Snf5 tumor suppressor couples chromatin remodeling, checkpoint control, and chromosomal stability

SNF5 is a core subunit of the SWI/SNF chromatin-remodeling complex. Mammalian SNF5 is essential for normal cell viability, and loss or mutation of the human SNF gene is the molecular basis for familial malignant rhabdoid tumorigenesis. Previous studies have suggested that SNF5 suppresses cancer by signaling through the p16Ink4a and retinoblastoma tumor suppressors to negatively regulate cell cycle progression from G0/G1 into S phase. A recent paper in *Genes & Development* (Vries et al., 2005) reports that human SNF5 also signals via the p16INK4a-Rb-E2F pathway to regulate chromosomal stability, suggesting a new function for this chromatin remodeling protein in tumor suppression.

SWI/SNF chromatin remodeling complexes utilize ATP to alter histone-DNA interactions and activate or repress the expression of numerous eukaryotic genes. In humans, SWI/SNF complexes contain approximately 10 subunits, including either the SNF2 family ATPase BRM (Brahma) or BRG1 (Brahma related gene 1). The ability of these subunits to bind to other known tumor suppressors, the loss of expression of these genes in some human tumor cell lines, and the increased cell proliferation and/or low level of tumor formation in genetically altered mice lacking these subunits has suggested a connection between SWI/SNF chromatin remodeling complexes and cancer. A third subunit, SNF5 (also know as INI1 or BAF47), is a core subunit of all human SWI/SNF complexes. SNF5 physically interacts with several tumor-related proteins and is mutated in children with malignant rhabdoid tumors (MRT) (Versteege et al., 1998). Subsequent analysis performed by several groups has documented germline mutations of hSNF5, with deletion or mutation of the remaining wild-type hSNF5 allele occurring in MRT of the kidney and brain. In addition, biallelic hSNF5 mutations have also been observed in sporadic renal rhabdoid tumors and in choroid plexus carcinomas, medulloblastomas, and central primitive neuroectodermal tumors (reviewed in Roberts and Orkin, 2004).

The ability of SNF5 to function as a tumor suppressor has been confirmed in studies utilizing Snf5-deficient mice. Snf5 null mice die at the peri-implantation stage in early embryogenesis. Mice haploinsufficient for *Snf5* undergo normal development, but 5%–35% later present with rhabdoid tumors and other tumor types, many of which are highly aggressive and metastatic (Roberts et al., 2000, Klochendler-Yeivin et al., 2000, Guidi et al., 2001). Where examined, tumorigenesis correlates with loss or inactivation of the remaining wild-type *Snf5* allele. More recently, Orkin and coworkers generated both an inactivating-conditional *Snf5* allele and a reversibly inactivating-conditional *Snf5* allele in mice (Roberts et al., 2002). Inhibition of *Snf5* expression in a variety of adult tissues resulted in bone marrow failure and rapid death, whereas sporadic inactivation of *Snf5* in hematopoietic tissues and in other organs resulted in rapid onset of lymphomas and rhabdoid tumors. Collectively, these mouse studies demonstrate that Snf5 suppresses tumor formation by some undetermined mechanism yet is absolutely required for the survival of nonmalignant cells.

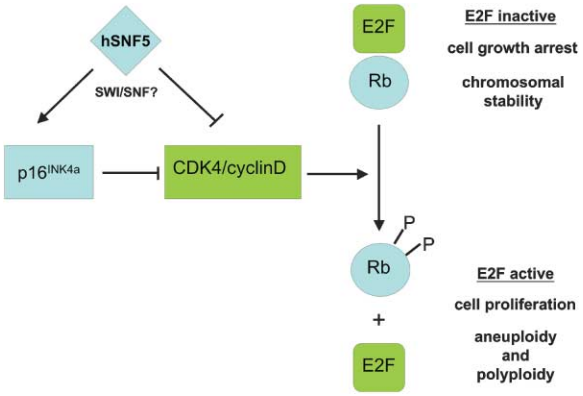

Figure 1. Modulation of the Rb tumor suppressor pathway by hSNF5

By increasing expression of p16 and inhibiting expression of cyclin D1, SNF5 promotes the inactivation of E2F transcription factors by Rb, thereby negatively regulating cell growth and maintaining numerical chromosomal stability. Tumor suppressor proteins are shown in blue.

Insight into the mechanism of Snf5-mediated tumor suppression has been provided by studies performed in cultured MRT cells. Reintroduction of hSNF5 into hSNF5-deficient human MRT-derived cell lines induces flat cell morphology, an accumulation of cells in G1/G0, and, in some cases, cell senescence or apoptosis (Ae et al., 2002, Versteege et al., 2002). These effects appear to be mediated through the retinoblastoma (Rb) tumor suppressor, since addition of Rb function is sufficient to restore G1 arrest to cells lacking Snf5 (Betz et al., 2002). Phosphorylation of Rb by cyclin-dependent kinases promotes entry of the cells into S phase by releasing Rb from E2F transcription factors, and MRT cells transduced with hSNF5 exhibit Rb hypophosphorylation and decreased expression of E2F target genes. Furthermore, chromatin immunoprecipitation experiments have indicated that hSNF5 alters Rb activity by inhibiting the expression of cyclin D1 (Zhang et al., 2002) or by activating the transcription of the $p16^{Ink4a}$ tumor suppressor gene (Oruetxebarria et al., 2004). Increased $p16^{Ink4A}$ (Ink4A = inhibitor of kinase 4A) activity inhibits cell cycle progression into S phase by preventing cyclin D1-CDK4 kinase induced phosphorylation of Rb, leaving the Rb corepressor complexed with E2F transcription factors (Figure 1).

The results suggest that hSNF5 suppresses tumor formation by regulating cell proliferation via the Rb cell cycle checkpoint. However, it is unclear whether the antiproliferative effects of hSNF5 are solely responsible for preventing cancer. A recent paper by the Verrijzer group explores this question further (Vries et al., 2005). In their study, the authors used an inducible promoter system to re-express

into hSNF null MRT cells either wild-type hSNF5 or mutant versions of hSNF5 that contain single amino acid substitutions previously identified in MRT samples. The results indicate that tumor-associated, mutant *hSNF5* genes are capable of reducing cell proliferation, though not as well as wild-type hSNF5, suggesting that failed growth arrest may not fully explain the association of these hSNF5 mutations with MRT. Multilobed nuclei were observed in both hSNF5 null MRT cells and non-MRT cells following induction of the expression of one of the cancer-associated *hSNF5* genes. This altered nuclear morphology was seen routinely in hSNF5 null MRT cells, but never in cells transduced with wild-type hSNF5. This led the authors to test whether expression of the mutant *hSNF5* promotes polyploidization. Using multicolor pq-COBRA-FISH analysis, it was determined that 10% of the hSNF5 null MRT cells were near tetraploid and 90% were numerically diploid, albeit with more than half of the cells bearing multiple chromosomal aberrations. Strikingly, induction of *hSNF5* expression in these cells for 96 hr resulted in a nearly 100% diploid population, whereas induction of a mutant hSNF5 led to centrosome and spindle amplifications and exacerbated the polyploidization and aneuploidization of the cells, suggesting a role for hSNF5 in mitotic checkpoint control. Although several of the tested hSNF5 mutations further increased the chromosomal instability of hSNF5 null MRT cells, not all cancer-associated hSNF5 mutations had this effect, indicating the presence of both gain-of-function and loss-of-function hSNF5 mutations in MRT.

In addition, the authors examined the karyotypes of noncycling and cycling hSNF5-expressing cells and found that the noncycling cells displayed significantly more chromosomal gains and losses that the mitotic cells, suggesting that the accumulation of diploid MRT cells following reintroduction of hSNF5 is due to the arrest or senescence of aneuploid cells in the population. Whole-genome expression profiling revealed changes to many E2F targets, including several genes known to be involved in mitotic control and regulation of ploidy. To test if the p16Ink4a-CDK4/cyclinD-Rb-E2F pathway was also involved in hSNF5-mediated regulation of ploidy, the authors expressed a p16Ink4a-insensitive CDK4 mutant in MRT cells and determined that the high level of aneuploidy and polyploidy in these transduced cells could not be reversed by induction of *hSNF5* expression.

The results of this study define a new critical function for hSNF5, thereby expanding previous models of hSNF5-mediated tumor suppression. The authors propose that loss of hSNF5 in normal cells results in reduced p16Ink4a levels and increased phosphorylation of Rb (by CDK4/cyclin D), leading to an increase in E2F levels (Figure 1). Intriguingly, loss of hSNF5 correlates with upregulation of E2F target genes that have been linked to increased cell proliferation and to mitotic defects and aneuploidy. The ability of hSNF5 to regulate cell proliferation and chromosomal ploidy in MRT cells indicates that loss of hSNF5 in cells can induce both the growth advantage and the chromosomal instability needed for tumor induction and progression. This work and previous studies reveal a convergence of the hSNF5 and Rb tumor suppressor pathways. What other mutations must occur in order for primary cells undergoing loss of SNF5 to retain viability and become cancerous remains to be determined. Furthermore, it is unclear if this is the only mechanistic pathway utilized by hSNF5 to regulate neoplasia. As part of the SWI/SNF chromatin remodeling complex, hSNF5 presumably assists in regulating the expression of many genes, and the absolute requirement of hSnf5 for cell viability and during early embryogenesis indicates that hSNF5 is likely playing multiple roles in regulating normal cell growth. Finally, it should be noted that there is scant evidence that hSNF5 is regulating tumorigenesis solely via the SWI/SNF complex. Indeed, recent evidence suggests that SWI/SNF complexes can function in the absence of hSNF5, suggesting that hSNF5-mediated suppression of MRT may not involve SWI/SNF (Doan et al., 2004). Clearly, more work will be needed to elucidate all of the functional roles of hSNF5 in tumor suppression.

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