EFFECTS OF AGE ON SUSCEPTIBILITY TO DIETHYLNITROSAMINE-INDUCED HEPATOCARCINOGENESIS IN CONNEXIN 32 DYSFUNCTIONAL TRANSGENIC RATS

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Background: Gap junctions have important roles in the maintenance of tissue homeostasis and the control of cell growth and differentiation. Connexins are subunits of gap junction channels, which allow intercellular exchange of small molecules, such as ions, second messengers, and cellular metabolites between contacting cells. In the liver, connexin 32 (Cx32) is a major gap junction protein. Concerning hepatocarcinogenesis, Cx32 protein decreases during chemical hepatocarcinogenesis in the rat liver and progression of human chronic liver disease, including cirrhosis and hepatocellular carcinomas. We previously established transgenic rats carrying a dominant negative mutant of Cx32 under control of albumin promoter (Tg rats), which have much decreased capacity for Gap junctional intercellular communication (GJIC), and they were susceptible to diethylnitrosamine (DEN)-induced hepatocarcinogenesis compared to littermate wild-type Sprague-Dawley (SD) rats (n-Tg rats). In this study, we examined whether aging affect GJIC function and susceptibility to DEN-induced hepatocarcinogenesis with the transgenic rat model.

Methods: (Experiment 1) Male Tg and n-Tg rats of 10, 30 or 85 weeks-old were given single intraperitoneal administration of 40 mg/kg DEN. Each group consisted of 8 rats and they were all sacrificed at 12 weeks after the treatment of DEN. GST-P positive foci were used as a maker for preneoplastic legions. Number and area of them were measured with a image analyzer. (Experiment 2) GJIC capacity in liver of male Tg and n-Tg rats (10 and 100 weeks-old) were measured by Dye-loading assay. Expressions of Cx32 protein in liver were visualized by immunohistochemical staining.

Results: (Experiment 1) At 10 and 30 weeks-old, the number and area of GST-P positive foci were significantly increased in the liver of Tg rats compared to n-Tg rats. On the other hand, at 85 weeks-old, both Tg and n-Tg rats had large number and area of GST-P foci and the difference in these values between Tg and n-Tg was not significant. (Experiment 2) Dye-loading assay revealed that abilities for GJIC of 10 weeks-old Tg rats were much decreased compared to those of same age of n-Tg rats. At 100 weeks-old, GJIC abilities of n-Tg rats reduced similar to those of Tg. In Tg rats, Cx32 were not expressed regardless of aging. However in n-Tg rats, Cx32 expressions located at hepatocyte membrane in 100 weeks-old were decreased compared to those in 10 weeks-old.

Conclusion: Ability of GJIC is decrease with aging, resulting in increasing susceptibility for DEN-induced hepatocarcinogenesis in wild-type rats.