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Predisposition to Renal Cell Carcinoma Due to Alteration of a Cancer Susceptibility Gene

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A single germ line gene mutation at a tumor susceptibility locus in a rodent model of hereditary human renal cancer caused a 70-fold increase in susceptibility to chemical carcinogenesis. A carcinogen that targeted both renal epithelial and mesenchymal cells caused an increase in tumors of epithelial origin in susceptible animals; the number of carcinogen-induced mesenchymal tumors was unaffected by the presence of the mutation at the susceptibility locus. Thus, this mutation defines a genetic locus for susceptibility to carcinogen-induced tumors and modulation of carcinogen susceptibility by this locus exhibits cell-type specificity.

THE DEVELOPMENT OF GENETIC markers to identify individuals predisposed to tumor development after occupational or environmental exposure to potential carcinogens will require an understanding of how specific genes determine susceptibility for the induction of cancer by chemical carcinogens. Knowledge of the proportion of susceptible individuals in the population and the relative cancer susceptibility of normal and predisposed groups will make it possible to estimate human risk from carcinogen exposure. In addition, it may be possible to limit the exposure of these susceptible individuals to potential carcinogens.

Tumor suppressor genes represent one class of cancer susceptibility genes in humans (*1*). Inheritance of a mutation in one allele of a tumor suppressor gene predisposes individuals to develop tumors after sustaining an additional spontaneous mutation in the remaining normal allele of that gene (*2*). It follows from this work that inheritance of a mutation in a susceptibility gene would also predispose to the induction of tumors by chemical carcinogens.

In human renal cell carcinoma (RCC), loss of heterozygosity of chromosome 3 occurs frequently (*3*), and inheritance of an alteration at this putative tumor suppressor locus in von Hippel–Lindau disease predisposes to the development of RCC (*4*). In rats, a single gene mutation [described by Eker and Mossige (*5*)] predisposes to multiple bilateral RCCs with an autosomal dominant pattern of inheritance, and animals carrying the Eker mutation serve as a model for hereditary RCC (*6*). Rats that are heterozygous for the gene defect develop spontaneous RCCs between 4 and 12 months of age (*7*), whereas rats that are homozygous for the wild-type allele rarely develop spontaneous RCC (<0.5%) (*8*). When homozygous, the mutation is lethal prenatally at 9 to 10 days of gestation (*7, 9*).

The hereditary tumors that develop in the Eker rat model have many similarities to their human counterparts: they have similar histology, are bilateral, overexpress transforming growth factor (TGF-α), and do not exhibit a high frequency of *ras* oncogene activation (*10*). Loss of sequences on rat chromosomes 4 (q11 through qter), 5 (monosomy), and 6 (q24) occur in these tumors and tumor-derived cell lines, suggesting that the location of the susceptibility gene may reside on one of these chromosomes (*11*). Animals carrying the Eker mutation develop hemangiosarcomas in the spleen (males and females) and uterine leiomyosarcomas as second primary tumors later in life (*12*). Vascular neoplasms (hemangioblastomas) and second primary tumors are also associated with RCC in human von Hippel–Lindau disease (*13*).

In rats carrying the Eker mutation, it is possible to test for carcinogen susceptibility (as measured by increased kidney tumor multiplicity) and cell type specificity in two distinct cell populations, renal tubular epithelial cells and renal mesenchymal cells. The carcinogen dimethylnitrosamine (DMN) induces both renal cell adenomas and carcinomas (renal cortical tumors = RCT), arising from tubular epithelial cells, and renal mesenchymal tumors (RMT), arising from stromal cells of the kidney (*14*). F1 offspring of heterozygous rats carrying the Eker mutation (*15*) were exposed to a single carcinogenic dose of DMN (30 mg per kilogram of body weight) at 16 weeks of age (*16*). The mutation segregates as a single-locus autosomal dominant; therefore, one-half to two-thirds of the carrier F1 rats would be expected to carry the Eker mutation. At 12 months of age, tumors were quantitated by light microscopy in each kidney of the carrier F1 rats and in kidneys of a control group of wild-type rats exposed under identical conditions (*16*).

Carrier F1 rats exposed to the chemical carcinogen showed a large increase in tumor number (Table 1 and Fig. 1). The number of spontaneous RCTs ranged from 1 to 14 tumors per animal in the unexposed carrier F1 males. In the DMN-exposed carrier F1 males, more than half had >16 tumors per animal, with one DMN-exposed animal having 90 RCTs. In wild-type males, the same dose of DMN was marginally carcinogenic (Table 1 and Fig. 1). Thus, after the background incidence of spontaneous tumors was subtracted (male F1 offspring of gene carriers developed an average of 2.8 RCTs per animal), a 70-fold increase in tumor susceptibility (23 versus 0.33) could be attributed to the presence of the Eker mutation in the carrier F1 animals (*17*). The combined effect of the mutation and carcinogen exposure was a three to four orders of magnitude increase in tumors relative to the spontaneous tumor frequency in wild-type rats (25.6 versus 0.005).

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In female rats, DMN induced fewer RCTs than in the males. In untreated carrier F1 females, fewer spontaneous tumors developed than in carrier F1 males (Table 1), but exposure to DMN increased tumor multiplicity as was observed in the males. Carrier F1 females exposed to DMN exhibited a 26-fold increase in tumor number relative to DMN-exposed, wild-type females (Table 1). However, there were more preneoplastic lesions (*18*) in DMN-exposed, carrier F1 females than in DMN-exposed, wild-type females (14 versus 0.4 lesions per animal, respectively), suggesting that quantitation of tumors at 12 months of age may have underestimated tumor development in female rats.

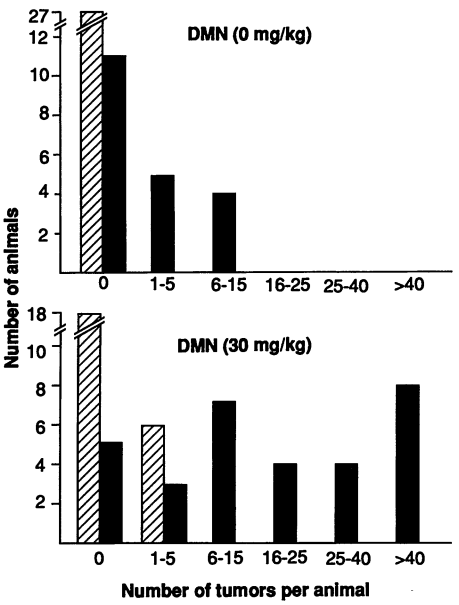

Fig. 1. Distribution of RCTs in male rats. The number of tumors per animal is shown for carrier F1 and wild-type animals exposed to either DMN or vehicle alone. Filled bars, carrier F1; hatched bars, wild type.

The effects of the germ line susceptibility mutation were cell type–specific, predisposing to tumor development in epithelial but not mesenchymal cells of the kidney, even in conjunction with a potent carcinogen challenge. Neither carrier F1 nor wild-type animals developed spontaneous RMT, and the induction of RMT by DMN showed no differences between carrier F1 and wild-type groups (Table 1).

In females, the Eker mutation predisposes to spontaneous second primary tumors of mesenchymal origin, leiomyosarcomas, in addition to RCTs. Therefore, it is clear that this susceptibility gene has the potential, at least in females, to influence the development of a tumor of mesenchymal origin. Female rats in the carrier F1 group could be definitively identified as heterozygotes, based on the presence of uterine leiomyosarcomas, or wild-type, based on the absence of RCTs (*12*). No differences in the number of RMTs between heterozygotes and wild-type females in the carrier F1 group were observed. The average number of RMTs per animal was 0.80 (n = 10) and 0.82 (n = 11) for heterozygotes and wild-type animals, respectively. Thus, although the stromal cells of the kidneys of the carrier animals contained the germ line Eker mutation and received a carcinogenic dose of DMN, the mutation appeared to have no effect on susceptibility to carcinogen-induced tumors in the renal mesenchymal cells.

Although inheritance of the germ line mutation predisposed to tumor development, the presence of the mutation alone was not sufficient to induce tumors. At least one additional somatic event was required for development of both spontaneous and carcinogen-induced tumors, as evidenced by the observation of focal tumors in a population of phenotypically normal kidney cells. Whether the carcinogen-induced alterations were the same or different from those that occurred in the spontaneous tumors and whether the normal allele of the Eker susceptibility gene was altered in either type of tumor remain to be determined.

Genetic determinants of susceptibility to spontaneous tumors may predispose to carcinogen-induced tumors in humans as well. Studies by the Late Effects Study Group (*19*) of pediatric oncology centers have indicated that survivors of childhood cancers run an increased risk of second malignant neoplasms associated with chemotherapy and radiation therapy after treatment of their primary tumors (*20*).

Tumor development after carcinogen exposure often exhibits tissue specificity. This study indicates that genetic susceptibility for chemical carcinogenesis also can be cell type–specific, even when inherited through the germ line. Future studies of the genetic basis of carcinogen susceptibility will have to take into account both the specific tissue and cell type affected by the carcinogen and genetic restrictions that may exist in various cell types that affect cancer susceptibility.

Table 1. Frequency of RCTs and RMTs in carrier F1 males and females versus wild-type males and females after exposure to DMN.

DMN dose (mg/kg)	Number of exposed animals	Renal cortical tumors			Renal mesenchymal tumors		
		Total number of tumors	Number of tumor-bearing animals	Average number of tumors per animal	Total number of tumors	Number of tumor-bearing animals	Average number of tumors per animal
			Wild-type males				
0	27	0	0	0	0	0	0
30	24	8	6	0.33	33	14	1.4
			Carrier F1 males				
0	20	56	9	2.8	0	0	0
30	31	801	26	25.8	27	15	0.87
			Wild-type females				
0	17	0	0	0	0	0	0
30	27	6	6	0.22	17	15	0.63
			Carrier F1 females				
0	15	24	6	1.6	0	0	0
30	32	165	19	5.2	25	16	0.78

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15. All rats were selectively bred from animals hysterectomy-rederived from animals carrying the Eker mu-

tation on a Long-Evans background and maintained pathogen-free in a barrier facility. Males were diagnosed as carriers at 12 months of age after we performed unilateral nephrectomy to detect tumors histologically in the excised kidney. Heterozygous male rats carrying the Eker mutation from a carrier breeding colony were bred to female rats of Eker stock, undiagnosed as to carrier status. Wild-type animals were bred from second generation tumor-free homozygous wild-type animals from Eker stock, maintained in a wild-type breeding colony.
16. The 16-week-old male and female animals were placed on a protein-free, high-carbohydrate diet for 96 hours before intraperitoneal injection of either saline diluent or 30 mg of DMN per kilogram of body weight dissolved in saline. All rats were observed for 8 months after injection, at which time all rats were euthanized and necropsied. A midsagittal section of each kidney was examined microscopically for quantitation of histological type, size, and number of renal neoplasms. At necropsy we examined all tissues and we noted no metastatic lesions by macroscopic observation or by microscopic examination of the lung and liver of any of the animals.
17. The carrier F1 group included both heterozygotes carrying the mutation and homozygous wild-type animals. Therefore, the impact of the Eker gene mutation on carcinogen susceptibility is in all likelihood an underestimate of the effect of this gene mutation.
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Evidence of a Role for Heterotrimeric GTP-Binding Proteins in Endosome Fusion

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Guanosine triphosphate (GTP)–binding proteins are required for intracellular vesicular transport. Mastoparan is a peptide component of wasp venom that increases nucleotide exchange in some classes of Gα subunits of regulatory heterotrimeric GTP-binding proteins (G proteins). Mastoparan and other compounds that increase nucleotide exchange by G proteins inhibited endosome fusion in vitro and reversed the effects of guanosine 5′-O-(3-thiotriphosphate) (GTP-γ-S), a nonhydrolyzable GTP analog. Addition of βγ subunits of G proteins to the fusion assay antagonized the stimulatory effect of GTP-γ-S, confirming the participation of G proteins. These results indicate that GTP-binding proteins are required for endosome fusion and in particular that a G protein is involved. Given the function of G proteins in signal transduction, these findings may provide insight into the mechanism by which endosomal vesicles become competent for fusion after their formation at the cell surface.

EVIDENCE THAT GTP-BINDING PROTEINS take part in vesicular transport comes from in vitro assays that reconstitute fusion between intracellular compartments and from the analysis of secretion-deficient mutants of yeast (*1, 2*). Non-hydrolyzable GTP analogs (such as GTP-γ-S) inhibit several steps of the secretory pathway reconstituted in vitro (*3*) and stimulate secretion in some preparations (*4*). In vitro endosome fusion can be stimulated or inhibited by GTP-γ-S, depending upon the assay conditions (*5, 6*). GTP-binding proteins behave as molecular switches that rapidly change from an active GTP-bound form to an inactive GDP-bound form. These proteins are regulated by interactions with other proteins that promote nucleotide exchange and GTP hydrolysis (7). Until recently, only monomeric GTP-binding proteins had been implicated in intracellular transport, such as the proteins YPT1, SEC4, ARF, and SAR1, which are required for secretion in yeast (*1, 2*). Moreover, several small GTP-binding proteins of the Rab family are localized on endosomal and secretory vesicles from mammalian cells (*8*). Rab 5 functions in fusion between early endosomes (*9*), and Rab 4 is apparently associated with a population of early endosomes that participate in transferrin receptor recycling (*10*). However, there are some indications that heterotrimeric GTP-binding proteins (G proteins) may also function in intracellular transport. Aluminum fluoride, which can activate G proteins but not monomeric GTP-binding proteins (*11*), can affect transport in the secretory (*12*) and endocytic pathways (*5, 13*). Also, the presence of Gα subunits of G proteins in specific intracellular compartments (*14*) and in rat liver fractions (*15*) suggests a function for G proteins in membrane trafficking. The experiments presented in this paper suggest a role for one or more G proteins in regulating endosome fusion.

G proteins are activated by ligand-stimulated receptors (*16*). Mastoparan is an amphiphilic tetradecapeptide toxin from wasp venom that accelerates nucleotide exchange in some α subunits (preferentially

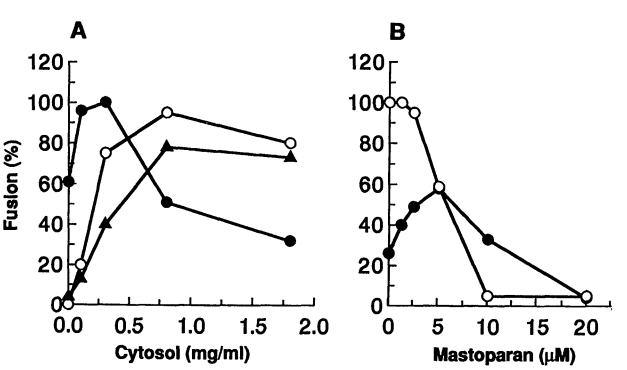

Fig. 1. Regulation of endosome fusion by mastoparan. Endosome fusion was assessed in a cell-free system with mannosylated antibody to dinitrophenol (DNP) and DNP-β-glucuronidase as probes (*27*). (**A**) Endocytic vesicles containing fusion probes were mixed with increasing concentrations of cytosol in the presence of 20 μM GTP-γ-S (●), 20 μM GTP-γ-S and 10 μM mastoparan (▲), or without additions (○). (**B**) Effect of increasing concentrations of mastoparan in the presence of GTP-γ-S (20 μM) at two different cytosolic protein concentrations, 0.1 mg/ml (○) and 2 mg/ml (●). Cytosol was prepared as described (*6*). Protein concentration was determined after gel filtration by the Bradford method (*28*). Incubations were carried out for 45 min at 37°C and the assay was stopped by cooling at 4°C. Values are expressed as percentages of the maximum fusion in the experiment.

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