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Genetic Changes during Mouse Skin Tumorigenesis

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This paper describes specific genetic changes involving chromosome 7 in mouse skin tumors, the most important consequence of which appears to be an alteration in the allelic balance of normal and mutant H-*ras* genes. The use of restriction-fragment-length polymorphisms in F_1 hybrid mice demonstrates that trisomy of chromosome 7 is an early event preceding papilloma formation, and further events, such as mitotic recombination, seem to occur during progression to malignant carcinomas. There is some evidence of a tumor-suppressor locus situated on chromosome 7.

H-*ras* Mutations in the Mouse Skin System

The mouse skin tumorigenesis system provides a useful animal model for multistage carcinogenesis. Initial treatment of the dorsal skin with a subcarcinogenic dose of a carcinogen, such as dimethylbenzanthracene (DMBA), induces a population of initiated cells. Subsequent treatment with a noncarcinogenic tumor-promoting agent, such as 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA), produces benign papillomas within 3 months. A proportion of these lesions spontaneously progress to give malignant carcinomas. This system therefore possesses at least three well-defined stages: initiation, promotion, and progression.

Mutations of the proto-oncogene H-*ras* have been found in both papillomas and carcinomas. The type of mutations found correspond closely with the known mutational specificity of the carcinogens used to initiate the skin tumors (*1*). Thus, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG)-induced tumors have G:C to A:T transition mutations, correlating with the mispairing properties of the major premutagenic lesion of MNNG, O^6-methylguanine. Likewise, DMBA induces a high proportion of adenine adducts (*2*) and gives rise to tumors with A:T transversions. Since the mice receive only a single initiating dose of carcinogen, it would appear that mutations in H-*ras* represent one kind of initiating event in mouse skin tumorigenesis. This conclusion is supported by the observation that Harvey murine sarcoma virus (HaMSV), which carries a mutationally activated H-*ras* gene, can substitute for the initiating carcinogen treatment in this system (*3*).

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However, not all mouse skin tumors carry mutationally activated H-*ras* genes, or, it would appear, any alternative dominantly acting transforming genes. Other pathways of tumor initiation must therefore exist in this system.

H-*ras* Allele Imbalance in Mouse Skin Tumors

The A:T to T:A transversion commonly found in DMBA-induced tumors introduces a novel *Xba* I site into the H-*ras* gene. Thus, on Southern transfers, it is possible to distinguish bands corresponding to mutant alleles from bands corresponding to normal alleles (*4*). By comparing the densities of the relative bands, it is observed that an allelic imbalance in favor of mutant alleles is common in carcinomas (*4*). This has recently also been shown to be the case for papillomas (P. Burns, R. Bremner, and A. Balmain, manuscript in preparation). The imbalance appears to represent some form of duplication of the mutant allele. In addition, a proportion of carcinomas also display homozygosity for the mutant allele.

Thus, it would appear that in addition to activating H-*ras* mutations, some form of gross chromosomal alteration of chromosome 7 is also a consistent, early event in mouse skin tumorigenesis.

Use of F_1 Hybrids to Characterize Chromosome 7 Abnormalities

It is difficult to study the nature of allelic imbalances or the loss of heterozygosity in inbred strains of laboratory mice because of the general lack of useful restriction-fragment-length polymorphisms (RFLPs). However, by generating F_1 hybrids between strains of mice carrying useful allele-specific markers on chro-

mosome 7 and using these animals for generating skin tumors, it has been possible to analyze the nature of the genetic events involved in the generation of allelic imbalance and homozygosity (Fig. 1). By looking at the behavior of RFLPs at loci proximal (*fes* and *Hbb*) and distal (*int*-2) to H-*ras* on chromosome 7, it has been possible to show that nondisjunction events, leading to trisomy, and mitotic recombination, leading to loss of heterozygosity, are mainly responsible for generating H-*ras* allelic imbalances (*5*). Trisomy of chromosome 7 in papillomas has also been observed using cytogenetic analysis of DMBA-induced papillomas (*6*).

It is interesting to note, however, that those tumors that do not have mutationally activated H-*ras* genes, e.g., the majority of MNNG-induced tumors, do not display abnormalities of chromosome 7 (Table 1).

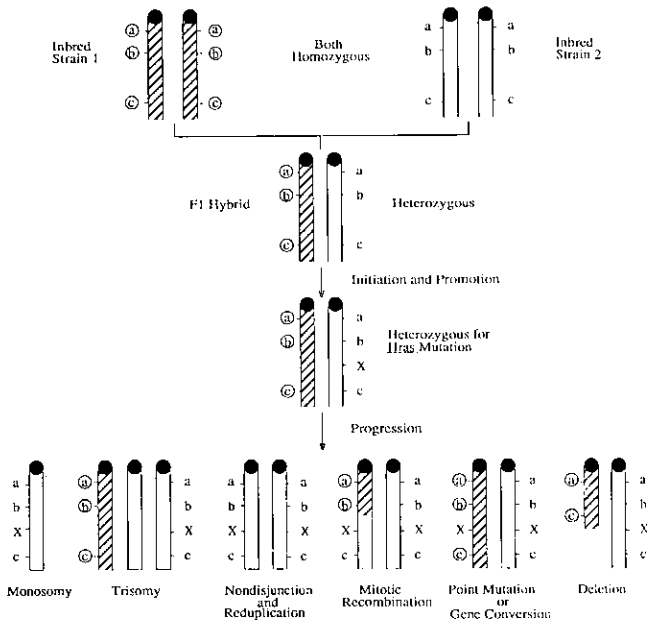

FIGURE 1. Use of F_1 hybrids to characterize chromosomal alterations.

Is Normal H-*ras* a Tumor-Suppressor Gene?

The observations concerning the generation of homozygosity or allelic imbalance on chromosome 7 have at least three possible explanations: *a*) the presence of normal H-*ras* alleles may suppress the effect of mutant alleles such that there may be a selective advantage, with respect to tumorigenicity, in losing a normal allele or duplicating a mutant allele; *b*) a simple increase in the number of mutant alleles, and concomittant increase in the expression of mutant p21 protein, may confer a selective growth advantage; and *c*) the loss of H-*ras* alleles may be coincidental with the loss of linked tumor-suppressor genes (the observed trisomy of chromosome 7 may represent the loss of a chromosome from a tetraploid cell).

Suppression by normal *ras* alleles could occur through competition with mutant alleles for either transcription factors at the DNA level or for effector molecules at the protein level. There is much conflicting evidence concerning this possibility. A recently discovered tumor-suppressor gene, K-*rev* (*7*), has strong sequence homology to *ras* and is able to induce reversion of transformed cells expressing mutant K-*ras*, perhaps through competition for cellular factors. Loss or underrepresentation of normal *ras* alleles has been observed in a wide variety of tumors (*8–10*), although this may simply be the consequence of chromosomal changes that increase the copy number of mutant *ras* alleles. Marshall and colleagues (*11*) have reintroduced normal N-Ras p21 protein into HT 1080 cells carrying mutant N-*ras* genes and failed to observe reversion. However, the parental HT1080 cells already express high levels of normal N-Ras p21 and may have adopted an alternative route to escape tumor suppression. Other studies have demonstrated the ability of normal *ras* genes to act in an oncogenic (*12*) or anti-oncogenic fashion (*13*).

It is unlikely that the level of expression of mutant *ras* is the sole or main determinant of tumorigenicity. Some tumorigenic cell lines express low levels of mutant *ras* together with high levels of normal *ras*, whereas other cell lines express high levels of mutant *ras* without becoming tumorigenic (*11*; Quintanilla et al., manuscript in preparation).

Table 1. Correlation between H-*ras* mutations and chromosome 7 changes.

Induction protocol[a]		Number of carcinomas analyzed	H-*ras* mutation/ chromosome 7 changes			
Initiation	Promotion		+/+	+/−	−/−	−/+
DMBA	TPA	19	19	0	0	0
DMBA	DMBA/TPA	1	1	0	0	0
MNNG	TPA	4	2	0	2	0
MNNG	MNNG	5	0	2	3	0
DMBA	DMBA	8	4	0	4	0

[a]DMBA, dimethylbenzanthracene; TPA, 12-*O*-tetradecanoyl-phorbol-13-acetate; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

A Tumor Suppressor on Chromosome 7

A good reason for looking for chromosomal changes involving mouse chromosome 7 is that regions of this chromosome are syntenic with regions on the short arm of human chromosome 11 (*14*). These regions appear to contain a number of potential tumor-suppressor loci, including the Wilm's tumor locus and a gene that predisposes for Wiedemann-Beckwith syndrome (*14*). In addition, loss of alleles around 11p15 is a common feature of a variety of human tumor types (*15–17*). Loss of heterozygosity at loci distal to *Hbb* on chromosome 7 was seen in four tumors in these studies. The H-*ras* locus was involved in three of these cases, and the alterations may therefore have served primarily to increase the copy number of mutant H-*ras*. However, in one case, the loss of heterozygosity only involved a marker distal to H-*ras*, supporting the proposal that a tumor-suppressor gene may be located on chromosome 7 in the mouse.

Conclusions

In the mouse skin tumorigenesis system, gross alterations of chromosome 7 leading to trisomy and loss of heterozygosity are a consistent feature of those tumors carrying mutationally activated H-*ras* alleles (Fig. 2). The observation that most benign papillomas carrying mutant H-*ras* genes are trisomic for chromosome 7 suggests that nondisjunction leading to trisomy is a relatively early event occurring during promotion. The tumor-promoting agent that is used in this laboratory, TPA, has been shown to induce aneuploidy in mouse epidermal cell lines (*18*) and may therefore be responsible for these changes *in vivo*. The nature of the selective advantage that these gross chromosomal changes confer on tumor cells is not known, nor is it known whether an increased copy number of mutant H-*ras* or a relative decrease in the copy number of normal H-*ras* is responsible. In addition to changes involving the gene dosage of H-*ras* allele, there is also some evidence from loss of heterozygosity studies for the presence of a tumor-suppressor locus distal to H-*ras*.

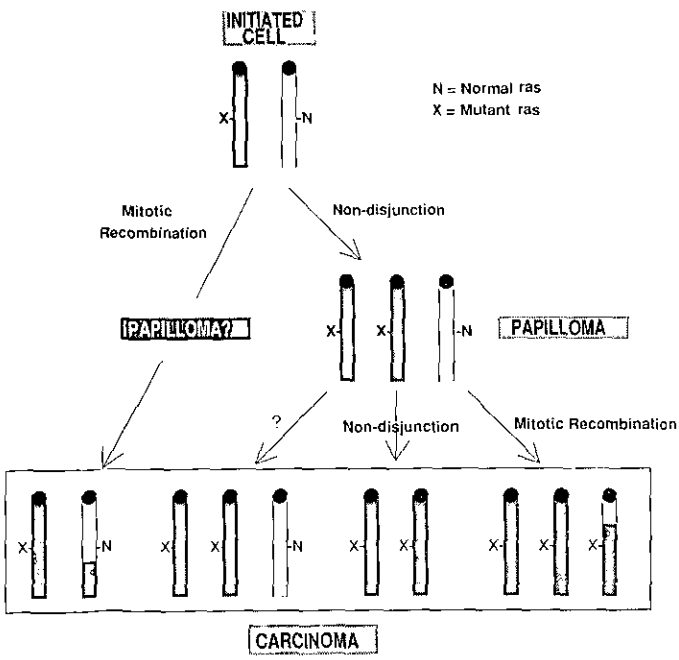

FIGURE 2. Chromosome 7 changes occurring during mouse skin tumorigenesis.

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