The p27Kip1 tumor suppressor gene: Still a suspect or proven guilty?

The p27Kip1 cyclin-dependent kinase inhibitor is considered to be a tumor suppressor even though somatic mutations in p27Kip1 are only rarely detected in human tumors. On the other hand, overwhelming evidence indicates that its hemizygous or posttranscriptional loss plays an important role in tumorigenesis. Based on these data, p27Kip1 was classified as a haploinsufficient tumor suppressor whose protein level has to be fine-tuned for optimal function. However, a recent study links germline mutations in p27Kip1 to multiple endocrine neoplasia syndrome in rats and humans, thus establishing p27Kip1 as a bona fide tumor suppressor gene.

p27Kip1 was identified as a cell cycle inhibitor responsible for growth arrest induced by TGF-β and contact inhibition (Polyak et al., 1994). It belongs to the Cip/Kip family of cyclin-dependent kinase inhibitors (CKIs) together with p21Cip1 and p57Kip2. Initially, p27 was thought to have an inhibitory effect against all cyclin-cdk complexes and was presumed to only negatively regulate cell cycle progression. Consistent with this, mice with homozygous deletion of the p27Kip1 gene are larger compared to their wild-type counterparts due to increased cell numbers in various organs, especially in the organs with normally high endogenous p27 levels (Sherr and Roberts, 1999). In addition, p27-deficient mice develop pituitary tumors resembling the phenotype of Rb-deficient mice, confirming that the growth and tumor suppressor function of p27 is mediated via its negative effect on the Rb pathway. Mice lacking one or both copies of p27 gene have increased susceptibility to carcinogen-induced tumorigenesis, but the wild-type p27 allele is always retained in these tumors formed in *p27*$^{+/-}$ animals, which has led to the conclusion that p27 is a haploinsufficient tumor suppressor. Subsequent studies further supported this hypothesis, and they revealed that decreased, but not completely eliminated, p27 protein levels could enhance tumorigenesis due to the positive effect of p27 on the assembly of cyclin D-cdk4 complexes and thus on cell cycle progression (Sherr and Roberts, 1999). The dual function of p27 in the regulation of cell proliferation and tumorigenesis was convincingly demonstrated in an erbB2/neu-induced mouse model of breast cancer in which heterozygous loss of p27 accelerated but nullizygous loss inhibited tumor formation (Muraoka et al., 2002). Similar results were obtained in a Nkx3.1/PTEN loss-induced prostate tumor model as well (Gao et al., 2004).

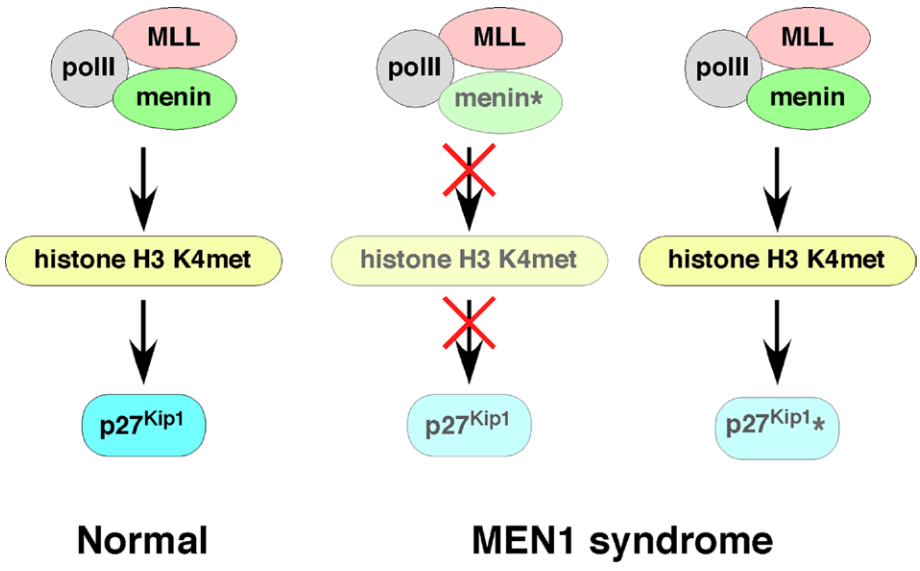

Figure 1. Model depicting the MEN1-p27Kip1 tumor suppressor pathway

In normal cells menin is part of a protein complex responsible for histone H3 lysine 4 methylation (histone H3 K4met), which is required for the expression of p27^{Kip1} in certain cell types, including endocrine cells. In MEN1 syndrome, due to mutation in either menin (menin*) or p27^{Kip1} (p27^{Kip1}*) cellular p27^{Kip1} protein levels decrease resulting in tumor formation.

The regulation of p27 levels is fairly complex and predominantly occurs posttranscriptionally, involving phosphorylation by various kinases, ubiquitin-mediated degradation, and nuclear export mechanisms (Koff, 2006). Since its discovery, p27 was presumed to be a tumor suppressor due to its growth-inhibitory function, but despite comprehensive large-scale mutation screening studies in various human tumor types, genetic alterations in p27 have been infrequently detected. However, the protein level of p27 is decreased in most human tumors, and this correlates with worse clinical outcome in multiple cancer types, including breast, colon, and prostate cancer (Koff, 2006). Thus, it was concluded that the inactivation of p27 during tumorigenesis rarely involves genetic mechanisms.

However, a recent study by Pellegata et al. published in *PNAS* identified p27Kip1 as a gene responsible for multiple endocrine neoplasia-like (MENX) syndrome in rats (Pellegata et al., 2006). MENX is inherited in an autosomal recessive manner, and affected animals develop bilateral pheochromocytomas, parathyroid adenomas, multifocal thyroid C cell neoplasia, paragangliomas, and pancreatic islet cell tumors. Linkage analysis placed the responsible gene in a ~3 Mb area of rat chromosome 4, and mutational analysis of candidate genes, including the *Cdkn1b* gene encoding p27Kip1, identified a homozygous 8 nucleotide insertion in exon 2 that leads to a frame shift after codon 177 and a p27 protein with a different C terminus (p27_G177fs). Similar to *p27*$^{-/-}$ mice, rats homozygous for this germline mutation were larger compared to their wild-type littermates, and their life span was statistically significantly decreased. Heterozygous rats demonstrated the same phenotype as wild-types confirming the recessive inheritance of MENX. Immunohistochemical analyses of various organs revealed extremely reduced

or absent p27 protein levels in multiple organs in mut/mut animals, but this did not correlate with cellular proliferation, suggesting that p27 may have a CKI-independent function in certain tissue types or not be required for the proliferation of these cells. Indeed, the C-terminal domain of p27 has been shown to be dispensable for cyclin-cdk complex binding and inhibition, and in some cell types p27 is involved in the regulation of Rac- or RhoA-dependent cell migration and Ras activation.

In humans, MEN syndromes are inherited in an autosomal dominant fashion and are due to germline mutations in the *MEN1* tumor suppressor gene (MEN1 syndrome) or the *RET* oncogene (MEN2A and MEN2B syndromes) (Marx, 2005). However, in ~30% of patients with MEN1 syndrome, mutations in *MEN1* could not be identified, but because genetic heterogeneity for the MEN1 phenotype was not expected, it was assumed that these patients were likely to still have a mutation in MEN1 that was not identifiable using currently employed methods. However, the rat MENX data raised the hypothesis that p27Kip1 could be responsible for the MEN1 phenotype in a subset of these patients. Thus, Pellegata et al. sequenced the human *CDKN1B* gene in multiple members of a suspected MEN1 family without an identifiable MEN1 mutation. In an affected member of this family, a 48-year-old woman with a pituitary tumor and primary hyperparathyroidism, a germline heterozygous TGG to TGA nonsense mutation at codon 76 leading to a truncated p27 protein (p27_W76X) was identified. This sequence alteration was not present in 380 healthy normal controls, thus it is unlikely to be a polymorphism. The same mutation was also detected in one of the patient's sisters, who was diagnosed with renal angiomyolipoma, a nonendocrine tumor frequently observed in MEN1 families. Two out of four unaffected family members analyzed were mutation negative, while two others were mutation carriers but symptom free, potentially due to their younger age. The proband's father had acromegaly, and her brother died at age 39 of hypertension, but blood samples were not available from these cases. Among all the tumors detected in the family members, only the renal angiomyolipoma diagnosed in the proband's older sister was available for molecular analysis. In this tumor the wild-type *CDKN1B* allele appeared to be maintained, and wild-type mRNA was detected, but the tumor cells demonstrated complete lack of p27 protein based on immunohistochemical analysis.

To address the potential mechanism underlying the decreased p27 levels and MEN phenotype in rats with the p27_G177fs and in humans with p27_W76X mutations, the authors exogenously expressed these proteins in rat fibroblasts and human MCF-7 and 293T cells together with wild-type and p27_G177X (nonsense mutation at codon 177 to determine if different C terminus influences function) controls and analyzed their levels and cellular localization. Both p27_G177fs and p27_W76X were expressed at significantly lower levels compared to wild-type p27, and p27_W76X was only detected in the cytoplasm and thus could not function as a cdk inhibitor. It is not clear if p27_W76X could act as a dominant negative and lead to the sequestration and downregulation of the wild-type protein, which could potentially explain the lack of p27 in tumors of heterozygous mutation carriers.

The link between p27Kip1 and MEN1 syndrome is not entirely surprising, since menin, encoded by the MEN1 gene, has been shown to participate in a complex that has histone H3 lysine 4 (H3K4) methylation activity in various human and mouse cells (Hughes et al., 2004). Furthermore, menin-dependent histone H3K4 methylation was required for the maintenance of p27 expression in pancreatic islet cells in *MEN1*$^{+/-}$ mice (Karnik et al., 2005). The histone H3K4 methylation promoting function of menin is thought to be required for its tumor-suppressive function, since several patient-derived mutants lost this activity.

The study by Pellagata et al. links genetic alterations in p27Kip1 to MEN syndrome in both rats and humans, suggesting that p27 could be one of the key downstream targets of *MEN1*'s tumor-suppressive function and germline or somatic mutations in p27Kip1 may play a role in a subset of MEN1 cases (Figure 1). However, several questions remain to be answered. To conclusively link the human p27_W76X mutation to MEN1 syndrome would require the analysis of more than one family and tumors that developed in the affected patients. Interestingly, the exact same truncating mutation (p27_W76X) was reported in one case of adult T cell leukemia/lymphoma, but in this patient the mutation was reported to be somatic and accompanied with the loss of wild-type allele in the tumor (Morosetti et al., 1995). Assuming that there are no technical issues, the lack of wild-type p27Kip1 allele loss in the heterozygous p27_W76X mutation carrier's renal angiolipoma accompanied by the loss of p27 protein in the tumor cells requires some explanation and further mechanistic studies. Most importantly, the possibility that p27_W76X represents a rare allelic variant has to be eliminated by sequencing the complete coding region of p27Kip1 in a large number of controls to determine the frequency of all nonsense mutations. However, regardless of these unresolved issues, p27Kip1 has finally entered the stage of bona fide tumor suppressor genes, and these new results are likely to stimulate new waves of mutation screens in a subset of MEN syndrome cases and in sporadic endocrine tumors.

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