Review

Deregulation of p27 by oncogenic signaling and its prognostic significance in breast cancer

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Abstract

p27 is a key regulator of progression from G1 to S phase. Although the gene encoding p27 is rarely mutated in human cancers, p27 is functionally inactivated in a majority of human cancers through accelerated p27 proteolysis, through sequestration by cyclin D–cyclin-dependent kinase complexes and by cytoplasmic mislocalization. Here we review mechanisms whereby oncogenic activation of receptor tyrosine kinase and Ras pathways lead to accelerated p27 proteolysis and p27 mislocalization in cancer cells. The prognostic significance of p27 in human breast cancer is also reviewed.

Keywords: breast cancer, cell cycle, cyclin-dependent kinase, deregulation, p27

Introduction

G1 progression is governed by cyclin-dependent kinases (Cdks) [1–3]. The Cdks regulate biochemical pathways, or checkpoints, that integrate mitogenic and growth inhibitory signals and coordinate cell cycle transitions [4,5]. The Cdks are regulated by both activating and inhibitory phosphorylation, by cyclin binding and by two different families of Cdk inhibitors. In early G1 phase, mitogens increase D-type cyclins, which bind and activate Cdk4 and Cdk6 [6]. The subsequent activation of cyclin E and cyclin A–Cdk2 complexes regulate S phase entry and progression. Two families of Cdk inhibitors regulate the cyclin–Cdk complexes [2,7,8]. The inhibitor of Cdk4 (INK4) family members, which include $p15^{INK4B}$, $p16^{INK4A}$, $p18^{INK4C}$, and $p19^{INK4D}$, specifically bind Cdk4 and Cdk6 and inhibit cyclin D association. Members of the kinase inhibitor protein (KIP) family, $p21^{CIP1}$, $p27^{Kip1}$, and $p57^{Kip2}$, bind and inhibit cyclin E-bound and cyclin A-bound Cdk2. Although p21 and p27 are major inhibitors of Cdk2, they also promote G1 progression by facilitating the assembly of cyclin D–Cdk4 and cyclin D–Cdk6 complexes [9,10]. In early G1, p27 assembles cyclin D1–Cdks in the cytoplasm and this facilitates the import of cyclin D1 complexes into the nucleus.

Regulation of the cell cycle inhibitor $p27^{KIP1}$

p27 was discovered in cells arrested by transforming growth factor-β (TGF-β), by contact inhibition, and by lovastatin [11–14]. p27 acts in G0 and early G1 to inhibit cyclin E–Cdk2. Assembly of cyclin D–Cdk complexes by p27 is activated in early G1 and involves changes in p27 phosphorylation [15]. Mitogenic growth factor signaling mediates a decrease in p27 protein levels. Whereas p27 mRNA concentrations are constant throughout the cell cycle, p27 concentrations are the highest in quiescent cells, decrease during G1 phase and are minimal in S phase [16]. p27 translation is maximal in quiescence and falls rapidly after exit from G0 [16,17]. p27 is also importantly regulated by proteolysis, with the p27 $t_{1/2}$ decreasing fivefold to eightfold with passage from G0 to S phase [18,19].

Transcriptional regulation

Although p27 regulation occurs predominantly at the levels of translation and protein stability, transcriptional

AFX = acute lymphocytic leukaemia-1 fused gene from chromosome X; Cdk = cyclin-dependent kinase; INK = inhibitor of Cdk; KIP = kinase inhibitor protein; MAPK = mitogen-activated protein kinase; MEK = MAPK kinase; PI3K = phosphoinositide 3-kinase; PKB = protein kinase B; PTEN = phosphatase and tensin homolog deleted on chromosome 10; SCF = Skp1, Cul1, F-box protein; TGF-β = transforming growth factor-β; TSC2 = tumor suppressor tuberin-2.

regulation of p27 has been demonstrated. Normal quiescent T cells express high concentrations of p27 mRNA and protein, both of which decline rapidly after T cell activation [20]. Regulation of p27 mRNA concentrations also occurs after androgen depletion in breast cancer cells [21], in normal prostate tissue, and benign prostatic hyperplasia [22]. In melanoma cells, interleukin-6 signaling activates signal transduction and activators of transcription-3 (STAT3) and increases p27 mRNA [23]. The p27 promoter contains binding sites for several transcription factors including Sp1, cAMP-response element, Myb, NFκB, and acute lymphocytic leukaemia-1 fused gene from chromosome X (AFX). AFX is a forkhead transcription factor recently shown to activate p27 transcription [24]. Phosphorylation of AFX by protein kinase B (PKB) inactivates this transcription factor and might thereby decrease p27 transcription. The relevance of transcriptional regulation of p27 to human cancers is unclear because most reduction of p27 in human cancers is thought to occur through proteolysis (see below and [7]).

Regulation of p27 localization

p27 localization is also cell cycle regulated: p27 is nuclear in G0 and early G1 and appears transiently in the cytoplasm at the G1/S transition [25]. The nuclear import of p27 depends on a bipartite nuclear localization signal in the carboxy-terminal region of the protein [26]. Interaction of p27 with the nuclear pore protein NPAP60 [27,28] is important in p27 nuclear import and might also regulate p27 export [27]. In response to mitogenic stimulation, at least part of the nuclear p27 pool undergoes nuclear export dependent on phosphorylation at serine 10 [25,29,30]. Human kinase interacting stathmin (hKis) can phosphorylate p27 at serine 10 [29]. p27 is bound to the exportin CRM1 in early G1, and binding of CRM1 to p27 increases with G1 progression [25]. p27 contains a nuclear export signal (NES) whose mutation decreases p27–CRM1 binding, nuclear export, and p27 degradation [25]. Active CRM1–RanGTP-mediated nuclear export of p27 is linked to cytoplasmic proteolysis of p27 in early G1.

Proteolytic degradation of p27

p27 proteolysis is regulated by at least two distinct mechanisms. In early G1, mitogens seem to activate an export-linked degradation mechanism that is followed in late G1 and S phases by a cyclin E–Cdk2-dependent degradation of p27. The late G1 and early S phase of p27 proteolysis is regulated by its phosphorylation at threonine 187 (T187) by cyclin E–Cdk2 [18,31–33]. Phosphorylation of p27 at T187 promotes the interaction of p27 with Skp2, the F box component of the SCF^{Skp2} (Skp1, Cul1, F-box protein) ubiquitin ligase. Once p27 phosphorylated on T187 is recognized by its SCF-type E3 ligase, composed of Skp1, Cul1, the F-box protein, Skp2 and Roc1, and the Cks1 cofactor [34–39], this complex then mediates the subsequent degradation of p27 by the 26S proteasome.

Recent data from T187A knock-in and $Skp2^{-/-}$ mice also suggest that p27 proteolysis in early G1 is independent of T187 phosphorylation [19,40]. In early G1, growth factors stimulate p27 proteolysis in a manner independent of T187 phosphorylation and possibly also of Skp2 [19,40]. This initial mitogen-stimulated p27 degradation in early G1 might be linked to p27 export [25] and would allow an incremental activation of cyclin E–Cdk2 that is then followed by rapid, progressive Cdk2 activation as cyclin E–Cdk2 mediates T187 phosphorylation-dependent p27 degradation in late G1 and S phase.

Deregulation of p27 in human cancers

The human $p27^{KIP1}$ gene resides on chromosome 12 p13. Loss of a single allele of $p27^{KIP1}$ confers increased susceptibility to chemical carcinogenesis in mice [41] and is not uncommon in a number of human malignancies [42–44]. However, $p27^{KIP1}$ does not follow Knudson's classic 'two-hit hypothesis' of tumor suppression: in tumors that show reduction to hemizygosity at the $p27^{KIP1}$ locus, silencing of the remaining allele is rare [42–44]. p27 is rarely mutated in human cancers. However, decreased concentrations of p27 protein might be implicated in human tumorigenesis or oncogenic progression in many human malignancies. Whereas p27 protein is expressed at high concentrations in all normal epithelia examined, including breast, prostate, ovary, skin, oral epithelium, and esophageal, gastric, colonic, and pulmonary mucosa, loss of p27 protein is frequently seen in carcinomas involving all these tissues [7]. Indeed, decreased concentrations of p27 protein have been observed in up to 60% of human carcinomas [7,45]. Multivariate analyses of p27 along with other known clinical and pathologic prognostic markers have shown that loss of p27 protein has independent prognostic potential in primary carcinoma of the breast, lung, colon, prostate, and many other malignancies including lymphomas, glioma, and melanoma [46–57] (reviewed in [7,45]). Although p27 function is rarely disrupted at the genetic level, it is frequently diminished in human cancers because of accelerated proteolysis, sequestration by other proteins, and cytoplasmic mislocalization. The next sections elaborate how the normal mechanisms of p27 regulation become disrupted in human cancers.

Accelerated p27 proteolysis

Most, if not all, cases of decreased p27 concentrations in human cancers are a reflection of accelerated proteolysis [7,45]. Lysates from a number of different tumor types show increased proteolytic activity toward p27 [47,52,58–60]. As noted above, a major mechanism of p27 proteolysis involves its recognition by SKP2, a component of the SCF^{Skp2} ubiquitin ligase. An association between decreased p27 protein and increased Skp2 concentrations has been observed in a subset of colorectal, prostatic, small cell lung, gastric, and oral carcinomas, and

in lymphomas [60–65]. In oral squamous carcinoma, tumors with increased Skp2 always showed decreased p27 immunostaining. However, most oral cancers with low p27 did not show increased Skp2. Thus, causes other than increased Skp2 mediate p27 loss in a majority of cases [66]. Increased Skp2 might account for p27 loss in a minority of breast and prostate cancers with low p27 (J Slingerland and L Kapusta, unpublished work).

Loss of p27 function can be mediated by oncogenic activation of multiple receptor tyrosine kinase and signal transduction pathways. These are reviewed below.

p27 sequestration by binding proteins

In human cancers, p27 can also be sequestered by associated proteins that reduce its ability to bind and inhibit cyclin E–Cdk2. Some human lymphomas show the sequestration of p27 into active cyclin D1–Cdk4 complexes, potentially decreasing the p27 available for cyclin E–Cdk2 inhibition [67]. Overexpression of c-Myc can induce one or more heat-labile proteins that bind p27 and impair its binding to cyclin E–Cdk2 [68]. This effect is independent of p27 degradation and, in at least some cell types, is not due to increased cyclin D1 or D2. In other cancers, oncogenic activation of c-*myc* leads to p27 sequestration through the upregulation of cyclin D1 and D2 concentrations [69,70]. c-Myc also represses the expression of several negative cell cycle regulators including *p15*, *p21*, and *p27* (reviewed in [71]). c-Myc activation can arise through oncogenic mitogen signal transduction. Cyclin D1 and c-Myc expression are induced early in G1 after growth factor stimulation through the mitogen-activated protein kinase (MAPK) pathway [72–74]. Furthermore, activation of the phosphoinositide 3-kinase (PI3K)/PKB pathway increases the translation of c-Myc [50] and cyclin D1, and stabilizes D-type cyclins [75–77].

Cytoplasmic mislocalization of p27

Tumors that retain abundant p27 often show p27 mislocalization in the cytoplasm away from nuclear cyclin–Cdk targets [7]. Cytoplasmic localization would prevent p27 from binding and inhibiting nuclear cyclin–Cdk targets [78]. Cytoplasmic p27 is observed in certain lymphomas, in up to 55% of ovarian cancers, 80% of thyroid tumors, and 35% of colon cancers, with decreased p27 concentrations in the more poorly differentiated, advanced forms of these neoplasms [50,79–81]. Localization of p27 in the cytoplasm has also been described in 48% of esophageal dysplasias and 26% of Barrett's associated adenocarcinoma, and is associated in the latter with reduced survival [52].

We and others recently reported cytoplasmic mislocalization of p27 in up to 40% of primary human breast cancers [78,82–84]. These three studies showed that PKB phosphorylates p27 at T157 in its nuclear localization signal region. Cells with genetic loss of *PTEN* (the gene encoding PTEN [phosphatase and tensin homolog deleted on chromosome 10]) or amplification of *HER2*/*neu* showed cytoplasmic mislocalization of p27 that was reversed by the PI3K inhibitor LY294002. Liang and colleagues [84] showed that p27 phosphorylation by PKB impaired the nuclear import of p27 *in vitro*. All three studies showed a consistent association between PKB activation and cytoplasmic p27 staining in primary breast cancers. Moreover, cytoplasmic p27, but not nuclear p27, isolated from primary cancers reacted with an anti-p27 antibody specific for phosphorylation on T157 [83]. Cytoplasmic p27 was correlated with tumor dedifferentiation (increased tumor grade) and poor prognosis [84]. Thus, oncogenic activation of the PI3K/PKB pathway and the PKB-dependent phosphorylation of p27 is probably one of the mechanisms underlying the cytoplasmic mislocalization of p27 in human cancers.

Although PKB-dependent T157 phosphorylation affected the subcellular localization of p27, transfection of constitutive PKB did not affect p27 protein concentrations in normal human mammary epithelial cells [84]. Activation of PKB and mislocalization of p27 were not statistically associated with decreased p27 concentrations in primary breast tumors. Thus, the increased p27 proteolysis observed after PI3K activation in many cell types might involve PI3K-dependent effectors other than PKB.

It is worth noting that cytoplasmic sequestration of p27 might not be equivalent to a loss of function of these Cdk inhibitors. Cytoplasmic p27 probably does not exist as a monomer. Cytoplasmic p27 might have functions other than Cdk inhibition; for example p27 might alter Ras function through the inactivation of GRB2 [85,86]. The phosphorylation event or events that alter the subcellular localization of p27 might also change KIP function.

Regulation of p27 proteolysis by receptor tyrosine kinase and Ras signaling

Ras effector pathways have key roles in p27 proteolysis [87–89]. The isolation of p27 and cloning of the *p27* gene by the Reed group was facilitated by p27 upregulation by lovastatin, a potent inhibitor of Ras [12]. In fibroblasts, dominant-negative *ras* transfection increased p27 concentrations and caused G1 arrest; moreover, Ras-mediated activation of the MAPK-stimulated p27 proteolysis [87,88]. MAPK kinase (MEK1) transfection increased p27 degradation, and a MEK1 inhibitor, PD98059, abolished Ras effects on p27 [88,90]. Although p27 contains several MAPK consensus sites and MAPK can phosphorylate p27 *in vitro* and reduce its ability to bind Cdk2 [88,91], it is not clear that p27 is a direct target of MAPK *in vivo* [88]. Constitutive Ras activation in epithelial cells can mislocalize p27 to the cytoplasm and increase p27 binding to Cdk6 [92]. This might reflect Ras-dependent PI3K/PKB activation. In some cell types, Ras-stimulated RhoA activation may mediate p27 proteolysis [72,93,94].

Several groups have shown that signaling dependent on Her2/ErbB2 and epidermal growth factor receptor can activate p27 proteolysis in a MEK/MAPK-dependent manner [95–97]. This is potentially of relevance to the accelerated p27 proteolysis observed in human breast and prostate cancers. Her2 overpression is observed in up to 30% of primary breast and other human cancers and is associated with increased tumor invasiveness and a poor patient outcome [98–100]. Primary breast cancers overexpressing Her2 exhibit decreased p27 concentrations [101,102]. Her2/ErbB2 overexpression can also upregulate c-Myc and D-type cyclins, and this might facilitate p27 sequestration in cyclin D–Cdk complexes and an associated increase in cyclinE–Cdk2 activity [103].

Constitutive expression of Bcr–Abl leads to the activation of p27 proteolysis and prevents the upregulation of p27 protein by TGF-β and by serum and mitogen deprivation [104]. Bcr–Abl is constitutively active in chronic myeloid leukemia cells and constitutively activates several key regulators of survival, proliferation, and adhesion including Ras, Src, PI3K, and MAPK kinases [105–108].

Activation of p27 proteolysis through the PI3K pathway

In addition to effects of PI3K/PKB on p27 localization noted above, several studies suggest that the PI3K pathway also regulates p27 protein stability. Activation of the PI3K pathway decreases p27 concentrations, and PI3K inhibition by LY294002, by kinase-dead PKB/Akt, or by the overexpression of PTEN, a lipid phosphatase that opposes PI3K activation, have all been shown to increase p27 concentrations in certain cell types [109–111]. PI3K activation increased p27 proteolysis in hematopoietic cells expressing BCR/Abl, in prostate cancer cells, and in PTEN-null embryonic stem cells [112–114]. PTEN might inhibit p27 proteolysis through the repression of *SKP2*, the F-box component of the SCF^{Skp2} ubiquitin ligase complex that mediates cyclin E–Cdk2-dependent p27 degradation. p27 concentrations were decreased and Skp2 was increased in *PTEN*-deficient mouse embryonic stem cells [115]. Restoring *PTEN* expression in a *PTEN*-deficient line and PI3K inhibition by LY294002 both decreased *SKP2* gene expression. However, PTEN mediates arrest in G0, and thus the effect of PTEN on Skp2 concentrations in these studies might also reflect the destabilization of Skp2 protein associated with G1 arrest. Skp2 stability is dependent on the cell cycle, and its proteolysis is maximal in quiescent cells [116]. In human prostate, Skp2 protein concentrations are inversely correlated with p27 and the PTEN tumor suppressor protein [117].

p27 stability may also be linked to the PI3K/PKB pathway through the PKB-dependent phosphorylation of tuberin, a tumor suppresor encoded by the tuberous sclerosis complex 2 (*TSC2*) gene. The *TSC2* gene is mutated in up to 50% of tuberous sclerosis patients. Tuberous slerosis family members develop benign hyperproliferative tumors with high frequency. *TSC2* mutations are also seen in up to 30% of high-grade astrogliomas and in human lung cancers (reviewed in [118,119]). Loss of functional TSC2 or tuberin protein is associated with increased cyclin E–Cdk2, p27 mislocalization and decreased p27 due to p27 proteolysis that is independent of phosphorylation at T187 [120]. PKB has been recently shown to phosphorylate tuberin (reviewed in [118,119]) and this relieves its inhibitory action on mTOR. Inhibition of mTOR by rapamycin is known to stabilize p27 [121,122]. Thus, the increased stability of p27 after inhibition of PI3K might result in part from inhibition of PKB, activation of TSC2 and subsequent inhibition of mTOR. It will be of interest to determine how downstream effectors of mTOR might regulate p27 proteolysis.

Although PI3K activation might decrease SKP2 transcription, and through PKB inactivate TSC2 and activate mTOR, the effects of this pathway on p27 stability are clearly dependent on cell type [84]. As noted earlier, activation of PKB alone might not suffice to trigger p27 degradation in some tumors. High PKB activity was not statistically associated with decreased p27 protein concentrations in primary breast cancers [84].

Activation of the PI3K effector PKB/Akt has been shown to phosphorylate p27 and to lead to its cytoplasmic sequestration in part by impaired nuclear import [78,82–84]. Cytoplasmic sequestration would tend to impair the cyclin E–Cdk2-dependent phase of p27 proteolysis because cyclin E–Cdk2 is nuclear. Moreover, because nuclear p27 export in early G1 involves p27 phosphorylation, the cytoplasmic p27 that accumulates in PKB-activated cells might not be appropriately conditioned for export-linked proteolysis. Cytoplasmic p27 in these tumors might be relatively stable. Thus, the net effect of the oncogenic activation of the PI3K pathway on p27 stability might be complex *in vivo*, and the relative importance of PI3K effector pathways in certain cell types and concomitant changes in other signaling pathways might lead to important differences, dependent on cell type and tumor, in p27 regulation by the PI3K pathway.

The prognostic role of p27 in human breast cancer

Several groups have reported p27 studies in primary breast cancers. The first studies that identified p27 as an independent prognostic indicator involved three different breast cancer populations [47,123,124]. Tan and colleagues [47] studied the prognostic significance of p27 in 202 patients with breast cancers less than 1 cm in size. A low concentration of p27 protein, defined as less than 50% of tumor nuclei staining positively by immunohistochemistry, was associated with a 3.4-fold increased risk of death ($P=0.0306$) on multivariate analysis.

In a study of 168 unselected breast cancers, Catzavelos and colleagues [123] found that low p27 (less than 50% of tumor nuclei staining positive) was a strong independent predictor of reduced disease-free survival with a 2.7-fold increased risk of disease relapse ($P=0.017$). Breast tumors with low p27 were shown to have high cyclinE/Cdk2 activity [123,125].

Porter and colleagues [124] assayed p27 in the primary breast cancers of 278 women under the age of 45 years. Decreased p27 concentration was a significant independent predictor of poor overall survival (relative risk 2.7; $P=0.01$). Patients whose breast cancers showed both low p27 and elevated cyclin E proteins had the highest mortality, but both of these factors were significant on multivariate analysis. An inverse correlation between cyclin E and p27 concentrations has been observed in breast cancers [125], whereas increased concentrations of cyclin D1 were associated with both higher p27 concentrations and low tumor grade in another study [46].

A decrease in p27 concentrations might precede tumor invasion [123,126,127], and invasive lobular carcinoma seems to show increased p27 staining compared with invasive ductal carcinoma [128,129]. Two studies have also found an association between low p27 together with Her2/neu overexpression and reduced disease-free survival [101,102].

Several subsequent studies of breast cancer patients have also shown that the frequent decrease in p27 correlates with poor patient outcome. These studies have confirmed the correlation between low p27 and high tumor grade, negative estrogen receptor status and low cyclin D1 expression, and some have shown that low p27 is correlated with increased Ki67 or a high S-phase fraction [102,125,130–134]. Whereas several studies confirm the independent prognostic significance of p27 [46,131,132, 135], others failed to find p27 to be prognostically significant on multivariate analysis [102,130,133,136]. The Gillet study used a different scoring method from most [130]. A study of 198 breast cancer patients with a 17-year median follow-up concluded that tissue expression of p27 might be important for predicting 5-year, but not longer (more than 10-year) breast-cancer-specific survival [136]. This observation was not supported by our study in node negative breast cancers (see below).

Although node-negative breast cancers have a better prognosis than node-positive cancers, up to 30% of node-negative patients suffer recurrence and death from breast cancer. It would be of value to define variables other than traditional histopathologic features (size, lymphovascular invasion, tumor grade, and estrogen receptor status) to help define which patients merit aggressive treatment and who could safely be spared adjuvant chemotherapy. The Porter study showed that p27 was prognostic in the node-negative patient group, but this lacked statistical power on multivariate analysis [124]. Wu and colleagues analyzed 97 patients with node-negative breast cancer. A decreased p27 concentration (less than 50% of tumor nuclei positive) was an independent prognostic factor with relative risks of 5.7 ($P=000.1$) and 3.7 ($P=0.049$) for disease-free and overall survival, respectively [132].

In an analysis of 118 Ashkenazi Jewish women with node-negative disease, a decreased p27 concentration conferred a 10-fold increased risk of disease relapse ($P=0.03$) [135]. Paradoxically, Barbareschi and colleagues [137] found high p27 concentrations to be prognostically significant in their node-negative subset analysis. We have recently completed a large study of 1057 prospectively accrued node-negative breast cancer patients with a median follow-up of 9 years. Decreased p27 concentration was strongly correlated with estrogen-receptor-negative status and high grade. With a scoring cut-off of 25% to define low/high p27 values, low p27 (less than 25% of tumor cells positive) was an independent prognostic factor with a relative risk of 1.54 ($P=0.02$) and this was durable over the extended median follow-up of 9 years (C Catzavelos and J Slingerland, manuscript in preparation). It is noteworthy that two prostate cancer studies have also used the lower threshold of p27 (less than 25%) to define the worst prognostic group [53,54].

The failure to find p27 to be of prognostic significance in some of the more recent reports might reflect differences in tumor fixation, p27 staining and scoring methods, and the prolonged storage time of the archival tumor blocks used in these studies. In our attempts to stain p27 in more than 800 tumors recovered during the 1970s, we needed to increase antigen retrieval; even with high concentrations of antibody, tumor staining was variable and unreliable (M Dowsett, A Alkarain and J Slingerland, unpublished work). Prolonged storage of tumor blocks and fixation differences might account for the different results published recently. For p27 to become part of the panel of prognostic tests applied in the routine evaluation of breast cancers at diagnosis, it will be necessary to establish a uniform methodology for tumor processing, staining, and scoring.

As noted above, p27 is detectable in the cytoplasm of up to 40% of primary breast cancers, and this has been consistently associated with PKB/Akt activation. Cytoplasmic p27 was correlated with tumor de-differentiation (increased tumor grade) and poor survival [84]. The presence of cytoplasmic p27 was not statistically correlated with low concentrations of nuclear p27 protein. Some cancers showed both decreased p27 concentrations and cytoplasmic p27 localization, and these had the lowest survival rate, whereas those with high p27 concentrations and nuclear localization fared the best [84]. Thus, inde-

pendent mechanisms might regulate the cytoplasmic mislocalization of p27 in some tumors and its accelerated degradation in others. p27 proteolysis and mislocalization are regulated by major downstream effectors of receptor tyrosine kinases, including PKB and MAPK. It will be of interest to determine the additional pathways that predicate one effect or the other, or, in some tumors, the coexistence of both p27 protein loss and cytoplasmic mislocalization. Taking into account the localization of p27 might be an additional variable that adds to the prognostic potential of p27 evaluation in breast cancers.

Conclusion

Since its discovery and cloning a decade ago [11,12,14,138], p27 has proved to be ubiquitously expressed and a frequent target for deregulation in cancer. The loss of a single p27 allele is not infrequent in human cancers. In mice, haploinsufficiency for p27 mediates an increased susceptibility to carcinogens. p27 function is impaired in a majority of human cancers through accelerated proteolysis, sequestration by other proteins, and an imbalance of mechanisms regulating nuclear import and export. Decreased p27 protein concentration is associated with cancer cell dedifferentiation and poor patient outcomes in a majority of breast cancer studies including node-negative disease. The broad clinical application of p27 in the prognostic evaluation of breast cancer will require the development of a consensus on methods of tumor fixation staining and scoring. As the growth factor signaling pathways that regulate p27 in normal cells and lead to its deregulation in cancers are elucidated, it is to be hoped that therapeutic avenues for the restoration of p27 function will emerge.

Competing interests

None declared.

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