How to decrease p27^{Kip1} levels during tumor development

***p27^{Kip1}*, a cyclin-cdk inhibitor, is a tumor suppressor. An overwhelming amount of data correlate p27 abundance to tumor prognosis in humans. Mouse models have supported the importance of decreasing p27 to tumor incidence. Inactivation of most tumor suppressors occurs at the level of gene mutation or silencing, but p27 is regulated posttranscriptionally, and how its level is reduced in cancer is largely unknown. Reports on a series of allelic mice with p27 mutations affecting different posttranscriptional regulatory pathways are emerging and being used to examine which pathways are necessary for p27 turnover associated with tumor development, with surprising results.**

Strong prognostic markers that differentiate patient outcomes when analyzing an intermediate stage disease are of utmost importance in making appropriate therapeutic evaluations. The inverse correlation between expression of the cdk inhibitor p27 and prognosis in a variety of human neoplasms, including those of the breast, colon, and prostate among others, is well known (Blain et al., 2003). Mice with an allele that reduces the ability of p27 to inhibit cyclin-cdk complexes (Δ51, a 51 amino acid deletion of the amino terminus of the protein) or a complete null are larger because of a defect in timely exit from the cell cycle. Establishing the causal nature between p27 abundance with tumor incidence, tumors develop in a wide variety of tissues more frequently and progress faster in these p27-deficient mice challenged by a variety of carcinogens, oncogenes, and the loss of other tumor suppressors (Blain et al., 2003). Although it is well established that p27 is a dosage-dependent tumor suppressor, two questions have remained—what does the reduction of p27 contribute to tumor development, and how is the level of p27 reduced to eliminate its tumor-suppressive function?

Gene mutation or silencing at the *p27* locus is rare, and abundance of p27 protein appears to be largely controlled by posttranscriptional mechanisms. The mechanisms controlling p27 abundance range from translational control observed in quiescent cells to proteolytic mechanisms operating at specific phases of the cell cycle or in specific subcellular compartments, such as the cytoplasm or nucleus. Focusing on protein turnover and localization, three pathways have been suggested. The best understood is the SCFskp2/cks1 nuclear ubiquitination-dependent turnover pathway in S and G2/M cells (Bloom and Pagano, 2003). In the absence of this pathway, p27 levels decrease as cells reenter the cell cycle but reaccumulate in S phase cells, ultimately leading to aneuploidy and problems in G2/M progression (Nakayama et al., 2004). Unexpectedly, mutating threonine 187 to alanine (T187A) prevents p27 ubiquitination by SCFskp2/cks1, but these cells do not share the mitotic phenotype of skp2-deficient cells (Kossatz et al., 2004). Restriction of this p27 turnover pathway to S and G2/M cells is explained by the growth-dependent accumulation of skp2 and the requirement for cyclin A-cdk2 to present p27 into the SCFskp2/cks1 complex (Hao et al., 2005; Zhu et al., 2004). Less understood is a pathway by which p27 is exported to the cytoplasm, where it may be degraded in a KPC1/2-induced, ubiquitin-dependent manner (Kamura et al., 2004). In this pathway, p27 is phosphorylated on serine 10, which promotes CRM1-dependent binding and nuclear export. This pathway operates in G1 cells or cells stimulated to reenter the cell cycle from a quiescent state. Additionally, Akt-dependent nuclear exclusion can be induced by phosphorylation of threonine 157 (Blain et al., 2003). However, while this mechanism explains the regulation of p27 in certain human tumors, this site is not conserved in the mouse.

Understanding which mechanisms are important for reducing p27 during tumor development has obvious importance. In a recent issue of *Genes and Development*, Besson and coworkers (Besson et al., 2006) provide convincing evidence for a serine 10-dependent regulatory mechanism affecting p27 tumor suppressive activity during lung tumor development. This is a notable first. Using urethane-induced carcinogenesis, they show that p27 is a haploinsufficient tumor suppressor—heterozygous mice develop tumors at the same frequency as the complete null and do not lose or inactivate the remaining allele, albeit the tumors in the heterozygous mice appear to progress at a slower rate. During this process, serine 10 phosphorylated p27 accumulates in the cytoplasm of lung tumor cells. Activated ras plays a role in this carcinogenic process, and the authors demonstrate that *K-rasV12* induces cytoplasmic accumulation of p27 and serine 10 phosphorylation, both of which can be increased further by addition of a proteasome inhibitor. The crux of the report of interest to the reader of *Cancer Cell* is that mutating serine 10 to alanine reduces the number of tumors and the rate at which they grow, and *K-rasV12* cannot induce cytoplasmic localization of p27S10A, arguing that this is a bona fide mechanism by which p27 function is reduced during oncogenesis. This mutation does not affect the size of the animal, spontaneous pituitary tumor development, or fertility (James Roberts, personal communication).

However, what may temper the momentum to link skp2-induced p27 turnover to cancer is that the authors mentioned the tantalizing (but still unpublished) observation that mutation of threonine 187, which eliminates SCFskp2/cks1-dependent turnover, failed to suppress tumor development. While it might be reasonably argued that a single example of this is not sufficient to diminish the importance of the skp2-p27 pathway to tumor development, most of the evidence linking skp2-induced p27 turnover to cancer is indirect. While *skp2* is oncogenic when overexpressed (Latres et al., 2001), skp2-induced p27 turnover is generally restricted to S and G2/M phase of the cell cycle, and skp2 has more substrates than p27 itself. Cancer is a disease reflecting defects in the choice cells make to enter or exit the cell cycle rather than an aspect of the irreversibility of the decision to enter the cell cycle.

Thus, this work may presage an interesting paradigm shift from an SCFskp2/cks1-centric mechanism regulating p27 abundance and tumor suppression activity toward a serine 10-dependent cytosolic localization mechanism. This may parallel or overlap the human-specific threonine 157-dependent cytosolic pathway (reviewed in Blain et al., 2003). Determining the mechanisms controlling serine 10 phosphorylation and the differ-

ences between G0 and G1 cells affecting the stability and localization of the phosphorylated p27 will prove of interest in understanding the role of p27 as a tumor suppressor. Additionally, given that enforced expression of skp2 leads to tumor development, one might wish to pursue additional targets of this E3 ligase that contribute to its oncogenicity.

Perhaps of lesser importance to cancer at this time, but likely to grow as more experiments are done, Besson also reports the generation of a mutant that will not bind to cyclin-cdk complexes. An allele with a four amino acid substitution was made that eliminates cyclin-cdk binding (ck–). The crystal structure of the SCFskp2/cks1 E3 ligase (Hao et al., 2005) and the reconstitution experiments with catalytically inactive cyclin A-cdk2 (Zhu et al., 2004) demonstrate that this mutation affects cyclin-cdk presentation of p27 to the ligase, and it is assumed that cyclin-cdk binding is also necessary for destabilization of the nonphosphorylated S10A protein in the nuclei of quiescent cells. Similar to mice with null or *Δ51* alleles, *ck–* mice are large, suggesting that cyclin-cdk binding is the functional reason for this phenotype, but the fertility and spontaneous tumor phenotype were not addressed.

The ck– mouse may play a role in unraveling cyclin-cdk-independent functions associated with p27 accumulation in tumorigenesis. Through sequences in the carboxyl half of p27, it can interact with rho (Besson et al., 2004) and Grb2 (Moeller et al., 2003). This interaction may affect these signaling pathways or their biologic outcomes during tumor development. A requirement for "some" p27 has been suggested for tumor development in mouse *ErbB2*/*Neu* breast (Muraoka et al., 2002) and *Pten*-deficient prostate (Gao et al., 2004) cancer models utilizing *p27Δ51* animals. However, these authors associated this function with appropriate regulation of cyclin D1 levels and kinase activity. A potential cytosolic function was largely unaddressed. While both the ck– mutant and the Δ51 mutant retain the carboxyl domains, ck– has the serine 10 phosphorylation site, which would permit a level of regulation not possible with Δ51. Thus, the ck– mutant might be able to address whether retention of p27 in the cytoplasm, if not completely eliminated by a pathway involving KPC-induced ubiquitination, has oncogenic cyclin-cdk-independent functions.

Armed with the resource of an allelic series of mice, we can now begin to understand which of the regulatory mechanisms determining p27 expression allow its function as a tumor suppressor. While these mice will have value for looking at cyclin-cdk-independent functions that may impact tumorigenesis, equally interesting will be their effects on cell differentiation and animal development.

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