【招待講演】

Tumor Suppressor Gene Mutations in Mice

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The advent of gene targeting technology in mouse embryonic stem (ES) cells has made it possible to introduce specific mutations into the murine germline. Over the past several years we have used this technology to mutate the murine homologues of a series of human genes implicated in tumor development, specifically the tumor suppressor genes *Rb*, *p53*, *Nf1* and *Nf2*. These mutant animals have been useful as models for certain human familial cancer syndromes caused by the inheritance of a single mutant allele of a given tumor suppressor gene, as a means to address the role for these genes in normal development, and as a source of primary cells and cell lines with which to examine tumor suppressor gene function *in vitro*. Recently, we have also developed a novel mouse strain carrying a targeted mutation in the *K-ras* proto-oncogene, which causes predisposition to tumors of the lung and other sites. This lecture will summarize our progress in the study of these mutant mouse strains, with a particular emphasis on their value as animal models of human tumor development.

In humans, inheritance of a defective allele of a tumor suppressor gene strongly predisposes to tumor development. Similarly, mice heterozygous for a mutation in *Rb*, *p53*, *Nf1* or *Nf2* are cancer prone. Importantly, however, although these animals exhibit increased cancer risk, the tumor types that they develop typically differ from those seen in the cognate human familial cancer syndrome. For example, while *Rb*+/- humans develop retinoblastoma, *Rb*+/- mice never develop this tumors; rather, these mice succumb to tumors of the intermediate lobe of the pituitary. Likewise, *Nf1*+/- mice do not develop the common symptoms of neurofibromatosis type 1, and instead develop myeloid

leukemia and pheochromocytoma. There are two general models to account for the differences in tumor spectra between tumor suppressor gene mutant humans and mice. First, the rate of loss of the wild-type allele of the tumor suppressor gene might differ in a tissue-specific manner between the two species. Alternatively, the effect of loss of function of the gene might differ between the two species, particularly due to the existence of overlapping or compensatory growth control pathways in a given tissue of one species. We have evidence in support of both of these models. For example, although *Nf1*+/- mice do not develop neurofibromas, chimeric mice composed in part of *Nf1*-/- cells do. Thus, in *Nf1*+/- mice, the loss of the wild-type *Nf1* allele in the appropriate cell type is likely to be rate limiting for tumor development. In the case of *Rb* mutation and retinoblastoma, we have discovered that the activity of the *Rb*-related protein *p107* influences the retinal phenotype caused by an *Rb* mutation. Specifically, while *Rb*+/- fail to develop any retinal pathology, animals with the genotype *Rb*+/-; *p107*-/- exhibit bi-lateral, multifocal retinal lesions that have been classified as dysplasia. Our data suggests that the presence of *p107* function in the developing retina of the mouse can in some way “compensate” for the loss of *Rb*. Additional results from our laboratory indicate that the *Rb* gene family (including *Rb*, *p107* and *p130*) have overlapping function in other contexts as well.

The continued development of histopathologically accurate animal models of human cancer is critical for establishing experimental systems for probing the mechanisms of tumor development as well as for testing therapeutic strategies that are targeted to specific organ

sites. This notwithstanding, any and all phenotypes caused by tumor suppressor gene mutations in mice can serve as a basis for understanding gene function and as a means to test therapeutic strategies that are directed toward the gene product or the pathway(s) in which it participates. Therefore, we have continued to study tumor phenotypes without consideration of the related human condition. For example, we are currently characterizing the effects of combined mutations in several tumor suppressor genes (e.g., *Nf1/p53* and *Nf2/p53*). These double mutant animals develop tumors (largely sarcomas) much more rapidly than the relevant single mutants, indicating a degree of cooperativity in the tumorigenic effects of these mutations. Moreover, because of the short latency of tumor development in these strains, they should be very useful for therapeutic evaluation.

Finally, we have utilized gene targeting technology to create a novel mouse strain carrying a “latent” oncogenic allele of the *K-ras* gene. Due to the structure of the mutation, the expression of the activated *K-ras* oncogene is prevented until the cell undergoes an intrachromosomal recombination event. This event is predicted to occur in sporadically and, at some frequency, in all tissues of the mouse, which should promote tumorigenesis broadly. Indeed, mice heterozygous for this mutation are highly cancer prone and develop a variety of tumor types. Molecular analysis of tumor DNA has confirmed that the expected recombination event occurred at some point during tumor development. We anticipate that this strain will be useful in screening anti-tumor agents directed against Ras. In addition, by crossing this strain to the tumor suppressor mutant strains, we may be able to improve certain tumor models.