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REVIEW

Cyclin-Dependent Kinase Inhibitor p21^{Waf1}: Contemporary View on Its Role in Senescence and Oncogenesis

V. S. Romanov[1,2]*, V. A. Pospelov[1,3], and T. V. Pospelova[1,3]

[1]*Institute of Cytology, Russian Academy of Sciences, Tikhoretsky pr. 4, 194064 St. Petersburg, Russia; fax: (812) 297-3541; E-mail: vsromanov@hotmail.com*
[2]*Leibniz Institute for Age Research – Fritz Lipmann Institute, Beutenbergstraße 11, D-07745 Jena, Germany*
[3]*St. Petersburg State University, Universitetskaya nab. 7/9, 199034 St. Petersburg, Russia*

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Abstract—p21^{Waf1} was identified as a protein suppressing cyclin E/A-CDK2 activity and was originally considered as a negative regulator of the cell cycle and a tumor suppressor. It is now considered that p21^{Waf1} has alternative functions, and the view of its role in cellular processes has begun to change. At present, p21^{Waf1} is known to be involved in regulation of fundamental cellular programs: cell proliferation, differentiation, migration, senescence, and apoptosis. In fact, it not only exhibits antioncogenic, but also oncogenic properties. This review provides a contemporary understanding of the functions of p21^{Waf1} depending on its intracellular localization. On one hand, when in the nucleus, it serves as a negative cell cycle regulator and tumor suppressor, in particular by participating in the launch of a senescence program. On the other hand, when p21^{Waf1} is localized in the cytoplasm, it acts as an oncogene by regulating migration, apoptosis, and proliferation.

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Cyclin-dependent kinase inhibitors (CKIs) play a crucial role in the regulation of the cell cycle in nontransformed cells and are implicated in suppression of cell proliferation under stress conditions caused by growth factor deficiency, DNA damage, heat shock, and exposure to heavy metals or antiproliferative cytokines, particularly TGF-β [1, 2].

CKIs are divided into two families, Ink4 and Cip/Kip. The first includes four proteins: p15^{Ink4b}, p16^{Ink4a}, p18^{Ink4c}, and p19^{Ink4d}. They have rather narrow specificity: by interacting with CDK4 and CDK6, they prevent complex formation between these kinases and D-type cyclins [3, 4]. The Cip/Kip family includes p21$^{Waf1/Cip1}$, p27^{Kip1}, and p57^{Kip2}, which are able to inactivate cyclin-dependent kinase 2 (CDK2). However, many other proteins implicated in various cellular processes are also targets for members of this family. The most studied member of the Cip/Kip family is p21^{Waf1}.

* To whom correspondence should be addressed.

FUNCTIONS OF p21^{Waf1} IN THE NUCLEUS

p21^{Waf1} as a negative regulator of the cell cycle. p21^{Waf1} was identified as a p53-dependent protein implicated in cell cycle checkpoints in G_1 and S phases by inhibiting activities of cyclin E-CDK2 and cyclin A-CDK2 complexes (Fig. 1) [5, 6]. Later, it was shown that p21^{Waf1} also causes cell cycle arrest in the G_2 and M phases [7-10]. This probably occurs due to the implication of p21^{Waf1} in transcriptional repression of Emi1, an inhibitor of the APC/C ubiquitin-ligase complex (Anaphase-Promoting Complex/Cyclosome). It was demonstrated on HCT116 cells that this results in APC/C-dependent degradation of A- and B-type cyclins and cell cycle arrest in the G_2 phase [11]. Studies demonstrating negative cell cycle regulation by p21^{Waf1}, as well as experiments on murine models with knockouts of distinct tumor suppressors, in which p21^{Waf1} inactivation results in increased tumorigenesis, enable classification of this CKI as a tumor suppressor. It is worth noting, however, that this conclusion was later revised [12-15].

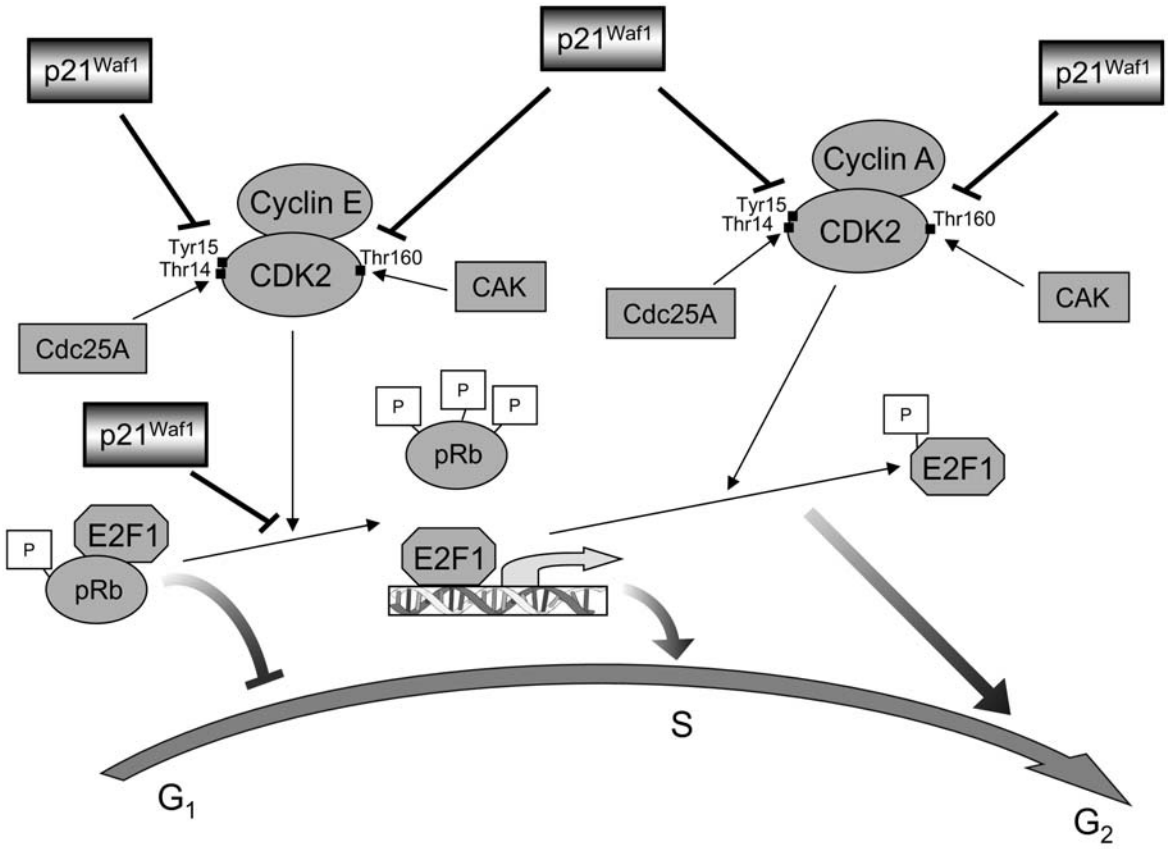

Fig. 1. Scheme demonstrating regulation of cyclin E-CDK2 and cyclin A-CDK2 complex activities that are responsible for cell transition from G_1 to S phase and progression through the S phase, respectively. The targets of cyclin E-CDK2 complexes are pRb family proteins, whose hyperphosphorylation leads to release of E2F family transcription factors, E2F1-DP1 dimers, which is required for expression of S phase-specific genes. The cyclin A-CDK2 complexes determine the further fate of E2F. The kinase of the cyclin A-CDK2 complex phosphorylates E2F1, which results in the loss of the DNA-binding capability of the E2F1-DP1 dimer whose timely inactivation is decisive for correct completion of the S phase and transition to the G_2 phase of the cell cycle. Inhibitory effect of $p21^{Waf1}$ is based on competition with CAK, Cdc25A, and pRb for binding with cyclin-CDK2 complexes (see text for details).

$p21^{Waf1}$ contains a CDK2 kinase-binding site (K-site) at the N-terminal region and cyclin A- and E-binding sites: Cy1 at the N-terminus (high affinity) and Cy2 at the C-terminus (low affinity). It was shown that all three sites are involved in the interaction of $p21^{Waf1}$ with cyclin E-CDK2 and cyclin A-CDK2 complexes [16, 17]. Crystallographic analysis demonstrated the interaction between the Cip/Kip family CKIs and cyclin-kinase complexes. An inhibitor initially interacts with a cyclin, and then – via the K-site – with CDK2 [18]. The inhibitory effect of $p21^{Waf1}$ is based on its binding to cyclin-kinase complexes, so that it shields the Thr160 residue of CDK2 from activating phosphorylation by CAK kinase (Fig. 1) [19]. Besides, the Cip/Kip family members prevent the activating dephosphorylation of Thr14 and Tyr15 of CDK2 by Cdc25A phosphatase, which interacts with cyclin-kinase complexes by a site homologous to Cy1. The inhibition of cyclin E-CDK2 and cyclin A-CDK2 complexes by $p21^{Waf1}$ is not possible after the activating dephosphorylation of CDK2 by Cdc25A. And otherwise, dephosphorylation of kinases cannot occur after formation of functionally inactive cyclin-CDK2-$p21^{Waf1}$ ternary complexes [20]. This can be explained assuming that cyclin-kinase complexes have the same binding sites for Cdc25A and $p21^{Waf1}$. Thus, the Cip/Kip family CKIs inhibiting cyclin-kinase complexes and CAK and Cdc25A proteins activating them continually compete for binding to cyclin-CDK2 (Fig. 1). Moreover, there are data on competition between $p21^{Waf1}$ and pRb family proteins (p107 and p130), which are CDK2 targets, for sites of binding with cyclin-CDK2 complexes [21, 22].

There are other $p21^{Waf1}$-mediated pathways of suppression of cell proliferation (Fig. 2). A site localized on the C-terminus of $p21^{Waf1}$ is responsible for interaction with a DNA-polymerase δ subunit, PCNA, and thereby for inhibition of DNA replication [23-26]. Besides, independently of cyclin-CDK2 complex inactivation, $p21^{Waf1}$ can regulate transcription by direct binding with E2F1 transcription factors to inhibit their activity [27]. $p21^{Waf1}$

can also inactivate other transcription factors, such as STAT3 and c-Myc, thus suppressing STAT3- and c-Myc-dependent transcription [28, 29]. Interestingly, the antiproliferative role of complex formation between transcription factors and p21Waf1 is not restricted to inactivation of these factors. In particular, the interaction between p21Waf1 and C/EBP-α transcription factors leads to stabilization of this CKI level due to suppression of its degradation [30]. Besides interactions with PCNA and transcription factors, p21Waf1 forms complexes with stress-activated Gadd45 family proteins, which likely also comprises part of the proliferation suppression program, but the role of this interaction remains unclear [31, 32].

Being a regulator of transcription factor activities, p21Waf1 is also implicated in control of gene expression at the level of regulation of transcription coactivators such as p300 and CBP. Cyclin E-CDK2 complexes bind with p300/CBP proteins and prevent their interaction with NF-κB, while p21Waf1, by inhibiting CDK2, causes p300/CBP release, thus providing NF-κB-dependent transcription [33]. Furthermore, p21Waf1 can regulate transcriptional activity of the coactivators p300 and CBP via their CRD1 domains, independently from cyclin E-CDK2. The mechanism of this p300/CBP activation remains largely unknown, but it was found that a mediator is required for manifestation of p21Waf1 effect on the CRD1 domain [34, 35]. Later, it was shown that p21Waf1 could directly bind to p300, thus regulating histone acetylation, an important process in DNA repair (Fig. 2). The p21Waf1 molecules interacting with PCNA and p300 in DNA damage regions prevent complex formation of these proteins and abolish the PCNA-dependent inhibition of HAT activity of p300, which is required for providing access of repair proteins to the damaged DNA [36].

The implication of p21Waf1 in DNA repair is not restricted to regulation of p300 activity [37]. p21Waf1 can directly interact with PARP1 enzyme, a marker of both single- and double-stranded DNA breaks that plays an important role in DNA repair via interacting with many repair proteins (Fig. 2) [38]. Fibroblasts with knocked out p21Waf1 demonstrate excessive accumulation of PARP1 in

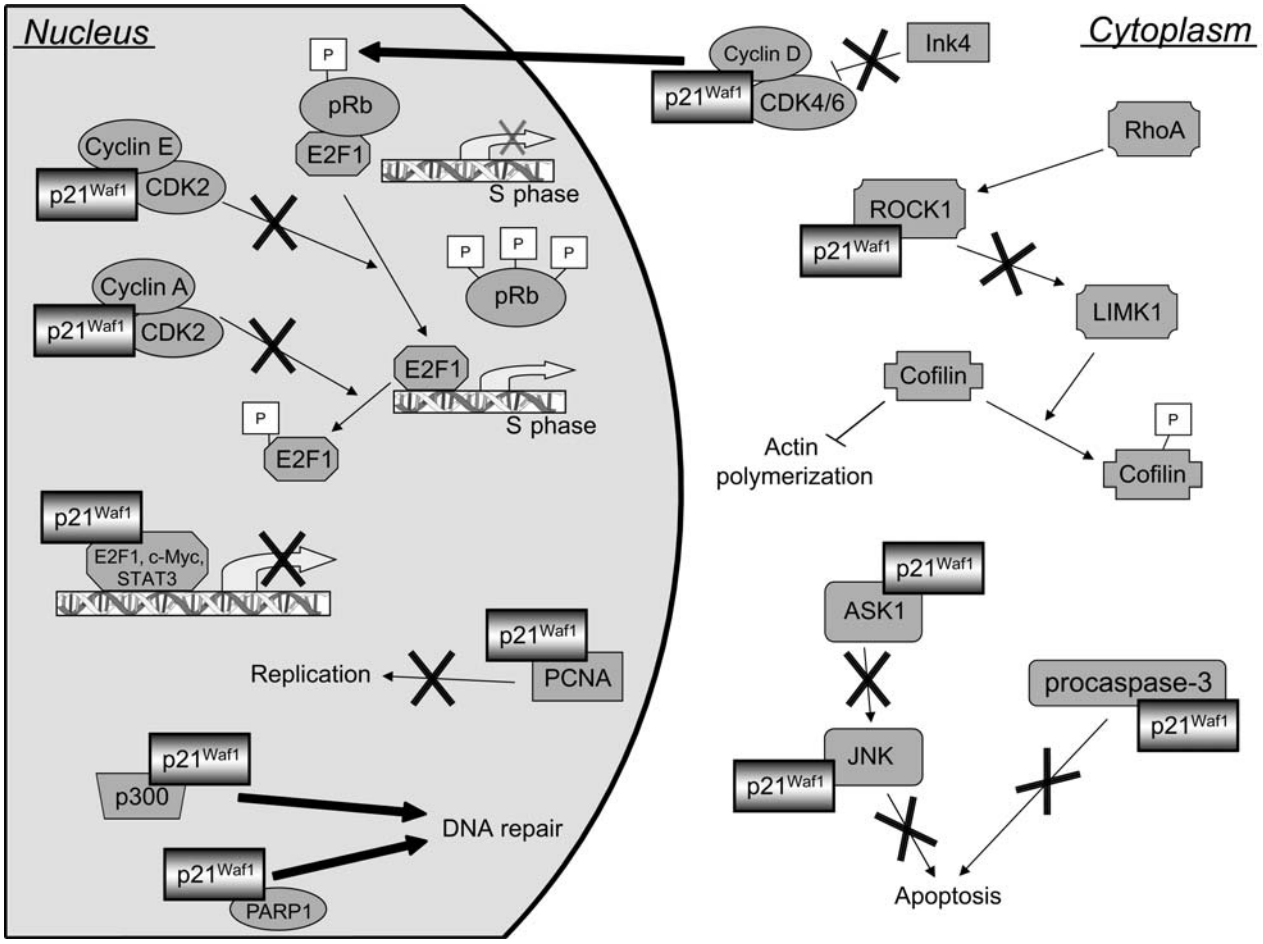

Fig. 2. Nuclear and cytoplasmic functions of p21Waf1. Depending on intracellular localization, p21Waf1 is implicated in regulation of the cell cycle, DNA repair, apoptosis, and reorganization of the actin cytoskeleton (see text for details).

DNA lesions, which counteracts access of repair factors such as XRCC1 and DNA polymerase β and decreases efficiency of DNA repair [39]. This suggests the involvement of $p21^{Waf1}$ in regulation of interaction between PARP1 and DNA repair proteins, with $p21^{Waf1}$ being a factor promoting efficient DNA repair. It is worth noting that $p21^{Waf1}$ regulating the precise action of the repair system and thus decreasing the probability of spontaneous mutations exhibits antioncogenic properties.

Thus, in the last two decades many data were accumulated on functions of $p21^{Waf1}$ as a negative regulator of the cell cycle and a tumor suppressor. It should be emphasized that all above-mentioned protein–protein interactions occur in the nucleus. To all appearances, it is the nucleus where $p21^{Waf1}$ is also implicated in the antitumor program of cellular senescence.

$p21^{Waf1}$ and cellular senescence. Since the program of cellular senescence is tightly associated with cell cycle arrest, a number of studies have been performed on the role of $p21^{Waf1}$ in this program. Nonetheless, its role is not completely elucidated.

According to a commonly accepted model, suppression of proliferation in senescent primary cells is initiated following p53-dependent transcriptional activation of the *CDKN1A* gene encoding $p21^{Waf1}$, which results in G_1 arrest. In many cell lines $p21^{Waf1}$ activation is temporary, and the level of this protein decreases after cell cycle arrest. In contrast, another CKI, $p16^{Ink4a}$, becomes upregulated, so this inhibitor was supposed to maintain cell cycle arrest in senescent cells [40-42]. Later, this strict distribution of the roles was revised because data appeared indicating that $p16^{Ink4a}$ is not an obligate protein for senescence of some cell types [43]. In particular, BJ human skin fibroblasts do not express $p16^{Ink4a}$, also after the activation of the cellular senescence program. Just inactivation of the p53-$p21^{Waf1}$ signaling pathway is required for canceling the cell cycle arrest of senescent BJ cells [44]. Besides, the miRNA- or siRNA-mediated knockdown of $p21^{Waf1}$ in HMEC epithelial cells, which also do not express $p16^{Ink4a}$, abrogates Ras-induced cellular senescence triggering, thus demonstrating the crucial role of namely $p21^{Waf1}$ in HMEC cell senescence [45]. Recently, Takeuchi and colleagues have demonstrated activation of both replicative and Ras-induced senescence of mouse embryonic fibroblasts (MEFs) with knocked out $p21^{Waf1}$ or $p16^{Ink4a}$, but obtained no evidence of senescence of MEFs with double knockout of these CKIs [46]. Thus, to all appearances $p21^{Waf1}$ and $p16^{Ink4a}$ fulfil interconvertible functions in the program of cellular senescence, and the implication of these CKIs in establishment of the senescence phenotype depends on their expression profiles and activities.

Despite the abundance of data implicating $p21^{Waf1}$ in the program of cellular senescence, mechanisms of its involvement in this process and contribution to establishment of senescence phenotype are not completely known. In human fibroblasts the $p21^{Waf1}$ level elevates through passages of primary cells [40, 41, 47]. This is associated with an increase in the portion of cells with drastically increased *CDKN1A* expression rather than with gradual accumulation of the protein in the entire population. Fast kinetics of elevation of $p21^{Waf1}$ expression coincides with the kinetics of its accumulation in response to DNA damage, which is consistent with a model of triggering replicative senescence resulting from DNA "lesion" in telomeric regions in the absence of telomerase activity [48]. Elevation of $p21^{Waf1}$ level in senescence correlates with ATM-dependent phosphorylation at Ser15 and activation of the transcription factor p53, as well as with accumulation of phosphorylated histone H2AX, a marker of double-stranded DNA breaks (nuclear γ-H2AX foci). Since the majority of γ-H2AX foci in replicative senescence are localized in telomeric regions, the elevation of $p21^{Waf1}$ expression most likely results directly from appearance of open chromosomal ends, which are recognized as double-stranded breaks [49].

To all appearances, elevation of $p21^{Waf1}$ level in telomere-independent senescence is also associated with accumulation of γ-H2AX foci. Recently, Passos and coworkers demonstrated the existence of a feedback loop between $p21^{Waf1}$ and reactive oxygen species (ROS) in ionizing radiation (IR)-induced senescence of human and murine fibroblasts. They proposed a model according to which the non-repaired double-stranded DNA breaks cause a p53-dependent accumulation of $p21^{Waf1}$, leading to activation of the Gadd45-p38-TGFβ signaling pathway, mitochondrial dysfunction, and gradual elevation of ROS level. High level of ROS, being a DNA damaging factor, leads to yet higher number of DNA breaks and causes accumulation of $p21^{Waf1}$ and γ-H2AX foci, which results in continuation of cell cycle arrest and activation of the cellular senescence program [50]. This model is of great interest because there are many reports on the correlation between high levels of ROS and cellular senescence [51-53]. It is worth noting that the model explains the multistage, rather than one-stage, character of the senescence program, although it does not answer the main question: how does elevated level of $p21^{Waf1}$ lead to the establishment of the characteristic senescence phenotype?

On the other hand, the data appear that accumulation of senescence-associated γ-H2AX foci might not be related to DNA breaks. Recently, a detailed analysis of γ-H2AX-containing foci in senescent cells was performed [54] that demonstrated the absence of single-stranded DNA breaks and repair enzymes therein. Besides, it was shown that cellular senescence of E1A/Ras-transformed rodent fibroblasts caused by long-term treatment with the HDAC inhibitor sodium butyrate (NaBut), as well as the IPTG-$p21^{Waf1}$-induced senescence of HT1080 human fibrosarcoma cells, are accompanied by accumulation of

γ-H2AX foci without DNA damage. The DNA comet assay revealed no DNA breaks in these senescent cells. Unlike DNA-damaging agents, particularly IR, NaBut causes formation of γ-H2AX foci lacking double-stranded DNA break markers, such as 53BP1 and phospho-ATM [55]. It should be emphasized that the NaBut-induced accumulation of γ-H2AX foci is p21^{Waf1}-dependent, as is evident from examination of E1A/Ras-transformants with knocked out p21^{Waf1} [56]. These data suggest cardinally different, not associated with DNA damage, nature of γ-H2AX focus formation in NaBut-induced cellular senescence.

In many human tumor cell lines, the overexpression of p21^{Waf1} leads to cell cycle arrest and appearance of cellular senescence markers such as SA-β-Gal activation and cell hypertrophy and flattening [57, 58]. Besides, cDNA array hybridization and gene expression assays suggest that the IPTG-induced overexpression of p21^{Waf1} in HT1080 cells leads to elevated expression of extracellular matrix proteins, such as fibronectin, PAI1, and integrin 3β, which are implicated in replicative senescence of primary fibroblasts [59]. The role of p21^{Waf1} in the NaBut-induced senescence of E1A/Ras-transformed murine fibroblasts (mERas) was also studied. It was shown that, unlike mERas p21$^{Waf1-/-}$, the program of cellular senescence is activated in full-genomic mERas cells, which is characterized by the appearance of markers such as activation of SA-β-Gal, accumulation of γ-H2AX foci, irreversibility of cell cycle arrest, and flattening and hypertrophy of the cells [56]. Some reports suggest that HDAC inhibitors have an antiproliferative effect, in particular due to the p53-independent activation of the *CDKN1A* gene promotor [60-62]. However, NaBut caused cell cycle arrest in mERas cells independently from the presence of p21^{Waf1}. The important difference is reversibility of the arrest in mERas p21$^{Waf1-/-}$, which is indicative of a crucial role of p21^{Waf1} in maintenance of cell cycle arrest in the NaBut-induced senescence of E1A/Ras-transformed MEFs [56].

Besides irreversibility of cell cycle arrest, hypertrophy is also a marker of cellular senescence [63]. Correlation between cell hypertrophy and p21^{Waf1} level has been reported in several studies. For example, in experimental diabetic nephropathy *in vitro* rat mesangial cells demonstrated hypertrophy correlating with elevated expression of p21^{Waf1}, but not other Cip/Kip family CKIs [64]. Experimental diabetes mellitus *in vivo* caused by streptozotocin was accompanied by hypertrophy of wild-type but not p21^{Waf1}-null murine mesangial cells [65]. p21^{Waf1} knockdown by antisense oligodeoxynucleotides (ODN) led to suppression of human mesangial cell hypertrophy caused by either high level of glucose in the culture medium or IGF1 stimulation [66]. Similar results were obtained on rat thoracic aortic smooth muscle cells: elevation of p21^{Waf1} expression was accompanied by cell hypertrophy, whereas suppression of p21^{Waf1} by ODN inhibited it [67]. Involvement of p21^{Waf1} in hypertrophy was also shown for murine adipocytes [68]. However, the mechanism of dependence of cell hypertrophy on p21^{Waf1} is not yet understood. Hypertrophy is a consequence of cell cycle arrest at high activity of mTOR kinase complex 1 (mTORC1) controlling protein synthesis via phosphorylation of key regulators of mRNA translation, namely the S6 kinase 1 (S6K1) and 4E-BP1 [63]. A correlation was shown in NaBut-induced senescence of mERas cells between p21^{Waf1}-dependent irreversibility of cell cycle arrest, hypertrophy, and activation of the mTORC1-dependent pathway. In mERas p21$^{Waf1-/-}$ cells compared with full-genomic mERas, elevation of phosphorylation of the mTORC1 cascade target, ribosomal protein S6 (rpS6), does not occur, as well as elevation of total protein and size of the cells. These data suggest involvement of p21^{Waf1} in regulating transformed MEF hypertrophy via control over the mTORC1 cascade [56]. In Blagosklonny's laboratory, it was shown that IPTG-induced overexpression of p21^{Waf1} causes hypertrophy of HT1080 human fibrosarcoma cells, which can be canceled by treatment with rapamycin, a specific inhibitor of mTORC1 [69, 70]. Taken together, these data suggest a dependence of cell hypertrophy and activity of mTORC1 on p21^{Waf1}, but the role of this CKI in regulation of the mTORC1 cascade remains unknown.

FUNCTIONS OF p21^{Waf1} IN THE CYTOPLASM

One can see from the previous section that many data demonstrate the antiproliferative role of p21^{Waf1} in regulation of the cell cycle. Nevertheless, alternative functions of this CKI in cytoplasm were reported, which change the oversimplified view on p21^{Waf1} as only a negative regulator of proliferation [12-14, 71, 72].

p21^{Waf1} and oncogenesis. It was shown *in vitro* that p21^{Waf1} knockout counteracts transformation of primary mouse embryonic fibroblasts, rather than facilitating it. Pantoja and Serrano have shown that, unlike p53- or p16^{Ink4a}-null MEFs, both the wild-type and p21^{Waf1}-null MEFs cannot be transformed by introduction of the *c-H-ras* oncogene. At high expression of constitutively active Ras, both cell lines maintain the shape of flattened non-transformed fibroblasts and cannot form colonies in soft agar and proliferate at clonal density [73]. Simultaneous introduction of *c-H-ras* and the complementary oncogene *E1A*, which leads to transformation of primary MEFs, cannot compensate for the absence of p21^{Waf1}. In particular, unlike full-genomic mERas, mERas p21$^{Waf1-/-}$ cells lack some features characteristic of the transformed phenotype, in particular they cannot proliferate at clonal density and divide independently on attachment to substrate [74].

Inactivation of p21^{Waf1} tumor suppressor function in human tumors is very rarely due to mutations or deletions

of the *CDKN1A* gene, but commonly occurs at the post-translational level [75]. Despite the absence of $p21^{Waf1}$ in some cancer types, its overexpression or cytoplasmic localization correlates with poor prognosis in malignant tumors of skin, pancreas, breast, prostate, ovary, cervix, and brain [14, 76-84]. The relocation of $p21^{Waf1}$ to the cytoplasm is mainly associated with its phosphorylation at the Thr145 residue located near the nuclear localization sequence (NLS) by Akt/PKB kinase [85, 86] and is accompanied by loss of its function as a negative regulator of the cell cycle [87, 88]. Elevation of Akt/PKB activity and phosphorylation of $p21^{Waf1}$ at Thr145 and its relocation from the nucleus to the cytoplasm correlate with poor prognosis in treatment of oncological diseases [89-92]. The relocation of $p21^{Waf1}$ from the nucleus to the cytoplasm apparently leads to the loss of its tumor suppressor activity and appearance of opposite, oncogenic, features.

The active involvement of $p21^{Waf1}$ in carcinogenesis was also supported by studies on murine models. It was shown that $p21^{Waf1}$-deficient mice compared with wild-type mice are not characterized by elevated susceptibility to tumor formation [93]. Moreover, $p21^{Waf1}$ knockout counteracts both appearance of thymus lymphomas in mice subjected to IR [94] and spontaneous formation of this tumor type in mice with knocked out p53 or ATM [95, 96]. Besides, $p21^{Waf1}$ knockout considerably decreases PDGF-induced formation of gliomas [97]. Recently, transgenic mice were generated overexpressing the Akt-phosphorylated at Thr145 form of $p21^{Waf1}$ in mammary epithelium. It was shown that this overexpression of cytoplasmic $p21^{Waf1}$ leads to accelerated tumor onset and promoted lung metastasis when compared with the wild-type mice [98]. Thus, both clinical studies and studies on murine models suggest that $p21^{Waf1}$ possessing tumor suppressor function in the nucleus can promote carcinogenesis when localized in the cytoplasm.

$p21^{Waf1}$ as a positive regulator of cell cycle. According to the contemporary view, cytoplasmic $p21^{Waf1}$ is implicated in positive regulation of the cell cycle. While three sites (Cy1, Cy2, and K) are responsible for the interaction of $p21^{Waf1}$ with cyclin E/A-CDK2, this CKI can also form complexes with cyclin D-CDK4/6, but exclusively via the Cy1 site [16]. Interestingly, Cip/Kip family CKIs do not counteract the CDK4/6 activating phosphorylation by CAK and do not inhibit activity of cyclin D-CDK4/6 complexes [99, 100]. Contrariwise, they are required for assembly of these complexes in the cytoplasm and their delivery into the nucleus (Fig. 2) [3, 72]. Knockout of $p21^{Waf1}$ in MEFs leads to a decrease in the content of cyclin D-CDK4 complexes, suppression of their activity, and decrease in cyclin D level in the nucleus. In cells with double knockout of two Cip/Kip CKIs, $p21^{Waf1}$ and $p27^{Kip1}$, cyclin D-CDK4 complexes virtually do not form, the level of cyclin D in the nucleus is considerably decreased, and CDK4-associated kinase activity determined by phosphorylation of pRb remains very low. Introduction of plasmids encoding $p21^{Waf1}$ or $p27^{Kip1}$ in these double knockout MEFs restores assembly of cyclin D-CDK4 complexes [101]. This is probably because Cip/Kip family members, when binding to cyclin D and CDK4/6, counteract the interaction between Ink4 family inhibitors and CDK4/6 and play the role of a frame for cyclin D-CDK4/6 complex assembly [102]. Already being in the nucleus, cyclin D-CDK4/6 sequestrate Cip/Kip family CKIs, thus preventing inactivation of cyclin E/A-CDK2 complexes [72, 103]. Thus, the cytoplasmic $p21^{Waf1}$ plays a role of positive regulator of the cell cycle. Obviously, it is a cause of enhanced proliferation in some cell lines in experiments on directed relocation of $p21^{Waf1}$ from nucleus to cytoplasm [87, 104, 105].

$p21^{Waf1}$ and actin cytoskeleton. It has been established that Cip/Kip family members, when localized in the cytoplasm, control reorganization of the actin cytoskeleton and cell migration [106]. In particular, it was shown that synthetic peptides identical to the C-terminus of $p21^{Waf1}$ uncouple integrins $\alpha_v\beta_3$ and focal contacts, thus inhibiting integrin-dependent melanoma cell flattening [107]. Besides, the cytoplasmic $p21^{Waf1}$, by binding to and inactivating Rho-associated kinase 1 (ROCK1), regulates the RhoA-ROCK1-LIMK1-cofilin cascade, whose activity is required for actin stress fiber and focal contact formation (Fig. 2) [108, 109]. Tanaka and associates reported that overexpression of $p21^{Waf1}$ with deletion of the NLS sequence in hippocampal neurons and neuroblastoma cells *in vitro* inhibits ROCK1 and causes remodeling and elongation of axons [108]. Later, it was shown that delivery of the TAT-fusion protein of cytoplasmic $p21^{Waf1}$ with the same deletion, locally after dorsal hemisection of the thoracic spinal cord in rats, significantly stimulated axonal regeneration and recovery of hindlimb function [110]. Another study suggests that elevation of $p21^{Waf1}$ expression in Ras-transformed NIH3T3 fibroblasts leads to inhibition of the RhoA-ROCK1-LIMK1-cofilin cascade [109]. $p21^{Waf1}$ is relocated to the cytoplasm and binds with ROCK1, which leads to a decrease in the phosphorylation level of cofilin and to a depolymerization of actin. Inactivation of the Ras-Raf-MEK-ERK cascade by MEK inhibitor U0126 results in a decrease in the level of $p21^{Waf1}$ and its relocation to the nucleus, which correlates with restoration of the actin cytoskeleton and suggests Ras-dependent regulation of intracellular localization of $p21^{Waf1}$ in NIH3T3 fibroblasts. Experiments on overexpression of $p21^{Waf1}$ with deleted NLS sequence in Ras-transformed NIH3T3 cells have shown that it is the cytoplasmic fraction of $p21^{Waf1}$ that is responsible for actin cytoskeleton disorganization. These experiments have also shown that $p21^{Waf1}$ inability to migrate to the nucleus leads to canceling the effect of U0126 on the cytoskeleton [109]. It should be noted that transformation of immortalized Swiss-3T3 fibroblasts with the *c-H-ras* oncogene leads to disorganization of the cytoskeleton and enhancement of cell migration, whereas overexpression of

LIMK1 followed by restoration of stress fibrils decreases mobility of the cells [111]. The Ras-dependent protein p21Waf1 is apparently implicated in this process; when localized in the cytoplasm, it inhibits the RhoA cascade and can stimulate migration of the transformed cells. p21Waf1 was shown to play a key role both in disorganization of the actin cytoskeleton and regulation of migration and invasion of E1A/Ras-transformed MEFs [74]. The presented data demonstrate an important role of p21Waf1 in regulation of the actin cytoskeleton and cell migration. It should be emphasized that, in the context of tumor cell migration and metastasis, such behaviour of this CKI suggests an oncogenic function of cytoplasmic p21Waf1.

p21Waf1 and apoptosis. Another function of cytoplasmic p21Waf1 is associated with regulation of apoptosis (Fig. 2) [72, 112]. This CKI is directly involved in inhibition of this antitumor program by means of binding with a number of proapoptotic proteins to inactivate them. The phosphorylation of p21Waf1 at Thr57 and Ser130 by ERK2 MAP kinase leads to its relocation from the nucleus to the cytoplasm [113], which is directly associated with protection of human primary fibroblasts from apoptosis following mild DNA damage [114]. The cytoplasmic p21Waf1 inhibits the JNK (SAPK) family MAP kinases, whose activation is a part of the cell death program initiated in response to stress agents [115]. The inhibition of the stress-activated MAP kinase cascade by cytoplasmic p21Waf1 also occurs via inactivation of upstream kinase, MAPKKK ASK1 [116]. Besides, it was shown on HepG2 human hepatoma cells that p21Waf1 forms a complex with procaspase-3, a precursor of caspase-3 that directly digests cell proteins. This interaction prevents processing and activation of caspase-3 and leads to suppression of Fas-mediated apoptosis [117]. Another report suggests that p21Waf1-procaspase-3 complex formation occurs on the surface of mitochondria following the phosphorylation of p21Waf1 by protein kinase A (PKA) [118]. It is worth noting that – by inhibiting apoptosis – the cytoplasmic fraction of p21Waf1 counteracts one of the main antitumor programs, thus contributing to carcinogenesis [13, 72, 119, 120]. In particular, overexpression of p21Waf1 protects SK-MEL-110 melanoma cells from p53-dependent apoptosis [121] and suppresses prostaglandin A_2-induced apoptosis of RKO colon carcinoma cells [122]. Induced overexpression of p21Waf1 causes resistance of glioblastoma cells to chemotherapeutics, such as 1,3-bis(2-chloroethyl)-1-nitrosourea and cisplatin [123], and protects H1299 lung adenocarcinoma cells, which do not express p53, from the cytotoxic effect of adriamycin or IR [124]. In contrast, the absence of p21Waf1 leads to considerable enhancement of HCT116 colon carcinoma cell apoptosis caused by treatment with NaBut or camptothecin [125, 126]. Recently, it was shown both *in vitro* and *in vivo* that there are antiapoptotic functions of cytoplasmic p21Waf1 that determine cases of ovarian carcinoma cell resistance to cisplatin [127].

Thus, the modern view of the role of p21Waf1 obviously differs from the primary concept that it is only a negative regulator of the cell cycle and a tumor suppressor; the functions of p21Waf1 are determined depending on its intracellular localization. In the nucleus p21Waf1 activity is directed to suppression of proliferation, while in the cytoplasm, where p21Waf1 is relocated following phosphorylation at distinct sites, it fulfils alternative functions. The presented data suggest oncogenic properties of cytoplasmic p21Waf1 and its implication in malignant transformation of cells.

Depending on intracellular localization, p21Waf1 is involved in different signaling cascades and cellular programs. Nuclear p21Waf1 fulfils the integrated negative regulation of the cell cycle by influencing cell proliferation at the levels of cyclin-kinase complexes, transcription factors and coactivators, and suppressing DNA replication. To all appearances, the negative regulation of proliferation determines the key role of p21Waf1 in the program of cellular senescence. Besides, nuclear p21Waf1 is also implicated in regulation of DNA repair. In contrast, cytoplasmic p21Waf1 facilitates cell proliferation, inhibits apoptosis, and by regulating reorganization of the actin cytoskeleton influences cell migration. These data suggest that p21Waf1 on one hand is a tumor suppressor, when localized in the nucleus, and on the other hand is an oncogene, when localized in the cytoplasm. Thus, in a determinate sense, p21Waf1 is two-faced Janus, because it can play opposite roles in oncogenesis depending on the intracellular localization. The presented data demonstrate an extremely broad range of processes regulated by this cyclin-dependent kinase inhibitor that, under closer examination, proves to be a regulator of a variety of cell programs, in which its implication might hardly be expected soon after its discovery.

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