157F, 163C, 175H, 179Y, 194R, 273H, 281G, 282W	Overexpression, reporter assay, ChIP-273H	Scian et al. 2004
hTERT	H1299, 10(3), Saos-2	143A, 157F, 163C, 175H, 179Y, 194R, 273H, 281G, not 282W	Overexpression, reporter assay, ChIP-273H	Scian et al. 2004
NF-κB2	H1299, 21PT, Saos-2	175H, 273H, 281G, 175H increased NF-κB activity	Gene expression array with ectopic expression, qPCR, reporter assay, EMSA	Scian et al. 2005
EBAG9, *ITGA6*, *E2F5*, *MCM6*, *C-SYN*	H1299	281G	Gene expression Array with ectopic expression, qPCR	Scian et al. 2005
Stathmin	Huh-7, HepG2, U138-MG	213Q, 220C, not wt p53	Knockdown, WB	Singer et al. 2007

Continued

Table 3. *Continued*

Gene	Cell line	Mutant	Method	Study
IGFBP3 and *CYP24A1* (VDR target genes)	SKBR3, H1299, SW480, MDA-MB-231	175H, 273H	ChIP-on-chip, overexpression, Southwestern blot, reporter assay	Stambolsky et al. 2010
hMMP-13	Saos-2	175H, 281G	Overexpression, WB, reporter assay	Sun et al. 2000
EGR1	H1299, PC3, SKBR3, HeLa	175H, 179E, 248W, 273H, 281G, not wt p53	Overexpression, gene expression array, ChIP, reporter assay, VEGF-induction by EGR1	Weisz et al. 2004
CXCL1 (GRO1)	SW480, MIA-PaCa-2, HCT116	273H/309S, 248W, 175H, not wt p53	siRNA, ectopic expression, ChIP	Yan and Chen 2009, 2010
Id2 (inhibition by mutant p53)	SW480, MIA-PaCa-2, HCT116	273H/309S, 248W, 175H, not wt p53	siRNA, ectopic expression, ChIP	Yan et al. 2008; Yan and Chen 2010
REGγ	H1299, UMSCC-1	175H, 248W, 273H; showed mutant p53 cooperation with p300 and negative regulation of Smad3	Ectopic expression, reporter assays, ChIP	Ali et al. 2013
Axl	H1299, H1437, H1048	175H, 267P, 273C, 273H, 273P, 281G; used mTAD1 mutants	siRNA, qPCR, +/− ChIP	Vaughan et al. 2012
VEGFR2	MDA-MB-231, MDA-MB-468	175H, 245S, 248W, 273H, 280K	siRNA, qPCR, +/− ChIP	Pfister et al. 2015
PDGFRβ	KPC mouse cells, MiaPaCa-2, BXPC3, CFPAC, A2.1, SW620, H1975, MBA-MB-231	155P, 172H (mouse), 220C, 242R, 248R, 280K	sIRNA, qPCR, +/− ChIP	Weissmueller et al. 2014
Nucleotide metabolism genes	HCC38, BT549, MDA-MB-231, MiaPaCa-2, H1299	175H, 248W, 249S, 273L, 280K	Ectopic expression, siRNA, qPCR, +/−ChIP	Kollareddy et al. 2015
Pla2g16	H76 (mouse), Saos-2	175H, 179R, 245S, 248Q, 273H	Ectopic expression, siRNA, qPCR, +/−ChIP	Xiong et al. 2014

Listing of known mutant p53-regulated genes (primarily resulting in increased expression), the cell lines in which the gene was found to be regulated, the p53 mutants that were used, the experiment system that was used, and the associated references. Note mutant p53 represses other genes, such as CD95 (Fas receptor) (Gurova et al. 2003; Zalcenstein et al. 2003), ATF3 (a CREB TF family protein) (Buganim et al. 2006), TGF-β receptor 2 (Kalo et al. 2007), caspase 3 (Wong et al. 2007), Id2 (Yan et al. 2008), and wild-type p53 target genes p21, GADD45, PERP, and PTEN (Vikhanskaya et al. 2007).

sponse element) may explain why wild-type p53 and mutant p53, despite binding to many of the same TFs (Table 2) differentially affect gene expression. Mutant p53 can even lead to diametric outcomes of gene expression, even though mutant p53 can still cooperate with wild-type p53 coactivators, such as the histone acetyltransferase p300 (Avantaggiati et al. 1997; Di Agostino et al. 2006). For instance, mutant p53, when located at genes that wild-type p53 activates, such as p21, GADD45, PERP, and PTEN, leads to their repression (Vikhanskaya et al. 2007). It is not immediately obvious why mutant p53 and wild-type p53 lead to diametric gene expression outcomes when they can interact with similar subsets of TFs and coactivators. One possibility is that wild-type p53 has interactions with the same transcription factors that mutant p53 binds, but when overexpressed or activated, wild-type p53 retains preference to its response element while mutant p53 is brought to different promoters (or in the case of wild-type p53-regulated genes, different sites within the promoter) by the transcription factor interactions leading to gene dysregulation.

In the following paragraphs, we speculate on possible mechanisms of mutant p53 transcriptional activity. Mutant p53 likely mediates transcription by co-opting sets of TFs to initiate gene activation at the transcription factor's location, as it is likely the transcription factor is DNA-bound. Coactivators recruited by the transcription factor or mutant p53 then stimulate gene expression. The extent that mutant p53 co-opts individual TFs (or other chromatin modulators) for target gene activation is unclear and may be dependent on the specific mutation in p53 and the active cell signaling pathways leading to subsets of active TFs in the cell. It is also possible that mutant p53, following recruitment by a transcription factor or chromatin modulator, recruits additional factors that can stimulate the function of the initial recruiting factor. It is possible that there are mechanisms for mutant p53 to change the state of chromatin, and these mechanisms may rely on mutant p53 recruitment to DNA through transcription factor binding followed by a change in chromatin architecture by known and unknown chromatin-modifying machinery that are recruited by the mutant p53–TF complex.

TFs may not need to be active to be recruited by mutant p53, although mutant p53 may need to be modified in a specific manner. Mutant p53 engagement may lead to conformational change of an inactive TF to a conformation that can bind DNA. In this mechanism, the presence of mutant p53 in a cell leads to transcription factor engagement by mutant p53. If these TFs were inactive and cytoplasmic, mutant p53 presence would shift their localization to the nucleus, forming a complex with mutant p53, the engaged transcription factor, and a coactivator such as p300. Indeed, cell context, perhaps through mutant p53 modifications, can alter mutant p53-coactivator binding (Di Agostino et al. 2006). Promoters known to be engaged by NF-Y and mutant p53 shift from containing the repressive HDAC1 to the activating p300 on DNA damage by doxorubicin (Di Agostino et al. 2006). Additionally, serine-6 and serine-9 phosphorylations are required for mutant p53-Smad binding (Cordenonsi et al. 2007; Adorno et al. 2009). Mutant p53 contact mutants can cooperate through NF-κB signaling to increase mutant p53 target gene activation (Solomon et al. 2012). It should also be considered that mutant p53 may refine transcription factor-binding specificities, a process termed latent specificity (a cofactor-induced change in DNA recognition) (Slattery et al. 2011). Significantly, mutant p53 impacts the expression of transcription factors such as NF-κB2 (Scian et al. 2005; Weisz et al. 2007; Cooks et al. 2013), c-Fos (Preuss et al. 2000), c-Myc (Frazier et al. 1998), EGR1 (Weisz et al. 2004), ID2 (Yan et al. 2008), ID4 (Fontemaggi et al. 2009), as well as chromatin modulators such as the MLL1 and MLL2 histone methyltransferases (Zhu et al. 2015) and the MOZ histone acetyltransferase (Zhu et al. 2015), all of which could influence mutant p53 transcriptional processes.

We recently showed that mutant p53 promotes SWI/SNF chromatin-remodeling complex activity (Pfister et al. 2015). Further, our results show that mutant p53 and SWI/SNF function to mediate nucleosomal remodeling at the *VEGFR2* promoter (Pfister et al. 2015).

Mutant p53 and SWI/SNF coordinately repress or increase transcription at approximately half of mutant p53 target genes (Pfister et al. 2015). As mutant p53 and wild-type p53 often mediate opposing effects on their interacting partners, in theory, mutant p53 could dysregulate normal SWI/SNF complex functions that wild-type p53 requires for transcriptional activities (Lee et al. 2002; Xu et al. 2007) by affecting its activity, interaction with other proteins, or chromosomal positioning. Mutant p53 has been proposed to facilitate transcriptional plasticity (Quante et al. 2012), and functional interaction of mutant p53 with a chromatin-remodeling complex like SWI/SNF that has broad genomic distribution (Euskirchen et al. 2011) may explain the ability of mutant p53 to mediate gene expression at multiple loci (see Fig. 1B). Because nucleosomal positioning is a critical factor in gene regulation, promoting or inhibiting transcription by regulating access to DNA-binding proteins (Wilson and Roberts 2011), mutant p53 interaction with SWI/SNF could mediate gene activation and repression (Pfister et al. 2015).

It must be emphasized that the mechanism of mutant p53-transcription factor and mutant p53-coactivator interaction is not well understood, and it is these interactions culminating in transcriptional changes that likely represent the majority of mutant p53 gain-of-function effects (versus cytoplasmic or other nontranscriptional mechanisms). This supposition is predicated on experimental data and that the majority of wild-type p53 effects are transcriptional. To understand mutant p53 gain-of-function transcriptional effects, the mechanisms for mutant p53 interactions with transcription factors, coactivators, and other chromatin regulators required for gene expression changes must be delineated. Novel coactivators and corepressors must be identified that could account for mutant p53 transcriptional outcomes. Future drug discovery targets rely entirely on the previous point—if a factor is found that is required for mutant p53 gene transactivation, such as a novel coactivator, then a drug could be developed that would be highly specific for the mutant p53–coactivator complex that would only exist in cells expressing mutant p53 gain-of-function mutants.

CONCLUDING REMARKS

We are headed toward molecular medicine whereby specific *TP53* mutations, combined with the other characteristics of that tumor, will determine clinical response. Knowing whether a tumor is *TP53* wild type or mutated is incomplete information. *TP53* wild-type tumors may or may not predictably function as if they expressed wild-type p53 depending on other aspects of that tumor. Likewise, all mutant p53 proteins are not alike. Hotspot mutants affect transcription in ways that are currently unpredictable, although common mechanisms of action could lead to new drug candidates. Pharmaceuticals that can target specific epigenetic machinery (and chemical modifications of such machinery) are likely to be powerful anticancer drugs in the future. Defining tumor-specific protein interactions, such as mutant p53 interactions with other proteins, are likely to be more therapeutically viable as the targeted interaction would only exist within the tumor. Signaling pathways that are activated by mutant p53 are also candidates for anticancer drugs. Defining the transcriptional changes mediated by mutant p53 in tumors is fundamental to the classification and treatment of tumors harboring mutant p53 and provides insight to the mechanism of mutant p53 target gene activation. Ultimately, we hope work in this direction furthers the goal of defining common vulnerabilities in mutant p53-expressing tumors that are common to multiple types of p53 alterations (so treatment options are simplified) that significantly enhance patient survival.

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