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p21 in cancer: intricate networks and multiple activities

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Abstract

One of the main engines that drives cellular transformation is the loss of proper control of the mammalian cell cycle. The cyclin-dependent kinase inhibitor p21 (also known as $p21^{WAF1/Cip1}$) promotes cell cycle arrest in response to many stimuli. It is well positioned to function as both a sensor and an effector of multiple anti-proliferative signals. This Review focuses on recent advances in our understanding of the regulation of p21 and its biological functions with emphasis on its p53-independent tumour suppressor activities and paradoxical tumour-promoting activities, and their implications in cancer.

Higher eukaryotes have evolved multiple checkpoint mechanisms to monitor and respond to cellular perturbations, halting cellular progression until errors are fixed or the environment becomes permissible to the faithful transmission of genetic material[1]. Perturbations in checkpoint mechanisms are detrimental to the integrity of the genome, promote cancer development[2] and significantly affect the efficacy of anticancer treatment[3]. The tumour suppressor protein p53 mediates the DNA damage-induced checkpoint through the transactivation of various growth inhibitory or apoptotic genes. Among these, the small 165 amino acid protein p21 (also known as $p21^{WAF1/Cip1}$) mediates p53-dependent G1 growth arrest[4,5]. Earlier studies supported the view that p21 suppresses tumours by promoting cell cycle arrest in response to various stimuli. Additionally, substantial evidence from biochemical and genetic studies indicates that p21 acts as a master effector of multiple tumour suppressor pathways for promoting anti-proliferative activities that are independent of the classical p53 tumour suppressor pathway (FIG. 1). Despite its profound role in halting cellular proliferation and its ability to promote differentiation and cellular senescence, recent studies suggest that, under certain conditions, p21 can promote cellular proliferation and oncogenicity[6]. Consequently, p21 is often misregulated in human cancers, but its expression, depending on the cellular context and circumstances, suggests that it can act as a tumour suppressor or as an oncogene (TABLE 1).

p21 mediates its various biological activities primarily by binding to and inhibiting the kinase activity of the cyclin-dependent kinases (CDKs) CDK2 and CDK1 (also known as CDC2) leading to growth arrest at specific stages in the cell cycle (FIG. 2). In addition, by binding to proliferating cell nuclear antigen (PCNA), p21 interferes with PCNA-dependent DNA

DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
APC | BIRC5 | CCNA2 | CCNB1 | CDC2 | CDKN1A | CHEK1 | KLF6 | Wnt4
UniProtKB: http://www.uniprot.orgAKT1 | AP4 | ARF | ATM | BAX | BMP2 | caspase 8 | caspase 10 | CBP | CDC20 | CDC25 | CDK1 | CDK2 | CDK4 | CDK6 | CDX1 | CDX2 | DDB2 | DTL | E2F1 | E2F3 | ERBB2 | ETO | IKKβ| INK1 | KLF4 | MAP3K5 | MAX | MYC | NEUROD1 | NGF | notch 1 | p21 | p27 | p53 | p57 | PCNA | procaspase 3 | RB | RBL1 | RBL2 | RHOD | SKP2 | STAT3
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polymerase activity, thereby inhibiting DNA replication and modulating various PCNA-dependent DNA repair processes. In this Review we discuss recent advances concerning the complex role of p21 in the development of cancer. We describe the various effector functions of p21 that allow it to exert its biological activities. We further describe our current understanding of the various mechanisms that control p21 expression, both transcriptionally and post-transcriptionally, and how deregulation of these mechanisms may contribute to tumorigenesis.

Effector functions of p21

p21 — a negative regulator of the cell cycle

p21-mediated growth inhibition has been attributed to two main activities that depend on two non-overlapping structural domains: the carboxy-terminal PCNA-binding domain and the amino-terminal CDK–cyclin inhibitory domain[7,8]. Through binding to PCNA, p21 competes for PCNA binding with DNA polymerase-δ and several other proteins involved in DNA synthesis, thus directly inhibiting DNA synthesis[9].

p21 belongs to the Cip and Kip family of CDK inhibitors that includes p21, p27 and p57. These inhibit the kinase activity of broad but not identical classes of CDK–cyclin complexes through their N-terminal homologous sequences. p21 also inhibits CDK activity indirectly by interfering with the activating phosphorylation of CDK1 and CDK2 in the activation segment by an unidentified mechanism[10–12]. p21 binds the cyclin subunit through a conserved Cy1 motif in the N-terminal half and through a weaker and redundant Cy2 motif in the C-terminal half[13]. It also interacts with the CDK subunit through a separate CDK-binding site in the N-terminal half[13]. Through its Cy motifs, p21 disrupts the interaction between CDK and substrates that bind to CDK–cyclin through similar Cy motifs, such as RBL1 (also known as p107) and RBL2 (also known as p130), retinoblastoma (Rb) family proteins and CDC25C[14–16]. CDC25C, a tyrosine phosphatase that dephosphorylates the cyclin B-bound CDK1 that is required for entry into mitosis, can in turn alleviate CDK inhibition by competing with p21 for cyclin binding through the Cy motif[16].

p21 inhibits cell cycle progression primarily through the inhibition of CDK2 activity, which is required not only for the phosphorylation of RB with the consequent release and activation of E2f-dependent gene expression, but also for the firing of replication origins and for the activity of proteins directly involved in DNA synthesis[17]. Although this activity is shared by other CDK inhibitors such as p27 and p57, biochemical and genetic evidence suggest that they have distinct roles in tumorigenesis[18]. Nevertheless, p21 is uniquely positioned to function as a central inhibitor of CDK2 that is activated in response to a variety of cellular and environmental signals to promote tumour suppressor activities (FIG. 1). Experimental evidence however, suggests that the proliferation of some human cancer cells does not require active CDK2 (REF. [19]). Moreover, targeted deletion of *Cdk2* indicates that CDK2 is dispensable for cell cycle inhibition by p21 (REF. [20]). CDK1, at least in some tissues, may be the crucial target of p21 in tumorigenesis[27] because p21 effectively inhibits the kinase activity of CDK1 both in unstressed cells and after genotoxic stresses, leading to growth arrest in the G2 phase of the cell cycle[21–26] (FIG. 2).

p21 and regulation of gene transcription

Microarray-based studies suggest that p21 expression positively correlates with the suppression of genes that are important for cell cycle progression and the induction of genes associated with senescence[28]. Although p21-induced changes in gene expression can be explained by the inhibition of CDK2 activity by p21, several studies support additional roles for p21 that are independent of CDK2 or RB. For example, p21 associates directly with

E2F1 and suppresses its transcriptional activity[29] (FIG. 2). In response to notch 1 activation, p21 suppresses E2F1-dependent *Wnt4* expression, thereby controlling cellular growth[30]. p21 also binds to and represses the transcription factor signal transducer and activator of transcription 3 (STAT3)[31], thereby inhibiting cytokine-stimulated and STAT3-dependent gene expression. Similarly, p21 represses MYC-dependent transcription by associating with the N-terminus of MYC and interfering with MYC–MAX dimerization[32]. In turn, MYC disrupts the PCNA–p21 interaction, thus alleviating p21-dependent inhibition of PCNA and DNA synthesis[32].

The ability of p21 to promote cell cycle inhibition may also depend on its ability to mediate p53-dependent gene repression, as p21 is both necessary and sufficient for p53-dependent repression of genes regulating cell cycle progression, including *CDC25C*, *CDC2*, *CHEK1*, *CCNB1* (which encodes cyclin B1), *TERT* (which encodes telomerase reverse transcriptase) and the anti-apoptotic gene *BIRC5* (survivin)[33,34]. *CDC2*, *CHEK1* and *TERT* are repressed by p21 through the inhibition of CDK2-mediated phosphorylation of RB- and E2f-dependent transcription[34–36]. Additionally, by inhibiting CDK2, p21 inhibits the induction of *CDC2* and *CCNB1* indirectly, as the expression of these genes at the G1/S transition is mediated by the NF-Y transcription factor following its phosphorylation by CDK2 (REFS [37,38]).

p21 also activates gene transcription by de-repressing p300–CREBBP (CREB-binding protein) [39]. Because p300–CREBBP cooperates with multiple factors to promote the transcriptional induction of *CDKN1A* (the gene encoding p21) in response to a variety of stimuli (see below), de-repression of p300–CREBBP by p21 seems to be part of a positive feedback loop that amplifies p21 expression. The p21-dependent activation of p300–CREBBP-driven gene transcription has a significant role in regulating oestrogen receptor-α (ERα)-dependent gene expression, thereby inducing the differentiation of ERα-positive cells[40]. This is important as p21 upregulation is sufficient to prevent the growth of ERα-positive breast cancer cells[41] and may affect the efficacies of anti-oestrogen treatments.

p21 — a modulator of apoptosis

Although best known for its growth-inhibitory functions, p21 also inhibits apoptosis, which might account for its paradoxical oncogenic activities[6] (discussed below). Through its ability to promote cell cycle inhibition, especially in the face of genotoxic insults or microtubule-destabilizing agents, p21 protects cells from apoptosis because an active cell cycle is required to sense these agents and trigger apoptosis. The cytostatic effect of p21 with the consequent inhibition of apoptosis, however, is counteracted by several mechanisms. For example, the cellular response can be switched from cell cycle arrest to apoptosis by the selective transcriptional repression of *CDKN1A*, the selective activation of pro-apoptotic genes or defects in p21 expression downstream of p53 (REFS [42–44]). Furthermore, and as discussed below, post-translational modifications of p21 such as its phosphorylation (which affects protein stability[45–47] or cytoplasmic localization[45,48] of p21) and its cleavage by caspase 3 (REF. [49]) also account for the differential effects on cell cycle arrest versus apoptosis.

p21 can protect against apoptosis in response to other stimuli such as those induced by growth factor deprivation, p53 overexpression or during the differentiation of monocytes[6]. Under these conditions, apoptosis does not depend on cell cycle progression, so the anti-apoptotic activity of p21 cannot be attributed to its cytostatic effects. Instead, it may rely on the ability of p21 to regulate gene transcription through its multiple protein–protein interactions or through its roles in DNA repair (described below). For example, cytoplasmically localized p21 binds to and inhibits the activity of proteins directly involved in the induction of apoptosis, including procaspase 3, caspase 8, caspase 10, stress-activated protein kinases (SAPKs) and apoptosis signal-regulating kinase 1 (ASK1, also known as MAP3K5)[6,50] (FIG. 2). Furthermore, p21 can mediate the upregulation of genes encoding secreted factors with anti-apoptotic

activities[6,50]. p21 also suppresses the induction of pro-apoptotic genes by MYC and E2F1 through direct binding and inhibition of their transactivation functions[50]. The potential requirement for CDK activity for the induction of pro-apoptotic genes by MYC or E2F1, however, cannot be ruled out. Knock-in mice expressing p21 mutants that cannot suppress the transcription of genes or that fail to bind to or inhibit the transactivation functions of MYC or E2F1 will help to elucidate the contribution of these different effector functions of p21 to blocking apoptosis.

Paradoxically, p21 might also promote apoptosis through both p53-dependent and p53-independent mechanisms under certain cellular stresses. Exactly how p21 promotes apoptosis is not clear, but might depend on both p53-dependent and p53-independent upregulation of the pro-apoptotic protein BAX, activation of members of the tumour necrosis factor family of death receptors or effects on DNA repair[51]. In several of the studies that indicated a pro-apoptotic role for p21, it was shown only that apoptosis concurred with induction of p21 without determining whether p21 is required for the induction of apoptosis. Thus, a careful analysis is needed to investigate the exact role of p21 under these conditions.

p21 and DNA repair

p21 has a significant role in modulating DNA repair processes. First, by inhibiting cell cycle progression, p21 allows DNA repair to proceed while inhibiting apoptosis. Secondly, p21 can compete for PCNA binding with several PCNA-reliant proteins that are directly involved in DNA repair processes[9] (FIG. 2). For example, p21 interferes with PCNA–DNMT1, which is required not only for DNA synthesis but also for DNA repair[52,53]. Additionally, a p21 or p21-derived PCNA-interacting peptide inhibits mismatch repair[54] and PCNA-dependent base excision repair[55] indicating that the p21–PCNA interaction is sufficient for p21 to inhibit these DNA repair processes. Moreover, p21 modulates translesion DNA synthesis, which is important for bypassing stalled replication forks, by inhibiting PCNA monoubiquitylation[56,57].

Recent evidence suggests that p21 may also regulate nucleotide excision repair (NER) although its exact role has been controversial[58]. Defects in NER genes account for the rare genetic disorder xeroderma pigmentosum, which is characterized by an increased frequency of skin cancer[59]. The xeroderma pigmentosum group E gene product DDB2, a significant player in recognizing DNA damage in NER and a component of the CRL4 (cullin–RING ligase 4) E3 ubiquitin ligase complex, promotes p53 degradation in ultraviolet-irradiated cells with the consequent downregulation of p21 (REF. [60]). Significantly, downregulation or deletion of *Cdkn1a* in NER-deficient *Ddb2*$^{-/-}$ mouse embryonic fibroblasts restores NER activity, suggesting that p21 represses NER activity[60]. Additionally, in ultraviolet-irradiated cells[61,62], as well as in several neoplastic cell lines irradiated with ionizing radiation[63], p21 is proteolytically degraded through the action of another member of the CRL4 E3 ubiquitin ligase family, CRL^{CDT2} (also known as DTL), by a mechanism that requires the physical interaction of p21 with PCNA. Thus, the CRL4 E3 ubiquitin ligases seem to promote NER by downregulating p21, both transcriptionally (through the degradation of p53 through DDB2) and post-transcriptionally (through PCNA-dependent degradation of p21 through CRL^{CDT2}). Given the significant role of the various DNA repair processes in protecting against cancer, future work using DNA repair animal models will be useful in elucidating the extent to which p21 modulates DNA repair processes and whether this activity contributes to its tumour-suppressing or tumour-promoting activities.

Transcriptional regulation of p21 and cancer

Oncogenic activation of CDKN1A transcription

The transcriptional regulation of p21 has been extensively studied[64]. In this Review we focus on recent advances in our understanding of the transcriptional activation and repression of *CDKN1A* (FIG. 3). In diploid, non-immortalized, non-transformed cells oncogenic Ras activates *CDKN1A* transcription through both p53-dependent and p53-independent mechanisms. The p53-independent transactivation of *CDKN1A* by activated Ras requires the transcription factor E2F1 (REF. [65]). E2F1 and E2F3 strongly activate *CDKN1A* transcription by binding to *cis*-acting elements between −119 to +16 of *CDKN1A*[66,67]. Raf, a downstream effector of Ras, also transactivates *CDKN1A* independently of p53 (REF. [68]). Oncogenic Ras and Raf, however, induce p21-dependent senescence[69,70] and other genetic mutations are necessary for bypassing oncogene-induced senescence, which, like apoptosis, is a significant barrier to tumorigenesis[71]. The significant role of p21 in promoting HRAS-induced senescence is underscored by the finding that *Cdkn1a* deletion cooperates with activated HRAS to promote tumours in mice[72–75]. If p21 inhibits CDKs, how does HRAS or Raf transform cells or promote tumours when it induces p21 and cellular senescence? The answer to this question came from the discovery that RHOD, a small GTPase and a downstream effector that is required for the transforming activity of HRAS[76,77], suppresses *CDKN1A* trans-activation in response to HRAS stimulation[78]. In fact, RHOD is dispensable for HRAS-induced DNA synthesis in serum-starved *Cdkn1a*$^{-/-}$ fibroblasts, indicating that the primary role of RHOD is to suppress p21 induction by HRAS[78]. Recent work suggest that the HRAS–ARF–p53–p21 senescence circuitry can be disrupted by the expression of ID1 (REF. [75]), a helix–loop–helix transcription regulator that is overexpressed in a number of solid tumours[79, 80] and whose expression positively correlates with advanced disease and poor prognosis in prostate[81,82], ovarian[83] and breast cancer[79]. ID1 appears to render cells refractory to growth inhibition by p21 (REF. [75]). How ID1 prevents growth inhibition despite high levels of p21 remains unclear. However, given that p21 expression is frequently increased in human cancer (TABLE 1), understanding the mechanisms by which growth inhibition is prevented despite high levels of p21 will provide significant insight into the development and progression of various human cancers.

p53-independent regulation of CDKN1A transcription

Besides mitogen-dependent transactivation through the HRAS–Raf–Mapk pathway, *CDKN1A* transcription is also activated by several nuclear receptors including retinoid receptors, vitamin D receptors and androgen receptors. These operate independently of p53 through binding to their cognate responsive elements in the *CDKN1A* promoter[64]. The transcription factors SP1, SP3, AP2, CCAAT/enhancer binding protein-α (C/EBPα), C/EBPβ, BETA2 (also known as NEUROD1), GAX (also known as MOX2), homeobox A10 (HOXA10), STATs and myoblast determination protein 1 (MYOD1) also control *CDKN1A* transcription and upregulate p21 in response to a plethora of stimuli and anticancer agents (FIG. 3). Several of the transcriptional inducers of p21, such as nerve growth factor (NGF), progesterone, Ca^{2+} or the transcription factors BETA2 and MYOD1, cooperate with the transcriptional co-activator p300–CREBBP to activate the *CDKN1A* promoter[64].

Several members of the Krüppel-like transcription factor (Klf) family, which are key transcriptional regulators of proliferation and differentiation[84], also regulate the transcription of *CDKN1A* by p53-independent mechanisms. These transcription factors bind to GC boxes and upregulate or downregulate target gene transcription. Of particular interest is KLF6, a tumour suppressor that is frequently inactivated or downregulated in human tumours including prostate[85,86], lung[87], hepatic[88] and colon[89]. KLF6 binds two GC boxes located about 120 bp upstream from the transcription start site of *CDKN1A* and cooperates with p300–CREBBP to activate *CDKN1A* transcription[85,90]. Interestingly, KLF6 also activates transcription of the

transforming growth factor-β (TGFβ) receptors[91], indicating that KLF6, TGFβ and p21 are in the same tumour suppressor pathway (FIG. 3).

KLF4, which is expressed in epithelial tissues, is frequently downregulated in gastrointestinal, colorectal and bladder cancers, and its tumour suppressor activities partially depend on its ability to induce p21 expression[92]. In colorectal cancer, KLF4 downregulation or inactivation is associated with a similar reduction in p21 expression[93]. In response to DNA damage, KLF4 is induced by p53 and synergizes with p53 to activate the *CDKN1A* promoter[94]. In fact, through induction of p21, KLF4 mediates a DNA damage-induced and p53-dependent G1/S checkpoint[95]. Intriguingly, KLF4 directly suppresses the *TP53* promoter and exhibits paradoxical oncogenic activities through its ability to suppress senescence in response to oncogenic HRAS activation[96]. Thus, a complex pattern of p21 and p53 regulation by KLF4 may determine the role of KLF4 in oncogenesis[92].

The transcription of *CDKN1A* is also regulated by CDX2, a member of the caudal-related homeobox gene family that is involved (along with CDX1) in intestinal development, proliferation and differentiation[97]. CDX2 is a tumour suppressor that is downregulated during colorectal carcinogenesis[98,99]. Ectopic CDX2 expression inhibits the proliferation of colorectal cancer cells, induces the differentiation of undifferentiated intestinal epithelial cells[99,100] and induces p21 in human colon cancer cells through transactivation of the *CDKN1A* promoter[101]. p21 is also downregulated during colorectal tumorigenesis (TABLE 1), which is probably a direct result of CDX2 downregulation or inactivation, as stronger expression of CDX2 and p21 is observed mostly in tumour patches with higher levels of differentiation[98,99,102,103]. Strikingly, CDX2 activates *KLF4* transcription[104] and the *CDX2* gene itself is regulated by another tumour suppressor gene, *APC* (adenomatous polyposis coli) [105]. Consistently, colon cancer cells with mutations in *APC* or *CTNNB1* (which endodes β-catenin) exhibit lower expression levels both of CDX2 and KLF4 (REFS [104,105]). Thus, the APC–CDX2–KLF4–p21 axis is a multilayered tumour suppressor pathway that regulates p21 expression (FIG. 3).

Repression of CDKN1A transcription and cancer

Whereas the deregulated expression of p21 in cancer often correlates with the loss of function of transcriptional activators of p21 (including p53), upregulation or gain of function mutations in genes that repress *CDKN1A* transcription may also contribute to cancer development. For example, it is likely that the transcriptional repression of *CDKN1A* by MYC (FIG. 3) plays a part in the development of tumours in which MYC is overexpressed. This may be important in ERα-positive breast tumours in which oestrogen-dependent upregulation of MYC and the subsequent downregulation of p21 promote cell proliferation, and disruption of the MYC–p21 circuit contributes to the resistance to anti-oestrogen therapies[106].

Interestingly, MYC induces the transcription of *AP4*, a transcription factor that is frequently increased in colonic progenitor cells and in colorectal cancer and is capable of repressing *CDKN1A* transcription[107]. Significantly, AP4 overexpression inhibits p53-mediated cell cycle arrest, sensitizes cells to DNA damage-induced apoptosis and can suppress TGFβ-dependent *CDKN1A* transactivation[107]. Abrogating the growth-inhibitory functions of TGFβ is a hallmark of many cancers[108], so it is tempting to speculate that AP4, and other factors that inhibit p21 post-transcriptionally and abrogate TGFβ-induced growth arrest, such as the newly identified microRNA cluster miR-106b-25 (REF. [109]), may contribute to the development of these cancers.

Post-transcriptional control of p21

Ubiquitin-dependent and ubiquitin-independent proteolysis of p21

Although much of the control of p21 is at the transcriptional level, recent work suggests that post-transcriptional control of p21 is equally important. In actively dividing cells, p21 is an unstable protein with a half-life of about 20 to 60 minutes. Newly synthesized p21 protein is protected from proteasomal degradation by the activity of FKBPL (also known as WISP39), an adaptor that recruits HSP90 to p21 (REF. [110]). Importantly, cells depleted of WISP39 fail to upregulate p21 in response to DNA damage, indicating that the transcriptional control of *CDKN1A* is insufficient to upregulate p21 after DNA damage in the absence of p21 stabilization[110].

Three E3 ubiquitin ligase complexes, SCF^{SKP2} (SKP1–CUL1–SKP2), $CRL4^{CDT2}$ (CUL4A or CUL4B–DDB1–CDT2 (DDB1 is DNA damage-binding protein 1)) and APC/C^{CDC20} (anaphase-promoting complex (APC)–cell division cycle 20), promote the proteolysis of p21 through the proteasome at specific stages in an unperturbed cell cycle (FIG. 4). SCF^{SKP2}, $CRL4^{CDT2}$ and APC/C^{CDC20} promote the ubiquitylation and degradation of p21 only when it is bound by complexes of CDK2 with cyclin E or cyclin A, PCNA, or complexes of CDK1 with cyclin A or cyclin B, respectively. p21 that is not bound to CDK or PCNA, however, is degraded independently of ubiquitin by interaction of its C terminus with the C8α subunit of the 20S proteasome[111,112], but this method of p21 degradation does not occur in all cell types[113]. Ubiquitin-independent proteolysis of p21 does not require the ubiquitin-binding 19S proteasomal lid and instead is dependent on the REGγ subunit of the proteasome[113,114]. Various factors and signalling molecules affect the stability of p21 to affect cell cycle progression. For example, TGFβ and bone morphogenetic protein 2 (BMP2) suppress the growth of human colon cancer cells partly owing to increased p21 protein stability, although the mechanism is poorly understood[115,116]. In response to oxidative stress, the activation of JNK1 (JUN amino-terminal kinase 1) promotes growth arrest by inhibiting p21 ubiquitylation[117–119]. Additionally, a number of tumour viruses regulate p21 stability and affect cell cycle progression and apoptosis (BOX 1).

Several proteins involved in the ubiquitin-dependent proteolysis of p21 are upregulated in a variety of human tumours, suggesting that p21 downregulation may account for some of the oncogenic properties of these proteins. For example, SKP2, an F box protein that is the substrate recognition factor of the SCF^{SKP2} E3 ubiquitin ligase complex, which is necessary for the degradation of p21 at the G1/S transition and during S phase of the cell cycle, is oncogenic and frequently upregulated in human cancers[120]. Similarly, CDT2, a substrate recognition factor for p21 degradation[61,121] by the $CRL4^{CDT2}$ ubiquitin ligase complex, is overexpressed in breast cancer[122] and in primary hepatocellular carcinomas, especially at advanced stages[123]. Finally, *CUL4A* (which encodes the CUL4A E3 subunit of the $CRL4^{CDT2}$ ubiquitin ligase complex) is overexpressed in breast cancers and hepatocellular carcinomas[124,125]. It will be of interest to test whether the upregulation of these oncogenes causes p21 downregulation and whether p21 downregulation contributes to their oncogenic activity.

Phosphorylation of p21 and its effect on stability and localization

Whereas the growth-inhibitory functions of p21 are associated with its nuclear localization, the anti-apoptotic or oncogenic activities of p21 (described below) are frequently associated with its cytoplasmic accumulation. In fact, cytoplasmic expression of p21 is common in human malignancies and correlates positively with aggressive tumours and poor prognosis (TABLE 1). Multiple protein kinases catalyse the phosphorylation of p21 to regulate its stability and localization in the cell[126]. Phosphorylation of p21 at Ser130 by CDK2–cyclin E, for example, promotes its binding to SKP2, leading to its ubiquitylation and subsequent proteolysis, and

thus promotes cellular progression at the G1/S transition and during S phase of the cell cycle[127].

Phosphorylation of p21 at Thr145 in the PCNA-binding site by AKT1 (also known as PKB) disrupts its binding with PCNA[45,128], induces its cytoplasmic accumulation and is required for ERBB2-mediated proliferation of breast cancer cells and breast carcinogenesis[48,129,130]. Similarly, the overexpression of the IKKβ (inhibitor of nuclear factor-κB kinase-β), which is seen in some human breast cancers, is associated with AKT1 phosphorylation and the cytoplasmic accumulation of p21 (REF. [131]) (FIG. 2). The cytoplasmic accumulation of p21 promotes cell survival through the inhibition of cytoplasmically localized apoptosis-related proteins, and promotes cellular proliferation through both the alleviation of CDK2 and PCNA inhibition and the assembly of the D-type cyclins (D1, D2 and D3) with CDK4 and CDK6 (FIG. 2). Because AKT1 phosphorylates and inhibits glycogen synthase kinase-3β (GSK3β), which phosphorylates cyclin D1 at Thr286 and promotes its degradation[132], AKT1-mediated assembly of complexes of cyclin D1 with CDK4 or CDK6 is facilitated by the stabilization of cyclin D1.

In endothelial cells, however, AKT1-mediated phosphorylation of p21 at Thr145 does not affect p21 localization, although it disrupts its interaction with PCNA, decreases CDK2 inhibition and promotes endothelial cell proliferation[128]. On the other hand, in serum-stimulated endothelial cells, GSK3β phosphorylates p21 at Thr57 and promotes its degradation[133] by an unidentified mechanism. The contradictory effects of AKT1- and GSK3β-mediated phosphorylation of p21 at Thr145 and Thr57, respectively, on the fate of p21 may be explained by cell type differences or additional cell type-specific modifications on p21. Furthermore, the regulation of p21 by AKT1 and GSK3β in endothelial cells may have a role in promoting neovascularization and metastasis.

In addition to Thr145, AKT1 phosphorylates p21 at Ser146, also leading to the stabilization of p21 and cell survival[45]. p21 can also be phosphorylated at Ser146 by protein kinase C (PKC). However, it is unclear whether this phosphorylation is catalysed by PKCδ to stabilize p21 (REF. [47]) or PKCζ to destabilize p21 (REF. [134]). The explanation for this contradiction may lie in the cellular context in which these PKC isoforms are activated and on other proteins that affect p21 phosphorylation.

Box 1

Exploitation of p21 stability by tumour viruses

Many viral proteins affect the stability or post-transcriptional regulation of p21, thereby affecting cellular proliferation. For example, the human papilloma virus (HPV) E6 protein can downregulate p21 independently of p53 (REFS [173–176]). Although E6 is essential for the oncogenic activity of HPV and has anti-apoptotic activities, under some conditions, such as DNA damage[175], E6 downregulates p21 to promote apoptosis[177]. The adeno-associated virus type 2, a helper-dependent human parvovirus, preferentially downregulates p21 protein in HPV-infected cells with a concomitant increase in cyclin-dependent kinase 2 (CDK2)–cyclin E activity but prevents further progression through S phase, thus favouring the replication of the adeno-associated virus type 2 (REF. [178]). The hepatitis C virus core protein inhibits p21 post-transcriptionally, alleviates CDK2 inhibition and contributes to hepatitis C virus-mediated tumorigenesis[179]. Finally, the K cyclin encoded by the human herpesvirus 8 promotes p21 phosphorylation at Ser130 by CDK6 without affecting its stability or nuclear –cytoplasmic localization[180]. Interestingly, although the phosphorylation of p21 at Ser130 by CDK2 targets it for ubiquitylation by the SCF^{SKP2} (SKP1–CUL1–SKP2) E3 ubiquitin ligase complex and degradation[127], p21 phosphorylation by CDK6–cyclin K prevents p21 association with CDK2, thus alleviating

a p21-imposed G1 arrest[180]. Although the mechanism by which these viral proteins affect p21 stability or activity is largely unknown, these findings demonstrate that targeting p21 is a common mechanism by which these viruses regulate cell cycle progression and apoptosis.

p21 deregulation in cancer

Much of our understanding about the role of p21 in cancer has come from knockout mouse studies combined with biochemical and functional analysis of cells in culture. Groundbreaking work came from the initial discovery of p21 as a potential mediator of the tumour suppressor activity of p53 (REF. [135]). Subsequent work showed that, although deletion of *Cdkn1a* in mice abrogated DNA damage-induced and p53-dependent growth arrest, it had no effect on p53-dependent apoptosis[4,5]. p21 could not, therefore, account for all the tumour suppressor activities of p53. Nevertheless, p21 is a major determinant of tumour protection by p53 (REF. [136]), as *Cdkn1a* deletion drastically accelerated tumour formation in mice expressing a mutant form of p53 (*Trp53*$^{R172P+/+}$) that is incapable of inducing apoptosis but retains partial growth arrest activity[137].

The first genetic evidence supporting a tumour suppressor activity for p21 came from the discovery that *Cdkn1a*$^{-/-}$ mice developed spontaneous tumours[138]. The late onset of these tumours (average age of 16 months) compared with those arising in mice deficient in other tumour suppressor genes such as *Trp53* (REFS [139,140]), *p16* (REF. [141]) or *Arf* (REF. [142]) suggests that the loss of *Cdkn1a* by itself is insufficient to promote malignancy. Although many human cancers such as colorectal, cervical, head and neck, and small-cell lung cancers are associated with reduced p21 expression (TABLE 1), the extreme rarity of loss-of-function mutations in *CDKN1A* in human cancer[143–145] argues that p21 may not be a classical tumour suppressor. Instead, p21 synergizes with tumour suppressors and antagonizes oncogenes to protect against cancer (TABLE 2). Furthermore, *Cdkn1a* deficiency accelerates the development of chemically induced tumours in mice[146–149]. Additional *in vivo* evidence for tumour suppressor activity for p21 comes from studies using the transplantation of *Cdkn1a*$^{-/-}$ cells in mice with defined genetic alterations. For example, although the leukaemogenic fusion protein AML1–ETO (AML1 is also known as RUNX1) does not promote leukaemia without secondary mutations, fetal liver haematopoietic cells isolated from *Cdkn1a*$^{-/-}$ mice and transduced with *AML1–ETO* promoted leukaemogensis when transplanted into mice[150]. *Cdkn1a* deficiency also cooperates with the co-expression of HRAS and MYC[151], the expression of BCR–ABL1 (BCR is breakpoint cluster region) (REF. [152]) or with *Ink4* deletion[153] to promote transformation and proliferation of cells in culture. Together, these data are consistent with the multi-step tumorigenesis theory and a role for p21 in this process.

A significant insight into the role of p21 in tumour suppression came from a study by Shen *et al.*[154] demonstrating a prominent tumour suppressor role for p21 in a genomically unstable background. *Cdkn1a* deficiency cooperated with the loss of the DNA damage checkpoint protein ATM (ataxia–telangiectasia mutated) in promoting aneuploidy that preceded tumour development[154]. Furthermore, although malignancies developing in the aforementioned *Trp53*$^{R172P+/+}$ mice retain stable genomes, lymphomas and sarcomas arising in *Trp53*$^{R172P+/+}$;*Cdkn1a*$^{-/-}$ mice had an earlier onset and exhibited chromosomal aberrations and marked aneuploidy[137]. The finding that p21 downregulation inversely correlates with microsatellite instability in colorectal cancer, irrespective of the p53 status[155,156], adds support to the conclusion that the loss of protection against genomic instability by p21 contributes to human malignancy.

p21 also promotes genomic stability in stem cells, both maintaining the self-renewal capacity of stem cells (BOX 2), and possibly contributing to its oncogenic potential (discussed below). For example, although haematopoietic stem cells (HSCs) derived from mice that are engineered to express PML–RAR (retinoic acid receptor) — the initiating oncogene of human acute promyelocytic leukaemia (APL)[157] — exhibit relatively moderate DNA damage foci, those derived from *PML–RAR;Cdkn1a*$^{-/-}$ mice exhibit a significantly higher rate of DNA damage foci, with more than 95% of cells exhibiting multiple foci per cell[158]. Thus, at least in the context of overexpression of this oncogene, p21 seems to limit DNA damage and protect against genomic instability in HSCs. Although there is currently no evidence to suggest that the increase in genomic instability in the absence of p21 in HSCs results in increased tumorigenesis, it is conceivable that the acquisition of additional genetic alterations, under these circumstances, may uncover a protective role for p21.

Oncogenic activities of p21

The simple view that p21 acts as a tumour suppressor has been complicated by the finding that p21 can exhibit oncogenic activities[6,159]. p21 is overexpressed in a variety of human cancers including prostate, cervical, breast and squamous cell carcinomas and, in many cases, p21 upregulation correlates positively with tumour grade, invasiveness and aggressiveness and is a poor prognostic indicator (TABLE 1). As mentioned above, in some of these cases p21 is cytoplasmic so its oncogenic function might be dependent on non-traditional cytoplasmic targets of the protein. Although there is little or no direct evidence to suggest that p21 upregulation contributes to the development of these cancers, it may affect the responsiveness to chemotherapy and radiotherapy[160].

The theory that p21 may function as an oncogene under certain circumstances is supported by a limited number of mouse genetic studies that showed that *Cdkn1a* deletion suppressed the development of spontaneous lymphomas arising in *Trp53*$^{-/-}$ (REF. [161]) and *Atm*$^{-/-}$ (REF. [162]) mice and radiation-induced lymphomas arising in wild-type[138] and *Trp53*$^{-/-}$ (REF. [161]) mice. Interestingly, lymphomas arising in *Cdkn1a*$^{-/-}$ mice exhibit a high rate of apoptosis, suggesting that the anti-apoptotic activity of p21 is pro-tumorigenic[6]. Why such an oncogenic activity is only manifested in lymphomas is unclear, but lymphocytes may be particularly sensitive to the anti-apoptotic activity of p21. Because p21 is crucial for cellular differentiation, it is possible that reduced tumorigenesis in the absence of p21 is due to a block in cell differentiation at a stage in which the cells cannot proliferate.

Box 2

The role of p21 in stem cells

Recent evidence suggests that p21 is crucial for maintaining stem cell potential by restricting stem cell self-renewal in various tissues[146,181–183]. This is best understood in the haematopoietic system where, under homeostatic conditions,*Cdkn1a*$^{-/-}$ mice exhibit increased absolute numbers and proliferation of haematopoietic stem cells[181].*Cdkn1a*$^{-/-}$ haematopoietic stem cells, however, rapidly lose their stem cell potential following serial bone marrow repopulation. Premature death, owing to haematopoietic cell depletion, ensues when these animals are exposed to acute genotoxic stress. Thus, restricted proliferation is a prerequisite for long-term stem cell potential and p21, through its ability to suppress the cell cycle, is a crucial determinant of stem cell pool persistence *in vivo*[181]. However, in response to cytokines, *Cdkn1a*$^{-/-}$ bone marrow progenitor cells exhibit decreased proliferation[184,185]. Consequently, it was hypothesized that p21 has distinct roles in subcompartments of the haematopoietic lineages, inhibiting the proliferation of stem cells but stimulating the proliferation of progenitor cells[181]. This dichotomy may reflect the differential role of p21 in inhibiting cyclin-dependent kinase (CDK) complexes in stem cells

but promoting the assembly of complexes of D-type cyclins with CDK4 and CDK6 (REF. [163]) in their progeny.

Although some studies suggest that the lack of p21, and the consequent increase in stem cell populations (for example, in keratinocyte stem cells) is strongly associated with increased susceptibility to carcinogenesis[146,149,186], a recent study suggests that it does not contribute to carcinogenesis[187]. Nevertheless, p21 was recently shown to be crucial for maintaining the self-renewal capacity of leukaemia stem cells that were derived from mice expressing the leukaemia-associated oncogene PML RAR (retinoic acid receptor) by protecting them from exhaustion in stressful conditions[158]. The results demonstrate that p21 is important for the maintenance, rather than the initiation, of at least a subset of malignancies. They also suggest that this activity of p21 may vary depending on the specific genetic alterations.

As discussed, p21 can also promote oncogenesis independently of its anti-apoptotic activity by promoting the assembly of complexes of cyclin D with CDK4 or CDK6 without inhibiting their kinase activity[163]. For example, p21 promotes oligodendrogliomas only when it can form complexes with cyclin D1 (REF. [164]). p21-mediated nuclear retention of cyclin D1 protects cyclin D1 from cytoplasmic degradation[165] and promotes its association with and activation of CDK4 and CDK6. In fact, constitutively nuclear cyclin D1 (cyclin $D1^{T286A}$) restored the development of oligodendrogliomas in *Cdkn1a*$^{-/-}$ mice only if the cyclin D1 could complex with CDK4 (REF. [4]). The sequestration of p21 by CDK4–cyclin D and CDK6–cyclin D may also promote oncogenesis by freeing CDK2 from inhibitory p21. This is demonstrated by the ability of T cell leukaemia virus type 1 (HTLV-1) to bypass the G1/S arrest through binding of p21 to CDK4–cyclin D2 and the consequent activation of CDK2 (REF. [166]). The ability of p21 (at low stoichiometric concentrations) to promote the activity of CDK4–cyclin D and CDK6–cyclin D may explain why tumour suppression by p21 varies with its expression level or the genetic background — the loss of a single *Cdkn1a* allele (but not homozygous deletion), for example, accelerated tumour growth in mice carrying the *Wnt1* transgene[167].

Outlook: targeting p21 for cancer therapeutics

Several anticancer agents such as histone deacetylase (HDAC) inhibitors function, at least partly, through their ability to promote the induction of p21 (REF. [168]). Other agents such as statins, which are routinely used to lower cholesterol levels, exhibit profound anti-proliferative capacity by inducing p21 (REF. [169]) and are being investigated for their anti-tumorigenic activities[170]. The complex network regulating p21 activity and biological functions, however, warrants caution with regard to its application for cancer therapy. The various effects of p21 on gene regulation and its role in genomic stability, apoptosis, senescence and DNA repair may not only contribute to cancer development but also profoundly affect the efficacy of DNA-damaging agents or other anticancer drugs that induce p21. The challenge lies in selectively inhibiting only the oncogenic activities of p21 and not its tumour suppressor functions. Therefore, the development of agents that interfere with the ability of p21 to assemble CDK4–cyclin D and CDK6–cyclin D complexes but retain its ability to suppress CDK2 or CDK1 may be an attractive line of investigation. Alternatively, it may be beneficial, instead of targeting p21 *per se*, to selectively target factors upstream or downstream of p21 that affect these particular aspects of p21 function. Drugs that can specifically inhibit the anti-apoptotic functions of p21 may be especially effective when combined with other drugs that are capable of inducing p21, such as DNA-damaging agents.

Significant recent advances have been made in elucidating the various players that are involved in p21 degradation and the various post-translational modifications that affect the stability and cellular localization of p21. Biochemical and structural studies of the various ubiquitin ligase

complexes directly involved in p21 proteolysis under different conditions will undoubtedly help the development of selective inhibitors for these ligases and provide a platform for the development of a new generation of anticancer agents. Furthermore, DNA-damaging agents that may selectively inhibit AKT1 activity may not only deprive tumours of the pro-survival functions of AKT1, they are also likely to destabilize p21 leading to augmentation of their apoptotic effects. This possibility is supported by a study in which the DNA-damaging agent aminoflavone induced apoptosis of MCF7 breast cancer cells only at concentrations at which it reduced AKT1 activity and destabilized p21 (REF. [46]).

An alternative therapeutic approach may take advantage of the ability of p21 to induce senescence in tumours. Recent work suggests that tumour regression can be achieved through the reactivation of senescence, for example by restoring p53 function[171] or through the inactivation of MYC in tumours with functional p53 (REF. [172]). Although MYC inactivation upregulated p21 only in a subset of tumours, the results demonstrate that activation of senescence is not only feasible but also a promising approach to tumour regression *in vivo*. Even in tumours that retain high levels of p21, it may still be possible to induce tumour regression through the reactivation of senescence. However, this possibility will require a greater understanding of the various players (such as that described for the transcription factor ID1) that can abrogate p21-induced senescence despite high levels of p21. p21 expression in these tumours can potentially be exploited for therapy by targeting ID1 or similar molecules, leading to the reactivation of senescence downstream of p21. Finally, advances in our understanding of the precise role of p21 in modulating DNA repair processes under various conditions are urgently needed and may shed more light on the role of p21 in the development and treatment of cancer.

At a glance

- p21 came into the spotlight as a mediator of p53 tumour suppressor activity and as an inhibitor of cell cycle progression owing to its ability to inhibit the activity of cyclin-dependent kinase (CDK)–cyclin complexes and proliferating cell nuclear antigen (PCNA).
- The tumour suppressor activity of p21 stems from its role in inducing growth arrest, differentiation or senescence. Recently, it has become apparent that p21 is stimulated by many pathways that are independent of p53.
- p21 directly regulates gene expression and other cellular events through protein–protein interactions that are independent of CDKs and PCNA.
- Multiple transcription factors, ubiquitin ligases, and protein kinases regulate the transcription, stability and cellular localization of p21 thereby regulating its activity.
- Recent data suggest a tumorigenic role of p21 in certain contexts that relies on its ability to suppress apoptosis and promote the assembly of type-D cyclins with CDK4 and CDK6.
- Given that p21 is a tumour suppressor, but that it behaves as an oncogene in certain cellular contexts, targeting p21 or factors regulating its activity for therapeutic intervention is a promising but challenging task.

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Glossary

Senescence

A state of permanent growth arrest in G1 that is associated with changes in cell shape, cell adhesion and gene expression

Cyclin-dependent kinase

(CDK). In association with their cyclin regulatory subunits, CDKs control progression through key cell cycle transitions

Activation segment

The phosphorylation at a specific amino acid is required for maximal enzymatic activity of many kinases. In human cyclin-dependent kinases 1 and 2, the residues are Thr161 and Thr160, respectively, and are located within the T loop of kinase subdomain VIII

p300–CREBBP

(p300–CREB-binding protein). Two transcriptional co-activators, each possessing a histone acetyltransferase and a bromodomain (which binds acetylated lysines), that interact with many transcription factors and activate gene transcription

DNMT1

(DNA (cytosine-5)-methyltransferase 1). An enzyme that has a significant role in methylating cytosine residues shortly after replication and DNA repair, and in the regulation of tissue-specific patterns of methylated cytosines

Mismatch repair

Corrects DNA replication errors (base–base or insertion or deletion mismatches) caused by DNA polymerase errors

Base excision repair

A DNA repair pathway that operates on small DNA lesions such as oxidized or reduced bases, fragmented or non-bulky adducts, or those produced by methylating agents

Translesion DNA synthesis

A mechanism during DNA replication in which the standard DNA polymerase is temporarily exchanged for a specialized polymerase that can synthesize DNA across base damage on the template strand

Nucleotide excision repair

A process that removes large DNA adducts or base modifications that distort the double helix and uses the opposite strand as template for repair

CRL4

A cullin–RING ubiquitin ligase (CRL), composed of DDB1 (DNA damage-binding protein 1), a CUL4A or CUL4B E3 ligase subunit, and RBX1. CRLs recognize their substrates by interacting with one of many substrate recognition factors collectively called DDB1- and CUL4-associated factors

GC boxes

GC-rich sequences and related GT or CACCC boxes. Krüppel-like transcription factors bind with varying affinities to these sequences (also termed as SP1 sites) to regulate gene transcription

F box protein

F box proteins contain at least one protein–protein interaction F-box motif (about 50 amino acids). SKP2, the first identified F-box protein, is one of the three SCF complex components that recognize substrates for destruction through the SCF^{SKP2} E3 ubiquitin ligase

Substrate recognition factor

(SRF). SRFs are integral components of some cullin–RING ubiquitin ligase complexes and dictate substrate specificity. For example, SKP2 and CDT2 are p27 and p21 SRFs for the CRL1 (cullin–RING ubiquitin ligase 1) and CRL4 ubiquitin ligase complexes respectively

Microsatellite instability

A condition manifested by damaged DNA due to defects in the normal DNA repair process and characterized by unstable sequences of repeating units 1–4 base pairs in length

T cell leukaemia virus type 1

A retrovirus that is believed to be the cause of a rare cancer of T cells, adult T cell leukaemia–lymphoma

Histone deacetylase

Histone deacetylases are enzymes that regulate chromatin structure and function through the removal of the acetyl group from the lysine residues of core nucleosomal histones

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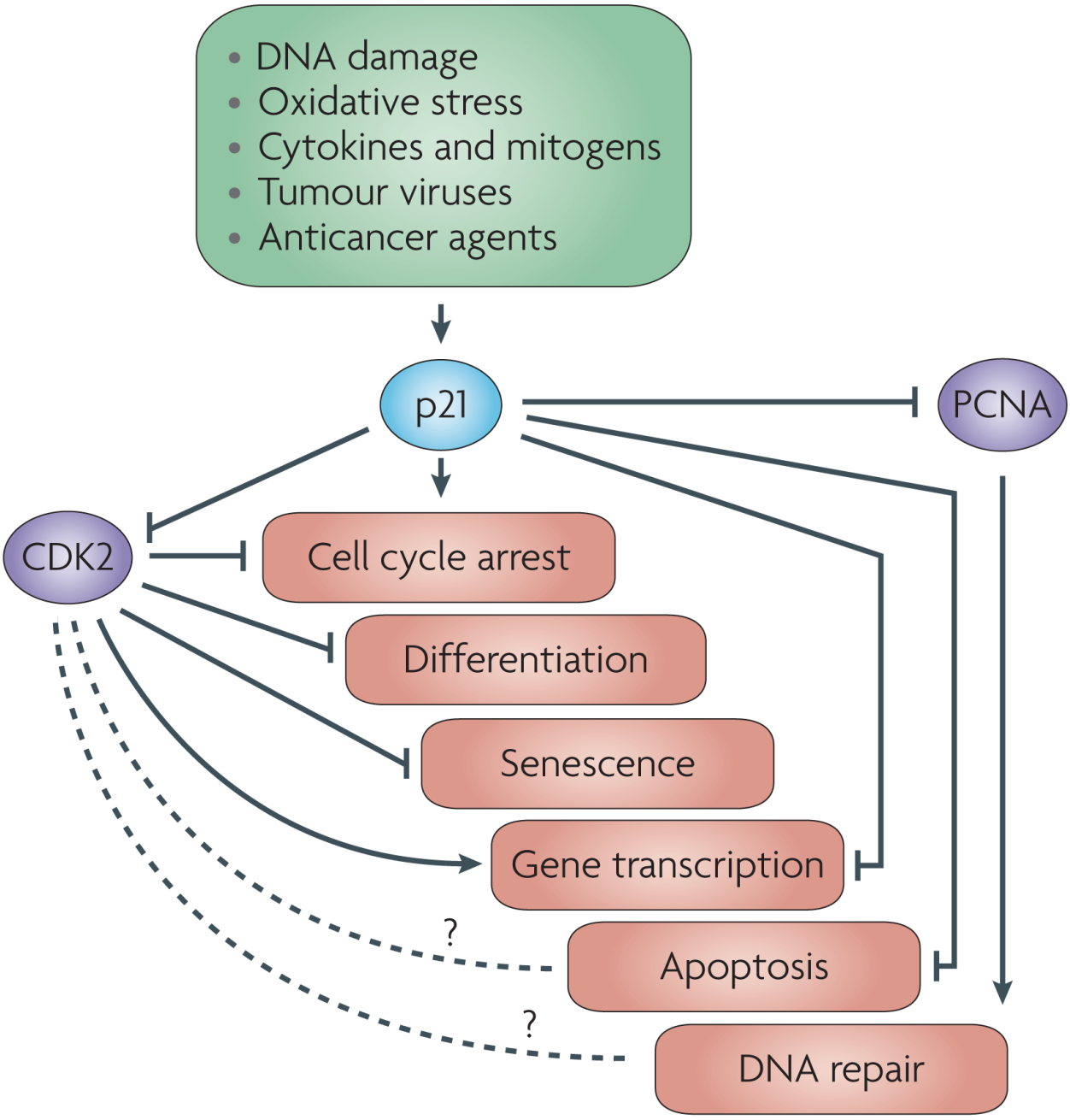

Figure 1. The central role of p21 in sensing and responding to a plethora of stimuli

p21 responds to a variety of stimuli to promote growth-inhibitory activities that depend primarily on its ability to inhibit the kinase activity of cyclin-dependent kinase 2 (CDK2). p21-induced cell cycle arrest also depends on its ability to inhibit CDK1. p21 can inhibit cellular proliferation independent of CDK2 inhibition by inhibiting proliferating cell nuclear antigen (PCNA), which is required for S phase progression. Some of the anti-proliferative activities of p21 rely on its multiple protein–protein interactions and its ability to regulate gene transcription. The various physiological responses triggered by p21 are interconnected. For example, cell cycle arrest induced by p21 promotes DNA repair by allowing sufficient time for the damaged DNA to be repaired before it is passed to daughter cells and is a major route

by which p21 exerts its anti-apoptotic activities. Similarly, the ability of p21 to regulate gene expression is important in promoting cellular senescence. The effect of p21 on gene transcription is generally inhibitory, but p21 can also activate gene transcription under certain conditions. The role of p21 in promoting DNA damage-induced and p53-dependent cell cycle arrest is well established and not the focus of this Review, and its role in mediating cellular responses to oxidative stress is well described[188].

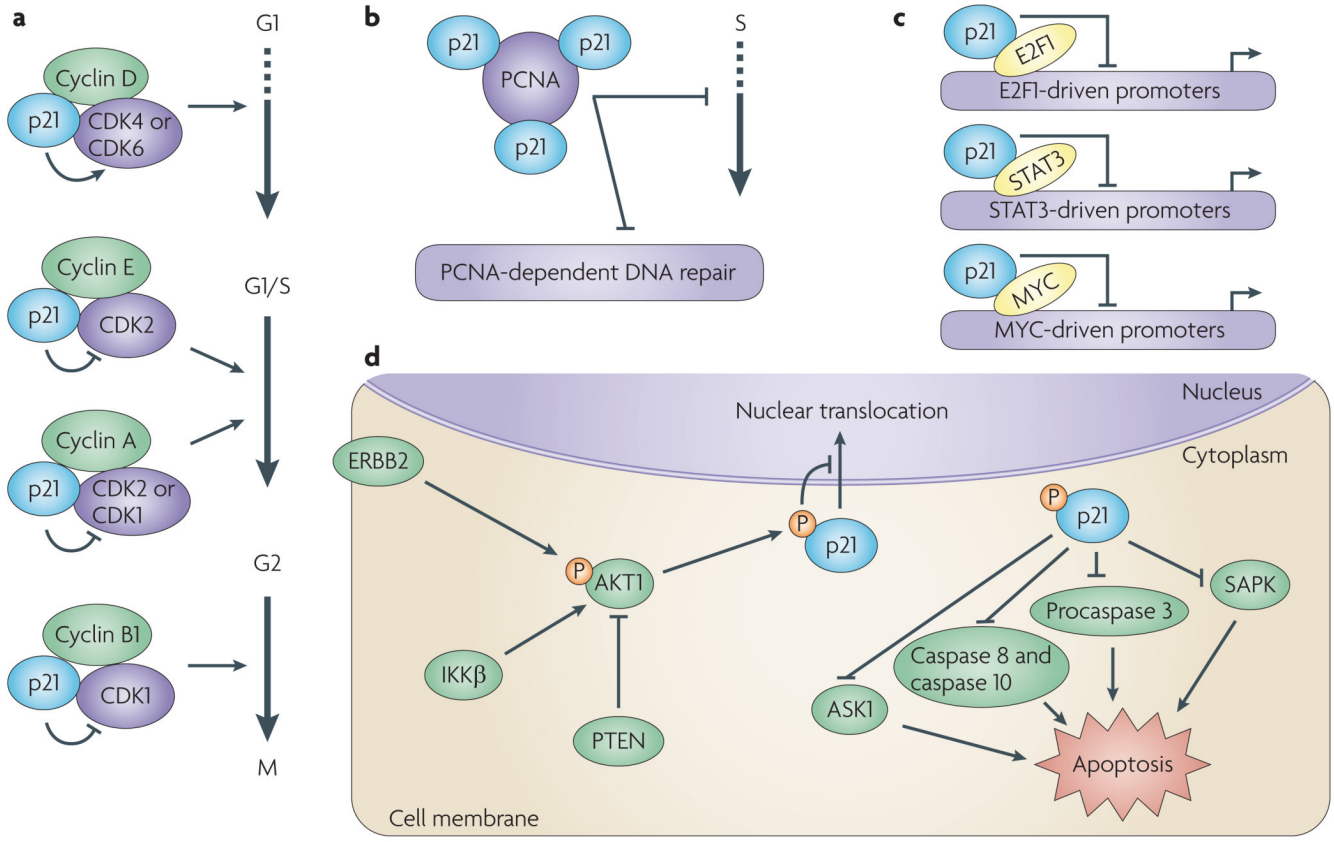

Figure 2. The molecular basis of p21 function in cancer

The figure shows activities of p21 in the nucleus and cytoplasm. **a** | Under certain conditions, p21 promotes the kinase activity of cyclin-dependent kinase 4 (CDK4) or CDK6 in complex with cyclin D, thus promoting progression through G1 (REF. [163]). p21 inhibits CDK2–cyclin E, with the consequent inhibition of CDK2-dependent phosphorylation of RB and the sequestration of E2F1, thus inhibiting E2F1-dependent gene transcription and progression into and through S phase. p21 also inhibits the kinase activity of CDK2–cyclin A and CDK1–cyclin A, which are required for progression through S phase and into G2 respectively. Additionally, p21 inhibits the kinase activity of CDK1–cyclin B1, thus inhibiting progression through G2 and G2/M. **b** | Through its carboxyl-terminal domain, p21 binds to and inhibits proliferative cell nuclear antigen (PCNA), thereby inhibiting processive DNA synthesis and modulating PCNA-dependent DNA repair pathways. **c** | p21 can inhibit the transcriptional activity of the transcription factors E2F1, STAT3 (signal transducer and activator of transcription 3) and MYC through direct binding and inhibition of their transactivation activity. This accounts for some of the anti-apoptotic effects of p21, which may contribute to its oncogenic activity. **d** | p21 phosphorylation at Thr145 by activated AKT1 (also known as PKB) downstream of ERBB2 (a member of the epidermal growth factor receptor family of receptor tyrosine kinases) or IKKβ (inhibitor of nuclear factor-κB kinase-β) signalling prevents the nuclear translocation of p21 (REFS [48,129,131]). Cytoplasmic p21 exhibits anti-apoptotic activity owing to the inhibition of proteins involved in apoptosis. Whether the phosphorylation of p21 by AKT1 only functions to retain p21 in the cytoplasm or is also required for its cytoplasmic activities is not clear. ASK1, apoptosis signal-regulating kinase 1, also known as MAP3K5; SAPK, stress-activated protein kinase.

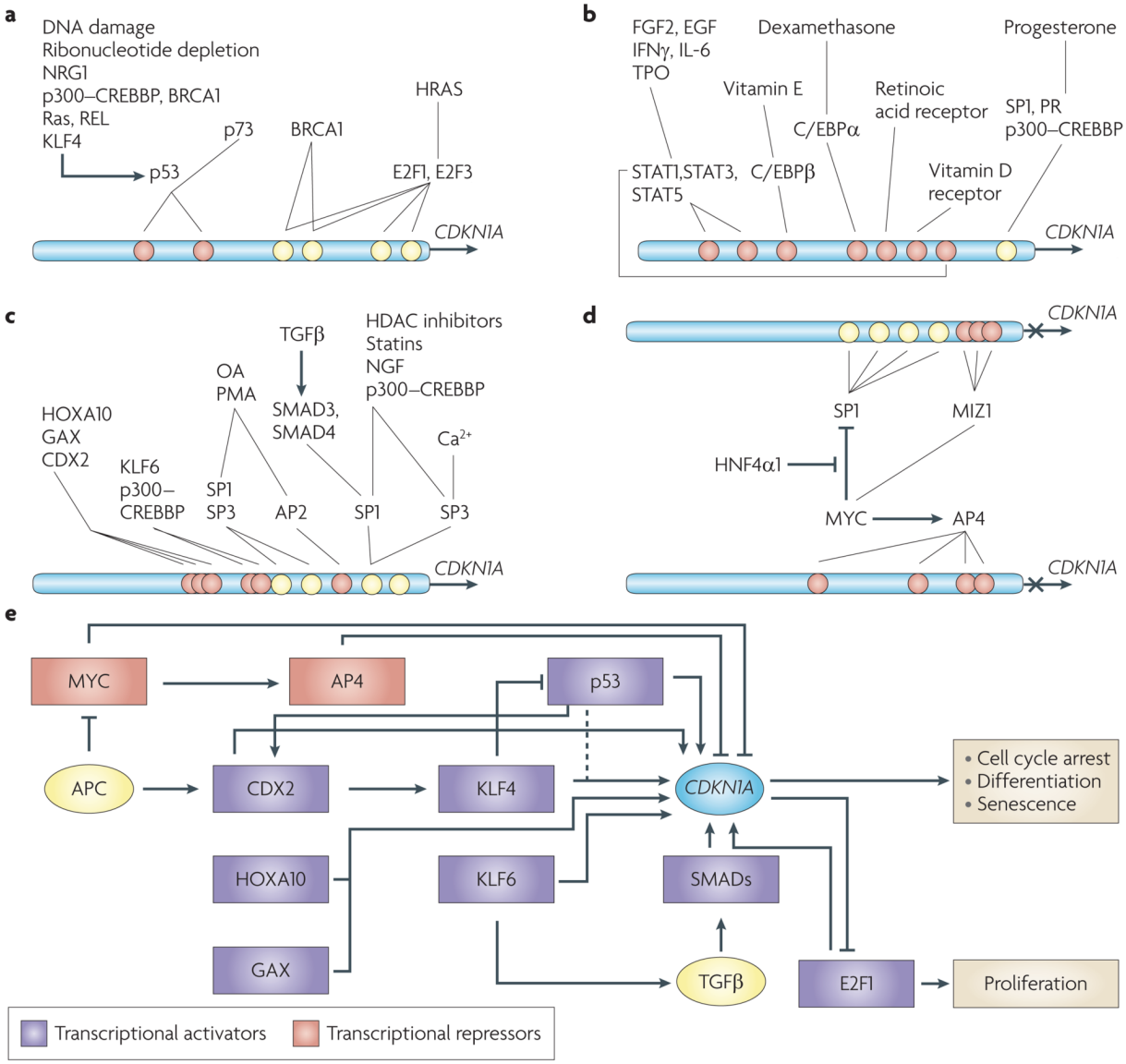

Figure 3. Transcriptional regulation of *CDKN1A* (the gene encoding p21)

Multiple signals and factors regulate transcription from the *CDKN1A* promoter. The four SP1-binding sites (yellow circles) in the proximal region of the *CDKN1A* promoter provide a relative reference for the position of other *cis*-elements (orange circles). **a** | Transcriptional activation of *CDKN1A* in response to a variety of stimuli, including DNA damage, are dependent on p53 and its family member p73. HRAS- and BRCA1-induced *CDKN1A* transcription, mediated by p53-dependent and p53-independent mechanisms, are also shown. **b** | Transcriptional activation of *CDKN1A* by growth factor and nuclear receptors. **c** | Activation of *CDKN1A* transcription by transcription factors and chemicals including anticancer agents (such as the histone acetyltransferase (HDAC) inhibitors) and drugs with anti-proliferative activity (such as statins). **d** | MYC represses *CDKN1A* transcription by binding to and inhibiting SP1 (REF. [189]), and this can be alleviated by the binding of the ligand-independent nuclear receptor hepatocyte nuclear factor 4α1 (HNF4α1) to SP1 (REF. [190]). In response to DNA damage, MYC is recruited to the *CDKN1A* promoter by MIZ1, and forms a ternary complex with the DNA methyltransferase DNMT3a, which represses *CDKN1A* transcription[191]. Additionally, AP4, a

basic helix–loop–helix protein and a transcriptional target of MYC, represses the *CDKN1A* promoter through binding to four proximal E-box motifs independently of MIZ1, SP1 or SP3 (REF. [107]). **e** | The *CDKN1A* transcriptional circuitry is shown, comprising transcription factors that upregulate (purple boxes) or downregulate (orange boxes) *CDKN1A* transcription under various conditions leading to growth arrest, differentiation or cellular senescence. Several of these factors function in transcriptional networks. APC, adenomatous polyposis coli; C/EBPα, CCAAT/enhancer binding protein-α; CREBBP, CREB binding protein; FGF2, fibroblast growth factor 2; GAX, also known as MOX2; HOXA10, homeobox A10; IFNγ, interferon-γ; IL-6, interleukin 6; KLF4, Krüppel-like factor 4; NGF, nerve growth factor; NRG1, neuregulin; OA, okadaic acid; PMA, phorbol-12-myristate-13-acetate; PR, progesterone receptor; STAT, signal transducer and activator of transcription; TGFβ, transforming growth factor-β; TPO, thrombopoietin.

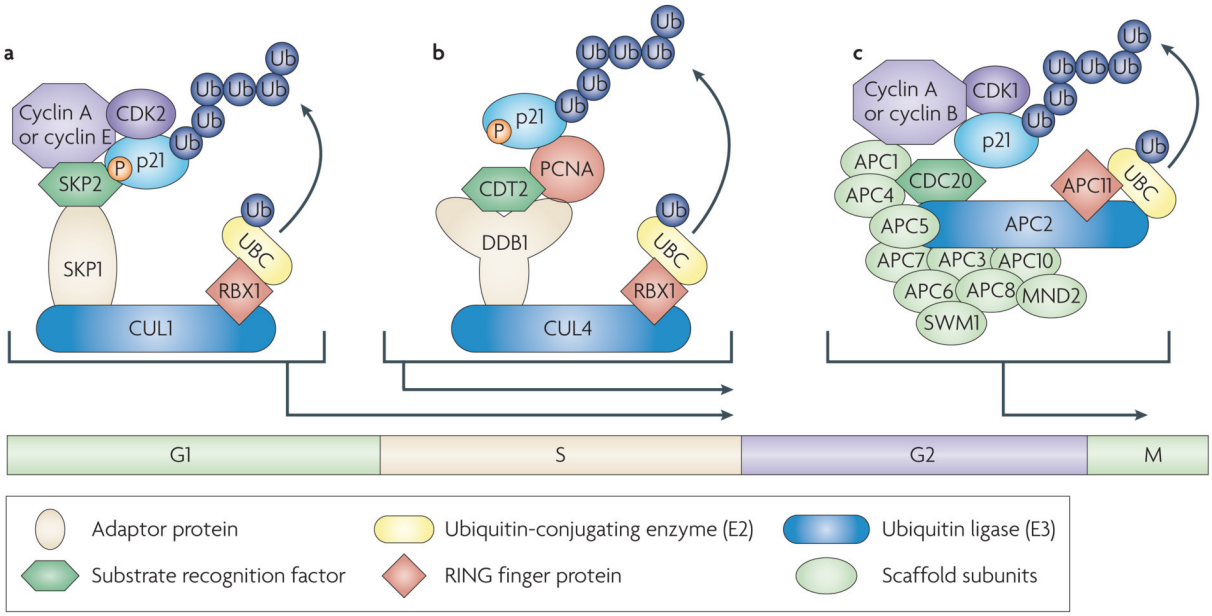

Figure 4. The p21 degradation cycle

The figure shows ubiquitin-mediated proteolysis of p21 during an unperturbed cell cycle. **a** | The SCF^{SKP2} (SKP1–CUL1–SKP2) E3 ubiquitin ligase complex promotes the ubiquitylation and degradation of p21 that is phosphorylated by cyclin-dependent kinase 2 (CDK2) at Ser130 at the G1/S transition and during S phase of the cell cycle, thus selectively de-repressing CDK2 kinase[127,192–194]. **b** | A second CRL (cullin-RING ligase), the $CRL4^{CDT2}$ (CUL4A or CUL4B–DDB1–CDT2 (DDB1 is DNA damage-binding protein 1)) E3 ubiquitin ligase complex, targets p21 for ubiquitin-dependent proteolysis specifically in S phase only when it is bound to proliferating cell nuclear antigen (PCNA)[61,62,121]. The $CRL4^{CDT2}$ ubiquitin ligase also targets p21 for degradation in response to low and moderate doses of ultraviolet irradiation[61,62] and after ionizing radiation[63] in a PCNA-dependent fashion. **c** | The degradation of p21 during G2/M is carried out by the APC/C^{CDC20} (anaphase-promoting complex (APC)–cell division cycle 20) E3 ubiquitin ligase complex, which recognizes CDK1–cyclin A- and CDK1–cyclin B-bound forms of p21, and is important for CDK1 activity necessary for mitosis[195]. The inhibition of APC/C^{CDC20} during spindle checkpoint activation results in the stabilization of p21, which inhibits CDK1 kinase activity and prevents premature entry of cells into mitosis. SKP2, CDT2 and CDC20 function as substrate recognition factors for the respective ubiquitin ligase complexes and bridge p21 to the rest of the E3 ligase. RBX1, RING box protein 1; UBC, ubiquitin-conjugating enzyme.

Table 1

p21 deregulation in human cancer

Tissue or cancer	Gene interaction or association	Description of association	Localization	Refs
Tumour-suppressive activity				
Colorectal cancer	*TP53*	p21 downregulation associates with p53 detection and the development of metastasis and poor survival	Not known	103
	TP53	p21 downregulation inversely correlates with high microsatillite instability irrespectively of the p53 status	Not known	155, 156
	None observed	Decreased p21 expression in dysplastic ACFs and adenomas; decreased p21 associated with lymph node and liver metastasis, and poor survival	Not known	102, 196,197
	KLF4	*CDKN1A* transcripts are downregulated and levels correlate with higher reductions in *KLF4* expression	Not known	93
Tonsillar carcinoma	HPV	p21 overexpression strongly associates HPV-positive tonsillar SCC with favourable prognosis	Not known	198
Gastric cancer	*TP53* and *TGFB1*	Those with p21-positive and p53-negative cancers had significantly higher survival curves; all p21- and p53-positive cases were TGFβ1 positive	Not known	199
Breast cancer	None observed	C94T of *CDKN1A* (Arg → Trp) with inability of p21 to inhibit CDK activity but intact ability to bind PCNA and promote CDK–cyclin association	Not known	200
Breast, gastric and ovarian cancers	*TP53*	Loss of p21 expression along with increased p53 detection associated with poor prognosis and decreased overall survival	Not known	206–208
Oesophageal and oral cancer	None observed	Polymorphism in codon 149 resulting in Asp to Gly substitution	Not known	201, 202
NSCLC	None observed	Reduced p21 expression in stage III compared with stage I or II	Not known	203
Cervical adenocarcinoma	None observed	p21 expression correlated with favourable prognosis	Not known	204
Pancreatic cancer	None observed	p21 is overexpressed in a subset of intraepithelial neoplasia	Not known	205
Laryngeal and oral carcinoma	None observed	p21 expression correlated with longer overall survival	Not known	209
Tumour suppressive and oncogenic activity				
Bladder carcinoma	None observed	p21 is a positive marker for invasive cancers, but is a negative prognostic marker in superficial cancers	Not known	210
Oncogenic activity				
Breast cancer	*CCNB1*, *TP53*	High cytoplasmic p21 levels were associated with high p53 and cyclin B and were significant predictors of poor prognosis	Cytoplasmic	211
	IKKB	Increased total and cytoplasmic p21 expression was observed in primary cancer and was associated with the expression of IKKβ	Cytoplasmic	131
	ERBB2	Positive correlation of ERBB2 expression with phosphorylated Akt and cytoplasmic p21; together these were associated with poor prognosis	Cytoplasmic	48,129, 130
HCC	HCV	Increased p21 expression correlates significantly with incidence in patients with HCV-associated chronic liver disease; cytoplasmic p21 associated with HCCs, especially in moderately and poorly differentiated HCCs	Cytoplasmic	212, 213
MM	*TP53*	Nuclear localization of p21 correlates with severity, with PCNA expression and p53 detection, and with poor survival	Nuclear	214
AML	None observed	High levels of p21 are observed and are associated with poor survival	Not known	215
Gliomas	*RB1*, *PCNA*	Overexpression of p21 in 50% of the cases, most notably in higher grades; p21 expression is an indicator of shortened disease-free survival and correlated loosely with PCNA and RB expression	Not known	216

Tissue or cancer	Gene interaction or association	Description of association	Localization	Refs
	None observed	Increased expression of p21 in various types of brain tumours	Not known	217
Prostate cancer	None observed	p21 overexpression associates with worst clinical outcome before and after androgen deprivation therapy	Not known	218, 219
Cervical carcinoma	None observed	Increased p21 expression significantly correlated with advanced stage	Not known	220,221
Ovarian cancer	None observed	Increased p21 associated with incidence, recurrence and metastasis	Not known	222
Oesophageal SCC	None observed	p21 is overexpressed; its expression associates with worst overall survival	Not known	223
Soft tissue sarcomas	None observed	Frequent p21 overexpression	Not known	224

ACF, aberrant crypt focus; AML, acute myeloid leukaemia; CDK, cylin-dependent kinase; HCC, hepatocellular carcinoma; HCV, hepatitis C virus, HPV, human papilloma virus; IKKβ, inhibitor of nuclear factor-κB kinase-β; *KLF4*, Krüppel-like factor 4; MM, multiple myeloma; NSCLC, non-small-cell lung carcinoma; PCNA, proliferating cell nuclear antigen; RB, retinoblastoma; SCC, squamous cell carcinoma; *TGFB1*, transforming growth factor-β1.

Table 2

Tumour phenotypes associated with *Cdkn1a* deletion in mice

Genotype	Tissues with *Cdkn1a* deletion	Tumour phenotype	Role	Refs
Rb1$^{+/-}$;*Cdkn1a*$^{-/-}$	All tissues	Accelerated tumour formation in *Rb1*$^{+/-}$ mice without affecting the spectrum of tumours (pituitary tumours, medullary thyroid tumours and pheochromocytomas)	Tumour suppressor	225
Trp53$^{515C/515C}$;*Cdkn1a*$^{-/-}$	All tissues	Accelerated the incidence lymphomas and sarcomas and resulted in the appearance of new sarcomas with aneuploidy and chromosomal aberrations	Tumour suppressor	137
Ink4c$^{-/-}$;*Cdkn1a*$^{-/-}$	All tissues	Increased frequency of pituitary adenomas and multifocal gastric neuroendocrine hyperplasia; development of lung bronchioalveolar tumours and hepatic nodular and parathyroid hyperplasia	Tumour suppressor	226
MMTV–HRAS;Cdkn1a$^{-/-}$	Mammary, salivary glands and lymphoid tissues	Dramatically increased the onset and frequency of tumours (mammary and salivary adenocarcinomas as well as benign Harderian hyperplasia with increased metastasis to the lungs and abdomen)	Tumour suppressor	72, 74
Apc$^{1638+/-}$;*Cdkn1a*$^{-/-}$	All tissues	Increased frequency and size of intestinal tumours; the effect is dependent on *Cdkn1a* dose.	Tumour suppressor	227
Muc2$^{-/-}$;*Cdkn1a*$^{-/-}$	All tissues	Increased the frequency and size of intestinal tumours and resulted in more invasive adenocarcinomas	Tumour suppressor	228
MMTV–Wnt1;Cdkn1a$^{-/-}$	Mammary, salivary glands and lymphoid tissues	Significant increase in mammary tumour growth rate in *MMTV–Wnt1;Cdkn1a*$^{-/+}$ mice, but no effect in *MMTV–Wnt1;Cdkn1a*$^{-/-}$ mice	Tumour suppressor	167
Wrn$^{\delta hel/\delta hel}$;*Cdkn1a*$^{-/-}$	All tissues	No effect on *Wrn*$^{\delta hel}$-dependent tumorigenesis	No effect	229
Cdkn1a$^{-/-}$	All tissues	Mice developed late spontaneous tumours of various origins, were less susceptible to radiation-induced carcinogenesis and developed delayed thymic lymphomas compared with wild-type littermates	Tumour suppressor and oncogenic	4, 138, 154
Ink4a$^{-/-}$;*Cdkn1a*$^{-/-}$	All tissues	Increase in fibrosarcomas and minor appearance of rhabdomyosarcomas; several tumour types arising in *Ink4a*$^{-/-}$ mice, such as lung adenomas and adenocarcinomas, were absent in *Ink4a*$^{-/-}$;*Cdkn1a*$^{-/-}$ mice	Tumour suppressor and oncogenic	230
Atm$^{-/-}$;*Cdkn1a*$^{-/-}$	All tissues	Increased frequency of tumour development and the appearance of sarcomas, myeloid leukaemia, hepatomas and teratomas; delayed appearance of thymic lymphomas	Tumour suppressor and oncogenic	154, 162
Trp53$^{-/-}$;*Cdkn1a*$^{-/-}$	All tissues	Reduced incidence of spontaneous and radiation-induced lymphomas	Oncogenic	161
MMTV–Myc;Cdkn1a$^{-/-}$	Mammary, salivary glands and lymphoid tissues	Decreased overall mammary tumour incidence; no effect on the onset or mean growth rate of tumours	Oncogenic	74

Apc, adenomatous polyposis coli; *Atm*, ataxia–telangietasia mutated; MMTV, mouse mammary tumour virus.