Commentary

Comparative Hepatocellular Cancer Genetics

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The use of rodents, especially mice, in cancer studies has been instrumental in establishing some key tenets of modern oncology. One example is the concept of multi-stage tumorigenesis. The two-stage skin carcinogenesis model and the preweanling liver tumor induction model were both instrumental in demonstrating the concepts of initiation, promotion, and progression.^{1,2} This was achieved by the judicious use of carcinogenic agents and tumor promoters and by observing their effects on tumor induction kinetics and malignant progression. In both systems, treatment with a single dose of a carcinogen results in tumor initiation. This is an irreversible, nearly instantaneous event, now known to be synchronous with a mutation in key cellular genes. The initiated cell, ie, one bearing a mutated oncogene such as *ras*, will clonally proliferate into a tumor if provided with a prolonged and persistent promoting stimulus. This tumor promotion phase can be reversible but may also involve genetic alterations. These early lesions, papillomas in skin and altered hepatic foci in the liver, are benign and their growth rate is a measure of tumor-promoting effectiveness. Following a variable latency, a percentage of these benign tumors progress to malignancy, forming squamous cell carcinoma and hepatocellular carcinoma respectively.

In addition to providing this insight into the natural history of cancer development, the mouse has been useful in understanding the genetic basis of cancer. There are at least two reasons to pursue the study of cancer genetics using mice. The first, using known or suspected cancer-related genes, is to learn more about the function of the gene product in the context of the natural history of tumor development through studies which are not possible in other systems, including the human. The second goal is to identify novel cancer-related genes using the unique attributes of mouse genetic analysis. It is the hope of many mouse cancer geneticists that genes so identified will turn out to be involved in human cancer.

Two simple principles inform these two approaches. One is the conservation of gene function through evolution. For example, *ras* and *p53* are very frequently implicated in both human and murine cancers, and presumably these proteins have similar functions in either

setting. The second is the conservation of linkage groups, or gene order along the chromosomes, through evolution. Thus the genome of the mouse and man share large regions of gene order, termed syntenic regions, greatly facilitating comparative genetic analysis.³ For example, a suspect gene may be identified through studies of linkage or loss of heterozygosity (LOH) or from chromosomal aberrations such as breakpoints or amplifications. If this gene lies within one of the conserved syntenic regions, it is simple and often informative to extrapolate between species.^{4,5}

Studying *ras* and *p53* in mouse models of cancer has provided a rich source of insight into their function. To cite just one example, genetic analysis of the two-stage skin carcinogenesis model has revealed that consistent genetic events occur at particular stages. Thus, mutation of the proto-oncogene *H-ras* can be an initiating event and the earliest tumors examined frequently contain mutant *ras*.⁴ In addition, mutation of the tumor suppressor *p53* is observed in carcinomas but not in precursor papillomas, indicating that inactivation of *p53* is important during malignant progression.⁵ Functional analysis of identified cancer-associated genes is also greatly aided by the use of transgenic or knockout mouse models. Studies such as these have greatly aided the functional analysis of the gene products and helped to reconstruct a genetic history of tumor development. The ideal mouse model would have similar genes mutated at similar stages of tumor development within the same tissue as occurs in human neoplasia. So far, the search for this ideal has met with success in limited settings.⁶

The study of liver cancer in mice, although providing valuable insight into the natural history of tumor development, has thus far not provided a close genetic model of human liver cancer. In mouse liver tumors, for example, mutation of *ras* is frequently observed even in the earliest lesions and is thought to be an initiating event.² However, human hepatocellular carcinoma rarely contains mutated

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ras on-cogenes. Conversely, mutation of p53 is observed frequently in human liver cancer, but rarely in mouse hepatic tumors.² Other frequent genetic events associated with malignant progression in both human and mouse liver cancer have been difficult to identify, although progress is being made.^{2,7} To accelerate this effort, new approaches and breakthroughs will be required.

A second major goal of murine cancer genetics, the identification of novel cancer genes, has met with varied success. Virally induced tumors in mice as well as other species have been useful for identifying cancer-related genes. In this approach, tumors are induced by viral infection, usually because the virus integrates adjacent to or within a cancer-causing gene, greatly facilitating the identification and cloning of the gene. This approach has met with great success in finding novel cancer genes, many of which have been shown subsequently to be involved in human cancer.⁸ However, up to now this approach has been fairly tissue-restricted, not applicable to skin or liver, for example, due to limits of viral infectivity. Recent developments in which transgenic mice were engineered to express viral receptors in specific tissues, thereby making them permissive for infection, may help to overcome this limitation.⁹

What other approaches are there to identifying novel cancer-associated genes and which approaches will help unravel the more genetically intractable cancers such as liver cancer? Variation in tumor susceptibility between inbred mouse strains provides a rich source of genetic variation with which to investigate the genetic basis of tumorigenesis. Susceptibility to liver tumor induction can vary more than 50-fold between inbred strains.¹⁰ Through backcrossing and classic linkage analysis, several loci that confer susceptibility or resistance to liver tumor formation have been accurately mapped.^{11,12} Progress is being made toward identification of these genes but to date none has been cloned. The primary difficulty is that phenotypic classification is ambiguous because tumor susceptibility is a quantitative trait. As the mouse genome project progresses and phenotyping becomes more refined, the pioneers who first mapped these genes should attain their goal of gene identification. An obvious question is whether the human orthologs of these genes will also contribute to human liver cancer.

A third approach to gene identification is genetic analysis of the tumors themselves. If tumors are derived from F₁ hybrid mice, polymorphic markers can be used to identify chromosomal regions or genes that have undergone LOH. These regions of LOH are thought to contain tumor suppressor genes and this approach has met with success in some human cancers and certain murine tumors. However, LOH is infrequently observed in murine liver tumors.² One reason for this may be that hepatocytes are frequently tetraploid or binucleate, which makes LOH much less likely to occur and/or harder to detect. Other mechanisms such as imprinting⁷ could also operate to inactivate tumor suppressor genes in liver tumor development.

A fourth approach, especially in hematopoietic tumors, is direct karyotyping of tumor cells. In both humans and mice this has helped to localize, and has contributed to cloning of, tumor suppressors associated with deletions or oncogenes associated with translocations. This approach has met with less success in solid tumors, due largely to the difficulty of obtaining good metaphase spreads for karyotypic analysis.

Sargent et al have overcome technical hurdles and obtained karyotypes of murine hepatocellular carcinomas.¹³ The tumors they analyzed arose in transgenic mice that overexpress both c-myc and transforming growth factor α in the liver. This model provides abundant material to analyze in a short time period and has been well characterized at the phenotypic level. Although the etiology of murine hepatocellular carcinoma is vastly different from that of human liver cancer, the results obtained by Sargent et al suggest that subsequent genetic alterations may be similar to those that occur in the human. They obtained metaphase spreads from normal and tumor-derived hepatocytes by short-term cultures obtained from collagenase-perfused tumor-bearing livers. To identify chromosomal aberrations in the tumor cells, they used a combination of fluorescence *in situ* hybridization with whole chromosome probes, single copy genes, and G-banded chromosomes. Consistent, non-random chromosomal aberrations in several chromosomal locations were observed in tumor cells, including translocations and deletions with consistent breakpoints and partial and complete loss of chromosomes. Also, some of the alterations were observed before the appearance of distinct neoplastic lesions; others were observed only in frank carcinomas. Thus it is likely that these alterations represent a sequence of genetic events that will help to reconstruct the genetic history of liver cancer. Finally, many of the murine chromosomal regions that display alterations are syntenic to human chromosomal loci that show LOH in human liver and other tumors. It remains to be shown which genes are relevant at these loci and whether the same genes are involved in both species; nonetheless, these results provide the groundwork for this endeavor.

It is worth pointing out that these alterations were not or could not be detected by standard LOH analysis. Conversely, LOH analysis can identify alterations not detectable by other techniques. Linkage and mapping studies identify yet another class of genes. In the future, comparative genome hybridization and other newly developing techniques will also contribute to the search for relevant cancer-associated genes; no single approach will suffice to identify all of them. A many-pronged approach will be required to achieve the ultimate aim of a complete genetic portrait of murine liver cancer to complement the known phenotypic picture. All of these efforts will be vindicated if similar genes are altered at similar stages in both humans and mice, providing a more robust model with which to test hypotheses about cancer etiology, the genetics and biology of tumor progression, and cancer intervention strategies. To this end the mouse holds great expectations.

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