Commentary

The cyclin dependent kinase inhibitor p27 and its prognostic role in breast cancer

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Abstract

p27 is an inhibitor of cyclin dependent kinase involved in the regulation of the cell cycle. In this commentary we discuss the current knowledge on p27 in breast cancer and its significance in predicting the outcome. p27 protein levels are high in most cases of breast carcinomas, are correlated with the levels of cyclin D1 and estrogen receptor, and could be a useful predictor of survival, because they are low in aggressive carcinomas. Immunodetection of p27 in breast tumors could be useful in the assessment of prognosis, especially in those cases in which the commonly used parameters are insufficient, and might ultimately influence the therapy of this disease.

Keywords: breast cancer, cell cycle, p27, survival, ubiquitination

Introduction

Cell cycle progression is governed by cyclin dependent kinases (CDKs) that are activated by cyclin binding and inhibited by CDK inhibitors [1]. CDKs regulate checkpoints that integrate mitogenic and growth inhibitory signals, coordinating cell cycle transitions. The passage from G_1 to S phase is regulated by the activities of cyclin D1/CDK4, cyclin E/CDK2, and cyclin A/CDK2 complexes. Cyclin B/CDK1 regulates the G_2–M transition. Two families of CDK inhibitors regulate the cell cycle. Members of the inhibitor of CDK4 (INK4) family, such as $p15^{INK4B}$ and $p16^{INK4A}$, bind specifically to and inhibit CDK4 and CDK6. In contrast, members of the kinase inhibitor protein (KIP) family ($p21^{CIP1}$, $p27^{Kip1}$, and $p57^{Kip2}$) have opposite effects on the function of different CDKs. In fact, whereas p27 and p21 have a negative effect on the activities of cyclin E/CDK2 and cyclin A/CDK2, they seem to activate cyclin D/CDK complexes. This is due to at least three different mechanisms: facilitation of the assembly of cyclin D–CDKs complexes, increased nuclear localization of these complexes, and increased stability of D type cyclins. In proliferating cells p27 is prevalently bound to cyclin D/CDKs, whereas in G_1-arrested cells p27 is found in complexes with cyclin E/CDK2. Therefore, the competition for p27 between cyclin D/CDKs and cyclin E/CDK2 complexes seems to be crucial for cell cycle progression, because cyclin D/CDKs can sequester p27 from the cyclin E/CDK2 complex and favor progression into S phase [1].

Protein p27 was initially discovered in cells arrested by transforming growth factor-α (TGF-α), by contact inhibition or by lovastatin [1]. Mitogenic factors cause loss of p27, whereas p27 levels and/or activity increase in response to differentiation signals, on loss of adhesion to extracellular matrix and on signaling by growth inhibitory factors such as TGF-α. Mice with p27 knockout develop multiorgan hyperplasia and pituitary tumors, supporting a

CDK = cyclin dependent kinase; INK4 = inhibitor of CDK4; KIP = kinase inhibitor protein; Ubc = ubiquitin-conjugating enzyme.

role for p27 in both proliferation and differentiation [1]. Furthermore, p27 haploinsufficient mice are more sensitive to malignant tumor induction by radiation and chemical carcinogens [2], and allelic haploinsufficiency for p27 is found in some human tumors [3–5].

Whereas p27 mRNA levels are constant throughout the cell cycle, p27 protein levels are high in quiescent cells and decrease during G_1 phase, reaching the lowest point in S phase [6]. The decrease in p27 protein levels observed in the passage from G_1 to S phase is due to a decrease in the p27 half-life, which in proliferating cells becomes 6–8-fold shorter than in quiescent cells. This shortening in half-life corresponds to an increased degradation via the ubiquitin–proteasome pathway [7].

During ubiquitination and subsequent degradation, the interaction between a ubiquitin-conjugating enzyme (Ubc) and a substrate is mediated by the action of a ubiquitin protein ligase. Ubiquitin ligases regulating the G_1 phase are called SCF complexes. The SCF ligases are each formed by at least four basic subunits: Skp1, a Cullin subunit (Cul1 in metazoans), an F-box protein, and the Roc1/Rbx1 protein [8]. Each SCF ligase joins a Ubc (Ubc3, Ubc4, or Ubc5) to specific substrates that are recruited by different F-box proteins. Many F-box proteins have been described for various substrates, and recent results have demonstrated that Skp2 is the F-box protein involved in p27 degradation. Indeed, cell cycle levels of Skp2 are inversely correlated with p27 levels, with Skp2 expression low in early/mid-G_1 and increasing in late G_1, in coordination with the decrease in p27 protein. Biochemical and genetic experiments have demonstrated that Skp2 is required for the ubiquitination and consequent degradation of p27 both *in vivo* and *in vitro* [9–11].

Biological and prognostic implications of p27 levels in breast cancer

Most normal epithelial tissues, including breast, prostate, lung, and ovary, express high levels of nuclear p27 protein, especially in the terminally differentiated layers. In contrast, p27 is virtually undetectable in proliferating cells, such as the basal layer of epithelia or lymphocytes in germinal centers.

A variable loss of p27 protein has been shown in many human tumors. Indeed, the loss of heterozygosity of the 12p13 locus encompassing the p27 gene is uncommon in human tumors [3–5]. However, p27 gene biallelic losses or mutations are very rarely found in breast carcinomas [12]. Thus, the decrease in p27 levels in some human tumors has been related to an increased and deregulated degradation. In fact, it has been shown that epithelial cancers, lymphomas, and brain tumors display high and often deregulated proteolytic activity of recombinant p27 protein *in vitro* [13–15].

In the last few years, interest has grown in understanding the role and the implications of p27 levels in breast cancers. The first three published studies tested the prognostic value of p27 in breast tumors in comparison with already well established parameters such as tumor stage, grade, nodal status, hormone receptor levels, and S-phase fraction. Porter *et al* [16] assayed both p27 and cyclin E levels in 246 primary breast cancers of women under 45 years of age. Patients whose breast cancers showed both low p27 and elevated cyclin E proteins had the highest mortality, and multivariate analysis demonstrated that both levels of p27 and cyclin E were independent predictors of overall survival. Catzavelos *et al* [17] studied a cohort representative of the breast cancer patient population. This study found low staining of p27 in 56% of the tumors, and loss of p27 was a strong independent predictor of decreased disease-free survival, associated with a 2.7-fold increased risk of disease relapse. Tumors with low p27 levels had higher cyclin E/CDK2 activities than those with high p27 levels. Tan *et al* [18] examined the prognostic value of p27 in 202 patients with breast cancers less than 1 cm in size (T1a,b) compared with clinicopathological features and other parameters such as p53, c-erb-B2, Ki-67, cdc25B, and the density of microvessels. This study showed that low p27 levels, defined as fewer than 50% of the tumor cells being positive for p27, were an independent risk factor on multivariate analysis and were associated with a 3.4-fold increased risk of death, particularly in node-negative tumors.

Subsequent studies on different groups of patients with breast carcinoma have confirmed many of these first observations. In fact, there is a general agreement that high p27 levels are usually correlated with low histologic grade, positive estrogen receptor status, high cyclin D1 expression, and low S-phase fraction [19–23]. Some studies have also compared p27 protein levels and cyclin E and/or cyclin D1 dependent kinase activities in breast cancers, finding an inverse correlation between cyclin E dependent kinase activity and p27 [24]. It has recently also been shown that the treatment with 4D5 antibody of the BT474 cell line derived from breast carcinoma induced a shift of p27 from cyclin D/CDK4 to cyclin E/CDK2 complexes and a consequent G_1 arrest, suggesting that the modulation of the levels and/or CDK binding of p27 could be helpful in controlling the growth of breast carcinoma cells [25]. However, the differences between studies become evident when the relation between p27 and prognosis is analyzed in an attempt to establish the importance of p27 as a single predictor of survival. Some studies have shown p27 to be an independent variable in multivariate analysis of survival [16–18]. Others proved p27 to be a prognostic factor only in univariate analysis, as compared with strong and well established prognostic factors such as grade, size, nodal status, and S-phase fraction [19,20,26]. Recently, two papers, one based on a

large group of consecutive breast carcinoma cases (512) [22] and the other on 148 grade I breast carcinomas [23], failed to find any prognostic relevance for p27 or cyclin D1. The discrepancies resulting from all these studies could be partly explained by considering the different antibodies used for the immunohistochemical stainings or the different criteria for p27 positivity used in the scoring systems. In addition, p27 seems to be correlated with the grading more than the staging, being less useful in a homogeneous category such as grade I carcinomas [23]. Alternatively, it has to be considered that p27 is not the only cell cycle inhibitor involved in breast carcinomas, an almost equivalent role being established for p21 in controlling the rate of cell cycle in cell lines derived from breast tumors [27,28].

Finally, loss of p27 might precede tumor invasion but is not related to particular subtypes of breast cancers. Catzavelos *et al* [17] observed a frequent loss of p27 in non-invasive ductal breast carcinoma *in situ*. In both the *in situ* and invasive components, lower p27 staining was observed in high-grade tumors. Similar studies conducted on less frequent subtypes of breast carcinomas, such as infiltrating lobular or apocrine carcinoma, did not show significant differences in p27 staining compared with the more common infiltrating ductal carcinoma [29,30].

In conclusion, a loss of p27 seems to have prognostic potential in breast tumors as for other types of tumor, such as lymphomas and lung, colon, ovary, and prostate carcinoma. This prognostic value could be especially useful in tumors in which the classical survival parameters are insufficient, such as in small and nonmetastatic breast cancers. This potential relies on the simplicity and reproducibility of the immunohistochemical stainings for p27 as well as on the growing number of studies on the deregulation of p27 function in human cancers. A better understanding of the mechanisms regulating p27 expression and its interaction with other oncogenes opens the possibility of selective control of its degradation, raising the hope for the generation of new and more effective drugs for breast cancer.

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