Chapter 15

The Role of p53/p21/p16 in DNA-Damage Signaling and DNA Repair

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Chapter Outline

1. INTRODUCTION

DNA damage as a mutagenic event threatens the integrity of genetic information. Thus, mammalian cells utilize a complex signaling network to detect, signal, and repair DNA damage with the aim to restore genomic stability. In case, the DNA damage is beyond repair, the damaged cell is eliminated from the proliferating cell pool by cell death or senescence. If DNA repair and cell death/senescence fail, mutations can accumulate in the genome, which can result in the deregulation of genes regulating cell growth, proliferation, and/or death, consequently increasing the risk for the development of cancer and other diseases [1,2]. Therefore, DNA mutations and genomic instability are the established hallmarks of cancer cells [3]. Furthermore, many commonly lost tumor-suppressor genes, including p53, p16^{INK4A}, RB, and BRCA1/2, play a role in the DDR and DNA-repair pathways which are among the most frequently compromised pathways in human cancers [4–6].

Given the biological significance of genomic integrity [2], the activation of cell-cycle checkpoints, apoptotic programs, and transcriptional changes are crucial end points of DDR signaling [7–9]. On the one hand, these cellular response mechanisms to DNA damage are crucial for tumor suppression by invoking cell-cycle arrest, senescence, and/or apoptosis [2,4,10,11]. On the other hand, the unrepaired DNA damage contributes to limitations in stem cell functionality and tissue homeostasis during aging [12] when the likelihood of cancer development and tissue dysfunction significantly increase with age because DNA damage accumulates gradually during cellular aging [13,14]. Different types of DNA damage can cause an acute or chronic DNA damage, which depending on the cellular context can result in reversible transient or irreversible permanent cell-cycle arrests. As an example of acute DNA damage, dysfunctional telomeres can cause a transient G1 cell–cycle arrest through the activation of p53 and its transcriptional target p21 [12], while continuous telomere dysfunction results in chronic DNA damage followed by the induction of p16 and pRB, thereby stabilizing senescence [12]. Thus, a detailed understanding of DDR involving p53, p21, and p16 also helps improve stem cell biology [12], in addition to their well-established actions in tumor suppression (see later).

Here, we summarize our current understanding of the key roles of p53, p21, and p16 in DNA-damage signaling and DNA-damage repair. In this regard, we are paying particular attention to the p53 tumor-suppressor protein and p21 as its effector protein, since p53 has been regarded as a prime example for the relationship between the DDR and tumor suppression [15–17].

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2. THE p53 TUMOR-SUPPRESSOR PROTEIN

Since the discovery and cloning of p53 in the late 1970s and early 1980s [17], the regulation and functions of p53 have been one of the most intensively studied areas of molecular cancer biology [16,18]. The p53 tumor suppressor is a central, versatile, and multifunctional player in the cellular DNA-damage response. Upon genotoxic stress, p53 is upregulated and induces transcriptional programs promoting transient cell–cycle arrest, permanent cell–cycle arrest in the form of senescence, DNA repair, and/or apoptosis [18–21]. The Tp53 gene is the most frequently mutated gene in cancer [18,22]. About 50% of all human cancers carry mutations in the p53 tumor-suppressor gene [23]. p53-deficient mice die with nearly 100% penetrance of cancer around 6 months of age [24–27], and patients suffering from Li–Fraumeni syndrome display an association with p53 mutations and cancer development [28,29]. Therefore, several researchers in the cancer research field have studied the consequences of the introduction of a normal p53 gene into tumor cells with mutant p53 which has the potential to restore their ability to undergo cell-cycle arrest, apoptosis, and/or the differentiation in response to DNA-damaging therapies [30]. The particular aim is to restore normal p53 function of the endogenous form with small molecules [30]. However, since p53 also acts in stem cell self-renewal and quiescence [12], these restoration approaches must be conducted with caution. Furthermore, p53 can exert pro- and anti-aging functions through the differential transcriptional regulation of apoptotic, senescence, and longevity target genes [31,32]. In this regard, a study of so-called "super-p53" mice showed that a moderately enhanced expression of p53 in combination with $p16^{INK4A}$/$p19^{ARF}$ can result in tumor-free life extension [33]. Further noteworthy, studies during 2010s challenged the view that the DNA damage–induced programs triggered by p53 are the sole and major mechanism by which p53 exerts its tumor-suppressor function [34–36].

2.1 p53 in the DNA-Damage Response

Already in the 1980s and 1990s, researchers observed that p53 was upregulated rapidly at the level of protein stabilization by genotoxic agents, including ultraviolet (UV) light, ionizing radiation (IR), and chemotherapeutics [37–40]. In response to DNA damage, p53 is extensively modified by phosphorylations and other posttranslational modifications [41]. Upstream regulators of p53 include the DDR kinases ATM, ATR, DNA-PK, CHK1, and CHK2. These modifications release p53 from the interaction with its negative regulator Mdm2 and promote the transcriptional activity of p53 [41], resulting in an increased transcription of p53 targets including the cyclin-dependent kinase (CDK) inhibitor (CKI) p21 [19,42]. Through the transcriptional induction of p21, p53 indirectly suppresses CDK activity, which in turn enables the activation of the pRB tumor-suppressor pathway [43]. The activated pRB reduces the activity of E2F, thereby decreasing the pro-proliferative expression of E2F target genes (Fig. 15.1).

Significantly, in response to DNA damage (and also other cellular stresses), the pure abundance of the p53 protein due to the inhibition of the Mdm2-mediated proteosomal degradation should not be considered as the sole determinant of p53 activity, although it normally can serve as a reliable readout of its activation [19]. As a transcription factor that can bind DNA in a sequence-specific manner [31], p53 is known to activate or repress the transcription of genes involved in cell-cycle arrest, DNA repair, apoptosis, metabolism, autophagy, and others [21,31], where depending on the cell system, p53 upregulation can trigger different biological effects [19,21]. Thus, monitoring a transcriptional readout is very suitable to complement measurements of p53 activity. Since in human cancers, most mutations in p53 affect the DNA-binding domain of p53 [44], it appears that the transcriptional function plays the main tumor-suppressive role of p53 in cancer cells, although p53 also displays nontranscriptional activities.

In case, the DNA damage is too extensive, p53 triggers cell death through intrinsic and extrinsic pathways [45,46]. In response to DNA damage, the upregulation of p53 can strongly induce apoptosis, but this function is cell type and DNA-damage dependent [32,39]. However, apoptosis can also take place without p53 [19]. Moreover, the extent of apoptosis in human lymphoma cells upon treatment with chemotherapeutics was dependent on the p53 status [47]. Collectively, these reports suggest that cell type, stress, and other signaling pathways determine whether p53 can induce apoptosis or not in response to DNA damage. Generally, p53 can promote apoptosis through three different routes: (a) transcriptional activation, (b) transcriptional repression, and (c) transcription-independent mechanisms. Considering the emphasis of this chapter, we refer the reader to an excellent summary of these three routes [19] for further information on the role of p53 in apoptosis induction. Note that various types of cellular stress, including DNA damage, can induce apoptosis by p53-dependent and p53-independent mechanisms, where apoptosis represents the primary response to DNA damage and telomere dysfunction in certain cell types [12,32].

Upon DNA damage, as an alternative to apoptosis, high p53 levels can induce a transient or permanent cell–cycle arrest [48–52]. In this context, p53 can facilitate either apoptosis or senescence in a cell type–dependent manner [49,53]. Although many factors have been shown to contribute to these different types of cell-cycle arrest (summarized in Ref. [19]),

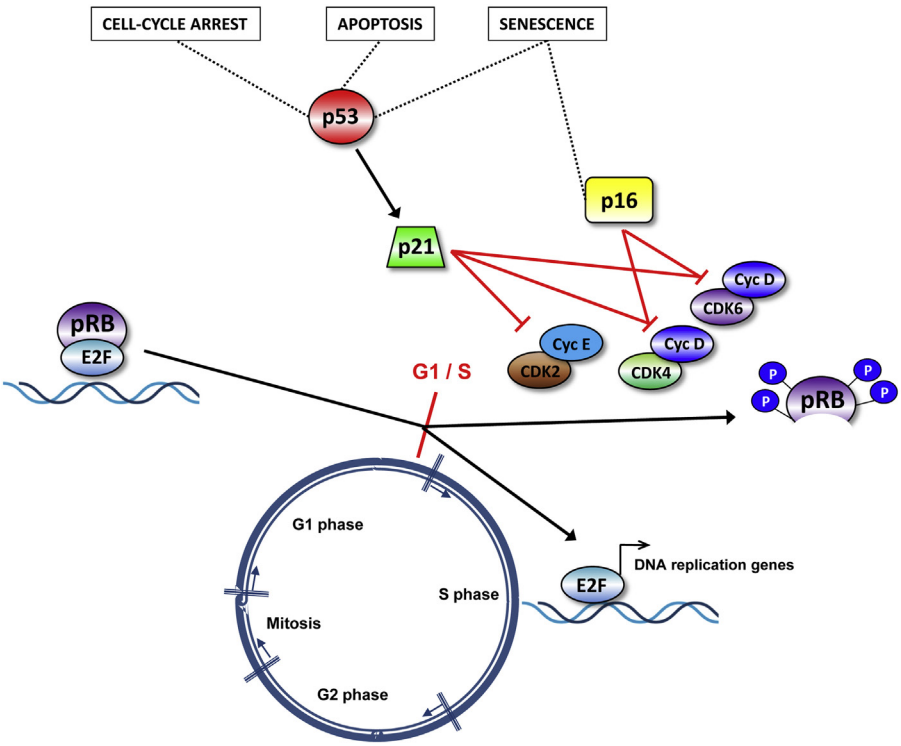

FIGURE 15.1 **p53/p21/p16 tumor suppressors in control of the G1/S cell–cycle checkpoint.** The tumor-suppressor proteins p53, p21, and p16 are major regulators of the G1/S cell–cycle checkpoint. Upon p53 stabilization, the transcription of p21 is induced, subsequently resulting in the inhibition of CDK4/6–cyclin D and CDK2–cyclin E complexes by p21. In response to senescence-inducing stimuli, p16 levels are elevated to inhibit the CDK4/6–cyclin D complexes. As a result of the inhibition of CDK–cyclin complexes, the hypo-phosphorylated form of pRB can accumulate, consequently inhibiting the transcription of S-phase promoting genes by E2F.

the parameters defining the choice between apoptosis, a transient cell–cycle arrest, and a permanent growth arrest (senescence) are not fully understood yet. In this regard, it is noteworthy that a sustained stabilization of p53 results in a permanent proliferation arrest through senescence, while pulses of stabilized p53 yield a transient, reversible cell–cycle arrest [54]. A misbalance between DNA damage and DNA repair normally results in higher p53 levels through pulses [55], causing a transient p53-dependent G1/S cell–cycle arrest [54]. This pulsatile behavior of p53 can be explained at least in part by ATM-induced activation of 53 followed by transactivation of Mdm2 and Wip1, two negative regulators of p53 [56]. This induction of Mdm2 and Wip1 counteracts p53 activity as a negative feedback loop. However, it still is important to fully understand these stabilization dynamics in the context of a variety of other distinct mechanisms that are known to stabilize p53 [41]. In this regard, the type of genotoxic stress also plays an important part since in contrast to radiation-induced DNA damage, UV-induced DNA damage triggers a sustained induction of p53 that does not seem to oscillate in waves [56].

Significantly, p53 can act in the G1/S and G2/M DNA-damage cell-cycle checkpoints (Figs. 15.1 and 15.2). These transient arrests prevent the amplification by replication and/or propagation by cell division of damaged DNA molecules. In particular, for the G1/S arrest in response to DNA damage, p53 is important. For example, p53-deficient mammalian cells display a lack of a G1/S arrest upon DNA-damage induction [57,58]. Considering that cells lacking p21 as a p53 effector display the same phenotype [59–61], it is well established that the p53-mediated upregulation of p21 is essential for the DNA damage–induced G1/S cell–cycle checkpoint, at least in mammalian tissue culture cells [62–64]. Mechanistically, p53 is a major effector of DDR kinase signaling [65], mediating a G1 cell–cycle arrest mainly through the transcriptional upregulation of the CKI p21 [16]. p21 subsequently inhibits the CDK2–cyclin E complex and consequently DNA replication, hence defining the p53/p21 pathway as a master regulator of the G1/S cell–cycle transition in the DDR [41].

Regarding the role of 53 in the G2/M DNA-damage checkpoint, one should note that the p53-mediated G2/M arrest involves the upregulation of various target genes with distinct functions which can negatively influence CDK1 activity (summarized in Ref. [19]). Although the induction of the G2/M arrest does not require p53 [38,42], p53 and its effector p21 seem to be required for the maintenance of the G2/M arrest [42,66]. In addition, p53 can directly and indirectly play a role in S-phase progression and DNA replication [19], where ATR and CHK1 can regulate the activation of p53 in response to DNA damage [67].

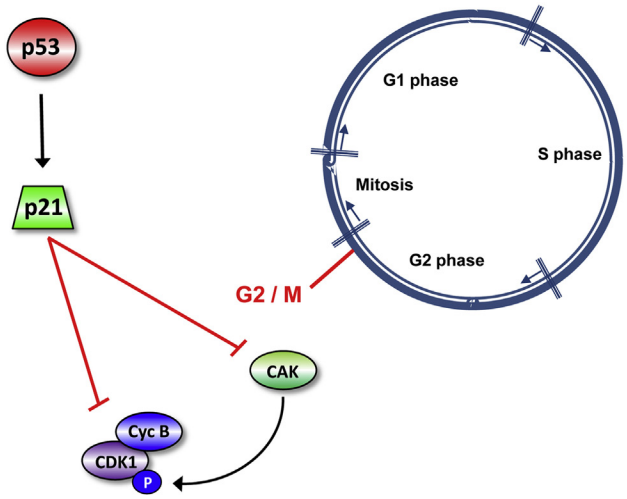

FIGURE 15.2 p53/p21 in control of the G2/M cell–cycle checkpoint. The p53 and p21 tumor-suppressor proteins can help to sustain a G2/M cell–cycle arrest in response to stress (eg, DNA damage). The stress-induced stabilization of p53 triggers an increased expression of p21. The elevated p21 levels can support a G2/M cell–cycle arrest through different routes, two of which are indicated here. On the one hand, p21 can inhibit the CDK-activating kinase (CAK) which interferes with the CAK-mediated activating phosphorylation of CDK1. Yet, on the other hand, p21 can directly inhibit the CDK1–cyclin B complex. For more details, please check the main text.

2.2 p53 in DNA-Damage Repair

p53-deficient cells display impaired nucleotide excision repair (NER) of UV-induced photoproducts [19]. p53 also appears to be involved in the base excision repair (BER) and mismatch repair (MMR) pathways (summarized in Ref. [19]). Moreover, p53 can regulate the repair of DNA double-strand breaks (DSBs) by homologous recombination (HRR) and nonhomologous end joining (NHEJ) [68]. Normal p53 can suppress HRR by binding to RAD51 and the BLM helicase [69–71], while mutant p53 can promote an increase in basal and DNA-damage induced RAD51 levels [72,73]. Thus, mutant p53 can cause a "hyper-recombination" phenotype [72,73] which potentially is related to the link between p53 and BRCA1 [74]. In the context of NHEJ repair and BER, it was reported that p53 can either suppress or promote these DNA-repair activities [19], suggesting that context- and cell system–dependent mechanisms must be carefully considered in this regard. Nonetheless, current evidence suggests that p53 facilitates DNA repair by at least three routes: (a) the transcription-dependent induction and maintenance of a transient cell–cycle arrest (mainly with p21/CIP1 upregulation to provide sufficient time for DNA repair), (b) the direct upregulation of the expression of DNA-repair genes (summarized in Ref. [19]), and (c) transcription-independent activities of p53 (see earlier and Ref. [19]). In addition, p53 can bind to damaged DNA, Holliday junctions, and heteroduplex joints in vitro [19], suggesting that p53 binding to abnormal DNA structures might also play a role in directing DNA repair, although these mechanisms are poorly understood.

2.3 p53 in Tumor Suppression and the DNA-Damage Response

Since the majority of mutations in p53 are likely to impair the transcriptional activity of p53 [44] and most cytotoxic clinical compounds induce the p53-mediated DDR, it was expected that the p53 status will have an influence on cancer progression and the outcome of cancer therapy. The initial xenograft experiments showed a clear correlation between the p53 status and apoptosis in response to DNA-damaging agents [75], but in spite of intensive efforts, the impact of the p53 status for a successful cancer therapy is yet to be fully understood (Ref. [19] and see later) with the current emphasis being on the development of small molecules that restore the normal p53 function of the endogenous form [30]. Before we close in the next subsection our summary of p53 with a discussion of p53 in the context DNA-damaging agents, we briefly summarize our current understanding of p53-mediated tumor suppression based on animal models. For an expert overview of p53-related animal models, we refer the reader to other reviews [76,77] since here, we focus on the role of p53-mediated DDR programs and tumor suppression.

As already mentioned, p53-deficient mice die of cancer with nearly 100% penetrance [24–27]. Radiation treatment of p53-deficient mice accelerated cancer formation even further [26]. However, follow-up studies revealed that the p53 status had no effect on cancer development triggered by radiation [78,79]. These studies rather revealed that p53 was required at later time points (after radiation treatment) to suppress tumor formation in mice [78,79]. Using mice expressing

transcriptionally dead p53, it was subsequently shown that the transcriptional activity of p53 was essential for the tumor-suppressive function of p53 [34,80], where p53-mediated tumor suppression was most likely a result of the expression control of more than one target gene [19,76], with the apoptotic function(s) of p53 being an important factor [81]. In this regard, one should further note that in addition to apoptosis, cell-cycle arrest and senescence can also play a part in p53-mediated tumor suppression [19].

Last but not least, it is noteworthy that three different studies during 2010s challenged the view that p53-mediated apoptosis, cell cycle arrest, and senescence are the sole effectors of p53 in preventing cancer [34–36]. In the first study, although in response to DNA damage, cells carrying mutant p53 responded like p53-null cells regarding apoptosis, cell-cycle arrest, and senescence, the corresponding mice did not develop spontaneous tumors over a period of 16 months [35]. In the second study, mice carrying deletions of three crucial p53 target genes were studied, revealing that despite defective DNA damage–induced apoptosis and senescence, mutant mice did not develop spontaneous tumors [36]. In the third study, mice expressing a p53 mutant unable to trigger an acute DDR displayed a significant suppression of tumor formation [34]. Collectively, these studies suggest that classical p53-mediated DDR programs are dispensable for the suppression of spontaneous tumor formation. For sure, in response to DNA damage, the p53-mediated apoptotic cell-cycle arrest and senescence programs play important roles, but these studies illustrate that other effector processes, such as possibly the p53-mediated regulation of metabolic and autophagic processes and others, are contributing significantly to the tumor-suppressive functions of p53.

It is also noteworthy that it could be speculated that p53 inactivation should result in genomic instability based on the central roles of p53 in the DNA-damage checkpoint [82]. Nonetheless, p53 deficiency in mammalian cells does not lead to aneuploidy [83,84], and in human precancerous lesions, genomic instability can be observed before the detection of p53 mutations [4,85]. Collectively, these findings suggest that the loss of p53 is not sufficient to induce genomic instability.

2.4 p53 and Targeted DNA-Damaging Cancer Therapy

Genomic instability is a hallmark of tumor development and progression [3]. Consequently, cancer cells must acquire the ability to tolerate an increased amount of DNA damage when compared to untransformed cells. Frequently, this is achieved by dampening/suppressing one or more DNA damage–repair/signaling pathways, which allows cancer cells to function and proliferate in the presence of DNA damage [86]. On the other hand, these mutated or compromised DNA-damage pathways, although contributing to cancer development, also represent a potential Achilles' heel for cancer therapeutics. Many commonly lost tumor-suppressor genes, including p53, $p16^{INK4A}$, RB, BRCA1/2, and ATM, play a role in the DDR- and DNA-repair pathways which are among the most frequently compromised pathways in human cancers [4–6]. Thus, the targeting of oncogenic signaling pathways in combination with DNA-damaging chemotherapeutics may offer an improved and more selective efficacy than single treatments alone. Considering advances in radiotherapy in 2015 and that DNA damage is the most critical factor in radiation-induced cell death [87], these approaches are not limited to chemotherapy but potentially also applicable to radiotherapy.

The increased selectivity of cancer cells to DNA-damaging agents indicates that cancer cells have rewired their DDR- and DNA-repair pathways, and consequently established new dependencies between cell-cycle checkpoints and survival pathways that are much less pronounced in normal cells. For example, the p53-regulated G1/S checkpoint is a predominant DNA-damage checkpoint in normal mammalian cells which is lost due to p53 mutations in many cancer cell lines [16] generally associated with resistance to chemotherapeutics [18]. In this context, synthetic lethal strategies are gaining more and more interest [86]. Upon the loss of p53 function, cancer cells are often completely dependent on the intra-S and G2/M DNA–damage checkpoints to arrest cell cycle after genotoxic chemotherapeutic stress. Thus, the interference with the intra-S and G2/M checkpoints has become a promising strategy to sensitize G1 checkpoint-deficient cancer cells to DNA-damaging therapy [86].

Compared to genetically matched controls, mammalian mutant or null-p53 tumor cell lines can display the elevated cancer-specific sensitivities to compounds associated with DNA-damaging cancer therapy [86], although this p53 dependency was not observed in all tumor types analyzed [74]. For example, in combination with DNA-damaging agents, CHK1 inhibition causes a by-pass of both the intra-S and G2/M DNA–damage checkpoints in p53-deficient cells (summarized in Ref. [86]). A synthetic lethal interaction between ATR, the upstream activator of CHK1 and p53 deficiency has also been observed, suggesting that ATR or CHK1 inhibition in combination with DNA-damaging agents could be beneficial for the treatment of p53-deficient tumor cells [86]. Most promising in this context might be the discovery of a synthetic lethal interaction between p53 and the ATM–CHK2 pathway, with the loss of ATM or CHK2 together with p53 causing the loss of the G1/S and G2/M checkpoints, consequently driving cancer cells into a mitotic catastrophe in response to genotoxic DNA damage [86]. In this context, it was also observed that a single loss of ATM or p53 can promote resistance to DNA-damaging chemotherapy, while their combined loss can cause an increased chemosensitivity [88]. In this regard, the

chemotherapy resistance of p53-proficient but ATM-deficient tumor cells may be reversible by DNA-PK inhibition. However, this will largely be dependent on the development of good pharmacological DNA-PK inhibitors [86,89]. As defined in more detail in Ref. [86], other kinases that can be used as interesting and promising clinical targets for the development of inhibitors that might display a synthetic lethality with p53 deficiency are the ATM- and ATR-activated kinases in the p38/MK2 pathway. The Wee1 kinase is also an attractive target since upon activation by CHK1, Wee1 inhibits the cell-cycle kinases CDK1 and CDK2 and thereby abrogates DNA damage–checkpoint activation [87,90]. Therefore, in ongoing and future clinical trials, the stratification of the p53 status together with functional assays of DNA-repair activities (eg, RAD51 foci formation as readout for HRR [91]) may provide valuable insights as stratification methods in the context of adjuvant chemotherapies including ATM and other kinase inhibitors [74]. However, these clinical analyses of the p53 status must include the determination of p53 single-point mutations since gain-of-function mutations have been described for p53 [92]. Specifically in the context of DNA-damage signaling, the most common p53 mutations, R248W and R273H, have been shown to exhibit gain-of-function properties by binding to MRE11, a key component of the DNA damage–sensing MRN complex [93], through which these p53 mutants can abolish early DDR signaling by negatively interfering with ATM activation in response to DSBs [94].

3. THE p21 TUMOR-SUPPRESSOR PROTEIN

In the 1990s, the $p21^{CDKN1A}$ tumor-suppressor protein was independently isolated as CDK-interacting protein 1 (CIP1) [95,96], wild-type p53-activated factor 1 (WAF1) [64], and senescent cell–derived inhibitor 1 (SDI1) [97]. p21 is also known under the synonyms MDA6, CAP20, and PIC1. The official gene name for the gene encoding p21 is CDKN1A (cyclin-dependent kinase tumor-suppressor protein inhibitor 1A). p21 CIP1 was identified as the first CDK inhibitor (CKI) since it can inhibit various CDKs [96,98–100]. p21 can inhibit CDK activity by blocking the ATP-binding site of CDK [101], by interacting with CDK, or by interfering with CDK phosphorylation [102]. Particularly, p21 blocks CDK2 activity which is required for the inactivation of pRB to release E2F to prepare for DNA replication [103,104].

As a major transcriptional target of p53, p21 is important for the response of cells to DNA damage [63,64]. Depending on its subcellular localization, p21 can perform different functions in a mammalian cell. For example, the nuclear p21 can inhibit CDK1 and CDK2 kinase activities, thereby blocking G1/S and G2/M cell–cycle progression (see Figs. 15.1 and 15.2 and later). p21 is further required for the induction of senescence, and it has an anti-apoptotic function where the anti-apoptotic activity of p21 is related to the cytoplasmic pool of p21 [103]. As a target of p53, p21 can also promote stem cell quiescence [12].

p21 is regulated by p53-mediated transcription, posttranslational modifications, degradation, and subcellular localization [103–105] Since these regulatory mechanisms are complexly interlinked, it is beyond the scope of this chapter to discuss them in detail. Thus, we refer the reader to reviews discussing these somewhat complex aspects in more detail [103–105]. For example, during normal cell–cycle progression, p21 is regulated by proteolysis mediated by E3 ubiquitin ligases including SCF^{SKP2}, APC/C^{CDC20}, and $CRL4^{CDT2}$, while upon ATR activation, p21 can be degraded in a ubiquitin-independent manner (summarized in Refs. [103,104]).

Besides functioning in the DDR and DNA repair, p21 has additional functions in cell motility and transcriptional control which are summarized elsewhere [103–105]. Here, we focus on summarizing specific roles of p21 in the DDR and DNA repair.

3.1 p21 in the DNA-Damage Response

Upon encountering DNA damage, a mammalian cell relies on three fundamental processes: (a) cell-cycle arrest to allow sufficient time for DNA repair, (b) the repair of DNA lesions, and (c) the induction of apoptosis or senescence in case the DNA damage is beyond repair. Significantly, p21 can play essential roles in all these processes [103].

p21 is a key mediator of cell-cycle arrest in response to DNA damage. $p21^{CDKN1A}$ is a DNA damage–inducible gene whose transcriptional induction can occur dependent on p53, but may also occur through p53-independent pathways. Thus, the induction of p21 expression is considered a paradigm of the cell response to genotoxic damage [106]. In response to DNA damage, the upregulation of p21 can cause a G1/S cell–cycle arrest, and it can also support the G2/M cell–cycle checkpoint [66,103] (Figs. 15.1 and 15.2).

In response to DNA damage, p53 accumulates in the nucleus driving the transcription of p21 and other response genes. Consequently, p21 accumulates in the nucleus causing the activation of the DNA-damage cell-cycle checkpoint in G1/S (Fig. 15.1). p21 functions as a potent inhibitor of CDKs which are major drivers of cell-cycle progression [96]. Particularly, p21 efficiently inhibits the CDK2–cyclin E and CDK2–cyclin A complexes which normally promote the G1/S cell–cycle

transition [95]. In response to DNA damage, CDK2 is inhibited by increased levels of p21, resulting in the accumulation of hypo-phosphorylated pRB and thereby sequestering the transcription factor E2F whose activity is required for the entry into the S phase [43]. Since p21-deficient mammalian cells display the lack of G1/S arrest in response to DNA damage [59–61], it is well established that the upregulation of p21 is essential for the DNA damage–induced G1/S cell–cycle checkpoint, at least in mammalian tissue culture cells [62–64]. Generally, it is recognized that p21-deficient cells lack the ability to arrest at the G1/S DNA-damage checkpoint. In addition, p21 has been shown to influence the G1/S checkpoint by interacting with PCNA, a cofactor of the DNA polymerases δ and ε which are required for DNA replication and DNA repair [103].

In addition to its role in the DNA-damage checkpoint in G1/S, p21 also plays a crucial role in the G2/S checkpoint [42,107,108]. Although the induction of G2/M arrest does not require p53 [38,42], p53 and its effector p21 seem to be required for the maintenance of G2/M arrest [42,66] which possibly also involves the interaction of p21 with the CDK1–cyclin B complex [103,107,109,110]. In G2, p21 can also inhibit the CDK-activating kinase (CAK) and consequently block the activating phosphorylation of CDK1 [110]. p21 can further mediate cyclin B degradation [111]. Other targets of p21 in G2 are CDK1–cyclin A and CDK2–cyclin A complexes [112]. Furthermore, by suppressing CDK activity, p21 can indirectly activate pRB, consequently reducing E2F activity, thereby decreasing the pro-proliferative expression of E2F target genes, such as the APC/C inhibitor Emi1. This can promote the premature activation of APC/C in G2, resulting in the degradation of cyclins A and B followed by a G2/M cell–cycle arrest [113]. Collectively, the upregulation of p21 can sustain the G2/M cell–cycle arrest through different routes (Fig. 15.2).

The upregulation of p21 can also impact S-phase progression and DNA replication [103,104]. p21 has also an important role in the induction, but not maintenance, of replicative and stress-induced premature senescence, with p21 upregulation serving as the first marker of replicative senescence [114]. p21 also plays a fundamental role in reversible cell-cycle exit (quiescence) by regulating CDK2 activity [115]. Furthermore, p21 plays an active role in inhibiting apoptosis summarized in Refs. [103,104] (see also later). We also refer the reader to the same papers [103,104] to obtain an overview of the roles of p21 in transcriptional regulation in response to DNA damage.

3.2 p21 in DNA-Damage Repair

Besides inducing a cell-cycle arrest, p21 appears to also have distinct functions in DNA repair [103,104]. Initially, it was suggested that p21 may play a role in DNA damage repair since p21 can interact with PCNA, a cofactor of the DNA polymerases δ and ε, required for DNA replication and DNA repair. Several studies indicate that p21 is involved in major DNA-repair pathways including NER, BER, HRR, and NHEJ, where the interaction of p21 with PCNA seems to play a role in NER and BER. Considering that the involvement of p21 in NER is currently based on the contrasting results, we refer the reader to reviews which elegantly summarized these apparently opposing findings [103,104].

p21-deficient cells displayed elevated PARP1 activity, a defective BER, and increased sensitivity to DNA-alkylating agents [103]. In this regard, in vitro experiments suggest that p21 specifically interferes with the activity of DNA polymerase δ, and p21 has been shown to inhibit the DNA damage–sensor protein PARP1 which is important in BER [103,104]. Collectively, these results suggest that p21 acts as a regulator in BER. p21 can also play a role in the two main DSB-repair pathways: HRR and NHEJ [103,104]. p21 can colocalize with components of the DNA damage–sensing MRN complex, a promoter of HRR together with CtIP, and can be recruited to sites of DSBs [103,104]. Specifically, the spatiotemporal analysis of GFP-tagged p21 revealed that p21 is rapidly recruited to regions containing DNA damage. By inhibiting CDK activity, p21 can favor HRR. In NHEJ, p21 is recruited to DNA lesions independently of key NHEJ factors including DNA-PK and Ku70/Ku80. In addition, p21 may also contribute to MMR and other DNA-repair pathways, but the mechanistic role(s) of p21 in DNA-repair processes remains poorly understood [103,104].

3.3 p21 and Tumor Suppression

Through its roles in DNA damage–checkpoint signaling and DNA repair, p21 can protect mammalian cells against the accumulation of DNA damage and subsequent genome instability. However, p21-deficient mice are not really prone to cancer development and are only subtly sensitive to radiation-induced cancer formation [60,116,117], although the loss of p21 can promote tumor development in mice carrying a p53 loss-of-function mutation or oncogenic RAS [19].

As discussed previously, depending on its subcellular localization, p21 can perform different functions in a mammalian cell. Nuclear p21 promotes cell-cycle arrests in G1/S and G2/M and possibly DNA repair (see earlier), while cytoplasmic p21 can inhibit apoptosis induction in response to DNA damage through inhibitory binding to pro-apoptotic factors [103,104]. Different levels of DNA damage may direct the response controlled by p21. Low levels of DNA damage can stabilize p21 leading to cell-cycle checkpoint activation, while high levels of DNA damage promote the downregulation of

p21 and consequently apoptosis. In this regard, although p21 can function as a tumor-suppressor protein by activating cell-cycle checkpoints (and possibly DNA repair), p21 can also play a role in tumor initiation by protecting the damaged cells from apoptosis. On the one hand, p21 deficiency enables the proliferation of cells carrying the damaged DNA promoting tumor progression. On the other hand, as anti-apoptotic factor p21 can act as an oncoprotein. In support of this notion, the elevated levels of p21 protein have already been described in different human cancer samples, frequently correlating with the invasiveness and malignancy of cancer [105]. Note that the deletion of p21 impairs the survival of leukemia stem cells, suggesting that in this context, p21 is required to maintain the self-renewal and quiescence capacities of cancer stem cells by protecting them from accumulating DNA damage and genomic instability, hence displaying an oncogenic activity [118]. Since p21 can also serve as an assembly factor for the formation of CDK4–cyclin D complexes [119,120] which may also contribute to the oncogenic properties of p21. Thus, it will be important to continue to decipher the context-dependent role of p21 in cancer prevention vs. initiation and maintenance in the context of cancer therapy [105,121]. In this regard, one should also note that p21 appears to have also context-dependent functions in stem cell biology, particularly in response to DNA damage and telomere dysfunction [12].

4. THE p16^{INK4A} TUMOR-SUPPRESSOR PROTEIN

Different stresses can induce senescence, including telomere dysfunction (eg, through replicative erosion), the induction of chronic or acute DNA damage (by UV radiation, IR, or genotoxic compounds), oncogene activation, and others [122,123]. Several signal transduction pathways are essential drivers of senescence, including p53–p21 signaling required for senescence induction and p16–pRB-signaling needed for senescence maintenance [123]. Here, we provide an overview of the roles of p16 in promoting senescence with a particular emphasis on DNA damage–signaling-induced senescence (see also Fig. 15.1).

p16 (also known as MTS1 and INK4A) was first discovered by yeast two-hybrid screens as a novel binding partner of CDK4 [124,125], and subsequently the full-length p16 was isolated [126]. Like p21 (see earlier), p16 also functions as CKI by specifically and directly binding to proto-oncogenic CDK4–cyclin D and CDK6–cyclin D complexes [125,127]. Consequently, pRB remains hypo-phosphorylated and keeps the S-phase initiating transcription factor E2F inactive, thereby stabilizing a G1/S cell–cycle arrest by activating the pRB checkpoint [43]. In support of this tumor-suppressive function, loss-of-function and overexpression studies showed that p16 functions as a tumor-suppressor protein [128]. Furthermore, it was observed that the CDKN2A locus which encodes the p16 protein is very frequently inactivated by deletions, point mutations, or promoter hypermethylation in melanoma, pancreatic carcinomas, leukemia, bladder cancer, head and neck carcinomas, and others [128]. However, in this context, one must note that the CDKN2A gene locus encodes for two independent tumor-suppressor genes, namely p16^{INK4A} and p14^{ARF}, through the use of alternative open reading frames [126]. Nonetheless, p16 inactivation by CDKN2A deletions in human cancers is likely the main event regarding tumor suppression since p16 functions in the regulation of CDK4, CDK6, and pRB [125,129]. Noteworthy, p16 can also be overexpressed in human cancers [130], in particular in the context of human papillomavirus (HPV)-transformed cells, where the elevated levels of p16 are considered a hallmark of HPV-positive cervical carcinoma and head and neck cancer [131]. Mechanistically, the HPV encoded oncoprotein E7 disrupts the function of pRB, consequently releasing pRB from its inhibitory role of E2F, hence allowing the entry into the S phase. Nevertheless, HPV-infected cells still upregulate p16 levels to block the proliferation of HPV-transformed cells, which, however, due to the deregulation of pRB by E7 is an unsuccessful attempt to stop the proliferation [132].

Generally, senescence is defined as an irreversible cell-cycle arrest that is associated with the secretion of a specific subset of growth factors, referred to as the senescence-associated secretory phenotype [133]. Mammalian cells undergoing senescence develop specific characteristics that distinguish them from other nondividing cell states, such as quiescence or terminal differentiation [134]. Senescence can be induced prematurely by DNA damage without telomere shortening, referred to as stress-induced premature senescence. In contrast, replicative senescence occurs as a response to telomere shortening. Moreover, senescence can be induced by the failure to repair DSBs. While replication-induced telomere erosion–dependent senescence is mainly triggered by the recognition of dysfunctional telomeres by the ATM-dependent DSB-signaling response driving p53-dependent mechanisms [135], DSBs have been observed in senescent cells independent of telomere erosion [13,14], suggesting that senescence can also result from nontelomeric DSBs, where the amount of unrepaired DSBs required to trigger senescence is dose-, damage-, and cell type–dependent [135].

Collectively, different stimuli can promote senescence, including telomere erosion which causes a permanent DDR and nontelomeric DNA–damage stress which can cause the persistent DDR activation through the misfired replication origins and replication fork collapses [135]. Thus, senescence can serve as an anticancer barrier [134]. In particular, in the context of oncogene-driven DNA damage, a specific type of stress-induced premature senescence has been recognized as a powerful

barrier to the malignant transformation of pre-cancerous lesions [10,11,85]. For example, hyperactivated oncogenes can trigger chronic DSB signaling by causing error-prone DNA replication, resulting in the initiation of senescence [10]. This type of premature senescence is known as oncogene-induced senescence. Furthermore, most human tumors display inactivating mutations of the p53 and/or p16–pRB pathways, which are central components of the senescence response [135], further supporting the notion that senescence is an important tumor-suppressive mechanism. Thus, senescence induction is considered a possible mechanism for cancer therapy [136].

In mammals, cellular senescence is regulated by two major mechanisms: the p16–pRB pathway and the p53–p21 pathway [114,135]. While p21 plays a role in the initiation of senescence [114], the state of permanent cell–cycle arrest is maintained by p16 [137]. Specifically, the p16–pRB pathway seems to be essential for the maintenance of senescence since the senescence phenotype is not reversible once senescence arrest has been fully established by the p16–pRB pathway [138,139].

Significantly, chromatin alterations are also a key feature of senescence accompanied by the formation of senescence-associated heterochromatin foci (SAHF) [135]. The p16–pRB pathway is required for the formation of SAHF structures in response to oncogene-induced senescence [135], where SAHF formation can serve as a barrier to chronic DSB responses, with the potential to help cancer cells to bypass cell death induction by DSB signaling [140]. Most likely, SAHF surround an unresolved DSB and suppress it from contributing to DSB-response signaling, which could be beneficial for normal tissue functionality but detrimental in the context of cancer cell survival [135]. Thus, it is possible that SAHF formation might assist in preserving genomic integrity, in addition to muting the DSB response which normally would trigger cell death mechanisms, and thereby remove cells containing excessive DNA damage. Thus, considering that the p16–pRB pathway is needed for the maintenance of senescence as an anticancer barrier (see earlier) and is possibly required for the suppression of DSB signaling to promote cancer cell survival, it is very likely that p16 may play context-dependent roles in cancer as already defined for p21 (see earlier).

Last but not least, one should further note that the role of p16 in the context of DNA damage has been linked to stem cell biology and aging in addition to cancer (summarized in Ref. [12]). For example, cell-intrinsic DNA damage in tissues can result in systemic alterations of the blood, thus accelerating normally age-dependent functional defects in hematopoietic stem cell pools [12]. Moreover, the functional impairment of somatic stem cells due to accumulated DNA damage and the consequent DNA-damage checkpoint responses can lead to defects in tissue maintenance. In this regard, p16 expression is significantly increased in various tissues during mouse and human aging, which possibly involves a decline in ATM functionality combined with the accumulation of DNA damage during aging. Even more importantly, since the age-dependent accumulation of DNA damage also occurs in stem cells, stem cell maintenance can be improved by the deletion of p16 in murine cells. Taken together, the deregulated expression of p16 can have detrimental effects on the functionality of stem cells as well as somatic cells [12].

5. CONCLUSION

In summary, many commonly lost tumor-suppressor genes, including p53, $p16^{INK4A}$, and RB, play roles in the DDR- and DNA-repair pathways. As summarized in this chapter, p53 acts as a central, versatile and multifunctional player in the cellular DDR. In response to DNA damage, p53 protein levels are upregulated, thereby inducing diverse transcriptional programs that can promote a transient cell cycle arrest, a permanent cell–cycle arrest in the form of senescence, DNA repair, and/or apoptosis. Considering that the Tp53 gene is the most frequently mutated gene in human cancers and that p53-deficient mice display nearly 100% penetrance of cancer development at the young age, it is not surprising that over the past decades, very intensive research efforts have focused on deciphering the key tumor-suppressive functions of p53. In this regard, it is now fully established that p53 plays important roles in the G1/S and G2/M DNA–damage cell-cycle checkpoints. In response to DNA damage, p53 as a transcription factor is stabilized, allowing p53 to drive the transcription of the p21 tumor-suppressor gene and other targets. This p53–p21 axis is essential for the induction of the G1/S arrest, while "only" being required for the maintenance of the G2/M arrest. In addition, p53 directly and indirectly (through its effector p21) can play distinct roles in supporting DNA repair. p21 also plays an active role in inhibiting apoptosis, which in a context-dependent manner can have cancer-promoting effects, in contrast to the general tumor-suppressive role of p21. Moreover, the p53–p21 pathway acts in the initiation of senescence. Conversely, the maintenance of cellular senescence is promoted by the p16–pRB pathway. Considering that the p53–p21 and p16–pRB pathways are among the most frequently compromised pathways in human cancers, more research is now needed to decipher which of their cell biological functions are essential for tumor suppression in vivo. Research aiming to translate the p53, p21, and/or p16 status into clinical cancer settings may help improve (maybe even optimize) the prediction of responses to radio- and/or chemotherapies. In the context of clinically developing and testing selective DDR inhibitors, the analyses of p53, p21, and/or p16 levels and mutations may even help open up completely novel anticancer approaches.

GLOSSARY

Acute DNA damage Severe and temporally limited DNA damage.
Aneuploidy The presence of an abnormal number of chromosomes in a cell.
Apoptosis The process of programmed cell death.
Cell-cycle checkpoint Specific control mechanisms in eukaryotic cells ensuring proper cell-cycle progression.
Cellular senescence An irreversible G1 cell–cycle arrest in which cells are refractory to growth factor stimulation.
Chronic DNA damage A type of DNA damage that persists for a long time or that regularly recurs.
DNA-damage checkpoint A cell-cycle checkpoint that is specifically activated upon the detection of DNA lesions.
DNA-damage response A complex network of cellular pathways that is responsible for the detection, signaling, and repair of DNA lesions.
E3 ubiquitin ligase An enzyme that catalyzes the transfer of ubiquitin from the E2 ubiquitin-conjugating enzyme to specific protein substrates.
Genomic instability (aka genetic or genome instability) Defined as a high frequency of mutations within the genome, where mutations can include changes in nucleic acid sequences, chromosomal rearrangements, and/or aneuploidy.
Genotoxic A damaging effect on a cell's genetic material.
Malignant transformation The process by which cells acquire the properties of cancer.
Mitotic catastrophe A cellular event in which a cell is destroyed during mitosis.
Permanent cell–cycle arrest An irreversible exit from cell-cycle progression.
Quiescence The state of a cell when it is not dividing as a consequence of a reversible cell–cycle exit.
Synthetic lethal interaction (aka synthetic lethality) A type of genetic interaction where the cooccurrence of two genetic events results in organismal or cellular lethality.
Transient cell–cycle arrest A fully reversible exit from cell-cycle progression.

LIST OF ACRONYMS AND ABBREVIATIONS

ATM Ataxia telangiectasia mutated
ATR ATM and Rad3 related
BER Base excision repair
CDK Cyclin-dependent kinase
CDKN1A CDK tumor-suppressor protein inhibitor 1A
CDKN2A CDK tumor-suppressor protein inhibitor 2A
CHK1 Checkpoint kinase 1
CHK2 Checkpoint kinase 2
CIP1 CDK interacting protein 1
CKI CDK inhibitor
DDR DNA-damage response
DNA-PK DNA-dependent protein kinase
DSB DNA double-strand break
HPV Human papillomavirus
HRR Homologous recombination repair
IR Ionizing radiation
MK2 MAPK-activated protein kinase 2
MMR Mismatch repair
MRN MRE11/RAD50/NBS1 complex
NER Nucleotide excision repair
NHEJ Nonhomologous end joining
pRB Retinoblastoma protein
SAHF Senescence-associated heterochromatin foci
SDI1 Senescent cell–derived inhibitor 1
UV Ultraviolet
WAF1 Wild-type p53-activated factor 1

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Author's Contributions

Yavuz Kulaberoglu, Ramazan Gundogdu, and Alexander Hergovich researched the literature and wrote the manuscript together. Yavuz Kulaberoglu and Ramazan Gundogdu created all figures. All authors read and approved the final manuscript.

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