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REVIEW ARTICLE

The role of DNA damage responses in p53 biology

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Abstract The tumour suppressor p53 is a central player in cellular DNA damage responses. P53 is upregulated and activated by genotoxic stress and induces a transcriptional programme with effectors promoting apoptosis, cell cycle arrest, senescence and DNA repair. For the best part of the last three decades, these DNA damage-related programmes triggered by p53 were unequivocally regarded as the major if not sole mechanism by which p53 exerts its tumour suppressor function. However, this interpretation has been challenged by a number of recent in vivo studies, demonstrating that mice which are defective in inducing p53-dependent apoptosis, cell cycle arrest and senescence suppress thymic lymphoma as well as wild-type p53 expressing animals. Consequently, the importance of DNA damage responses for p53-mediated tumour suppression has been questioned. In this review, I summarize current knowledge on p53-controlled DNA damage responses and argue that these activities, while their role has certainly changed, remain an important feature of p53 biology with relevance for cancer therapy and tumour suppression.

Keywords P53 · DNA damage · Tumour suppression · Cancer therapy · Apoptosis · Cell cycle arrest · Senescence · DNA Repair

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Introduction

DNA damage affects the integrity of the genetic information in a cell and thus can be mutagenic. To counteract this, mammalian cells have developed a sophisticated network of complex signalling programmes that respond to DNA damage with the aim of restoring genomic integrity (Fig. 1). This can be either achieved by DNA repair or elimination of the damaged cell from the organism/proliferating cell pool through induced cell death or senescence. If both types of programmes fail, mutations can manifest in the genome and, if a damaged cell proliferates, be duplicated during DNA replication and cell division. With repeated failures and the accumulation of too many mutations, there is a high risk that genes which regulate cell growth and death are disrupted in their proper function. This can lead to cancer.

Indeed, the prevalence of mutations and genomic instability—that is a propensity to easily acquire more mutations—are hallmarks of cancer cells and common features across the vast majority of cancers (Hanahan and Weinberg 2011; Roberts and Gordenin 2014). Therefore, cellular DNA damage responses have been firmly established as a crucial component of the mechanisms that suppress cancer from developing. Consistently, genes that control DNA damage response programmes are particularly frequently mutated and disrupted in cancer.

The Tp53 gene, encoding for the tumour suppressor protein p53, has, for a long time, been regarded a prime example illustrating the crucial relationship between DNA responses and tumour suppression (Lane 1992; Vogelstein et al. 2000). Tp53 is the most frequently mutated gene in cancer and a central player in cellular DNA damage responses (Kandoth et al. 2013; Vousden and Lane 2007). Numerous studies have demonstrated that p53 can induce transient cell cycle arrest,

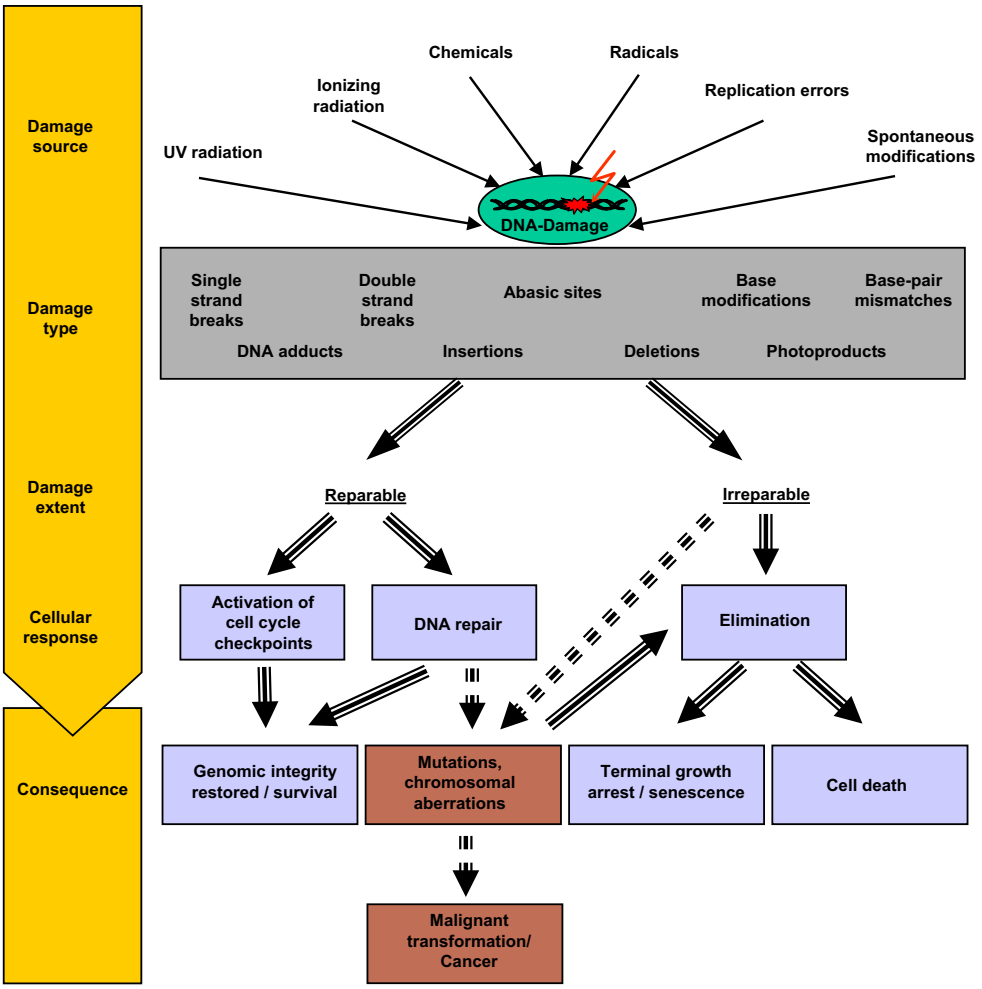

Fig. 1 Cellular responses to DNA damage. Different sources induce different types of DNA damage. Depending on the type and extent of damage cells respond in two fundamentally different ways. Transient activation of cell cycle checkpoints coupled with DNA repair can restore genomic integrity. Such cells survive and resume their normal life cycle. In cases where repair is not completely accurate and/or the damage too severe, cells can be eliminated from the organism by cell death programmes. Alternatively, their growth is irreversibly stopped by the senescence programme (terminal growth arrest). If DNA repair is not accurate and elimination programmes fail, mutations and chromosomal aberrations manifest in the genome. This can result in malignant transformation and cancer

senescence and apoptosis in response to genotoxic stress, while cells deficient for p53 show significant defects in their response to DNA damage (Vousden 2006; Vousden and Prives 2009). There is also no doubt on the potent tumour-suppressive function of p53 which is revealed impressively by, for example, the fact that p53-null mice die quickly and with nearly 100 % penetrance of cancer (mostly thymic lymphomas) at around 6 months of age (Donehower et al. 1992; Jacks et al. 1994; Kemp et al. 1994; Purdie et al. 1994). Moreover, patients suffering from Li-Fraumeni syndrome, a condition often associated with germline p53 mutations, have a much higher incidence of developing cancer than the normal population (Malkin et al. 1990).

For the best part of the last thirty-five years since the discovery of p53, DNA damage-response-related functions of p53 have been regarded the primary if not sole relevant mechanism to exert its potent tumour-suppressive effect. However, as it turns out, this view was too simplistic. Indeed, a number of recent studies described efficient p53-mediated tumour suppression in vivo despite severe defects in the mechanisms or genes involved in p53-mediated DNA damage responses. In this article, I will review data supporting the different views on the importance of p53-mediated DNA damage responses with a focus on cancer therapy and tumour suppression and discuss the implications for our understanding of p53 and its role in cancer biology.

Activation of p53 by DNA damage

The idea that p53 is involved in cellular DNA damage responses relates back to observations made in the 1980s

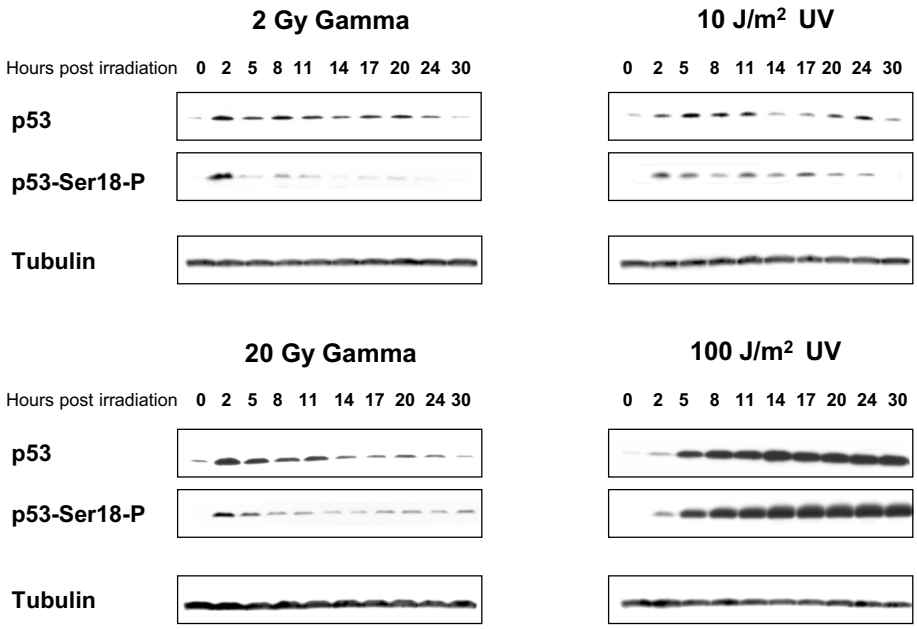

Fig. 2 DNA damage-induced activation of p53. Upregulation of p53, primarily caused by stabilization of the protein, and phosphorylation at serine 15 (serine 18 in mouse) are hallmarks of DNA damage-induced activation of p53 and can be assayed by Western blot analysis. Shown here are Western blot analyses of NIH3T3 cells treated with a low and high dose of UV and ionizing radiation, respectively. Note that the extent and type of DNA damage have an effect on the extent and kinetics of p53 stabilization and phosphorylation. Figure modified from Speidel et al. (2006)

and 1990s, showing that p53 is massively upregulated by ultraviolet (UV) light (Maltzman and Czyzyk 1984), ionizing radiation (Kastan et al. 1991; Lowe et al. 1993b) and chemotherapeutic agents (Fritsche et al. 1993), all causing different types of DNA damage. Upregulation of p53 occurs rapidly (within 30–60 min) and mainly at the level of the protein, which is stabilized (Fritsche et al. 1993; Kastan et al. 1991; Maltzman and Czyzyk 1984). Many years later, we know that more or less all DNA damaging agents (and beyond that, many other stimuli that cause cellular stress) lead to p53 upregulation.

In addition to being stabilized and upregulated, p53 is also heavily modified upon DNA damage, including phosphorylation of serine 15 by the ATM and ATR kinases [reviewed in (Meek and Anderson 2009)]. These two events (stabilization and phosphorylation at serine 15) are well-established measures of DNA damage-induced p53 activation and can be assayed by Western blot analysis (Fig. 2). Moreover, phosphorylation of p53 contributes to its upregulation, because DNA damage-induced stabilization of p53 is primarily caused by disruption of its complex with Mdm2—a ubiquitin E3 ligase that targets p53 for proteasomal degradation (Haupt et al. 1997; Kubbutat et al. 1997). In normal unstressed cells, p53 protein levels are kept very low, providing an efficient way to keep p53 in check. Post-translational modifications of both p53 and Mdm2, including phosphorylation of p53 at serine 20 and phosphorylation of Mdm2 at serine 395, are rapidly triggered upon DNA damage, disrupt the p53-Mdm2 complex and inhibit Mdm2 by other means (Chehab et al. 1999; Cheng et al. 2009; Hu et al. 2012; Maya et al. 2001; Momand et al. 1992; Oliner et al. 1993; Shieh et al. 1997). Because p53 is then no longer targeted for proteasomal degradation, its levels rise quickly. However, it should be noted that p53 has also some activity in unstressed cells, as reflected by its presence at the p21 promoter and the different expression profiles of unstressed isogenic wild-type and p53-null cell pairs (Allen et al. 2014; Espinosa et al. 2003; Tang et al. 1998). Notably, too, some cancerous cell lines display readily detectable levels of p53 protein without showing obvious signs of its anti-proliferative activity, cell cycle arrest or apoptosis in particular. Together, this suggests that the pure abundance of the p53 protein is not the sole determinant of its activity. Nevertheless, upregulation of wild-type p53 in response to DNA damage is normally a reliable indicator of its activation.

Usually, p53 activity relates to changes in expression of p53-responsive target genes. A protein that can bind DNA in a sequence-specific manner (Bargonetti et al. 1991; El-Deiry et al. 1992; Kern et al. 1991), p53 transactivates or represses an ever growing list of genes and micro-RNAs that operate in numerous pathways, including apoptosis, cell cycle arrest, DNA repair, metabolism, autophagy, differentiation and others (Vousden and Prives 2009). The direct role of p53 in inducing any of these significant cell fate-determining processes has been confirmed in many experimental systems, most simply by overexpression and knock-down studies. These studies also highlighted the importance of the context, as overexpression of p53 would, for example, induce apoptosis in some cell systems but cell cycle arrest in others (Agarwal et al. 1995; Chen et al. 1996; Liu et al. 1995; Polyak et al. 1996; Shaw et al. 1992; Stewart et al. 1995; Yonish-Rouach et al. 1991).

Transcriptional functions are clearly the main mode of action for the p53 protein against which the majority of

cancer cells select. Indeed, most p53 mutations found in human cancers affect codons in the central DNA binding domain of p53 and thus impair its transcriptional activity (Olivier et al. 2010). However, it is worth noting that p53 exerts activity also through non-transcriptional mechanisms, in particular protein–protein interactions. These activities have been particularly well characterized in the context of apoptosis induction (Speidel 2010). As discussed further below, p53 activities triggered by DNA damage have high relevance from a clinical perspective. This applies not only to their role in tumour suppression but also in the context of cancer therapy with cytotoxic agents, most of which trigger activation of p53.

P53-Mediated apoptosis

P53 can be a strong inducer of apoptosis in response to DNA damage. This function is dependent on the cell type as well as type and extent of the DNA damaging agent. Thus, the apoptotic function of p53 becomes most obvious in cell systems that are completely dependent on functional wild-type p53 for apoptosis in response to certain stressors. Mouse thymocytes provide a prominent example. These cells respond to ionizing radiation with massive apoptosis but only when harbouring wild-type p53 (Clarke et al. 1993; Lowe et al. 1993b). Similarly, human colon carcinoma cells (HCT116 cell line) undergo apoptotic cell death when treated with 5-fluorouracil, but an isogenic p53-deficient cell line is protected (Bunz et al. 1999). Clearly, however, apoptosis can occur also in the absence of p53, and in many cases, the loss of or inactivating mutations in p53 will even increase susceptibility of cells to apoptosis induced by cytotoxic agents. This can be seen in fibroblasts receiving high-dose gamma irradiation or the above-mentioned HCT116 cells after treatment with adriamycin (Bunz et al. 1999; Speidel et al. 2006). In these systems, wild-type p53 promotes a pro-survival response, cell cycle arrest/senescence in particular (discussed further below) whereas p53-null cells die (Bunz et al. 1999; Speidel et al. 2006). There will also be cases where a certain stressor will induce apoptosis in a particular type of cell regardless of the presence of wild-type p53. However, the presence of functional wild-type p53 may modulate the signalling pathways leading to cell death and sometimes change the efficiency of apoptotic cell destruction. For example, high-dose UV-irradiation induces apoptosis in mouse fibroblasts with wild-type p53, p53-null or mutant p53 genotype, but only wild-type and certain mutant p53 lines will show activation of Bax as measured by its translocation and aggregation in the outer mitochondrial membrane (Speidel et al. 2006). Furthermore, the extent of apoptotic cell death in a panel of human lymphoma cell lines that were treated with chemotherapeutic drugs depended on the cells' p53 status with wild-type p53-expressing lines being generally more sensitive than mutant p53 or p53-deficient cell lines (Fan et al. 1994). Together, the exact circumstances, i.e. type of cell, stress and other signalling pathways, will determine whether or not p53 will induce apoptosis in response to DNA damage.

Mechanistically, p53 can promote apoptosis through three distinct ways: transcriptional activation, transcriptional repression and transcription-independent mechanisms.

Among p53-responsive target genes that are upregulated by p53 and known to promote apoptosis are Puma (Nakano and Vousden 2001; Yu et al. 2001), Noxa (Oda et al. 2000a) and Bax (Miyashita and Reed 1995 (all belonging to the Bcl-2 family—the master controller of the intrinsic pathway of apoptosis), CD95/Fas (Muller et al. 1998; Owen-Schaub et al. 1995) and DR5 (Wu et al. 1997 (part of the extrinsic receptor-mediated pathway of apoptosis) and other factors, including Apaf1, Perp and Pidd (Attardi et al. 2000; Lin et al. 2000; Moroni et al. 2001; Robles et al. 2001). Puma and Noxa appear as the major target genes mediating DNA damage-induced and p53-dependent apoptosis as demonstrated by a number of knockout studies in which deficiency in these genes caused protection against apoptotic cell death (Jeffers et al. 2003; Nakano and Vousden 2001; Oda et al. 2000a; Shao et al. 2010; Villunger et al. 2003; Yu et al. 2003, 2010). Significantly, fibroblasts and thymocytes derived from Puma/Noxa double knockout mice showed similar resistance to apoptosis as cells from p53-null mice when treated with agents that induced p53-mediated cell death (Michalak et al. 2008).

In addition to the effects related to upregulation of proapoptotic target genes, p53 promotes apoptosis by direct protein–protein interactions with members of the Bcl-2 family at the cytosol and mitochondrial membrane (Chipuk et al. 2004; Leu et al. 2004; Mihara et al. 2003). These non-transcriptional activities lead to activation of either Bax or Bak and thereby promote mitochondrial membrane permeabilization and apoptosis (Speidel 2010). Because p53 accumulates primarily in the nucleus after genotoxic stress (Fritsche et al. 1993), it seems that a high extent of p53 stabilization leading to high cellular levels of p53 protein is required for the cytosolic and mitochondrial function (Speidel et al. 2006). This mode of apoptosis induction may be specifically used under certain physiological conditions, such as in response to high-dose UV-irradiation (Speidel et al. 2006) when it would be fatal if a severely damaged cell required normal operation of transcription and translation to carry out the apoptotic programme. In addition, it may also be employed to complement and augment the transcriptional response of p53 (Chipuk et al. 2005; Erster et al. 2004).

The significance of p53-mediated transcriptional repression in apoptosis induction is not completely clear. However, p53 response elements are present at the promoters of at least two bona fide anti-apoptotic factors, Bcl-2 and survivin, and both genes have been shown to be negatively regulated by p53 (Haldar et al. 1994; Hoffman et al. 2002; Mirza et al. 2002; Miyashita et al. 1994). Presumably more significant, p53 regulates microRNAs and thereby represses the expression of genes in an indirect manner [reviewed in (He et al. 2007b; Hunten et al. 2013; Otsuka and Ochiya 2014)]. Members of the mir34 family (mir34a, in particular) have clear relevance for p53-mediated apoptosis induction, as they are upregulated upon DNA damage in a p53-dependent manner and silence several bona fide anti-apoptotic genes, including Bcl-2 (Bommer et al. 2007; Chang et al. 2007; He et al. 2007a; Raver-Shapira et al. 2007; Tarasov et al. 2007). Consistently, downregulation of miR34-a attenuated p53-dependent apoptosis, whereas overexpression could promote cell death in some cell systems (Chang et al. 2007; Raver-Shapira et al. 2007; Tarasov et al. 2007). However, cells derived from miR34-deficient mice showed a normal p53 response suggesting that gene regulation through the miR-34 family provides a redundant form of regulating DNA damage responses (Concepcion et al. 2012).

P53-Mediated cell cycle arrest, senescence and DNA repair

While some cells respond to high p53 levels with apoptosis, numerous others will stop their growth, either transiently or permanently. Like with apoptosis, this can be observed in systems with enforced overexpression of p53 but also in response to DNA damage (Agarwal et al. 1995; Di Leonardo et al. 1994; Heinlein et al. 2010; Speidel et al. 2006; Stewart et al. 1995; Sugrue et al. 1997; Taylor et al. 1999). Which parameters decide whether activated p53 induces transient cell cycle arrest, permanent growth arrest (senescence) or cell death is still not fully understood—despite much effort [reviewed in (Carvajal and Manfredi 2013)]. However, several factors, including p53-levels, modification, type and extent of stress, co-factors, microenvironment and/or cell type specifics appear to play a role (Carvajal and Manfredi 2013; Chen et al. 1996; Das et al. 2007; Kracikova et al. 2013; Oda et al. 2000b; Speidel et al. 2006). There is also some evidence that in many cell types, cell cycle arrest is the primary p53 response with the induction of (p53-dependent) apoptosis being more 'difficult' and requiring additional signals, such as higher p53 concentrations and cooperative binding of several p53 tetramers (Chen et al. 1996; Kracikova et al. 2013; Schlereth et al. 2010; Speidel et al. 2006).

Traditionally, cell cycle arrest has been seen as an immediate response to DNA damage that gives cells time to conduct DNA repair and, if this is not successful, targets cells to either apoptosis or halts the cell cycle permanently by the senescence programme (Massague 2004; Medema and Macurek 2012; Weinert and Hartwell 1988). DNA repair activities have therefore been associated with cell cycle arrest, and several proteins, including the p53 targets p21 and Gadd45a, that induce cell cycle arrest have also distinct functions in DNA repair (McDonald et al. 1996; Smith et al. 1994, 2000; Stivala et al. 2001).

P53 can impair progression through the cycle by several mechanisms and induce arrest at the G1/S border (G1 arrest) and the G2/M border (G2 arrest) as detailed below. In addition, p53 can have an impact on S-phase and attenuate replication (Dutta et al. 1993; Ogryzko et al. 1997; Waga et al. 1994; Zhou and Prives 2003). While p53-dependent transient cell cycle arrest is already triggered by relatively low or moderate damage, more severe damage can either result in apoptosis or a terminal growth arrest, also known as cellular senescence. The latter can be observed in a variety of cells derived from solid tumours after treatment with DNA damaging drugs and is driven to a major part by p53 and its target gene p21 (Chang et al. 1999a, b). Cellular senescence is also induced by high-dose gamma irradiation in human and mouse fibroblasts where it seems completely dependent on functional wild-type p53 and p21 (Speidel et al. 2006; Suzuki et al. 2001). Moreover, there is clear evidence for p53-dependent senescence occurring as a DNA damage response in vivo as demonstrated, for example, in Eμ-myc-driven lymphomas treated with the chemotherapeutic agent cyclophosphamide (Schmitt et al. 2002).

A transient arrest in response to DNA damage stops cells at the G1/S and/or G2/M borders, thereby preventing the amplification of damaged material by replication and cell division, respectively. P53 has been shown to promote cell cycle arrest in G1 and G2 through upregulation of several micro-RNAs, including miR-34a-c and miR-192/215. In turn, these micro-RNAs repress a number of important factors necessary for cell cycle progression resulting in transient cell cycle arrest or senescence (Braun et al. 2008; Georges et al. 2008; He et al. 2007a; Tarasov et al. 2007).

P53 has a particularly crucial role in DNA damage-induced G1 arrest. This is reflected by the fact that fibroblasts that lack functional wild-type p53 are also deficient in displaying G1 arrest in response to DNA damage (Kastan et al. 1992; Lowe et al. 1993a). The same phenotype is observed in cells (MEFs and HCT116) lacking the p53 target gene p21 (Brugarolas et al. 1995; Deng et al. 1995; Waldman et al. 1995). Hence, p53-mediated transcriptional upregulation of p21 is essentially required for DNA damage-induced G1 arrest, at least in cell culture

settings (Dulic et al. 1994; El-Deiry et al. 1993, 1994). P21 is an inhibitor of cyclin-dependent kinases (CDKs) which are the drivers of cell cycle progression (Xiong et al. 1993). In particular, p21 strongly inhibits the Cyclin 2-CDK2 kinase which promotes transition from G1 into S-phase (Harper et al. 1993). Upregulation of p21 that is induced by p53 in response to DNA damage therefore leads to an efficient G1 arrest (Dulic et al. 1994; El-Deiry et al. 1993, 1994). In addition, another target gene, Ptprv, encoding for a transmembrane tyrosine phosphatase contributes to p53-induced G1 arrest in response to DNA damage and its deficiency resulted in a compromised ability of MEFs to induce G1 arrest in responses to gamma radiation (Doumont et al. 2005). However, the mechanism by which Ptprv exerts its cell cycle regulatory function remains to be elucidated.

P53 plays also a role in inducing G2 arrest, and this is mediated by upregulation of various target genes, including p21, Gadd45alpha, 14-3-3σ, Btg2, PCBP4/MCG10, GTSE-1/B99 and reprimo (Bunz et al. 1998; Hermeking et al. 1997; Kastan et al. 1992; Niculescu et al. 1998; Ohki et al. 2000; Rouault et al. 1996; Utrera et al. 1998; Zhu and Chen 2000). The mechanisms by which upregulation of these target genes effect G2 arrest are very diverse and include interactions with CDK1 (Gadd45alpha and Btg2; Ryu et al. 2004; Wang et al. 1999; Zhan et al. 1999), sequestration of Cyclin B1 and CDK1 (14-3-3σ; Chan et al. 1999), regulation of p21 mRNA stability (PCBP4/MCG10; Scoumanne et al. 2011) as well as other yet to be clarified means. In addition, there is evidence that p53 inhibits G2-M progression through direct repression of genes such as CDK1, Cyclin B1, cdc25 and others (Azzam et al. 1997; de Toledo et al. 1998; Passalaris et al. 1999; Spurgers et al. 2006; St Clair et al. 2004; Taylor et al. 1999). Moreover, p53 represses cell cycle progression also through upregulation of several microRNAs as mentioned before. Clearly, however, induction of G2 arrest in response to DNA damage does not require p53 and can be observed in p53-deficient cells (Bunz et al. 1998; Hirose et al. 2001; Kastan et al. 1991; Passalaris et al. 1999). Nevertheless, p53 and its target genes p21 and 14-3-3σ appear to be required to sustain an arrest in G2 (Bunz et al. 1998; Chan et al. 1999; Hirose et al. 2001).

The importance of p53 for DNA repair is reflected by the defects of p53-deficient cells. This was first noted in UV-irradiated fibroblasts where damage is repaired via the nucleotide excision repair (NER) pathway and UV-induced photo-products persist longer when p53 is not functional (Ford and Hanawalt 1995, 1997; Smith et al. 1995; Therrien et al. 1999; Wang et al. 1995). Similarly, compromised p53 function affected removal of DNA-adducts induced by tobacco carcinogens (Lloyd and Hanawalt 2000, 2002; Wani et al. 2000). However, p53 is also involved in the base excision repair (BER) and mismatch repair pathways (Chen and Sadowski 2005; de Souza-Pinto et al. 2004; Offer et al. 1999, 2001a, b; Scherer et al. 2000; Seo et al. 2002; Zhou et al. 2001; Zurer et al. 2004). Similarly, p53 regulates the repair of DNA double-strand breaks by homologous recombination (HR) and non-homologous end-joining (NHEJ) [reviewed in (Gatz and Wiesmuller 2006; Sengupta and Harris 2005)]. Of note, p53 can either suppress or promote DNA repair activities, revealing a somehow irritating complexity. This becomes particularly obvious with regard to the NHEJ processes that repair most DNA double-strand breaks in mammalian cells (Lieber et al. 2003). Here, p53 was shown to either stimulate (Lin et al. 2003; Tang et al. 1999; Yang et al. 1997) or inhibit (Akyuz et al. 2002; Bill et al. 1997; Bristow et al. 1998; Dahm-Daphi et al. 2005; Okorokov et al. 2002) DNA end-joining. Similarly, p53 would either enhance or reduce the activity of 3-methyladenine (3-MeAde) DNA glycosylase, an important enzyme in the BER pathway, and this was dependent on the type of DNA damage (Zurer et al. 2004). In addition, the phosphatase Wip1, also known as PPM1D, is induced by p53 in response to genotoxic stress and inhibits BER (Lu et al. 2004), although there is compelling evidence from several laboratories that p53 stimulates BER (Offer et al. 1999; Seo et al. 2002; Zhou and Prives 2003). Clearly, there are plausible reasons as to why a tumour suppressor would not only facilitate DNA repair but suppress it under certain conditions. The latter makes sense to efficiently kill cells after severe DNA damage, limit error-prone repair and/or switch off increased repair activity at completion of the DNA damage response.

Besides facilitating DNA repair by inducing and maintaining cell cycle arrest, p53 can promote DNA repair directly by upregulating the expression of DNA repair genes. These include Gadd45a (Smith et al. 1994), DBB2 (Hwang et al. 1999), XPC (Adimoolam and Ford 2002), p53R2 (Nakano et al. 2000; Tanaka et al. 2000), KARP-1 (Myung et al. 1998), thymine DNA glycosylase (da Costa et al. 2012) and MGMT (Grombacher et al. 1998; Rafferty et al. 1996). Notably, MGMT, a gene that is essential for converting the mutagenic DNA lesion O^6-methylguanine back to guanine, can also be downregulated by p53 as a means to efficiently kill cells in response to DNA damaging agents, alkylating drugs in particular (Grombacher et al. 1998; Harris et al. 1996). Similarly, p53 can suppress transcription of 3-methyladenine (3-MeAde) DNA glycosylase, an important enzyme in the BER pathway, in response to nitric oxide (Zurer et al. 2004). In addition to the mentioned genes, p53-responsive elements have also been identified in other genes related to DNA repair, including FANCC (Liebetrau et al. 1997), MSH2 (Scherer et al. 1996, 2000), MLH1 (Chen and Sadowski 2005) and PMS2 (Chen and Sadowski 2005; Gatz and Wiesmuller 2006).

Equally important for its role in DNA repair are transcription-independent activities of p53 (Sengupta and Harris 2005). P53 facilitates DNA repair in particular through direct protein–protein interactions with repair proteins such as the helicases XPB and XPD (Wang et al. 1995), the ribonucleotide reductase p53R2 (Xue et al. 2003), DNA polymerase beta (Zhou et al. 2001) and the homologous recombination factor RAD51 (Linke et al. 2003; Sturzbecher et al. 1996). In addition, p53 was shown to bind to damaged DNA in vitro in a non-sequence-specific manner via its C-terminus (Jayaraman and Prives 1995; Lee et al. 1995; Reed et al. 1995). Furthermore, p53 binds Holliday junctions and heteroduplex joints, which are both intermediate DNA structures of homologous recombination (Dudenhoffer et al. 1998; Janz and Wiesmuller 2002; Lee et al. 1997). Such binding of p53 to 'unusual' DNA structures has been associated with DNA repair; however, the underlying mechanisms remain incompletely understood. Much of this is caused by limitations in the methodology to assess the repair of chromatin-packed DNA in vivo.

The role of p53 responses in cancer therapy

The vast majority of cancer patients receive treatment with DNA damaging agents such as radiation and chemotherapeutic drugs. The preferred result of this treatment is the induction of cancer cell death as this outcome irreversibly eliminates these cells from the body. Most of the clinically used cytotoxic agents will activate p53 and induce p53-mediated DNA damage responses in cancer cells expressing wild-type p53. Because the vast majority of p53-mutations found in cancer patients impair the proper function of the p53 protein (Olivier et al. 2010), it is to expect that the p53 status has an influence on the outcome of cancer therapy. While early xenograft studies in mice have shown a clear correlation between p53 status and apoptotic cell death after treatment with adriamycin or gamma radiation (Lowe et al. 1994), the question of whether human wild-type p53 cancers respond generally better to therapy than cancers with mutated or deleted p53 does not have an easy general answer. This has mainly two reasons: as discussed before, p53 can either promote or suppress cell death with the outcome depending on the cell type and therapeutic agent. Secondly, the term 'mutant p53' describes a quite heterogeneous group of proteins that have biological activity themselves (Brosh and Rotter 2009). Although the vast majority of mutations found in cancer patients cluster in the DNA binding domain of p53 and hence impair its transcriptional activity, the exact type of mutation can make significant differences with regard to cellular DNA damage responses. Some mutations leave the p53 proteins with residual wild-type p53 activity. This could, for instance, mean that apoptotic functions are compromised but the ability to induce cell cycle arrest is retained (Ludwig et al. 1996; Rowan et al. 1996). Moreover, some mutations result in a 'gain of function', being it unique transactivation activities (e.g. induction of the multidrug resistance 1 gene by mutant p53 (Chin et al. 1992)) or interactions with other cellular proteins, such as p63, p73 or AMP-activated protein kinase (Brosh and Rotter 2009; Lozano 2007; Zhou et al. 2014). Together, this complexity makes it difficult to dissect which effects with regard to cancer therapy are truly related to a loss of wild-type p53 function rather than a consequence of biological activity exerted by the various mutated p53 proteins. Consequently, the real impact of p53 for the success of cancer therapy is not finally understood yet.

Many clinical studies have been conducted to determine whether and for which cancers the p53 status has prognostic and/or predictive relevance and this has been reviewed in detail by Brosh and Rotter (2009) and Tchelebi et al. (2014). A full, annotated and regularly updated list of studies that have assessed p53 mutations and their association with prognosis is available at the IARC Tp53 database (http://p53.iarc.fr/) (Petitjean et al. 2007). Of note, many older studies must be interpreted with caution. These studies would have determined the p53 status simply by means of the staining intensity in immunohistochemical analysis of tumour samples. An established practise in times when DNA sequencing was not as easy and cost-effective as it is today, this approach followed the simplified and therefore sometimes false concept that mutant p53 is more stable and thus more abundant than wild-type p53. More recent studies that used sequencing to determine the p53 status provide a more reliable basis for analysis of the prognostic value of p53.

As it turns out, there is a clear trend for certain cancer types, suggesting that mutations in p53 indeed segregate with poor prognosis. This is true for haematologic malignancies as well as cancers of the breast and colorectum (IARC database, version R17 from November 2013; Petitjean et al. 2007). Not surprisingly, this association is weaker for other tumour types, such as cancers of the bladder, brain or lungs. Inconsistencies and conflicting results may be caused by looking at different groups of tumours (e.g. 'non-small cell lung cancer' versus 'lung adenocarcinoma'), different treatment regiments or differences in other yet to be clarified parameters. Clearly, some studies do not support an influence of p53 status on overall patient survival and therapy response at all. Notably too, for many cancers, the prognostic impact of p53 mutations has not been thoroughly studied at all. Table 1 shows the number of studies on the prognostic value of p53 mutations and their results as downloaded from the most current version (R17 from November 2013) of the IARC database (Petitjean et al. 2007).

Table 1 Studies investigating the prognostic value of Tp53 mutations in various cancers

Tumour site	Number of studies reporting that TP53 mutations are		
	Related to bad prognosis	Related to good prognosis	Not related to prognosis
Bladder	5	–	4
Bones	1	–	1
Brain	3	2	4
Breast	29	1	6
Colorectum	16	–	8
Oesophagus	2	–	2
Head and neck	9	–	5
Haematologic	12	–	–
Liver	3	–	–
Lungs	8	–	7
Ovary	7	1	3
Pancreas	1	1	1
Prostate	1	–	1
Soft tissues	2	–	–
Stomach	1	–	2
Renal pelvis	1	–	–
Uterine corpus	2	–	1

Only studies that determined the p53 status by sequencing and had a cohort size of 50 or more patients were included. Data summary derived from the IARC Tp53 database (version R17 from November 2013, accessed December 2014; Petitjean et al. 2007)

Whereas a considerable number of studies have looked at the relationship between p53-status and survival, less is known about the impact that p53 mutations have on the direct and immediate response to chemotherapy. The IARC database (version R17) lists only 38 peer-reviewed studies that have explicitly looked at the response to chemotherapy in vivo. Eleven studies concern breast cancer and seven colorectal/rectum cancer, whereas other malignancies have been analysed by less than five studies each. A closer look at the breast cancer studies reveals strong heterogeneity in the patient cohorts, treatment regimens and measures of chemotherapy response or resistance (Table 2). These differences can explain some apparent discrepancies in the results. More importantly, this comparison highlights the need for more studies that assess therapy resistance in a standardized manner. This will be an essential requirement to decide on optimal therapy for individual patients. Notwithstanding these limitations, it seems clear that the disruption of p53 has a measurable and mostly negative impact on the success of cancer therapy in many cases.

While conventional chemo and radiation therapies are still the most important treatment for the majority of cancer patients, considerable efforts are made to substitute or complement these therapies with non-genotoxic agents. In this context, p53 has been regarded as an extremely promising target (Brown et al. 2009). Ideally, pharmacologic induction of p53-mediated DNA damage responses in a non-genotoxic way could induce apoptosis but reduce the side effects of treatment with conventional radiation and chemotherapy. Currently, more than 20 different p53-activating drugs are under pre-clinical and clinical evaluation and pursued by different companies [reviewed in (Khoo et al. 2014)]. These agents aim to either re-activate mutant p53 and thereby restore wild-type p53 functions in mutant p53-expressing cells or activate p53 in cancer cells expressing wild-type p53. The majority of drugs currently in clinical trials (all phase 1) are so-called Mdm2 antagonists (Khoo et al. 2014). These drugs disrupt the interaction of p53 and Mdm2 and thereby trigger stabilization and activation of wild-type p53. Although very promising, it is important to note that use of some of these drugs (e.g. nutlin-3) but not all of them (e.g. RITA) may also induce de novo p53 mutations and drug resistance, as demonstrated in tissue culture experiments (Aziz et al. 2011; Cinatl et al. 2014; Michaelis et al. 2011, 2012).

The role of p53-mediated DNA damage responses in tumour suppression

Any cellular programme that restricts the growth of cells with potentially mutagenic lesions and restores genomic integrity qualifies in principle as tumour suppressive. Because p53 triggers many of these programmes (transient cell cycle arrest, DNA repair, apoptosis and senescence), it seemed rather safe to assume that these activities of p53 (all of them being classic DNA damage responses) were responsible for its tumour suppressor function. Indeed, this concept remained unchallenged until recently. Many animal models were created to prove which target gene and/

Table 2 Association of TP53 mutations with response to chemotherapy in breast cancer

Reference	Cohort size	Cohort	Treatment	Measure of therapy success	P53 mutations associated with
Chrisanthar et al. (2008)	109	Primary, locally advanced breast cancer	Epirubicin	Anything other than progressive disease after therapy	Therapy resistance
Bertheau et al. (2007)	80	Noninflammatory breast cancers	Epirubicin + cyclophosphamide (neoadjuvant)	Complete response	Good therapy response
Kandioler-Eckersberger et al. (2000)	35	Advanced breast cancer (T3, T4)	5-FU + Epirubicin + cyclophosphamide (neoadjuvant)	Partial or complete response (Tumour diameter)	Therapy resistance
Kandioler-Eckersberger et al. (2000)	32	Advanced breast cancer (T3, T4)	Paclitaxel	Partial or complete response (Tumour diameter)	Good response
Harris et al. (2006)	145	Advanced metastatic breast cancer	Paclitaxel	Partial or complete response	No association
Geisler et al. (2003)	35	Locally advanced breast cancer	5-FU + mitomycin (neoadjuvant)	Anything other than progressive disease after therapy	Therapy resistance
Geisler et al. (2001)	90	Locally advanced breast cancer	Doxorubicin	Partial or complete response (Tumour diameter)	Therapy resistance (significant only for mutations affecting the L2/L3 domain)
Berns et al. (2000)	202	Advanced breast cancer	Tamoxifen	Partial or complete response or prolonged stable disease	Therapy resistance
Berns et al. (2000)	41	Advanced breast cancer	Various up-front treatments, including CMF (22 patients), CAF (16 patients and platinum-containing therapy (2 patients)	Partial or complete response or prolonged stable disease	Therapy resistance

Only studies that determined the p53 status by sequencing and had a cohort size larger than 30 patients were included. Studies that suggest an association between p53 mutations and therapy resistance are shaded. Data are derived from the IARC Tp53 database (version R17, November 2013; Petitjean et al. 2007)

or programme regulated by p53 is responsible for its powerful tumour-suppressive effect. In these models, either target genes of p53 were deleted or the p53 gene itself mutated to impair some or all of its transcriptional activities. The details on most relevant animal models addressing p53-mediated tumour suppression have been summarized and expertly reviewed in two excellent articles (Bieging and Attardi 2012; Bieging et al. 2014) and hence do not need to be replicated here. In the paragraphs below, I rather focus on the aspects relevant for the role of p53-controlled DNA damage response programmes.

When assessing the tumour-suppressive effect of individual p53 targets and/or programmes in vivo, three approaches/read-outs were used. Firstly, spontaneous tumour formation was assessed. Here, p53-null mice provide the benchmark. These mice succumb to cancer with almost 100 % penetrance around 6 months of age with most of them developing thymic T cell lymphoma and occasionally B cell lymphoma or sarcoma (Donehower et al. 1992; Jacks et al. 1994; Kemp et al. 1994; Purdie et al. 1994). Consequently, mouse models with a deficiency in a target gene/cellular programme regulated by p53 were studied with regard to the onset of early thymic lymphoma development. The second approach relies on mouse models where the formation of cancer is driven by oncogenes including Eμ-myc (B cell lymphoma), $Kras^{G12D}$ (non-small cell lung cancer), SV40 T-antigen/T_{121} (brain cancer) or other gene defects that confer predisposition to cancer and where p53 is known to counteract tumorigenesis. Here, enhanced tumorigenesis or quicker progression of

cancer (relative to wild-type control animals) would reveal the contribution of a specific p53-regulated target gene/mechanism to tumour suppression. The third approach is of immediate significance when discussing the role of DNA damage responses because it directly uses DNA damage, mostly gamma irradiation applied to very young mice (2 days–7 weeks old) but also UV-irradiation and chemicals, as a cancer-initiating stimulus. Significantly, irradiation of mice heterozygous (p53+/−)or deficient for both alleles of p53 (p53−/−) with low doses of ionizing radiation (1–4 Gy) showed a marked acceleration of tumorigenesis when compared to unirradiated controls (Kemp et al. 1994). Hence, this early study provides one of the clearest and most direct demonstrations for the importance of p53-mediated DNA damage responses for tumour suppression in vivo. However, in this context, it must be noted that the immediate response to acute DNA damage triggered by p53 might actually not contribute much to tumour suppression as suggested by studies with mouse models, in which p53 could be selectively activated and/or inactivated at different time points before or after irradiation (Christophorou et al. 2006; Hinkal et al. 2009). Both studies reported that the absence or presence of p53 *during* radiation treatment had no effect on radiation-induced lymphoma latency. Rather, p53-activity is required at much later time points to counteract the proliferation of abnormal cells with activated oncogenes. Therefore, persistent low levels of DNA damage and/or oncogene activation—both of which can of course be a consequence of a more severe genotoxic insult—appear crucial to trigger p53-mediated suppression of radiation-induced tumours in mice (Christophorou et al. 2006; Hinkal et al. 2009).

Conclusions that appear as conflicting at the first view have been drawn regarding the significance of the classic DNA damage response programmes apoptosis, cell cycle arrest and senescence for p53-mediated tumour suppression. At least in parts, this is due to the different experimental approaches used to dissect the importance of individual p53-regulated target genes and mechanisms for tumour suppression. However, two things seem clear: (1) the transcriptional activity of p53 is crucially important for its tumour suppressor function. This was demonstrated most compellingly in mice expressing a transcriptionally dead mutant p53 ($p53^{25,26,53,54}$). These mice, in which both transactional activation domains (TADs) of the trp53 gene were mutated, resembled p53-null animals and did not show any protection against cancer formation (Brady et al. 2011; Jiang et al. 2011). (2) None of the existing knockout mice with deficiency in one or more p53-responsive target genes fully phenocopies p53-null mice (Bieging and Attardi 2012). Therefore, the most important target(s) with regard to tumour suppression have not been identified yet, or p53-mediated tumour suppression is achieved by the combination of the many processes and genes controlled by p53.

Clearly, there is strong evidence supporting an important contribution of the classic DNA damage response programmes to p53-mediated tumour suppression. For example, such evidence comes from mice, in which the p53 gene was mutated to impair its apoptotic activity but not its ability to induce cell cycle arrest, senescence and other effector programmes in response to DNA damage. This was done in independent laboratories using different strategies. The team around Gigi Lozano engineered a mutp53 R172P mouse that is the homologue of a missense point mutation found in cancer patients (R175P; Liu et al. 2004). Apoptosis deficiency was achieved by another modification in the Braithwaite laboratory. Here, mice expressing a deletion mutant of p53 (mΔpro p53) that lacks the proline-rich domain were studied (Slatter et al. 2010). Similar to the mutp53 R172P model, cells from these mice could induce p53-mediated cell cycle arrest but not apoptosis in response to DNA damage (Liu et al. 2004; Slatter et al. 2010). A third and again different approach was taken recently by a team led by Thorsten Stiewe. They generated mice expressing a cooperativity mutant of p53 ($p53^{E177R}$) (Timofeev et al. 2013). This mutant p53 protein is impaired for interactions between adjacent p53 DNA binding domains in the tetrameric p53-DNA complex and thereby defective in transactivating proapoptotic target genes (Schlereth et al. 2010). When compared to p53-null mice, all of these mutant p53 mice (R172P, mΔpro p53 and $p53^{E177R}$) showed a longer latency and reduced incidence of thymic lymphoma but eventually succumbed to other malignancies (Liu et al. 2004; Slatter et al. 2010; Timofeev et al. 2013). Together, these studies support the conclusion that apoptotic activities are important for p53-mediated tumour suppression in vivo because the deficiency in p53 apoptosis results in some form of cancer. However, the complete or partial protection against spontaneously developing thymic lymphoma in these mouse models (R172P, mΔpro p53 and $p53^{E177R}$) highlights the fact that other activities of p53 (not apoptosis) are responsible for suppressing this type of cancer in mice.

In addition to apoptosis, also cell cycle arrest and senescence were shown to contribute to p53-mediated tumour suppression. For example, loss of p21—the crucial target gene for DNA damage-induced and p53-dependent G1 arrest and senescence—accelerated tumour development in mutp53 (R172P) and MMTV/v-Ha-ras transgenic mice (Adnane et al. 2000; Barboza et al. 2006), provoked renal carcinomas in Apc knockout mice (Cole et al. 2010) and increased sensitivity to carcinogen-induced cancer formation (Jackson et al. 2002). However, it should be noted that p21-null mice are generally not tumour-prone and only subtly sensitized to irradiation-induced cancer (Deng et al.

1995; Jackson et al. 2003; Martin-Caballero et al. 2001). Conversely, mice deficient in Gadd45a—another p53 target gene involved in cell cycle regulation and DNA repair—were markedly sensitized to gamma and UV-radiation-induced cancers (Hildesheim et al. 2002; Hollander et al. 1999). Interestingly, loss of gadd45a accelerated tumorigenesis in ras-driven mammary tumours but delayed cancer development in myc-driven breast cancer (Tront et al. 2006, 2010). Hence, different mechanisms are required for tumour suppression, and parameters such as cancer type, tissue and cancer-driving signal determine what is necessary to counteract cancer development and progression. Accordingly, it makes sense that specific target genes or DNA damage response programme controlled by p53 are crucial for tumour suppression under some conditions but not required in other settings. Results from the many other studies dissecting p53-mediated tumour suppression in vivo [reviewed in (Bieging and Attardi 2012)] fit into this concept as well.

Whereas the studies highlighted above are consistent with the idea that p53-mediated apoptosis, senescence and cell cycle arrest are important for tumour suppression, a few other reports challenged the view that these programmes are the sole effectors in preventing cancer (Brady et al. 2011; Li et al. 2012; Valente et al. 2013). Spectacular insights came from a model recently established by Wei Gu's group (Li et al. 2012). The so-called $p53^{3KR}$ mice have three mutations in the p53 gene preventing p53 from being acetylated at three lysines (K117, K161, K162) that are crucial for transactivating a substantial subset of p53-target genes. Cells from these mice behaved like p53-null cells with regard to the induction of cell cycle arrest, senescence and apoptosis upon treatment with DNA damaging agents. Surprisingly, $p53^{3KR}$ mice did not develop thymic lymphoma or any other spontaneous tumours when monitored up to 16 months (Li et al. 2012). This unexpected result suggested that the classic DNA damage responses triggered by p53 were completely dispensable for suppression of spontaneous tumours. A similar conclusion was made on the basis of another mouse model, in which not p53 was modified but three crucial target genes, Puma, Noxa and p21, deleted. Cells from these mice were defective for p53-dependent and DNA damage-induced apoptosis and senescence, but again the mice did not develop spontaneous tumours (Valente et al. 2013). Similarly, an earlier mouse model expressing a mutant p53 protein that is unable to elicit responses to acute DNA damage (p53 L25Q; W26S, also referred to as $p53^{25,26}$) showed a remarkable ability to suppress tumour formation (Brady et al. 2011).

Clearly, these studies demonstrate compellingly that for suppression of spontaneous tumours in mice, the classic DNA damage response programmes regulated by p53 are dispensable. Hence, other cellular programmes and/or target genes that have been underappreciated or not identified yet appear to play a powerful role in tumour suppression. Their identification is currently a major focus of several laboratories with regulation of metabolism and/or the coordination of DNA repair emerging as particularly interesting effector processes (Berkers et al. 2013; Li et al. 2012; Timofeev et al. 2013; Valente et al. 2013). What the aforementioned studies do not support (and also do not claim) is the conclusion that p53-mediated apoptosis, senescence and cell cycle arrest are *generally* dispensable for tumour suppression, although the titles '*Tumor suppression in the absence of p53-mediated cell-cycle arrest, apoptosis, and senescence*' (Li et al. 2012) and '*p53 Efficiently Suppresses Tumor Development in the Complete Absence of Its Cell-Cycle Inhibitory and Proapoptotic Effectors p21, Puma, and Noxa*' (Valente et al. 2013) may suggest so. Indeed, it can be expected that both of the mentioned mouse models ($p53^{3KR}$ and puma−/−, noxa−/−, p21−/−) will show impaired tumour-suppressive activity in another context such as oncogene activation and/or exposure to DNA damage. Notably, the puma single-gene knockout mouse showed enhanced tumorigenesis in myc-driven cancers (Michalak et al. 2009). Together, the numerous existing mouse models underscore the somewhat trivial, yet often underappreciated concept that, depending on the context, effective tumour suppression requires different genes and cellular programmes. The classic p53-mediated DNA damage response programmes play certainly an important part, but other effector processes, p53-dependent and independent ones, are necessary complements to suppress cancer.

Concluding remarks

P53 is a very complex and multi-faceted protein with many functions to which DNA damage-related responses provide only one albeit crucial component. Despite the many studies carried out in the past, our knowledge still has substantial gaps. This is illustrated for example by the lack of compelling data on the predictive power of p53 mutations with regard to therapy response and survival in most cancers. A central aspect in all areas of p53 research is the crucial importance of the cellular context. We can only understand p53 if parameters like cell type and stress signal are taken into account. With these being considered, many apparently conflicting results are no longer inconsistent (as discussed for the tumour suppressor function) but simply reflect the extreme versatility and multi-functionality of p53.

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Conflict of interest The author declares that he has no conflict of interest.

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