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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, influ-

enza 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.

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

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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 I collagen (P 1 P) production. Treatment with  $10^{-8}$  M concentrations of OCT, calcitriol and ATRA for 72 hours significantly increased ( $P < 0.05$ ) ALP, GLA-OC and P 1 P 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

selection of cells with increased metastatic properties from the parent POS canine osteosarcoma cells. The procedure selected medium sized and polygonal cells, and were named highly metastasizing POS (HMPOS) cells. HMPOS cells produced numerous and large masses of lung metastases with various sizes and replacement of lung tissues 12 weeks after implantation as compared to POS cells.

Secondly, treatments of HMPOS cells *in vitro* morphologically elongated and increased ALP activity and staining of cells. HMPOS tumor growth *in vivo* were significantly inhibited when OCT and ATRA were given subcutaneously three times a week for 5 weeks (1.0 ( $\mu\text{g}/\text{kg}$  bw)). The subcutaneous tumors of the control mice consisted of osteoblastic

cells and isolated chondroblastic cells, but formed several areas of osteoid and increased amount of collagen tissue in all treated mice. Microscopic metastatic nodule developed only in two from six mice treated with ATRA. Metastasis was not seen in the mice treated with OCT or OCT + ATRA.

In conclusion, this study demonstrated that inhibition of growth *in vitro* and *in vivo* of the POS canine osteosarcoma cells and its clonal cells, and its pulmonary metastasis *in vivo*, was induced by these drugs and suggest that both its differentiating and resulting apoptotic inducing activities may be responsible for the antitumor effects. These drugs may be useful in the clinic as an adjunct to canine osteosarcoma therapy.

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## Bovine nuclear transfer using embryonic blastomeres and cumulus cells

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This study was conducted to improve the recipient cytoplasm preparation, and to find out the most suitable donor-recipient cell-cycle phase coordination for nuclear transfer (NT).

First, the effect of recipient oocyte quality on the development after NT using embryonic blastomeres as nuclear donors was investigated. Embryos reconstituted from good

quality oocytes showed significantly higher developmental rate to the blastocyst stage than those from poor ones. The second study was designed to develop a new efficient enucleation technique. The chromatin material of 41% of metaphase II and 100% of the activated oocytes was located adjacent to the first and second polar body, respectively. Enucleation after activation resulted in a higher enu-