

**A GENETIC SCREEN FOR TUMOR SUPPRESSOR GENES IN THE MOUSE**

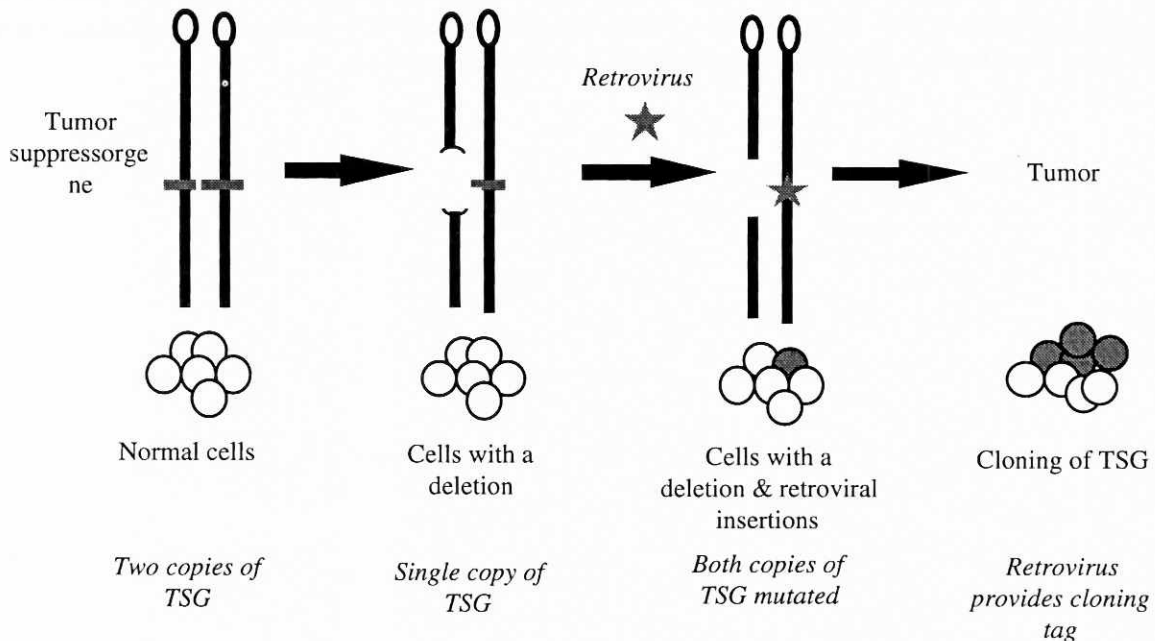
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The array of different cell types which constitute the adult organism exhibit very different capacities of self renewal. During embryonic development controlled growth of the multiple lineages which emerge is critical to the developing organism to ensure that morphogenesis is not disturbed. The growth potential of each cell is determined by signals which are a consequence of its lineage differentiation program (cell autonomous), are determined by its immediate cellular environment (cell-cell communication) and are responses to physiological signals emanating from other tissues in the organism. Any cell which loses its ability to respond to these signals may re-enter the cycle or cycle at an abnormally high rate, ultimately giving rise to a tumor. In the organism negative growth control signals will have a variety of specific consequences on a cell, such as cycle arrest, terminal differentiation or programmed death. The specific pathway will depend on the lineage and the signal received.

Tumors are a manifestation of genetic change(s) causing a loss of growth control. Many genes are known to alter growth control when mutated and these genes act at a variety of different points in the signal transduction cascade. One class of genetic alterations identified in tumors are loss of function mutations which affect both alleles of a gene because the mutations are recessive the genes affected by these mutations are known as negative oncogenes or tumor suppressor genes. It is the lack of these specific gene products which is associated with the loss of growth control. Like the dominant oncogenes, the protein products of tumor suppressor genes are functionally diverse. Because tumor suppressor genes are defined by *loss of function* assays the identification and cloning of these genes is difficult and has mostly relied on *dominantly* inherited familial cancer predisposition syndromes and positional cloning.

The cloning of the genes associated with familial cancer syndromes is only the initiation point for revealing their function. The recent availability of techniques to generate loss of function mutations in mice (Bradley *et al.*, 1992) has allowed the role of tumor suppressor genes to be evaluated during embryonic development (Matzuk and Bradley, 1994). The consequence to a mouse embryo of removing a specific gene product during development varies widely and clearly depends upon the function(s) of the gene product in any particular tissue and compensatory mechanisms that might exist. In the case of tumor suppressor genes identified through familial human genetics it is likely that compensatory mechanisms do not exist in those specific cell types that are susceptible to neoplastic growth.



We and others have described mouse strains with mutations in tumor suppressor genes generated through the application of gene targeting techniques in ES cells (Donehower *et al.*, 1992; Lee *et al.*, 1992; Matzuk *et al.*, 1992; Sands *et al.*, 1995). The unifying characteristics of these animals is the susceptibility of the heterozygous and/or homozygous mutants to neoplastic disease. Most of the tumor suppressor genes which have been mutated in mice were initially isolated by positional cloning approaches starting from human familial cancer pedigrees. To a great extent these mice have been good phenocopies of the equivalent human disease, although in some cases the tumor predisposition is quite distinct in the different species. This has demonstrated that many of the tumor suppressor genes identified in humans also serve the same physiological process in the mouse. Thus mice provide a sensitive *in vivo* assay system for tumor suppressor gene function. The generation of knockout mice has uncovered the tumor suppressor function of some genes which were not previously believed to have such a function (Matzuk and Bradley, 1994).

In addition to inherited mutations, important genetic lesions are also acquired somatically and clonally selected during tumor growth. As the availability, chromosomal density and informativeness of markers has improved it has become possible to examine large portions of the genome for loss of heterozygosity (LOH) by comparing matched samples of DNA from normal tissues and the tumor. There are many regions of the human genome which are observed to exhibit LOH in sporadic human tumors. These regions are hypothesized to contain tumor suppressor genes, but the genetic intervals are usually so large that gene identification cannot be realistically tackled by positional cloning.

We have established that mice with mutations in tumor suppressor genes are susceptible to tumors, thus mice of this type are both a relevant and a potent assay system for this class of gene. To utilize heterozygous mice in a genetic screen for tumor suppressor genes we have developed a novel technology which allows us to screen 200-300 genes at a time. This is accomplished by constructing very large deletions (up to 3cM) at specific locations in the mouse genome using loxP/Cre induced recombination combined with a powerful positive selection system for these rare recombination events (Ramírez-Solis *et al.*, 1995). By constructing mice with segmental haploidy that correspond to specific regions which are syntenic with regions which exhibit LOH in human tumors we can directly test for the existence of tumor suppressor genes in the relevant interval. We are developing insertional mutagenesis strategies to identify tumor suppressor genes in these large genetic intervals.

## References

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