Genetic Predisposition to Transplacentally Induced Renal Cell Carcinomas in the Eker Rat

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Abstract

N-Ethyl-N-nitrosourea-induced transplacental renal carcinogenesis in the rat results primarily in Wilms' tumors, apparently because primitive nephroblasts are the preferred target. Our question is whether N-ethyl-N-nitrosourea-induced mutations in the fetal kidney would increase the number of adult-type renal cell carcinomas in the Eker rat, which is heterozygous for a mutation that predisposes to renal cell carcinoma. Surprisingly, renal cell tumors but no Wilms' tumors began to appear from as early as 1 week after birth. Thus, the inheritance of a renal cell carcinoma mutation determines the specificity of tumor histology even with in utero carcinogenesis.

Introduction

For embryonal tumors of children such as retinoblastoma and the Wilms' tumor (nephroblastoma), the age-specific incidence peaks in early life, then declines, and falls to such low levels that these tumors are never, or only very rarely, found in adults. The explanation for this phenomenon evidently lies in the observation that these tumors arise from cell types that are normally present in embryonic, fetal, or early postnatal tissue but thereafter characteristically differentiate into postmitotic cells. Thus, the retinoblast is a precursor cell for neural and photoreceptor cells in the retina and the nephroblast for the nephrons. The oncogenic event(s) must occur during the period when target cells are still present and before the postmitotic state is reached (1). It is well known that ENU-induced transplacental renal carcinogenesis in the rat results primarily in Wilms' tumors around 5 months of age and not RCs (adult type), apparently because primitive nephroblasts are the preferred target (2, 3). To examine this specificity of tumor type further, we undertook transplacental carcinogenesis in the Eker rat model of dominantly inherited RC. Our question is whether ENU-induced mutations in the Eker rat would affect the development of RCs even with in utero carcinogenesis.

Hereditary RC in the rat, originally reported by R. Eker in 1954, is an example of a Mendelian dominantly inherited predisposition to a specific cancer in an experimental animal (4). At the histologic level, RCs develop from multiple stages from early preneoplastic lesions (e.g., atypical tubules, which begin to appear around 4 months of age) to adenomas in virtually all heterozygotes by the age of 1 year (5, 6). The homozygous mutant condition is lethal at around 10 days of fetal life (6). Ionizing radiation induces additional tumors with a linear dose-response relationship, suggesting that in heterozygotes two events (one inherited and one somatic) are necessary to produce tumors and that the predisposing gene is a tumor suppressor gene (6). Recently, the predisposing gene of the Eker rat was mapped to the proximal part of chromosome 10 (7, 8). Rat chromosome 10 currently has no syntenic relationship to human chromosome 3p (9), the presumed site of the putative tumor suppressor gene responsible for human RC (10) and the locus of the von Hippel-Lindau disease, which predisposes to multiple RCs (11). The Wilms' tumor gene (WT1) is located on rat chromosome 3 (12). Thus, the Eker rat might involve a novel tumor suppressor gene. As mentioned above, our question is whether a second, chemically induced mutation in the fetal kidney would increase the number of RCs in this experimental system.

Materials and Methods

Animals. Founder rats carrying the Eker mutation were kindly provided by A. G. Knudson. Eker rats were bred on a Long Evans background by brother x sister mating and maintained pathogen-free in the Animal Facility of Cancer Institute since 1991. All animals were housed and treated in accordance with institutional guidelines. Eker rats were diagnosed as carriers by detecting microscopic kidney tumors following unilateral nephrectomy around 6 months of age.

Experimental Procedure. Several normal female rats (+/+; 8 weeks of age), which were mated with male rats carrying the Eker mutation (RC/+; Long Evans strain), received a single i.p. carcinogenic dose of ENU (80 mg/kg of body weight; Nacalai Tesque, Inc., Kyoto, Japan) at the 15th day of gestation. In the rat at this stage, the kidney is metanephric after beginning to form around the 13th day of gestation (13). Pregnancy was confirmed by demonstration of sperm in the vaginal smears. The day when sperm were noticed was designated as gestation day 0. For sequential morphological observation in the kidneys, 10, 10, 11, 15, 12, and 45 offspring were euthanized and necropsied at 0, 1, 2, 3, 8, and 17 weeks of age, respectively. Control rats [+/+ x noncarrier male rats (+/+); total 78 offspring] were exposed to ENU under identical conditions and analyzed in parallel for the different stages of tumorogenesis.

Histology. A mid sagittal section of each kidney was examined microscopically for histology. Tissues were fixed in 10% formalin, and a routine histological examination was carried out on hematoxylin and eosin-stained paraffin sections.

Results and Discussion

The mutation segregates as a single-locus autosomal dominant, and therefore 50% of the offspring would be expected to carry the Eker mutation (RC/+; +/+; 1:1). Actually, among 93 offspring in the experimental group exposed to ENU, 49 (49 of 93; 53%) were found to have multiple renal tumors, and 44 (44 of 93; 47%) were negative, which is not significantly different from the expected 50%. Noncarrier animals exposed to ENU never developed RCs until 17 weeks of age. No nervous system tumors developed by 17 weeks of age in either group of animals.

At birth, no recognizable histological changes were apparent in the kidneys of the experimental group in comparison with the control rats. At 1 week of age, preneoplastic lesions (e.g., atypical tubules) began to appear in the differentiating renal proximal tubules (Fig. 1A). At 2 weeks of age, bilateral cystic lesions (atypical tubules and/or atypical hyperplasia) were macroscopically recognized on the surfaces of the kidneys (Fig. 1B). At 3 weeks of age, adenomas appeared (Fig. 1C).
At 8 weeks of age, RCs developed. At 17 weeks after birth, no macroscopic Wilms' tumors had developed in either group of animals, although microscopically very rare small foci of blastemal cells (early stage of Wilms' tumor) were found equally in both groups (Fig. 1D). No RMTs developed in this experimental system.

Whereas in humans renal development is complete at the time of birth, in rats a substantial amount of development takes place after birth (14–16). S-shaped bodies can still be found after 1 week, and thymidine incorporation does not decrease to background levels until 15–35 days after birth (15). In the human, a fully functional kidney develops during the last stages of embryogenesis. In contrast, terminal differentiation of the majority of the nephrons in the rat kidney is achieved during the first week following birth. This may be the reason why neoplastic lesions start to appear from 1 to 2 weeks.

The number of rate-limiting events is considered to be two for renal tumors in the Eker rat (6). The inheritance of the RC mutation reduces the required number of carcinogenic events, as evidenced by the present findings of renal lesions under conditions where ENU produces no tumors in controls and determines the specificity of tumor histology even with in utero, carcinogenesis, where nephroblasts are the preferred target (2, 3). In the rat, the 15th day of gestation is metanephric (13). Nephroblasts might be the targets for development of both kinds of tumor in ENU-induced transplacental renal carcinogenesis. Our data thus support the hypothesis that the Wilms' tumor gene may control differentiation of nephroblasts (metanephric stem cells) into renal tubular stem cells, while the Eker gene (RC gene) may control terminal differentiation of these stem cells but permit differentiation of nephroblasts to renal cells (Fig. 2).
Our study confirms that genetic susceptibility to chemical carcinogens can be cell type specific, as earlier reported by Walker et al. (17). In their experiment, rats carrying the Eker mutation and noncarrier control rats were exposed to the renal carcinogen DMN at 16 weeks of age. DMN, which is carcinogenic for both interstitial stromal cells and tubular epithelial cells of the kidney, induces RMTs and RCs, respectively, from these two cell types. When exposed to DMN, the number of RCs in animals carrying the Eker mutation increased at 12 months of age. However, the number of RMTs induced in the carrier group by DMN was the same as in the DMN-exposed noncarrier group. Therefore, the germline mutation at this locus dramatically affected susceptibility to carcinogen induction of tumors in the renal tubules but had no effect on the susceptibility of the mesenchymal cells of the same organ.

Our present study basically differs in two main respects from the previous report (17): (a) the target was the rudimentary kidney as opposed to the fully developed organ; and (b) neoplastic lesions began to appear very much earlier (e.g., only 1 week after birth), conceivably due to the very high rate of cell replication exerting a promotional effect during perinatal life. Without transplacental carcinogen application, the earliest we found microscopic preneoplastic lesions (e.g., atypical tubules and atypical hyperplasia) was at 4 months in the Eker rat following unilateral nephrectomy (5, 6). Now we can diagnose the Eker carrier state earlier and macroscopically before weaning.

Finally, isolation of the predisposing Eker gene located on rat chromosome 10 would provide an opportunity to understand differentiation and carcinogenesis of renal tubules. Thus, the Eker rat presents a valuable model for studying both normal development and tumorigenesis in the kidney.

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References


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