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Commentary

New insights into the tumor suppression function of $P27^{Kip1}$

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Tuberous sclerosis complex (TSC) is an autosomal dominant disease characterized by mental retardation, seizures, and tumors involving many organs, including the kidney, brain, heart, and skin (1). Linkage analyses identified two major disease loci on chromosomes 9 (TSC1) and 16 (TSC2), and both of these genes have been cloned (2, 3). These genes act like classic tumor suppressor genes: patients inherit a mutant germline allele and the remaining functional allele is inactivated in TSC-associated tumors (2–6). Mutations in these genes also occur in cases of sporadic TSC. The Eker rat contains a germline insertion within the rat TSC2 gene and provides an animal model for TSC (7, 8). Although the mutation is embryonic lethal when rats are homozygous, rats heterozygous for the Eker mutation develop spontaneous kidney tumors and are hypersensitive to carcinogen and radiation-induced renal carcinomas (9).

TSC1 encodes hamartin, a 1,164-aa protein of unknown function (3). The TSC2 gene product, termed tuberin, is a GTPase activating protein that activates the ras family GTPases Rap1a and Rab5 *in vitro* (2, 10, 11). Hamartin and tuberin physically interact, suggesting that these two tumor suppressors may lead to TSC through the same biochemical pathway (12). Early studies suggested that tuberin may negatively regulate cell proliferation, but it was not until the studies by Soucek *et al.*, in this issue of the *Proceedings* (56), that a possible direct link between tuberin and cell cycle regulatory proteins was uncovered (13). They find that in tuberin-null cells derived from homozygous Eker rat embryos, the p27kip1 cyclin-dependent kinase inhibitor is inactivated as a consequence of being mislocalized in the cytoplasm. Thus tuberin may directly impact a key negative regulator of cell division.

A group of protein kinases called cyclin-dependent kinases (CDKs) regulate progression through the cell cycle (14). The CDK holoenzyme contains catalytic and regulatory (cyclin) subunits, and each phase of the cell cycle has a characteristic profile of cyclin–CDK activity. Two classes of proteins called CDK inhibitors negatively regulate the cell cycle by binding to and inhibiting CDKs (15). The INK4 proteins (p15, p16, p18, and p19) specifically inhibit the CDK4/6 kinases, whereas the Cip/Kip proteins (p21cip1, p27kip1, p57kip2) can target most cyclin–CDK complexes.

P27kip1 was first identified as an inhibitor of cyclin E–CDK2 (16, 17). Overexpression of p27 in cultured cells arrests the cell cycle. In general, p27 expression is highest in quiescent cells and declines as cells reenter the cell cycle. Many antiproliferative signals lead to p27 accumulation, including mitogen/cytokine withdrawal, cell–cell contact, and agents such as cAMP and rapamycin (15). In fact, p27 modulation may be an essential component of mitogen-dependent cell cycle entry and exit (18). The crystal structure of p27 bound to cyclin A–CDK2 revealed that p27 inserts itself deep within the CDK catalytic site, blocking ATP access (19). These data led to a simple model in which antiproliferative stimuli up-regulate p27, followed by tight CDK inhibition and cell cycle arrest. The key role of p27kip1 in regulating cell proliferation is reflected in the p27 knockout mouse, which exhibits gigantism (because of increased cell number), female sterility, and increased tumorigenesis (see below) (20–23).

Multiple posttranscriptional mechanisms regulate p27 abundance. P27 may be degraded by the ubiquitin–proteasome system, and high proteolytic activity has been demonstrated in extracts prepared from S-phase cells, as well as from colorectal and non-small cell lung cancers. (24–27). Translational control also regulates p27 abundance. Increased p27 translation rates are found in arrested (G_0) versus growing cells, and the accumulation of p27 in G_0 cells may result largely from the increased association of p27 mRNA with polyribosomes (28, 29). P27 is also regulated by phosphorylation, and phosphorylation of p27 by cyclin E–CDK2 leads to its turnover (30, 31). The relative contribution of proteolytic and translational control to p27 regulation in various physiologic contexts and the biochemical consequences of p27 phosphorylation remain largely unknown.

P27 expression and/or function may also be affected by dominantly acting oncogenes. Several groups have reported that c-*myc* overexpression overcomes a p27-mediated cell cycle arrest (32–34). Ras activity, either alone or in concert with c-*myc*, may also down-regulate p27 (35–37). Interestingly, the adenovirus E1A protein, which functions like c-*myc* in some transformation assays, may also inactivate p27. However, two groups have reported very different mechanisms of action for E1A: (*i*) direct p27 binding and inactivation and (*ii*) p27 bypass in the absence of a physical p27/E1A interaction (38, 39).

The most recently proposed mechanism of p27 regulation is subcellular compartmentalization. P27 appears to interact with its targets in the cell nucleus, and mislocalization of p27 in the cytoplasm might inactivate p27 by sequestering it away from relevant cellular targets (40). In fact, cytoplasmic mislocalization of p27 has been reported in human tumors and cell lines (41). A recent study of Barrett's-associated esophageal adenocarcinoma found subcellular cytoplasmic localization of p27 in more than half of esophageal adenocarcinomas (42). These tumors contained high amounts of p27 but maintained a high proliferative rate, suggesting that the p27 may be inactive.

In their current study, Soucek *et al.* (56) demonstrate that loss of the tuberin protein is associated with p27 mislocalization in the cytoplasm resulting in (*i*) a failure of p27 to inhibit the cell cycle, even when overexpressed, and (*ii*) decreased p27 abundance caused by increased proteolysis (although this may not involve the proteasome). Because nucleo-cytoplasmic transport is regulated by the ran GTPase, it is tempting to speculate that tuberin's GTPase-activating activity is directly involved in p27 localization. However, no nuclear transport role for either rab5 or rap1a has been described to date.

Abbreviations: TSC, tuberous sclerosis complex; CDKs, cyclin-dependent kinases.

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Although the loss of tuberin expression clearly affects p27 localization and stability in Eker rat cells, it is too early to know just how intimately p27 is related to the pathogenesis of TSC. Certainly the phenotypes of the Eker rat and p27-null mouse are not at all concordant. Thus either the physiologic consequences of mislocalized p27 in the Eker rat are distinct from that of p27 loss in the mouse or tuberin-dependent effects on molecules other than p27 must contribute to the Eker rat phenotype. In fact, it is not yet clear that p27 inactivation directly contributes to the cell cycle anomalies associated with tuberin loss and/or overexpression.

Genes that inhibit cell proliferation are excellent candidates for tumor suppressor genes. However, although single allelic p27 loss has been observed in primary tumors, homozygous inactivation of the p27 gene is extremely rare (43–45). The complex posttranscriptional regulation of p27 suggests that mechanisms other than direct mutation might down-regulate p27 in tumor cells. This, in fact, seems to be the case, and p27 expression has now been examined in many human tumors.

Evidence that p27 may be involved in human tumor progression comes largely from studies that have directly measured the expression of p27 protein in clinical tumor samples using immunohistochemical assays. Although limited in their ability to provide mechanistic information, the cumulative data from these studies indicate that low or absent p27 protein in tumor cells is an important clinical marker of disease progression in many tumor types. The evidence is strongest in breast cancer, for which at least three relatively large studies in independent populations show significantly decreased overall, or disease-free, survival in women whose tumors lack p27 (46–48). Importantly, the two breast studies that have analyzed subgroups of breast cancer patients found low p27 levels associated with a significantly elevated risk of mortality in women with early stage (lymph node-negative) disease (46, 47). Because there are relatively few clinicopathological factors on which to base treatment decisions in this group of women, the demonstration of prognostic significance for p27 expression could eventually result in improved treatment strategies.

The number of tumor types that have been studied for expression of p27 has steadily increased and the data across types are strikingly consistent. In two independent studies of prostate cancer, low p27 was associated with high grade and identified as predictor of treatment failure after prostatectomy (49, 50). The prognostic value of p27 protein expression has been demonstrated in colorectal cancer where absence of p27 in the primary tumor was associated with an elevated relative risk of dying (26). In a group of patients with non-small cell lung cancer, low tumor expression of p27 corresponded to an overall survival rate of 14% compared with 25% survival in high expressors (51). Data concerning p27 expression from studies of melanoma, oral squamous cell carcinoma, and Barrett's esophageal adenocarcinoma involve only a small number of tumors but consistently support the association of low p27 with poor clinical outcome (42, 52, 53). Although it has been suggested that the prognostic value of low p27 is attributable to the correlation with high cell proliferation, the data in human tumors to support this explanation are mixed, and the association may be tissue-type specific (26, 54, 55). In addition to the assessment of p27 as a prognostic marker, a few studies have examined the relationship of p27 expression and tumor development by comparing levels of p27 expression in precursor and invasive lesions. In studies of oral, breast, and Barrett's-associated preinvasive and invasive cancers, reduction in the level of p27 is associated with increasing degree of malignancy (26, 48, 53).

In summary, although there is a large body of evidence supporting a role for p27 in human cancer, these data are only correlative and do not identify p27 loss as a causal event in multistep tumorigenesis. To date, the only direct evidence that p27 is a tumor-suppressor gene comes from studies in p27-null mice. Although p27-null animals develop pituitary adenomas with nearly 100% penetrance, they do not exhibit the widespread cancer syndromes seen in mouse strains with deletions of tumor suppressors such as p53 or INK4A. P27-null mice, however, are hypersensitive to radiation and chemical-carcinogen-induced tumorigenesis. Remarkably, the rate of tumor formation in these animals depends on the p27 gene copy number (p27 $-/-$ > p27 $+/-$ > p27 $+/+$) (23).

How might we best interpret the role of p27 in human cancers? The circumstantial evidence implicating p27 as a human tumor suppressor is rapidly accumulating, and the link between p27 function and TSC possibly provides additional independent support for this hypothesis. The clearest signature of a tumor suppressor, inactivating p27 point mutations in a human tumor cell with p27 loss of heterozygosity, has not yet been detected. The observation that p27 haploinsufficiency renders mice hypersensitive to carcinogens raises the possibility that p27 single allele loss in human cancers may function similarly, but this remains to be shown. The developing data in human tumors suggest that p27 may prove to be a useful clinical tool even before the mechanisms of p27 inactivation are completely understood.

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