bryonic lethality and hematopoiesis in Td^{ho} males, and hyperkeratosis in Td^{ho} adult females.

In humans, it is also demonstrated that the mutations of EBP result in X linked dominant chondrodysplasia punctata (CDPX2). Therefore, Td^{ho} mutant mouse is expected to be one of useful animal model for CDPX2.

The original papers of this thesis appeared in "Mamm. Genome", Vol. 8, 578-580 (1997) and "Lab. Anim. Sci. ", Vol. 50, 8-12 (2000).

Molecular characterization and antiviral activity analysis of the interferon-inducible *Mx* gene in mice

Hee Kyung Jin

Laboratory of Experimental Animal Scienece,
Graduate School of Veterinary Medicine,
Hokkaido University, Sapporo 060-0818, Japan

The mouse genome contains two related interferon-regulated genes, *Mx 1* and *Mx 2*. *Mx 1* codes for the nuclear 72-kDa protein that interferes with influenza virus replication after interferon treatment. On the other hand, the *Mx 2* gene is nonfunctional in all laboratory mouse strains examined, since its ORF is interrupted by insertional mutation and a subsequent frame shift. In the present study, the characterization and identification of IFN-inducible *Mx 1* and *Mx 2* genes in the feral-origin strains have been demonstrated.

On part 1, several mouse strains established from feral-origin mice were tested to determine their *Mx 1* + or *Mx 1* − allele status with PCR-RFLV, sequence analysis, RT-PCR and immunofluorescence staining. All of the mouse strains originating from feral-origin mice were found to uniformly carry the *Mx 1* + allele. Therefore, it is conceivable that the *Mx 1* + allele in feral-origin populations serves a function against some pathogens related to orthomyxoviruses. The PCR-RFLV and sequence analysis allowed to classify the *Mx 1* + alleles of the laboratory and feral-origin mouse strains into distinct classes. RT-PCR and immunofluorescence staining demonstrated that the *Mx 1* transcripts and proteins were induced by IFN α / β in macrophages from feral-origin mouse species.

On part 2, the author demonstrated that *Mx 2* mRNA of cells from the feral-origin mouse strains NJL (*Mus m. musculus*) and SPR (*Mus spretus*) differed from the nonfunctional mRNA of the laboratory mouse strains tested. The *Mx 2* mRNA of the feral-origin strains contained functionally a single long ORF consisting of 656 amino acids. The author further showed that Mx 2 protein in the feral-origin strains was expressed upon interferon treatment and localized to the cytoplasm much like the rat Mx 2 protein, which inhibited VSV replication. Furthermore, transfected 3 T 3 cell lines of laboratory mouse origin expressing *Mx 2* from the feral-origin strains acquired slight resistance to VSV.

In the study of part 3, the author has demonstrated that the embryonic fibroblastic

cells from feral origin strains (NJL and SPR) expressed 74 kDa Mx 2 protein, which also prevented the accumulation of viral transcripts and proteins of hantaviruses in transfected Vero cells expressing *Mx 2* gene constitutively. Furthermore, these transfected cells showed significantly lower titers of the virus than control cells. On the other hand, influenza virus replication was not affected by the expression of Mx 2 protein in Vero cells.

A wide range of genetic characters from feral-origin mice would be useful in a laboratory animal model for infectious disease studies.

The original papers of this thesis appeared in "Biochem. Genet.", Vol. 36, 311-322 (1998) and "J. Virol.", 73, 4925-4930 (1999).

A study on the differentiation therapy of canine osteosarcoma with vitamin D and retinoids

Edward F. BARROGA

Laboratory of Veterinary Surgery,
Department of Veterinary Clinical Sciences
Graduate School of Veterinary Medicine,
Hokkaido University, Sapporo 060-0818, Japan

The possibility of inducing normal differentiation of canine osteosarcoma cells into osteoblasts may reverse the disruption of their differentiation and result to the inhibition of growth and reduction of the malignant behavior. This was initially ascertained in a series of three *in vitro* experiments. Firstly, Treatment with 10^{-10}-10^{-8} mole (M) concentrations of calcitriol, 22-oxa-calcitriol (OCT), cholecalciferol, all-trans retinoic acid (ATRA) and 9-cis retinoic acid (9-cis RA) for 48-120 hours culture changed the morphology of POS canine osteosarcoma cells, POS 53B (chondroblast type), POS 53C (undifferentiated type) and POS 53D (osteoblast type) cells to cells that were elongated and spindle shaped; increased number of cytoplasmic organelles and pronounced nuclear activities; and inhibited the growth of POS cells dose dependently ($P<0.05$).

Secondly, functional differentiation was investigated *in vitro via* bone differentiation markers: alkaline phosphatase (ALP) staining, intracellular ALP activity, gammacarboxy glutamic acid-osteocalcin (GLA-OC) production and type 1 collagen (P1P) production. Treatment with 10^{-8} M concentrations of OCT, calcitriol and ATRA for 72 hours significantly increased ($P<0.05$) ALP, GLA-OC and P1P of these tumor cells except POS 14A.

Thirdly, apoptosis was also induced on POS cells in vitro by all drugs at a concentration of 10^{-6} M at 48 hours, 10^{-7} M at 96 hours, 10^{-8} and 10^{-9} M at 120 hours after incubation with the drugs.

A series of two experiments were consequently undertaken to evaluate the inhibitory effects of these drugs *in vivo*. Firstly, a highly metastasizing model of canine osteosarcoma to the lungs in nude mice was established by