Characterization of a naturally-occurring p27 mutation predisposing to multiple endocrine tumors.

BACKGROUND: p27Kip1 (p27) is an important negative regulator of the cell cycle and a putative tumor suppressor. The finding that a spontaneous germline frameshift mutation in Cdkn1b (encoding p27) causes the MENX multiple endocrine neoplasia syndrome in the rat provided the first evidence that Cdkn1b is a tumor susceptibility gene for endocrine tumors. Noteworthy, germline p27 mutations were also identified in human patients presenting with endocrine tumors. At present, it is not clear which features of p27 are crucial for this tissue-specific tumor predisposition in both rats and humans. It was shown that the MENX-associated Cdkn1b mutation causes reduced expression of the encoded protein, but the molecular mechanisms are unknown. To better understand the role of p27 in tumor predisposition and to characterize the MENX animal model at the molecular level, a prerequisite for future preclinical studies, we set out to assess the functional properties of the MENX-associated p27 mutant protein (named p27fs177) in vitro and in vivo.

RESULTS: In vitro, p27fs177 retains some properties of the wild-type p27 (p27wt) protein: it localizes to the nucleus; it interacts with cyclin-dependent kinases and, to lower extent, with cyclins. In contrast to p27wt, p27fs177 is highly unstable and rapidly degraded in every phase of the cell-cycle, including quiescence.
It is in part degraded by Skp2-dependent proteasomal proteolysis, similarly to p27wt. Photobleaching studies showed reduced motility of p27fs177 in the nucleus compared to p27wt, suggesting that in this compartment p27fs177 is part of a multi-protein complex, likely together with the degradation machinery. Studies of primary rat newborn fibroblasts (RNF) established from normal and MENX-affected littermates confirmed the rapid degradation of p27fs177 in vivo which can be rescued by Bortezomib (proteasome inhibitor drug). Overexpression of the negative regulators microRNA-221/222 plays no role in regulating the amount of p27fs177 in RNFs and rat tissues. CONCLUSION: Our findings show that reduced p27 levels, not newly acquired properties, trigger tumor formation in rats, similarly to what has been observed in mice. The molecular characteristics of p27fs177 establish MENX as a useful preclinical model to evaluate compounds that inhibit p27 degradation for their efficacy against endocrine tumors.