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Title	In-vitro culture of mouse preantral follicles and its application to the analysis of transforming growth factor- β 1 expression in the developing follicles
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previously isolated from aborted fetuses, we used PCR procedure to amplify a *N. caninum* internal transcribed spacer 1 (ITS 1) DNA fragment. A DNA fragment of expected size (279 bp) was amplified from all strains, but no DNA fragments were amplified from a DNA extract of *T. gondii*. These results proved that BT-3 and other 4 isolates are *N. caninum*, and genomic differences for ITS 1

were not found among the isolates. Immunological reactivities of the 5 isolates of *N. caninum* with different sera from seropositive dogs, cattle and mice were investigated. No immunological differences were observed among the 5 isolates. Therefore, any one of these isolates can be used as antigen for the indirect fluorescent antibody test.

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In-vitro culture of mouse preantral follicles and its application to the analysis of transforming growth factor- β 1 expression in the developing follicles

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This study was conducted to determine the normality of cultured follicles including the developmental competence of their oocytes after *in-vitro* culture and to apply the culture system to the analysis of transforming growth factor- β 1 (TGF- β 1) expression in the developing follicles in mice.

First, mouse follicles isolated from ovaries were classified into two categories (types 4 and 5a) and were cultured *in vitro*. Oocytes derived from both types of *in-vitro* grown follicles had similar maturation (70 and 62%) and cleavage (30 and 35%) rates. Blastocysts were, however, only obtained from the oocytes derived from the type 5a follicles.

The second study was designed to determine the expression of TGF- β 1 in mouse follicles grown *in vivo* by immunohistochemical staining of ovarian serial sections. Positive TGF- β 1 staining in the granulosa and theca

cells was initially observed immediately before the time of antrum formation (type 5b follicles). The proportion of follicles with positively stained granulosa cells increased with follicular development from early antral (type 6) to preovulatory (type 8) stages (13 to 82%). The proportion of follicles with positively stained theca cells did not show a clear trend (~30%) before ovulation, but increased in retarded type 7 (79%) and unovulated type 8 (55%) follicles during the ovulation period.

The third study was conducted to determine the relationship among growth, steroid production and immunolocalization of TGF- β 1 *in vitro* using preantral follicles of types 5a and 4 as models for normally developing and retarded follicles, respectively. At the time of antrum formation, follicular diameters were similar between the follicles originated from both types; however, antral follicles from the

type 5a showed larger number of granulosa cells, higher estradiol-17 β production and higher proportion of follicles with TGF- β 1 positive granulosa cells.

In conclusion, this study showed that type 5a follicles could develop normally *in vitro* and produce oocytes with developmental competence to the blastocyst stage. Results in the analysis of TGF- β 1 immunolocalization

in ovarian and *in-vitro* grown follicles indicated that normally developing follicles express TGF- β 1 in the granulosa cells around the time of antrum formation. Furthermore, the usefulness of the present follicle culture system in the investigations of follicular physiology was demonstrated.

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A fundamental study on the gene therapy of human bladder cancer using the dominant negative H-*ras* mutant N 116 Y

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To clarify the significance of the Ras guanine-nucleotide exchange reaction in human bladder cancer, I investigated the expression of epidermal growth factor (EGF) - receptor, growth-factor receptor binding protein 2 (Grb 2), and son of sevenless (Sos) proteins upstream of Ras in several human bladder cancer cell lines. Grb 2 and Sos proteins were overexpressed in all cell lines, suggesting that the Ras guanine-nucleotide exchange reaction was significant for the proliferation of human bladder cancer cells. These results led us to postulate that the dominant negative H-*ras* mutant N 116 Y (N 116 Y *ras* mutant), which has been suggested to be an inhibitor of Ras guanine-nucleotide exchange reaction, could be a potential tool of cancer gene therapy for human bladder cancer. To demonstrate the therapeutic effects of

N 116 Y *ras* mutant on human bladder cancer, I performed the experiments as follows.

First, I transfected an efficient N 116 Y *ras* mutant expression vector, pZIP-N 116 Y, into 3 human bladder cancer cell lines (T 24, UMUC-2, KU-7) by lipofection procedure. Thus the high expression of the N 116 Y *ras* mutant, under the control of the murine leukemia virus (MLV) retroviral promoter, significantly suppressed the growth of all human bladder cancer cell lines examined.

Next, to investigate the effects of N 116 Y *ras* mutant at a low expression level, I established stable transfectants of UMUC-2 cells in which the N 116 Y *ras* mutant was constitutively expressed under the control of the hMT IIa promoter. I examined the cellular morphology and the ability to grow in soft agar of the N 116 Y *ras* mutant transfectants.