Prevention of hereditary carcinogenesis

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Abstract: Cancer is a heritable disorder of somatic cells. The environment and heredity both operate in the origin of human cancer. Hereditary cancers in animals provide valuable experimental models for understanding the mechanisms of disease, and the development of the therapeutic treatments which can be translated into human patients, as well as how environmental factors interact with cancer susceptibility genes. Here, we show the example for cancer prevention in \( Tsc2 \) gene mutant hereditary renal carcinogenesis by introducing IFN-\( \gamma \) transgene.

Key words: \( \text{TSC2; IFN-}\gamma; \text{renal tumor; tuberous sclerosis; cancer prevention.} \)

Introduction. Hereditary cancers have been important in the understanding of carcinogenesis, even though only a small proportion of cancers belong to this group. Hereditary cancers in animals provide valuable experimental models for the study of problems in carcinogenesis; e.g., cell stage and tissue/cell-type specific tumorigenesis, multistep carcinogenesis, species-specific difference in tumorigenesis, modifier gene(s) in carcinogenesis, cancer prevention, and the development of the therapeutic treatments which can be translated into human patients, as well as how environmental factors interact with cancer susceptibility genes. Such a naturally occurring hereditary cancer in rat was described by Reidar Eker in 1954.

The Eker rat model of hereditary renal carcinoma (RC) is an example of Mendelian dominantly inherited predisposition to a specific cancer in an experimental animal. We found that the homozygous mutant condition is lethal at around the 13th day of fetal life. Heterozygotes, renal carcinomas develop from early preneoplastic lesions (phenotypically altered renal tubules, which begin to appear at two months of age) to adenomas around the age of one year; penetrance for this RC gene is virtually complete. Finally, we identified a germline retrotransposon insertion in the rat homologue of the human tuberous sclerosis (\( \text{TSC2} \)) gene. To the best of our knowledge, this was the first isolation of a Mendelian dominantly predisposing cancer gene in a naturally occurring animal model.

Human tuberous sclerosis is an autosomal dominant multisystem disorder caused by a mutation either in the \( \text{TSC1} \) or \( \text{TSC2} \) gene, characterized by phacomatosis with manifestations that include mental retardation and seizures. The phenotype in human differs from that in the Eker rat, except for the occurrence of RCs (in human, angiomyolipomas are more common), although subependymal, subcortical hamartomas and cortical tuber in the Eker rat were recently reported. Thus, the same gene shows diverse phenotypes between species, although we do not have any good explanation for this difference. Similar phenomena have been observed in knockout mice for a number of tumor suppressor genes. To elicit insights into species-specific tumorigenesis caused by \( \text{Tsc2} \) gene inactivation, we generated a \( \text{Tsc2} \) knockout mice. Mice heterozygous for \( \text{Tsc2} \) mutation developed RCs but no angiomyolipoma, with a complete penetration as seen in the Eker rat, although there is still species-specific difference between rat and mouse. In comparison with the rat system, one of the advantages of the mouse system is the availability of various genetically modified lines for genetic cross experiments. Because nothing is known about prevention for the tumorigenesis of human tuberous sclerosis, the animal models have great potential for that. In this paper, we show an example of disease prevention.
Materials and methods. Knockout mice heterozygous for the Tsc2 mutation and transgenic mice carrying IFN-γ transgene [C57BL/6-TgN (IFN-γ) 5 Imeg; provided from Dr. K. Yamamura] were described previously. Genotyping analyses were carried out using PCR. Conditions for PCR and primers for the mutant Tsc2 allele were described previously. Primers for the detection of IFN-γ transgene were GAINTI (5'-GCCTCTGCTAACCATGTTCA-3', in the rabbit β-globin intron sequence) and INFG4 (5'-GATGCAGTGTGTAGCGTTCA-3', in the IFN-γ coding sequence). Sequence of amplified products (180 bp) was confirmed by direct sequence analysis. Histological examinations of tissues were carried out after fixation by 10 % buffered formaline.

Results and discussion. We mated the IFN-γ transgenic mice with Tsc2 gene knockout mice. Surprisingly, renal carcinogenesis was dramatically suppressed in Tsc2 gene knockout mice, both macro- and microscopically (Table I and Fig. 1). These transgenic mice specifically expressed the IFN-γ in the liver, because of the link to liver specific serum amyloid P component gene promoter, and developed chronic hepatitis. Values of circulating IFN-γ in these transgenic mice were more than 100 pg/ml, while in non-transgenic mice IFN-γ could not be detected. IFN-γ is a pleiotrophic cytokine and suppressions of tumor developments have been reported using various systems, and various mechanisms were considered, e.g., immunity and non-immunity such as cell arrest and apoptosis. Previously, we reported that the capacity to stimulate allogenic and antigen-specific T lymphocytes, as well as the ability to produce IL-12 and to process soluble protein antigens, was significantly higher in dendritic cells from the IFN-γ transgenic mice compared to the normal mice. It is noted that lymphoid infiltration was lacking in the kidneys of these IFN-γ transgenic mice (Fig. 1). Long-term application of IFN-γ is feasible, because chronic systemic administration of low dose IFN-γ is already being given to Mycosis fungoides (cutaneous T cell lymphoma) patients.

On the other hand, recent studies have implicated a possible relationship of TSC genes to the PI3kinase-
Akt/PKB-S6K signaling pathway in Drosophila, although its biological data is lacking.19 Thus, the evaluation of our finding might also be helpful in understanding the mechanisms of disease, as well as the development of new therapeutic treatments for TSC patients.

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**References**