Cell. Mol. Life Sci. 65 (2008) 3255 – 3264
1420-682X/08/203255-10
DOI 10.1007/s00018-008-8296-7

Cellular and Molecular Life Sciences

Review

Post-translational regulation of the tumor suppressor p27^{KIP1}

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Received 26 May 2008; accepted 17 June 2008
Online First 19 July 2008

Abstract. Mitogenic signals stimulate cell division by activating cyclin/cyclin-dependent kinase (CDK) complexes. Their timely regulation ensures proper cell cycle progression. It is therefore not surprising that cyclin/CDK complexes are integrators of multiple signals from both the extracellular environment and intracellular cues. Important regulators of cyclin/CDKs are the CDK inhibitors that have attracted attention due to their association with disease. p27^{KIP1} is a CDK inhibitor that controls CDK activity throughout the cell cycle. As a CDK inhibitor, p27^{KIP1} has tumor suppressor activity. Besides CDKs, p27^{KIP1} regulates additional cellular processes, including cell motility, some of which seem to mediate oncogenic activities of p27^{KIP1}. These activities of p27^{KIP1} are regulated through multiple phosphorylation sites, targeted by several signal transduction pathways. Understanding functions and regulation of p27^{KIP1} will be important to determine which isoform of p27^{KIP1} has anti- or pro-tumorigenic activities. Such knowledge might be of prognostic value and may offer novel therapeutic windows.

Keywords. Cancer, CDK, cell cycle, CKI, cyclin, kinase, proteasome, signal transduction.

Introduction

Cell proliferation is tightly controlled by cyclin-dependent kinases (CDKs) that function sequentially during the cell cycle. These kinases phosphorylate and thereby regulate key substrates involved in cell cycle progression [1]. Particularly relevant are distinct checkpoints that separate individual phases of the cell cycle. Importantly, at these checkpoints both positive and negative signals are integrated. These signals retrieve information generated by cytokines and hormones interacting with cellular receptors and by signals produced in response to intracellular cues [2, 3]. This requires CDKs to be highly regulated, involving the interaction with additional proteins and post-translational modifications. Essential positive regulators of CDKs are cyclins, the regulatory subunits of cyclin/CDK kinase complexes, that are expressed periodically during the cell cycle. Furthermore, CDKs are controlled by phosphorylation that either stimulates or represses catalytic activity [4]. Finally, CDK inhibitors (CKIs) have been shown to interact with distinct cyclin/CDK complexes, thereby interfering with their catalytic activity [5]. p27^{KIP1} (p27) is a CKI that was originally identified as a protein capable of inhibiting G1 cyclin/CDK complexes. Furthermore, p27 was discovered as a protein whose expression is induced by different growth inhibitory agents, including tumor growth factor beta [6–9]. Thus, p27 links proliferative and anti-proliferative signals and controls the transition from the G1 into the S phase of the cell cycle.

Being associated with one of the central interfaces that control the cell cycle, it is not surprising that p27 itself is a highly regulated protein. The differential expres-

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sion of the gene and the protein as well as several distinct post-translational modifications have been uncovered as mechanisms that control the activity of p27 [10–14]. In this review we summarize the latest findings regarding post-translational modifications and their role in controlling p27 function. In addition, the consequences of these modifications for human diseases, in particular cancer, are highlighted.

A short summary of the role of p27 in tumorigenesis

Early studies had suggested that p27 mainly operates by interfering with cyclin/CDK complexes, suggesting a role as tumor suppressor. Indeed, many tumors show decreased levels of p27, consistent with this suggestion. However, the available evidence indicates that p27 does not behave as a classical tumor suppressor. In addition to tumor suppressor activities, it appears that p27 also possesses oncogenic properties and, as a consequence, p27 is regulated in a complex manner to accommodate both anti- and pro-tumorigenic activities [12, 14]. A number of mouse models have revealed that, depending on the level of expression and on the distinct p27 alleles expressed, different degrees of susceptibility to tumor formation are measurable. $p27^{-/-}$ mice show hyperplasia in multiple organs, develop adenomas of the intermediate lobe of the pituitary gland and demonstrate an increased susceptibility to tumor formation induced by chemical carcinogens and irradiation [15–17], altogether these alterations are relatively mild. In contrast, $p27^{+/-}$ mice are, at least under some circumstances, more susceptible to tumor development compared to the $p27^{-/-}$ animals [18–20], in line with the evolving concept that p27 is not a classical tumor suppressor. Importantly, the wild-type allele of p27 is retained in the tumors of $p27^{+/-}$ mice, leading to the suggestion that p27 is a haploinsufficient tumor suppressor. Recent evidence supports a role for p27 as oncoprotein. Mice with a p27 variant that cannot interact with cyclin/CDK complexes (p27-CK^{-}), and thus is unable to interfere with cell cycle progression, develop tumors in multiple organs at frequencies higher than seen with $p27^{+/-}$ mice [21]. One conclusion that was drawn from these studies is that some function or functions of p27, beyond the regulation of cyclin/CDK complexes, are enhancing tumorigenesis.

The analysis of human tumors has resulted in similar conclusion, *i.e.*, p27 is not a classical tumor suppressor since both alleles are rarely inactivated. It was observed early in the analysis of p27 that in some tumors the levels of nuclear p27 are low with a corresponding increase in cytoplasmic p27. In breast cancer in particular, this subcellular redistribution of p27 is associated with poor clinical outcome [22–24]. More recently, similar conclusions have been reached for a number of additional human tumors [12], suggesting that the subcellular redistribution is relevant for tumorigenesis. Altogether, these studies indicate altered regulation of p27 in a substantial number of tumors. Indeed, accumulating evidence and epidemiological studies demonstrate that the detailed analysis of p27 protein expression and subcellular localization has both prognostic and therapeutic implications [12].

In summary, a strong case for a dual role of p27, both in tumor suppression and promotion, has been established in recent years. While in normal cells the tumor suppressor activity is activated in response to antiproliferative signals, thus tilting the balance towards cell cycle blockade and inhibition of proliferation, tumor cells have developed strategies to down-regulate these functions of p27. However, other activities that appear to be pro-tumorigenic are not affected or even enhanced, resulting in a stimulation of tumor development. In this respect the functions of p27 in the cytoplasm, including its role as a regulator of the cytoskeleton, will be important to understand in detail [25–27]. The p27 balance is fine-tuned by multiple post-translational modifications that affect the function of p27 by altering protein-protein interactions, affecting subcellular localization and modulating protein stability, as discussed below.

Regulation of p27 in G0

In cells arrested in the G0 phase of the cell cycle, p27 is expressed at high levels in the nucleus and contributes to the maintenance of the quiescent state [28, 29]. However, mechanistically it has not been fully clarified how p27 functions in G0. One suggestion is that it prevents premature activation of cyclin-dependent kinases and thus blocks unscheduled entry into G1. But clearly other, CDK-independent functions of p27 might be relevant that will need to be explored in the future, including the role of p27 in cell migration. The high expression level is due to enhanced p27 mRNA translation and stabilization of the protein as a result of phosphorylation at serine 10 (S10) and threonine 198 (T198) [30–32] (Fig. 1). The mutation of S10 to aspartate or glutamate, residues that mimic the phosphorylation site, results in an increase in p27 stability in G0 cells, while the non-phosphorylatable mutant p27-S10A is less stable than the wild-type protein. One of the kinases that has been ascribed to phosphorylate S10 in quiescent cells is MIRK/DYRK1B. Its depletion by RNA interference decreases the phosphorylation of p27 at S10 and

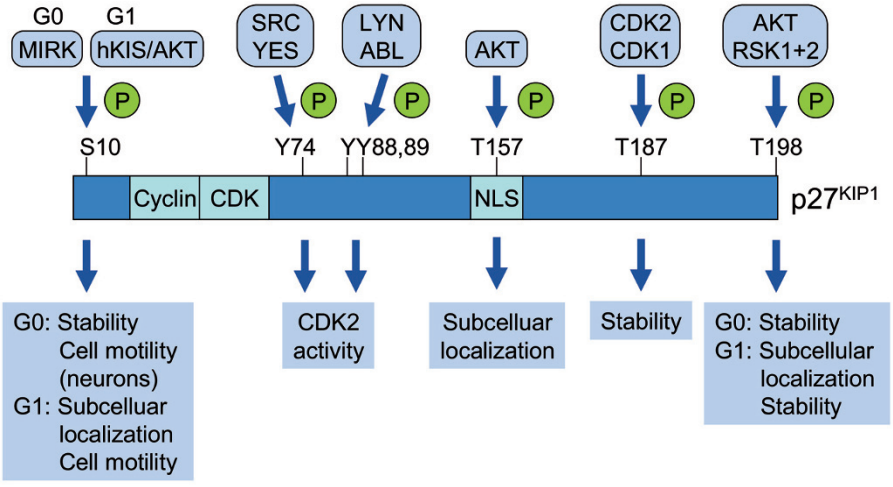

Figure 1. Summary of the phosphorylation sites observed in p27 with known kinases that modify individual sites. Furthermore, the functions associated with these post-translational modifications are indicated. For details regarding individual phosphorylation sites, see text.

concomitantly destabilizes p27 [31]. These findings have been confirmed using murine knock-in models carrying $p27^{S10A/S10A}$. The half-life of the $p27^{S10A}$ protein is reduced in serum-starved mouse embryonic fibroblasts (MEFs). Furthermore, $p27^{S10A}$ protein levels were reduced in different tissues of these knock-in animals [30, 33]. Thus, the stability of p27 is controlled through S10 phosphorylation in quiescent cells.

S10 is not the only phosphorylation site that contributes to p27 protein stability in resting cells. Phosphorylation of the C-terminal T198 is also implicated in p27 stability in quiescent cells. Immortalized $p27^{-/-}$ MEFs were complemented with genomic fragments encoding either wild-type p27 or p27-T198A [32]. The half-life of $p27^{T198A}$ was strongly reduced in quiescent cells. Presently it is unclear which kinase or kinases phosphorylate Thr198 during G0.

How do phosphorylation of S10 and T198 stabilize p27 in resting cells? It was suggested that S10 phosphorylation interferes with the binding of p27 to cyclin/CDK complexes, which may determine the rate of p27 turnover. Dephosphorylation of S10 would then promote the assembly of heterotrimeric p27/cyclin/CDK complexes, thereby promoting p27 degradation. Indeed in support of this model, immunoprecipitation of p27-S10A was abolished by a monoclonal antibody recognizing an epitope within the cyclin/CDK binding region of p27, suggesting enhanced interaction with cyclin/CDK complexes in comparison to p27 wild-type and p27-CK^-, a mutant that does not interact with cyclins and CDK2. However, the association of p27-S10A with cyclin D1 was comparable to the interaction with wild-type p27 in serum-starved cells [30]. One interpretation of these findings is that an unknown factor or factors exist, which bind p27 in the region of its cyclin/CDK-binding domain and are responsible for the degradation of p27 in arrested cells. This is consistent with the observation that p27-S10A/CK^- was stabilized relative to p27-S10A. Of note is that, in resting cells, p27 has been found preferentially in a monomeric form, suggesting that it is not tightly associated with any larger complex [34, 35]. Presently, the mechanism underlying the stabilization of p27 due to T198 phosphorylation in G0 cells remains to be determined. Also it is unclear, whether phosphorylation at S10 and T198 cooperate in the stabilization of p27 in G0 cells or whether one or the other phosphorylation is sufficient to achieve maximal p27 stability. In summary, although p27 appears to be important for resting cells, its regulation by phosphorylation affecting stability and possibly other p27-linked functions is still only poorly understood.

Regulation of p27 by mitogenic signals

After mitogenic stimulation and entrance into the G1 phase of the cell cycle, D-type cyclin/CDK4 and 6 complexes and subsequently cyclin E/CDK2 complexes are activated and contribute to inactivating the restriction point. A critical target is the retinoblastoma (RB) tumor suppressor protein that is phosphorylated by these CDK complexes, resulting in its release from E2F transcriptional regulators. The inactivation of the RB protein and the activation of E2F transcription factors are a prerequisite for the transition from the G1 into the S phase of the cell cycle [36, 37]. p27 participates in the regulation of this transition. First it stimulates the initial phase of this process by enhancing the assembly of D-type cyclin/CDK complexes [38, 39]. Then p27 blocks further progression by inhibiting the kinase activity of cyclin E/CDK2 complexes [5].

How is the function of p27 regulated? After mitogenic stimulation the phosphorylation of p27 on S10 and T198 is maintained. However, S10 is now modified by different kinases compared to the G0 phase. The first S10 kinase identified was kinase interacting stathmin (KIS), a nuclear enzyme that is activated in response by mitogens during G0–G1 transition [40]. Several lines of experimentation suggest that KIS is an important regulator of p27. Whereas the overexpression of KIS overcomes a p27-induced cell cycle blockade, KIS knockdown enhances growth arrest. Thus, early in G1, phosphorylation at S10 inhibits p27 with regard to its function as a CKI, but stimulates its function as assembly factor of cyclin D/CDK complexes and enhances translocation of these complexes into the nucleus [38]. Mechanistically this phosphorylation triggers the export of p27 from the nucleus into the cytoplasm by a CRM1-mediated export pathway [40–43]. Upon transport into the cytoplasm, p27 becomes a substrate for the KPC Ubiquitin ligase complex [44], probably if not interacting with cyclin D complexes. The KPC complex contains with KPC1 a RING-finger domain-dependent E3-ubiquitin ligase that poly-ubiquitinates p27 and results in its degradation early in G1. It appears that beyond the phosphorylation-induced nuclear export of p27 no further signal is required for its KPC-dependent poly-ubiquitination [44, 45]. Together these findings suggest that phosphorylation of S10 during G1 has a different functional consequence as compared to G0. While in resting cells this modification mediates stability, in early G1 S10 phosphorylation results in nuclear export and possibly in cytoplasmic degradation of p27. Serum-induced cell cycle entry in the presence of leptomycin B, an inhibitor of CRM1, prevents the export of p27 from the nucleus. Under these conditions the stability of p27 depends on S10 phosphorylation, indicating that this modification remains to be a stability determinant for nuclear p27 [42]. Thus, it appears that upon mitogenic activation of cells, the S10-P-driven export becomes the dominant consequence and indirectly determines the stability of p27. In addition to KIS, two other kinases, AKT and ERK2, were reported to phosphorylated p27 on S10 [46, 47]. Although it remains to be determined whether these kinases are relevant in cells, it demonstrates that S10 is an important site in mediating regulation of p27. AKT and ERK2 are regulated by prominent signal transduction pathways, *i.e.*, the PI3K/PTEN/AKT and the RAS/RAF/ERK pathway, which control multiple cellular activities in response to broad spectrum of extracellular signaling molecules, including many growth factors and cytokines [48, 49]. Since KIS is also activated in response to mitogens, although the precise pathway(s) involved have not been determined thus far, these different kinases provide a link between extracellular signals and the control of p27 function. Thus, it appears that p27, although not essential for mouse development [15–17], is a target of multiple signaling pathways in orchestrating the complex transition from G0 into G1 and subsequently S phase of cells.

In addition to S10, T198, which is phosphorylated in G0, is also subject to modification early in G1. At least three kinases have been demonstrated to phosphorylate p27 at T198, including AKT, RSK1 and RSK2 [46, 50]. As discussed for the S10 kinases, these three are also activated in response to mitogenic stimuli, including the PI3K/PTEN/AKT and the RAS/RAF/ERK pathways. Thus, it appears that T198 and S10 phosphorylation are at least in part co-regulated in early G1. After mitogenic stimulation of arrested cells the majority of p27 associates with cyclin D/CDK complexes in the cytoplasm, a function possibly stimulated by S10 phosphorylation as discussed above. In stable cell lines carrying the T198A mutation, most p27-T198A was found in CDK2 rather than CDK4 complexes, unlike observed for wild-type p27 [32]. Interestingly, despite high expression in resting cells, p27 is unable to assemble exogenously expressed cyclin D1 and CDK4 into complexes [51]. But the simultaneous exogenous expression of cyclin D1 and constitutively active MEK leads to the sequestration of p27 into cyclin D1/CDK4 complexes and allows the activation of cyclin E and A-dependent CDK2 complexes [52]. Therefore, the phosphorylation-dependent binding of p27 to CDK4 complexes could be relevant for the assembly of D-type-cyclin/CDK4 complexes in response to mitogenic signals and thus promote progression through G1. In contrast, loss of phosphorylation at T198 would then have the opposite effect since interaction with CDK2 complexes will inhibit the cell cycle in late G1. In support of these findings, the phosphorylation patterns of p27 are distinct when bound to either cyclin D or cyclin E [53]. Together, these studies suggest that the timely phosphorylation of both T198 and S10 are critical to control p27 function in regulating the activities of cyclin/CDK complexes.

The preferential binding of p27-T198A to cyclin E/CDK2 complexes delays the activation of its kinase activity during progression through G1 and, as a consequence, entry into S phase is retarded in cells expressing this mutant form of p27. In addition, the shift in complex formation stabilizes p27-T198A during the G1 phase in comparison to wild-type p27. These observations indicate that phosphorylation at T198 is also necessary to allow down-regulation of p27 after mitogenic stimulation [32].

A third phosphorylation is induced in response to mitogenic stimuli at T157. The main kinase that appears to modify this site is AKT. Interestingly T157 is located within the nuclear localization sequence (NLS) of p27, suggesting that this modification is also involved in regulating the subcellular distribution of p27. Indeed, phosphorylation at T157 and T198 cooperate to enhance cytoplasmic localization of p27 [54–57]. Phosphorylation at these two sites induces the interaction with different 14-3-3 proteins. This interaction competes with binding of p27 to Importin-α5, resulting in cytoplasmic localization of NLS-phosphorylated p27 [46, 50, 58, 59]. Interestingly the T157 phosphorylation site is present only in human but not rodent p27, indicating that this adds an additional level of regulation in humans cells, because 14-3-3 proteins binds to human p27 only when both sites are phosphorylated [58].

The findings summarized above suggest a model in which early in G1 phosphorylation at S10, T157, and T198 control the subcellular localization and the stability of p27. Disregulation of these phosphorylations would result in altered CDK activities, *i.e.*, delayed accumulation of p27 in the cytoplasm would slow the activation of cyclin D/CDK4 complexes and, in parallel, would inhibit newly formed cyclin E/CDK2 complexes and thus disregulate cell cycle progression.

The most recent additions to signal-dependent modification of p27 have been several reports that demonstrate phosphorylation on tyrosine residues. Tyrosine kinases of the SRC family, including SRC and LYN, and BCR-ABL, the tumor-associated and activated form of the Abelson kinase, were found to phosphorylate three distinct tyrosine residues in the central region of p27 [60–62]. SCR family kinases as well as ABL are components of several different signal transduction pathways that are closely associated with transmembrane receptors [63–65]. Thus, these kinases are candidates to control p27 function in close proximity to activated receptors. Three tyrosine residues, Y74, Y88, and Y89, are the sites that are modified by the kinases mentioned above (Fig. 1). Importantly these phosphorylation sites are in the CDK binding region of p27. A major consequence of the phosphorylation of these tyrosines is that p27 becomes unstable and thus provides an additional link between mitogenic signals and inhibition of the function of p27 as a CKI. How does tyrosine phosphorylation affect stability? p27 is an intrinsically unstructured protein that becomes structured when it interacts with cyclin/CDK complexes [66]. The crystal structure of the cyclin/CDK binding portion of p27 with cyclin A/CDK2 revealed that p27 interacts with both cyclin A and CDK2 [67]. Importantly, part of the CDK2 binding domain inserts into the catalytic cleft and prevents ATP binding. This structure of p27, referred to as the 3_{10}-helix, is ejected from the catalytic cleft as a result of Y88 phosphorylation [61]. The consequence of this structural alteration is that tyrosine-phosphorylated p27 is a poor inhibitor of CDK2, thus partially restoring kinase activity [60, 61]. In turn this activates the negative feedback loop between p27 and cyclin E and A/CDK2 complexes, as described below, that depends on yet an additional phosphorylation site at T187. Together these findings suggest that tyrosine phosphorylation of p27 modulates protein stability independent of the above-discussed mechanisms that work in association with S10, T157, and T198 phosphorylation.

Regulation of p27 at the G1 to S phase transition

In addition to controlling the activity of p27 in G0 and in early G1, a prominent regulation of this CKI has been uncovered in late G1, at the transition into S phase. p27 protein levels significantly decrease once cyclin E/CDK2 is activated in late G1. This is the result of decreased p27 protein stability. The analysis of this process has revealed that the altered stability depends on the phosphorylation of T187 in p27. This site is modified by cyclin E and A/CDK2 complexes and phosphorylated T187 provides a binding site for the SCF^{SKP2} (Skp-Cullin-F-box) E3 ubiquitin ligase complex. The result of this interaction is poly-ubiquitination of p27 and its subsequent proteasomal degradation [68–71]. In general the SCF complexes depend on the F-box components that function as substrate receptors [72]. In some cases these interactions are driven by distinct phosphorylations on the substrate, thus creating binding sites for the receptor subunits of the SCF complexes [72]. Recent studies on the interaction of p27 phosphorylated at T187 with the SCF^{SKP2} complex demonstrated that, in addition to SKP2, a further subunit of the complex, CKS1, is required [73]. Unlike for other phosphorylated substrates, T187-P is recognized by the phosphate-binding domain of CKS1 [74]. Thus, both SKP2 and CKS1 interact with p27-T187-P to induce poly-ubiquitination. The importance of T187-P for p27 degradation was further demonstrated by generating mice that express p27-T187A. Indeed, this protein was stable during S and G2 phases of the cell cycle [75]. Surprisingly, the consequences of the lack of p27 turnover were quite modest. A possible explanation is that p27 levels in late G1 are sufficiently low to proceed into S phase as a result of the different mitogenic signal-regulated p27 degradation earlier in G1. In addition tyrosine phosphorylation of p27 may

allow sufficient CDK2 kinase activity for cell cycle progression, albeit at a slower rate.

Function of p27 in S and G2 phases of the cell cycle

Early on in the analysis of p27 it was suggested that this CKI is capable of also inhibiting G2-M phase cyclin/CDK complexes [9]. However, the analysis of the role of p27 in these later phases of the cell cycle has been neglected probably as a result of the prominent role p27 plays in the control of processes early in the cell cycle.

The knockout of SKP2, which is important for the T187-P-dependent poly-ubiquitination and degradation of p27, results in mice that are smaller with a generalized hypoplasia [76]. This is the opposite of the phenotype observed in $p27^{-/-}$ animals [15–17]. In addition, in $skp2^{-/-}$ animals, polyploidization and centrosome over-duplication is observed in some tissues, suggesting that the lack of SKP2 affects distinct processes during replication or cell division. In an effort to define which of the many SKP2 targets might be relevant for the phenotypic effects observed, $skp2^{-/-}$ and $p27^{-/-}$ animals were crossed. The analysis of these animals showed that the generalized hypoplasia is most likely the result of the increased p27 levels. Furthermore, the double KO animals do not show the alterations in ploidy and centrosome number [77, 78]. Since in $skp2^{-/-}$ MEFs an increased association of p27 with both CDK1 and CDK2 was observed with a concurrent inhibition of cyclin A and cyclin B complexes (but not cyclin E/CDK2 complexes), processes during replication, in G2 and at the transition from G2 into mitosis are disturbed. Thus, it was concluded that the inhibition of CDK complexes that act late in the cell cycle are disregulated in the $skp2^{-/-}$ animals. Consistent with this interpretation, the block to reentry into the cell cycle of $skp2^{-/-}$ hepatocytes is reverted in double KO cells [78].

A role for p27 in the regulation of cyclin A and B kinase complexes has also been deduced from studies using MEFs derived from animals with a triple KO of the pocket proteins RB, p107, and p130 [79]. Upon serum starvation many of these cells undergo apoptosis but the surviving cells are blocked in late G2 dependent on the tumor suppressor p53. In these cells p27 is up-regulated, interacts with cyclin A and B complexes and inhibits the associated kinase activities. The induction of p27 upon mitogen deprivation is dependent on p53. It will be interesting to determine by which mechanism.

What are the functions of p27 in the cytoplasm?

Several pathways and regulatory molecules are associated with controlling the subcellular localization of p27. Furthermore, the cytoplasmic location of this CKI in many tumors is indicative of a poor prognosis. One function associated with cytoplasmic p27 is its stimulatory role in activating cyclin D/CDK complexes [38, 39]. This could be relevant for transformation, although many tumors have a deregulated restriction point that may make this function of p27 less critical. Furthermore, cytoplasmic p27 is sequestered away from nuclear cyclin/CDK complexes required for cell cycle progression beyond the restriction point. However, as outlined above the p27-CK^- mutant protein, which is unable to interact with cyclin/CDKs, has transforming properties. These findings suggest that p27 has additional functions in the cytoplasm that might contribute to its oncogenic activities.

One of the functions that have been suggested to be relevant for tumor formation is the ability of p27 to regulate the actin cytoskeleton. $p27^{-/-}$ MEFs display an increased number of stress fibers and focal adhesions and, as a consequence reduced motility. Importantly, the p27-CK^- mutant is as potent as the wild-type protein in reverting this effect [80]. Thus, these effects are independent of the CKI function of p27 but require signals that result in the cytoplasmic localization of the protein. Mechanistically these effects are at least in part due to the ability of p27 to interact with Rho GTPases that are critical to regulate actin dynamics and cell motility [81]. It has been shown that p27 interacts with RhoA and interferes with its function. This enhances actin depolymerization and reduces the formation and the stability of stress fibers [11, 26, 80]. Furthermore, p27 interacts with RAC, another Rho family GTPase, in hepatocytes and stimulates migration [82].

Deregulated phosphorylation of p27 promotes tumor formation

The importance of phosphorylation in controlling p27 functions raises the question, which role phosphorylation plays during tumor development to inactivate or modulate p27. The inactivation of p27 is not due to mutations of the p27 gene or epigenetic silencing like a classical tumor suppressor. Instead p27 is inactivated in cancer by altered post-translational modifications that result in down-regulation of the p27 protein, its exclusion from the nuclear compartment and/or its sequestration into cyclin/CDK complexes [12, 14].

The reduction of p27 expression has been associated with the development of human tumors originating from organs including lung, breast, colon, ovary, esophagus, thyroid and prostate. In most human tumors the loss of p27 results from altered proteasomal degradation. Consistent with these observations, an increasing number of studies demonstrate a direct inverse correlation between reduced p27 and increased SKP2 levels, the latter being a key component of one p27 ubiquitin ligase complex [83–87]. The importance of Skp2-mediated degradation of p27 in tumorigenesis was investigated recently using p27-T187A knock-in mice [88]. In mouse models for lung and colon cancer, no difference in tumor incidence or overall survival is measurable compared to wild-type mice. Inhibition of the T187-dependent phosphorylation-induced degradation of p27 does not prevent p27 down-regulation in lung tumors. The lack of Ubiquitin-dependent degradation is apparently compensated by decreased *p27* mRNA abundance in lung epithelial cells, resulting in reduced p27 protein expression. In the colon cancer model the frequency of intestinal adenomas is similarly unaffected by the p27-T187A mutation, but the progression of intestinal adenomas to carcinomas is inhibited [88]. Thus, in the mouse model summarized above only minor effects of the T187A mutant of p27 are measurable.

The phosphorylation of p27 by the SRC family kinases acts in the same pathway as SKP2 to inactivate the repressive function of p27 on cyclin E/CDK2. Resent studies show a correlation between an increase of SRC family kinase expression and activity and a decrease level of p27 in primary breast cancer cell lines and in BCR-ABL transformed chronic myelogenous leukemia cells [60, 89]. In both studies inhibition of the tyrosine kinases restores p27 expression and inhibits cell proliferation.

The p27 activity is also targeted by cytoplasmic mislocalization and has been observed in primary human breast [54–56], colon [90], ovarian [91], thyroid [92] and esophageal cancers [93]. Cytoplasmic p27 appears to directly correlate with poor long-term survival and tumor grade in Barrett's associated adenocarcinoma of the esophagus and breast carcinoma [54–56, 93]. Cytoplasmic sequestration of p27 inhibits the activity of p27 as CKI, since this prevents the interaction of p27 with its nuclear cyclin/CDK targets. The pathways regulating p27 translocation into the cytosol during G1 progression, as discussed above, are also involved in the p27 mislocalization in tumor cells.

In a mouse model of urethane-induced tumorigenesis p27-S10-P accumulates in the cytoplasm of lung tumor cells. Using the knock-in mouse strain expressing p27-S10A, the number of tumors and their growth rate is reduced [30]. Consistent with this observation is that the developing tumors in these animals show predominantly nuclear p27-S10A staining. The cytoplasmic localization of wild-type p27 seems to be dependent on the activation of K-RAS, which is a hallmark of urethane-induced lung tumors [94]. The overexpression of activated K-RAS in MEFs induces the accumulation of wild-type p27 in the cytosol, while it has little or no effect on the localization of p27-S10A [30].

In human breast and thyroid cancer, the cytoplasmic sequestration of p27 is regulated by increased signaling through the PI3K/AKT pathway, which phosphorylates p27 on T157 and T198 [54, 56, 92]. In breast and thyroid cancer with constitutive AKT activation, p27 is phosphorylated at T157 and T198 and is localized predominantly in the cytoplasm [56, 57]. Approximately 40 % of primary breast tumors display cytoplasmic p27 staining, which is highly correlated with AKT activation. The subgroup of patients with breast cancer who have the best prognosis have strong, exclusively nuclear, p27 staining, while patients with low, but cytoplasmic expression in tumors have a poor prognosis [54, 56].

Conclusion

The emerging concept strongly supports a role of p27 in tumor development. This is associated with a loss of its function as a CDK inhibitor. However, tumor-promoting activities, possibly by affecting cell motility, have also been uncovered. p27 is extensively regulated by post-translational modifications that control many aspects of the molecular activities of p27. It appears that the pathways that regulate these modifications in normal cells are also critical for controlling p27 in tumor cells. Thus, the level of modification and the properties of p27 are a reflection of the tumor-associated disregulation of specific signal transduction pathways. The precise molecular characterization of p27 will therefore be relevant to delineate the properties of tumors and will be important to obtain more precise prognostic information. It is the hope that the molecular information that has been obtained in recent years will additionally have an impact on the treatment of cancer patients. Reactivation of p27, either through direct targeting or through controlling the associated signal transduction pathways, may be beneficial to fight tumors.

Acknowledgement. The work in our laboratory is supported by grants from the Interdisciplinary Center for Clinical Research "BIOMAT." within the faculty of Medicine at the RWTH Aachen University, the START program, and the Deutsche Forschungsgemeinschaft DFG.

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