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Perspective

Mutant p53 in Cancer: New Functions and Therapeutic Opportunities

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Many different types of cancer show a high incidence of *TP53* mutations, leading to the expression of mutant p53 proteins. There is growing evidence that these mutant p53s have both lost wild-type p53 tumor suppressor activity and gained functions that help to contribute to malignant progression. Understanding the functions of mutant p53 will help in the development of new therapeutic approaches that may be useful in a broad range of cancer types.

p53 is one of the most intensively studied tumor suppressor proteins, with mutations that lead to loss of wild-type p53 activity frequently detected in many different tumor types. Perturbations in p53 signaling pathways are believed to be required for the development of most cancers, and there is evidence to suggest that restoration or reactivation of p53 function will have significant therapeutic benefit. For the first 10 years of investigation, p53 was considered to be the product of an oncogene, with many studies describing proliferative and transforming activities for p53. This mistake in the initial classification of p53 was the result of a simple error; the *TP53* gene that had been cloned and used in the initial experiments encoded a mutant version of the wild-type gene. The tumor suppressor credentials of wild-type p53 are no longer in doubt, but the early studies provided a tantalizing hint of what has become an extremely active area of study—the suggestion that mutations in p53 can result in both loss of wild-type activity and gain of a novel transforming function. Moving in a circle in the past 30 years, we have come back around to considering that p53, albeit mutant versions of p53, can function as oncoproteins. In this review, we highlight recent progress in our understanding of how mutant p53 functions, discuss the avenues that are being explored to target mutant p53 tumors, and explore future directions for mutant p53 research.

TP53 is the most commonly mutated gene in human cancer (Kandoth et al., 2013). Alterations have been found in virtually every region of the protein (Leroy et al., 2013), but only a handful of the most frequently occurring mutations have been studied in depth for their contribution to cancer progression. In some cases, frameshift or nonsense mutations result in the loss of p53 protein expression, as seen with other tumor suppressors. However, more frequently, the tumor-associated alterations in p53 result in missense mutations, leading to the substitution of a single amino acid in the p53 protein that can be stably expressed in the tumor cell. These substitutions occur throughout the p53 protein, but most commonly cluster within the DNA binding region of p53, with six "hotspot" amino acids that are most frequently substituted. These mutations generally lead to a loss or diminution of the wild-type activity of p53, and because p53 normally acts as a tetramer, these mutant proteins may also function as dominant negative inhibitors over any remaining wild-type p53. Indeed, in a mouse model, the expression of mutant p53 has been shown to dampen (but not prevent) the therapeutic response to restoration of wild-type p53 (Wang et al., 2011). However, it is becoming clear that at least some of these mutant p53 proteins give rise to a more aggressive tumor profile, indicating that they have acquired novel functions in promoting tumorigenesis.

Gain of Function of Mutant p53

The concept that mutant p53 may show a neomorphic gain of function (GOF) was first suggested 20 years ago (Dittmer et al., 1993), when the introduction of mutant p53 into p53 null cells was shown to give rise to a new phenotype. Since then, a large number of publications have demonstrated many GOFs in numerous cell lines with a variety of p53 mutations, summarized in Table 1. The GOF acquired by mutant p53 is further supported by the finding that patients carrying a *TP53* missense mutation (leading to expression of a mutant p53 protein) in the germline have a significantly earlier cancer onset than patients with mutations in *TP53* that result in loss of p53 protein expression (Bougeard et al., 2008; Zerdoumi et al., 2013). Consistently, in vivo experiments showed that mice expressing mutant p53 display a tumor profile that is more aggressive and metastatic than p53 null or p53 wild-type mice (Doyle et al., 2010; Lang et al., 2004; Morton et al., 2010; Olive et al., 2004), although some tissue specificity of this effect has been suggested by further studies showing that introduction of similar p53 mutations in the lung did not reveal any detectable GOF activity over p53 loss (Jackson et al., 2005). Nevertheless, numerous in vitro and xenograft models have confirmed the ability of mutant p53s to drive enhanced invasion and motility, with evidence that mutant p53 can enhance signaling through receptors such as transforming growth factor β (TGF-β) receptor, epidermal growth factor receptor, and MET (Adorno et al., 2009; Grugan et al., 2013; Muller et al., 2009, 2012; Sauer et al., 2010; Wang et al., 2013a). In part,

Table 1. The Different GOF Roles of Mutant p53 in Cells

Mutation	Cell Line	Mutant p53 Expression	Reference
Invasion			
R172H (human R175H), 175H	PDAC	endogenous (also stable/transient)	Muller et al., 2012
R175H	KLE	endogenous (also stable/transient)	Dong et al., 2009
R175H, R273H, R248Q, R280K,	H1299	stable/transient	Adorno et al., 2009; Coffill et al., 2012; Muller et al., 2009; Noll et al., 2012; Yoshikawa et al., 2010
G266E	MDA MB435	endogenous	Yeudall et al., 2012
R273H	A431	endogenous	Muller et al., 2009
R280K	MDA MB231	endogenous	Coffill et al., 2012; Girardini et al., 2011; Muller et al., 2009
Increased (Altered) Migration[a]			
R172H	MEF	endogenous	Adorno et al., 2009
R175H, H179L, R248Q, R273H, D281G	H1299	stable/transient	Adorno et al., 2009; Muller et al., 2009, 2012; Noll et al., 2012; Yeudall et al., 2012
R175H, R248Q	HEC-50	stable/transient	Dong et al., 2012
R248Q	HEC-1	endogenous	Dong et al., 2012
R248W	HCT116$^{-/-}$	endogenous	Muller et al., 2012
R249S	KNS-62	endogenous	Vaughan et al., 2012b
R267P	H1437	endogenous	Vaughan et al., 2012b
R273H	HT29, A431, U373, SNB19	endogenous	Huang et al., 2013; Muller et al., 2012
R280K	MDA MB231	endogenous	Adorno et al., 2009; Girardini et al., 2011; Li et al., 2011a
Proliferation, Propagation of Cell Cycle			
P278S	ABC1	endogenous	Vaughan et al., 2012a
R172H (human R175H)	MEF	endogenous	Lang et al., 2004
R175H	SK-BR3, VMRC	endogenous	Bossi et al., 2006; Vaughan et al., 2012a
R175H, R248H	BE-13	stable/transient	Hsiao et al., 1994
R175H, R273H, D281G	H1299	stable/transient	Liu et al., 2011; Scian et al., 2004b)
C176F, P223L, R273H, R282Q	PC-3	stable/transient	Shi et al., 2002
M246I	H23	endogenous	Vaughan et al., 2012b
R248W, D281G	10(3)	stable/transient	Loging and Reisman, 1999; Scian et al., 2004a
R249S	KNS-62	endogenous	Vaughan et al., 2012a
R267P	H1437	endogenous	Vaughan et al., 2012a; Vaughan et al., 2012b
R273C	H1048	endogenous	Vaughan et al., 2012b
R273H	HT-29, MDA MB468, H2405	endogenous	Bossi et al., 2006; Gurtner et al., 2010; Vaughan et al., 2012a; Wang et al., 2013a
R273H/ P309S	SW480	endogenous	Bossi et al., 2006; Yan et al., 2008
R273H/ R248W	Mia-Paca-2	endogenous	Yan et al., 2008
R280T	SWO-38	endogenous	Lin et al., 2012
Drug Resistance/Avoidance of Cell Death			
A135V, R248W, R273H	M1/2 cells, LN-308	stable/transient	Li et al., 1998; Matas et al., 2001; Pohl et al., 1999; Trepel et al., 1998
R175H	MEC, 10(3), HEC-50	stable/transient	Dong et al., 2012; Murphy et al., 2000; Pugacheva et al., 2002
R175H	SK-BR3	endogenous	Bossi et al., 2006; Di Agostino et al., 2006; Vaughan et al., 2012b
R175H, P223L + V274F	Pc-3	stable/transient	Gurova et al., 2003; Zalcenstein et al., 2003

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Table 1. *Continued*

Mutation	Cell Line	Mutant p53 Expression	Reference
R175H, R245S, R273H, D281G	Saos-2	stable/transient	Atema and Chène, 2002; El-Hizawi et al., 2002; Kawamata et al., 2007; Tsang et al., 2005; Wong et al., 2007
R175H, R248W, R273H	SKOV-3	stable/transient	Buganim et al., 2006; Liu et al., 2011; Pugacheva et al., 2002
R175H, R248W, R273H	H1299	stable/ transient	Blandino et al., 1999; Di Como et al., 1999; Pugacheva et al., 2002; Zalcenstein et al., 2006
Y220S	fibroblasts	stable/transient	Capponcelli et al., 2005
M237?	T98G	endogenous	Wang et al., 2013b
R248Q	HEC-1	endogenous	Dong et al., 2012
G266E	MDA MB435	endogenous	Vaughan et al., 2012b
R273?	U138	endogenous	Wang et al., 2013b
R273C	C33A, H1048	endogenous	Liu et al., 2011; Vaughan et al., 2012b
R273H	C33A	endogenous	Liu et al., 2011
R273H	HT-29, MDA MB468	endogenous	Bossi et al., 2006; Vaughan et al., 2012b
R273H/ P309S	SW480	endogenous	Bossi et al., 2006; Di Agostino et al., 2006
R273H/ R248W	Mia-Paca-2	endogenous	Do et al., 2012
V143A, R175H, R248W, R273H	Hep3B	stable/transient	Schilling et al., 2010
Anchorage-Independent Growth/Anoikis			
Y126C, R175H, H214R, G245S, R273C, R273H, V273F, R280T, R282Q	SAOS-2	stable/transient	Dittmer et al., 1993; Shi et al., 2002; Sun et al., 1993
P151S	TU-138	endogenous	Xie et al., 2013
Increased Colony Formation			
V143A	BEAS-2B	stable/transient	Gerwin et al., 1992
V143A, R175H, R248W, R273H	H1299	stable/transient	Kalo et al., 2012; Liu et al., 2011; Weisz et al., 2004
V143A, Y163C, R175H, L194R, R273H, D281G, R282W	10(3)	stable/transient	Scian et al., 2004a
G144P, R158H, Y163N, H168Y, V173L, Y234C, R248W	REF[b]	stable/transient	Smith et al., 1999
C174Y	Saos-2	stable/transient	Preuss et al., 2000
R172H (human R175H)	MEF	endogenous	Lang et al., 2004
R175H	SK-BR3	endogenous	Bossi et al., 2006
C194T	T47D	endogenous	Nguyen et al., 2013; Vikhanskaya et al., 2007
A220G	Huh-7	endogenous	Vikhanskaya et al., 2007
R270C	IP3	stable/transient	Halevy et al., 1990
R273H	HT-29, MDA MB 468, U373, SNB19	endogenous	Bossi et al., 2006, 2008; Huang et al., 2013; Wang et al., 2013a
R273H	MCF10A[b]	stable/transient	Nguyen et al., 2013
R273H/ P309S	SW480	endogenous	Bossi et al., 2006; Yan and Chen, 2009, 2010; Yan et al., 2008
R273H/ R248W	Mia-Paca-2	endogenous	Yan and Chen, 2009; Yan et al., 2008
Genomic Instability			
R172H (human R175H)	primary mouse oral tumor	endogenous	Acin et al., 2011
R175H	MEC	stable/transient	Murphy et al., 2000
R175H, R248W, R273H	MEF	stable/transient	Agapova et al., 1996
N236S (human N239S)	MEF	endogenous	Jia et al., 2012

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Table 1. *Continued*

Mutation	Cell Line	Mutant p53 Expression	Reference
R248W	primary mouse cells	endogenous	Song et al., 2007
R248W, R273H	K562 KMV	stable/transient	Restle et al., 2008
Spheroid Disorganization/Mammary Architecture Disruption			
R273H, R280K	MDA MB 468, MDA MB231	endogenous	Freed-Pastor et al., 2012
R175H, G245S, R248W, R273H	MCF10A[b]	stable/transient	Zhang et al., 2011
Stem Cell Dedifferentiation/Propagation			
V143A, R175H, R273H	10(3)	stable/transient	Yi et al., 2012
R172H (human R175H)	MEF	endogenous	Sarig et al., 2010
Xenograft Growth (Cell Line Injected Subcutaneously or in the Mammary Fat Pad)			
V143A, R175H, R248W, R273H, R281D, D281G	(10) 3	stable/transient	Dittmer et al., 1993; Lányi et al., 1998
R172H (human R175H)	primary mouse oral tumor	endogenous	Acin et al., 2011
R175H, R273H,	H1299	stable/transient	Liu et al., 2011
N236S (human N239S)	MEF	endogenous	Jia et al., 2012
R267P	H1437	endogenous	Vaughan et al., 2012a
R273C	H1048	endogenous	Vaughan et al., 2012b
R273H	HT29, MDA MB 468	endogenous	Bossi et al., 2008; Wang et al., 2013a
P278S	ABC1	endogenous	Vaughan et al., 2012a
R280K	MDA MB 231	endogenous	Adorno et al., 2009
R280T	SAOS-2	stable/transient	Sun et al., 1993
Intravenous Injection (Formation of Lung Metastasis)			
R175H, R248G, R213G	BE-13[c]	stable/transient	Hsiao et al., 1994
C236F	D3S2	endogenous	Adorno et al., 2009
R280K	MDA MB231	endogenous	Adorno et al., 2009
Elongated Cell Morphology/EMT			
C135Y, R175H, R273H	HEC-50	stable/transient	Dong et al., 2012
V143A	HCT116$^{-/-}$	stable/transient	Roger et al., 2010
R175H	H1299	stable/transient	Adorno et al., 2009
R175H, R273H	10(3)	stable/transient	Gloushankova et al., 1997
R248Q	HEC-1	endogenous	Dong et al., 2012
R273H	SW620	endogenous	Roger et al., 2010
R175H, G245S, R248W, R273H	MCF10A[b]	stable/transient	Zhang et al., 2011
Polyploidy			
V143A	NHF3 cells[b]	stable/transient	Gualberto et al., 1998
R248W, R249S, R175H	H1299	stable/transient	Noll et al., 2012
Angiogenesis			
Δ126	T24	endogenous	Zhu et al., 2013
R175H[d]	H1299	stable/transient	Fontemaggi et al., 2009
Y220S	fibroblasts	stable/transient	Capponcelli et al., 2005
Cell Survival			
V157F	Hs578T	endogenous	Braicu et al., 2013
C194T	T47D	endogenous	Lim et al., 2009
P223L/V274F	DU-145	endogenous	Zhu et al., 2011
R273H	MDA MB468, U373, SNB19	endogenous	Huang et al., 2013; Lim et al., 2009
R273H	H1299	stable/transient	Kalo et al., 2012

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Table 1. *Continued*

Mutation	Cell Line	Mutant p53 Expression	Reference
R280K	MDA MB231	endogenous	Ali et al., 2013; Hui et al., 2006
R280T	5637	endogenous	Zhu et al., 2013
Mammosphere Formation			
R175H	MESC, HEC-50	endogenous	Lu et al., 2013; Dong et al., 2012
R248Q	HEC-1	endogenous	Dong et al., 2012

The different cellular processes in which mutant p53 has been shown to play a role are indicated. Literature was selected based on the following search criteria in Pubmed: "Mutant p53" and "Gain of Function" or "Mutant p53" and "acquired functions." Only studies in which a clear gain of function effect was shown are included (i.e., mutant p53 compared to a p53 null in the same cell line). These comprise studies in which mutant p53 was overexpressed in a p53 null cell line and compared to a vector control, or studies in which endogenous mutant p53 was knocked down or knocked out compared to control cells. Studies describing the activity of mutant p53 in cells that express wild-type p53 are not included to avoid complications from possible dominant negative effects. Indicated are the different mutations, cell lines, endogenous expression, or stable/transient transfection, and the references. The studies in this table were manually selected from >400 publications and we apologize to those authors whose papers we have inadvertently missed.

[a]Increased (altered) migration comprises wound scratch assays, scattering, migration in three-dimensional culture conditions, and Boyden chamber migration (frequently referred to as transwell invasion without addition of a matrix such as Matrigel).

[b]Cells were depleted for endogenous wild-type p53 expression.

[c]These are T cell acute lymphoblastic leukemia cells and therefore increased hematological disease rather than promoted lung metastases.

[d]H1299 cells expressing p53 R175H promoted the angiogenesis of HUVEC cells.

these responses reflect an ability of mutant p53 to promote integrin/RCP driven recycling (Muller et al., 2009, 2012) or increase the expression of growth factor receptors (Sauer et al., 2010; Wang et al., 2013a). Although mutant p53s have generally lost the ability to bind consensus p53 DNA binding regions in target gene promoters, their activity appears to reflect an ability to regulate gene expression directly (Weisz et al., 2007), although cytoplasmic and mitochondrial activities of mutant p53 in regulating apoptosis and autophagy have also been described (Chee et al., 2013; Frank et al., 2011; Morselli et al., 2008). Whereas various different mutant p53s can bind directly to DNA with some degree of selectivity (Brázdová et al., 2013; Göhler et al., 2005; Quante et al., 2012) and may thereby directly control the transcription of some genes (Weisz et al., 2007), there is increasing evidence that an indirect effect on gene expression through binding to other transcription factors underlies the novel activities of mutant p53s. For example, several studies have revealed a role for TAp63, a p53 family protein and transcription factor, which interacts with mutant but not wild-type p53 (Gaiddon et al., 2001; Strano et al., 2002). By inhibiting TAp63, mutant p53 can regulate a pro-invasive transcription program that includes regulation of the expression of Dicer, DEPDC1, Cyclin G2, and Sharp1 (Adorno et al., 2009; Girardini et al., 2011). The Dicer regulation by mutant p53 may be of particular importance, because several miRNAs that can in turn regulate genes involved in invasion have been described to be regulated by mutant p53, although this may not always involve TAp63 or Dicer inhibition (Dong et al., 2012; Neilsen et al., 2012; Tucci et al., 2012; Wang et al., 2013a).

Mutant p53 inhibition of TAp63 can be modeled by deletion of TAp63, which results in an aggressive tumor profile and metastases similar to that seen in mice expressing mutant p53 (Su et al., 2010). However, a direct comparison of mutant p53 expression with loss of TAp63 in a mouse model of pancreatic ductal adenocarcinoma (PDAC) showed that loss of TAp63 is less potent in inducing metastases, suggesting that mutant p53 does more than inhibiting TAp63 (Tan et al., 2013). This is not surprising, because mutant p53 interacts with a wide variety of other proteins, resulting in interference in a multitude of cellular pathways, some of which are likely to contribute to metastasis (Freed-Pastor and Prives, 2012; Muller and Vousden, 2013; Walerych et al., 2012). Besides inhibiting p63, mutant p53 inhibits and interacts with other proteins including the MRE11-Rad51-NSB complex, p73, and SP-1 to induce genomic instability, chemoresistance, or proliferation (Chicas et al., 2000; Gaiddon et al., 2001; Song et al., 2007). Furthermore, mutant p53 can also promote the function of proteins including SREBP, NF-Y, VDR, ETS2, or NRF2, resulting in increased proliferation, cholesterol synthesis, accumulation of reactive oxygen species, and enhanced cell survival (Do et al., 2012; Freed-Pastor et al., 2012; Kalo et al., 2012; Liu et al., 2011; Stambolsky et al., 2010). All of these proteins and pathways affected by mutant p53 are thoroughly described in three recent reviews (Freed-Pastor and Prives, 2012; Muller and Vousden, 2013; Walerych et al., 2012).

More recent studies are identifying further GOF activities of mutant p53, such as a role in cell reprogramming and expansion or in the maintenance and interaction with tumor stroma. Wild-type p53 was characterized as a suppressor of somatic stem cell reprogramming, the process in which differentiated somatic cells can be reprogrammed into a pluripotent stem cell to allow for unlimited expansion (Kawamura et al., 2009; Marión et al., 2009). Loss of p53 promoted the dedifferentiation of somatic cells and some, but not all, mutant p53s could potentiate the reprogramming (Sarig et al., 2010; Yi et al., 2012). An expansion of hematopoietic and mesenchymal stem cell progenitors is also seen in mutant p53 R248Q transgenic mice (Hanel et al., 2013). Consistently, in breast tissue with a *Wnt* transgene, loss of wild-type p53 generally promoted the formation of one distinct tumor, whereas mutant p53 R175H expression promoted the initiation of multiple different tumors that could be expanded in mammosphere assays (Lu et al., 2013). Together, these data suggest that mutant p53 can initiate tumor formation by promoting the generation and expansion of pluripotent stem cells.

The role of stroma tissue, including extracellular matrix, proteases, cytokines, immune cells, epithelial cells, and cancer-associated fibroblasts (CAFs), in tumorigenesis has become very evident (Pietras and Ostman, 2010). CAFs, the most abundant cell type in the stroma, secrete cytokines, hormones, and growth factors including hepatocyte growth factor and TGF-β (Bhowmick et al., 2004; Ostman and Augsten, 2009), both of which have been shown to mediate mutant p53-dependent invasion and metastasis (Adorno et al., 2009; Muller et al., 2012). In addition, a recent report highlights an important function for mutant p53 in promoting the inflammatory environment of colorectal tumors by prolonging NF-κB activation and cell survival (Cooks et al., 2013). It seems clear, therefore, that the presence of a mutant p53 in tumor cells will have an influence on how the tumor and stromal cells interact. In co-culture experiments, H1299 cells (regardless of p53 status) upregulated interferon-β (IFN-β) secretion in CAFs. This would normally cause inhibition of cell migration, but mutant p53-expressing tumor cells counteracted this response by enhancing STAT phosphorylation to promote invasion (Madar et al., 2013). Although interesting, these experiments are difficult to interpret, because the IFN-β secreted by the fibroblasts also reduced mutant p53 expression (Madar et al., 2013). Alternatively, it is possible that *TP53* mutations occur in the stroma surrounding tumors to promote tumor growth (Narendran et al., 2003; Patocs et al., 2007). Mutant p53-expressing fibroblasts were shown to promote tumor growth better than p53 null fibroblasts, suggesting that mutant p53 has a pro-oncogenic GOF role not only in tumor cells, but also in stromal cells (Addadi et al., 2010). However, whether stromal cells that have sustained mutations in p53 are prevalent, and how they are affected by (or affect) tumor cells remains unclear.

Are All Mutant p53s the Same?

Although most experimental studies have focused on the activity of a few most commonly detected p53 mutations that are clustered at codons 175, 245, 248, 249, 273, and 282, almost every codon within the DNA binding domain of p53 has been found to be mutated in cancer. Mutations have also been found in other domains, but their contribution to carcinogenesis is largely unknown (Leroy et al., 2013). Different tumor types show different spectra of *TP53* mutations—in some cases, reflecting the mutagenic event was thought to contribute to that type of cancer (e.g., aflatoxin and liver, UV light, and skin) or geographic variation in other cases. The frequency of missense mutations also differs in different subclasses of tumors of the same organ. For example, luminal breast cancers almost all carry point mutations in *TP53*, while alterations resulting in p53 truncations were more frequently detected in basal breast tumors (Dumay et al., 2013).

Whereas p53 mutants are often considered to be equivalent, evidence is accumulating to indicate that different mutants show a distinct profile with respect to loss of wild-type p53 activity, the ability to inhibit wild-type p53, and the acquisition of gain of function (Table 1; Halevy et al., 1990; Petitjean et al., 2007). The large number of p53 mutations complicates such analyses, as does the realization that different mutants may function differently in different tissues, potentially reflecting differences in the expression of targets of mutant p53 such as TAp63. To date, mutant p53s have been considered in two different categories: the first affecting amino acids that contact DNA and so preventing wild-type transcriptional activity without dramatically affecting the conformation of the p53 protein (known as contact mutants), and the second comprising mutations that clearly disrupt the three-dimensional structure of the protein (termed conformational mutants). Data from cell lines suggest that conformational and contact mutants can cooperate via different mechanisms with the H-Ras signaling pathway, leading to similar gene expression profiles and tumorigenesis (Solomon et al., 2012). However, this classification of mutants is clearly an oversimplification, because different mutations can lead to subtly different alterations in the structure and conformational stability of the p53 protein (Joerger and Fersht, 2007). Various mouse models have shown that both conformational and contact mutants can promote metastasis compared to p53 null mice. These differences appear to be dependent on the nature of the substitution, but caution should be taken when interpreting data from mouse models using different strain backgrounds that are being studied in different laboratories, and in some cases mutate the mouse gene and in others examine humanized TP53 sequences in the mouse. Models of R172H or R270H (prototype examples of a conformation and a contact hotspot mutation, equivalent to R175H and R273H in humans) both showed GOF activity (Lang et al., 2004; Olive et al., 2004), whereas no GOF was seen in R246S (the mouse equivalent of human R249S) and the humanized G245S mutant p53 mouse models, although the R246S could dominant-negatively inhibit wild-type p53 to promote cell survival after radiation exposure (Hanel et al., 2013; Lee et al., 2012). R248Q (humanized) p53 knock-in mice showed an earlier onset of tumor formation with a significantly reduced lifespan compared to p53 null mice (Hanel et al., 2013), although this reduction in overall survival was not evident in any of the other mutant p53 models. Consistently, Li-Fraumeni patients carrying an R248Q mutation display an earlier onset of cancer compared to inherited null mutations or the G245S mutation (Hanel et al., 2013). These findings suggest that the R248Q p53 functions in a different manner than other p53 mutants that have been studied so far. Remarkably, not only the position of the mutation, but also the nature of the substitution may influence the activity of the resulting mutant protein. For example, both R248Q and R248W are structural mutants, but the humanized R248W p53 knock-in mouse does not display reduced lifespan or earlier disease onset (Song et al., 2007). Understanding the consequences of each p53 mutation in relationship to disease progression and response to therapy therefore promises to be an extremely complex undertaking.

Consequences of Mutant p53 Expression to Tumor Therapy

The realization that loss of p53 and expression of mutant p53 may not be analogous has also raised the question of whether the presence of a mutant p53 protein may affect the response to therapy. Whereas there is evidence that the presence of mutant p53 may dampen the response to restoration of wild-type p53 (Wang et al., 2011), reflecting a dominant negative activity of mutant p53, more recent studies have indicated that the retention of wild-type p53 can be detrimental to the therapeutic response in breast cancer. This effect is seen in tumors that express both mutant and wild-type p53 alleles (Jackson et al., 2012). Such studies highlight the possibility that in some

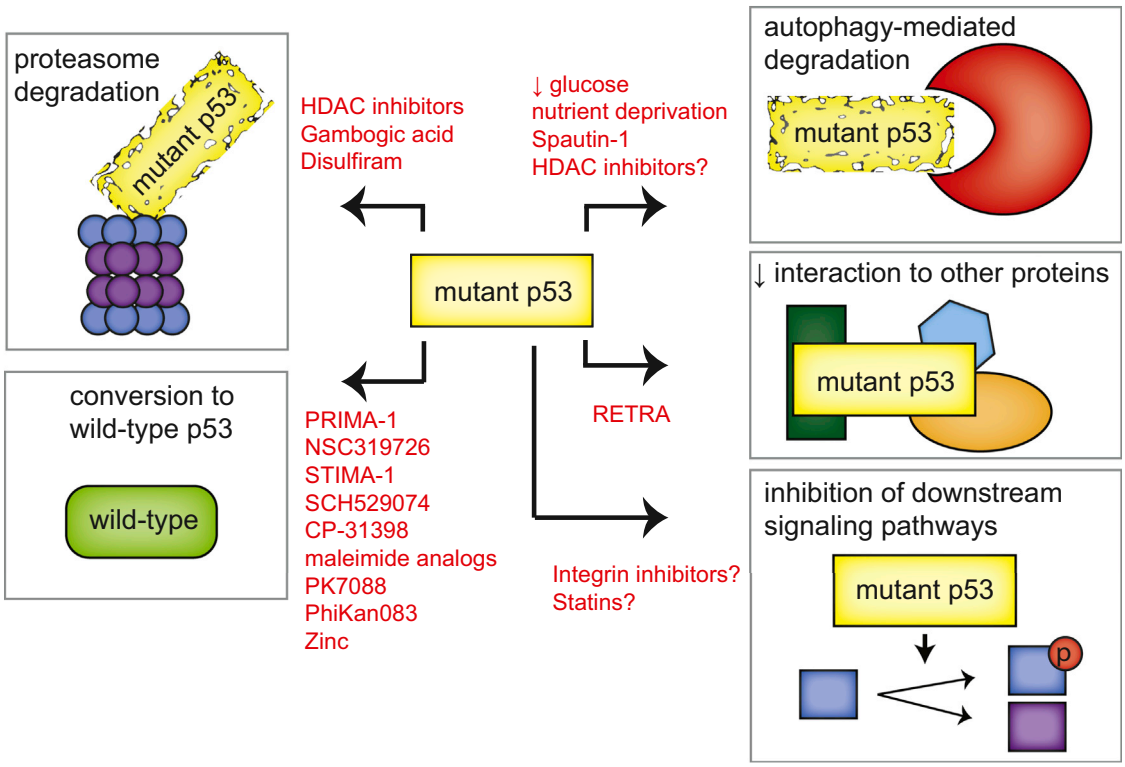

Figure 1. Strategies that Are Currently Being Explored to Target Mutant p53
Depicted in red are schematics of the strategies that are currently being explored to target p53 mutant-expressing cancers. These strategies include promotion of mutant p53 degradation through the proteasome and autophagy pathways, restoration of wild-type p53 activity, interference with the interaction between mutant p53 and other proteins, and interference in signaling pathways downstream of mutant p53.

tumor types wild-type p53 can be dominant over mutant, and that studies of patient response based on p53 status must take into account heterozygosity at the *TP53* locus, as well as the presence of mutant or wild-type p53 (Jackson and Lozano, 2013).

Therapeutic Strategies to Restore Wild-Type Activity to Mutant p53

With so many different mutations and phenotypes it is not surprising that a variety of strategies are being explored to target tumors expressing mutant p53s (summarized in Figure 1). Wild-type p53 is a potent inducer of apoptosis and senescence when expressed in tumor cells, making the reactivation of some level of wild-type function in mutant p53 (which is generally expressed at high levels in cancer cells) an attractive therapeutic avenue. Interestingly, loss of wild-type function introduced by some destabilizing tumor-derived mutations can be rescued by additional point mutations that serve to stabilize the conformation of p53 protein, showing that the loss of structure is intrinsically reversible (Joerger and Fersht, 2008). In addition, a variety of compounds that might restore wild-type p53 function have been characterized and are reviewed in several recent publications (Lehmann and Pietenpol, 2012; Maslon and Hupp, 2010; Wiman, 2010). Small molecules that bind to a site in p53 formed in the Y220C mutant (PhiKan083 and PK7088) function by stabilizing the structure of this mutant p53, and so increasing the level of p53 with a wild-type conformation and activity (Boeckler et al., 2008; Liu et al., 2013). Other compounds bind to multiple mutant p53 proteins (e.g., PRIMA-1, or the soluble derivative PRIMA-met/APR-246, CP-31398, and SCH29074; Bykov et al., 2002; Demma et al., 2010; Foster et al., 1999), interacting with the DNA binding domain, thereby promoting proper folding of the mutant protein and restoration of p53 function. However, the precise mechanistic function of these compounds and others, such as maleimide analogs and STIMA-1, remain to be elucidated (Bykov et al., 2005; Zache et al., 2008).

Whereas wild-type p53 requires binding to the metal ion Zn(2+) to fold correctly (Loh, 2010; Verhaegh et al., 1998), the R175H p53 mutant was found to be impaired in zinc binding (Butler and Loh, 2003). Loss of metallothioneins that chelate and store intracellular zinc promotes a wild-type conformation of misfolded p53 (Puca et al., 2009) and addition of zinc to the conformational mutants G245C and G245D p53 partially restored the wild-type conformation (Pintus et al., 2013). The potential use of zinc to recover wild-type folding has therefore been explored and this approach has been shown to restore chemosensitivity to anticancer drugs in cells expressing endogenous mutant p53 (Puca et al., 2011). In addition, the thiosemicarbazone metal ion chelator NSC31926 was found to restore wild-type function in a variety of different mutant p53-expressing cell lines, possibly through increasing the bioavailability of zinc to (mutant) p53 (Yu et al., 2012).

Of all the compounds that restore wild-type activity, the most progress has been made with PRIMA-1 analogs, with the

demonstration of safety in a phase I clinical study (Lehmann et al., 2012). PRIMA-1 is rapidly converted to other compounds, including MQ, which can bind to both mutant p53 and wild-type p53 (Lambert et al., 2009), although the precise mechanisms underlying the p53 reactivation are currently unknown. Under some circumstances, p53 can adopt an unfolded conformation and behave like a mutant p53 protein to promote invasion (Trinidad et al., 2013). Unfolded wild-type p53 seen in tumor cells grown under hypoxia (Gogna et al., 2012) could be restored by PRIMA-1 treatment (Rieber and Strasberg-Rieber, 2012). It will therefore be interesting to explore whether both wild-type and mutant p53 tumors might benefit from PRIMA-1 treatment.

Therapeutic Strategies to Promote Mutant p53 Degradation

An alternative approach to targeting mutant p53 is to remove the proteins by enhancing turnover (Figure 1). Both wild-type and mutant p53 can be targeted for proteasomal degradation in otherwise normal cells by the ubiquitin ligase MDM2. Inhibition of MDM2 in response to stress underlies the activation of wild-type p53, but is also thought to lead to the overexpression of mutant p53 seen in cancer cells. Indeed, stress induced stabilization of mutant p53 seems to be a prerequisite for its GOF (Suh et al., 2011). In addition to MDM2, another chaperone-associated E3 ubiquitin ligase, CHIP, was shown to be important for mutant p53 degradation (Esser et al., 2005; Lukashchuk and Vousden, 2007). To be stabilized, mutant p53 interacts with the Hsp70 and Hsp90 chaperone complex that requires an interaction with HDAC6 for proper functioning (Li et al., 2011b). Abrogation of HDAC6 binding results in the dissociation of the heat shock proteins from mutant p53 and allows for mutant p53 degradation by MDM2 and CHIP (Li et al., 2011b). HDAC inhibitors such as SAHA show promise in destabilizing mutant p53 by preventing HDAC6 from interacting with Hsp90 (Li et al., 2011a). However, SAHA and the pan-HDAC inhibitor NaB were recently shown to not only regulate mutant p53 stability, but also its transcription via the p53 activator HoxA5 (Yan et al., 2013). This activity was not confined to mutant p53 and also extended to decreasing wild-type p53 expression (Yan et al., 2013), indicating that care should be taken to determine the p53 status of tumors when HDAC inhibitors are used as therapeutic agents. Small molecule activators of SIRT1 have also been shown to lead to the deacetylation of p53 and reduction of overall mutant p53 levels (Yi et al., 2013). In other studies, Stathmin—a transcriptional target of wild-type p53 and mutant p53 (through the regulation of miR-223)—promoted mutant p53 activity by regulating phosphorylation and stability in ovarian cancers (Sonego et al., 2013).

Autophagy also plays a role in mutant p53 degradation. Macro-autophagy is the process by which intracellular contents such as proteins or organelles are engulfed and degraded through lysosomes. This can provide a means to recycling intracellular content, providing an alternative energy source to allow cells to survive transient starvation, and also functioning to remove damaged or excess organelles (Mizushima et al., 2008). The role of autophagy in cancer is complex and can both promote and inhibit tumor development, depending on the targets of the autophagic process and the timing during tumor evolution (Liu and Ryan, 2012). Macro-autophagy induced by glucose restriction selectively promoted mutant p53 degradation, whereas wild-type p53 was stabilized under similar conditions (Rodriguez et al., 2012). The degradation of mutant p53 was promoted by proteasomal inhibition and depended on functional autophagy machinery (Choudhury et al., 2013; Rodriguez et al., 2012). Glucose starvation combined with confluent growth conditions could promote mutant p53 degradation by a specialized form of autophagy known as chaperone-mediated autophagy (Vakifahmetoglu-Norberg et al., 2013). In contrast to the findings of Rodriguez et al. (2012), degradation of mutant p53 via this specialized autophagy pathway was enhanced by inhibition of macro-autophagy (Vakifahmetoglu-Norberg et al., 2013), suggesting conditional aspects to glucose deprived mutant p53 degradation. Furthermore, both mutant and wild-type p53 can inhibit autophagy when localized in the cytoplasm (Morselli et al., 2008; Tasdemir et al., 2008), indicating that the relationship between autophagy and mutant p53 is complex.

Therefore, while targeting mutant p53 for degradation seems feasible, there remains a concern as to how effective simple removal of mutant p53 (without replacement by degradation-resistant wild-type p53) might be in driving a therapeutic response. Some comfort has been provided by many studies showing reduction of mutant p53 levels (either by siRNA or spautin treatment) results in increased apoptosis, indicating that these cells may have become dependent on mutant p53 for their survival (Table 1; Ali et al., 2013; Braicu et al., 2013; Huang et al., 2013; Hui et al., 2006; Lim et al., 2009; Vakifahmetoglu-Norberg et al., 2013; Xie et al., 2013; Zhu et al., 2011, 2013). However, whether decreasing mutant p53 levels is sufficient as a means of therapy in vivo and in the long term requires confirmation.

Targeting Mutant p53 Regulated Pathways

Instead of targeting mutant p53 directly, another approach is to identify commonalities in the mechanisms through which mutant p53 proteins function and to target and exploit these downstream pathways (Figure 1). Despite the clear differences between mutant p53s, a large number of them interact and inhibit p63 and p73. A small molecule named RETRA, identified by serendipity in a screen to identify drugs to stabilize wild-type p53, has been suggested to destabilize the p73 mutant p53 interaction (Kravchenko et al., 2008). RETRA-induced release of p73 resulted in the activation of p73 target genes and a concomitant decreased tumor cell survival and suppression of xenograft tumor growth (Kravchenko et al., 2008). Whether RETRA impairs the interaction of mutant p53s with other target proteins has not been reported, but this could be a more general approach to block the oncogenic effect of mutant p53s that share binding partners.

Downstream pathways activated by mutant p53 may also be targets for therapeutic intervention. An attractive possibility here is the cholesterol synthesis pathway through which mutant p53 disrupts the morphology of mammary tumors (Freed-Pastor et al., 2012). Inhibition of cholesterol synthesis restored the morphology and decreased survival of mutant p53 cells (Freed-Pastor et al., 2012). This is of particular interest because statins (cholesterol inhibitors) are among the most commonly prescribed drugs worldwide to prevent cardiovascular diseases and have shown promise as preventive anticancer agents (Singh

and Singh, 2013). It will therefore be interesting and relatively straightforward to determine the utility of statins as a therapeutic strategy for mutant p53 tumors.

Finally, several studies have described a role for mutant p53 in enhancing receptor tyrosine kinase (RTK) signaling (Adorno et al., 2009; Muller et al., 2009; Sauer et al., 2010; Wang et al., 2013a). A multitude of inhibitors of the kinase activity of RTKs or their downstream mediators have been described, including EGFR inhibitors, MET inhibitors and MAPK inhibitors. Selective efficacy of these compounds in the treatment of mutant p53 expressing cancers remains to be explored. The specific role of RTK and integrin recycling may also provide an additional attractive target, since various integrin antibodies and drugs that inhibit integrin recycling are currently on the market and have shown some promise as anticancer agents (Desgrosellier and Cheresh, 2010).

Future Directions

A number of hurdles still need to be overcome before the studies of mutant p53 can be translated into clinical practice. While there is clear evidence that mutant p53 promotes various oncogenic responses, the relative importance of survival, motility, invasion, and metabolic changes, or the critical pathways through which these responses are mediated remain unclear. How different mutations affect p53 function also remains underexplored, as does the comparative importance of loss of wild-type, dominant-negative, and GOF phenotypes. The fact that most mutant p53s are expressed at very high levels in cancer cells (leading to the immunohistochemical detection of p53 being used as a proxy for the presence of mutant p53) makes these proteins tremendously attractive therapeutic targets, and the efficacy of inhibiting the activity of these mutant p53s or even re-establishing some wild-type function, as described above, holds great promise. Such approaches depend, however, on designing efficient mechanisms through which to target mutant p53, an understanding of the activities and function of the many different mutants, and the capacity to identify which mutation a tumor carries (the latter likely to be the most easily attainable goal).

Maybe a more effective approach will be to explore the possibility of synthetic lethality as a therapeutic strategy. Recently, a computational approach using gene expression from the NCI-60 panel, the GBM (glioblastoma multiforme) project and the TCGA (the cancer genome) project revealed a number of genes and pathways that may result in synthetic lethality when targeted in mutant p53-expressing tumors (Wang and Simon, 2013). The majority of these genes were involved in the cell cycle, perhaps reflecting the loss of wild-type p53 function, and an interesting candidate identified in several of the data sets is polo-like kinase 1 (PLK1), which is involved in the regulation of mitosis. PLK1 was found to be upregulated in breast cancers with mutant p53 expression; the presence of both coincided with a worse prognosis than cancers with either PLK1 upregulation or mutant p53 expression alone (King et al., 2012). Because PLK1 can be inhibited by a variety of compounds (Strebhardt, 2010), it will be interesting to follow up this lead.

Conclusions

Recent data reveal that mutant p53 is not just one protein, but a multitude of proteins that can contribute to a wide range of oncogenic processes. Designing drug strategies to target mutant p53 tumors is therefore highly challenging and will require a deeper understanding of the degradation pathways, interaction partners, and downstream signaling pathways in mutant p53 cells. However, we are optimistic that our ever-expanding knowledge of mutant p53 function will translate into some useful therapeutic strategies in the future.

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