Figure legends

Fig. 1. Enhanced green fluorescent protein (EGFP)-expressing mouse B16F1 melanoma cells after transfection with liposome containing an EGFP plasmid (pEGFP-C1). EGFP was expressed in approximately 30% of the cells treated. Fluorescent (left) and confocal (right) photomicrographs were taken from the same field. Original magnification, x2,000.

Fig. 2. Growth inhibition of cultured mouse B16F1 melanoma cells transfected with liposome containing the murine IFN-beta gene. Twenty four hours after aliquots of 2×10^4 B16F1 cells were inoculated in each well, 15 µl of phosphate-buffered saline (PBS), recombinant murine IFN-beta (muIFN-beta); liposome containing the lacZ gene [lip(pCH110)], or liposome containing the murine IFN-beta gene [lip(pSV2muIFN-beta)] were added to the medium. Incubation was continued for additional 2 or 5 days and the number of viable cells was counted using a hemocytometer. *$p<0.05$ compared with PBS and lip(pCH110). **$p<0.05$ compared with muIFN-beta. All values are means $\pm$ SEM.

Fig. 3. Morphologic changes in cultured mouse B16F1 cells transfected with liposome containing the murine IFN-beta gene. Under a video enhanced contrast-differential interference contrast microscope, we observed that approximately 30% of the cells displayed bleb formation and abnormally bright nucleoli by 24 hr after transfection. By 48 hr the same population had shrunk, developed large membrane outpouchings (ballooning). Original magnification, x 2,000.

Fig. 4. Liposome-mediated expression of murine IFN-beta in subcutaneous melanoma. Lane 1-murine IFN-beta standard (1000 IU); 2- lipsome containing the lacZ gene-injected; 3 and 4- liposome containing the murine IFN-beta gene-injected

Fig. 5. Growth inhibition of mouse B16F1 subcutaneous tumors treated with liposome containing the murine IFN-beta gene [lip(pSV2muIFN-beta)]. Animals were injected intratumorally with 75 μl of either phosphate-buffered saline (PBS), recombinant murine IFN-beta (muIFN-beta; 1000 IU), lacZ control [lip(pCH110)], or lip(pSV2muIFN-beta). Tumor sizes were measured for a period of 21 days. $*p<0.05$ compared with PBS, muIFN-beta, or lip(pCH110). All values are means ± SEM.

Fig. 6. Immunocytochemistry for immune cells infiltrating mouse B16F1 subcutaneous tumors. A; NK cells. B; CD4, CD8, and macrophages. Representative photomicrographs from tumors 7 days after treatment with liposome containing the murine IFN-beta [lip(pSV2muIFN-beta)] or phosphate-buffered saline (PBS). Natural killer cells, CD4 T and CD8 T lymphocytes, and macrophages were stained with antibodies to NK1.1, mouse CD4, mouse CD8, and F4/80, respectively. The tissues were counterstained with hematoxyline. Immunocytochemistry in the PBS-treated tumors was similar to those in the tumors treated with recombinant murine IFN-beta and liposome containing the lacZ gene (data not shown).

Fig. 7. The effect of in vivo natural killer (NK) cell depletion on the growth of subcutaneous tumors treated with liposome containing the murine IFN-beta gene [lip(pSV2muIFN-beta)]. NK cells were depleted by injecting the mice intraperitoneally with 25 μl anti-asialoGM1 antibody 1 day before and every 7 days after lip(pSV2muIFN-beta) injection. The NK cell-depleted (NK^-) animals were injected intratumorally with 75 μl of lip(pSV2muIFN-beta). Tumor sizes were measured and compared with NK cell-undepleted (NK^+) animals treated with lip(pSV2muIFN-beta) or PBS (the same data shown in Fig. 5 are used.). $*p<0.05$ compared with the PBS-treated group and the lip(pSV2muIFN-beta) treated NK^- group. There was no statistical significance between these two groups at any time. All values are means ± SEM.

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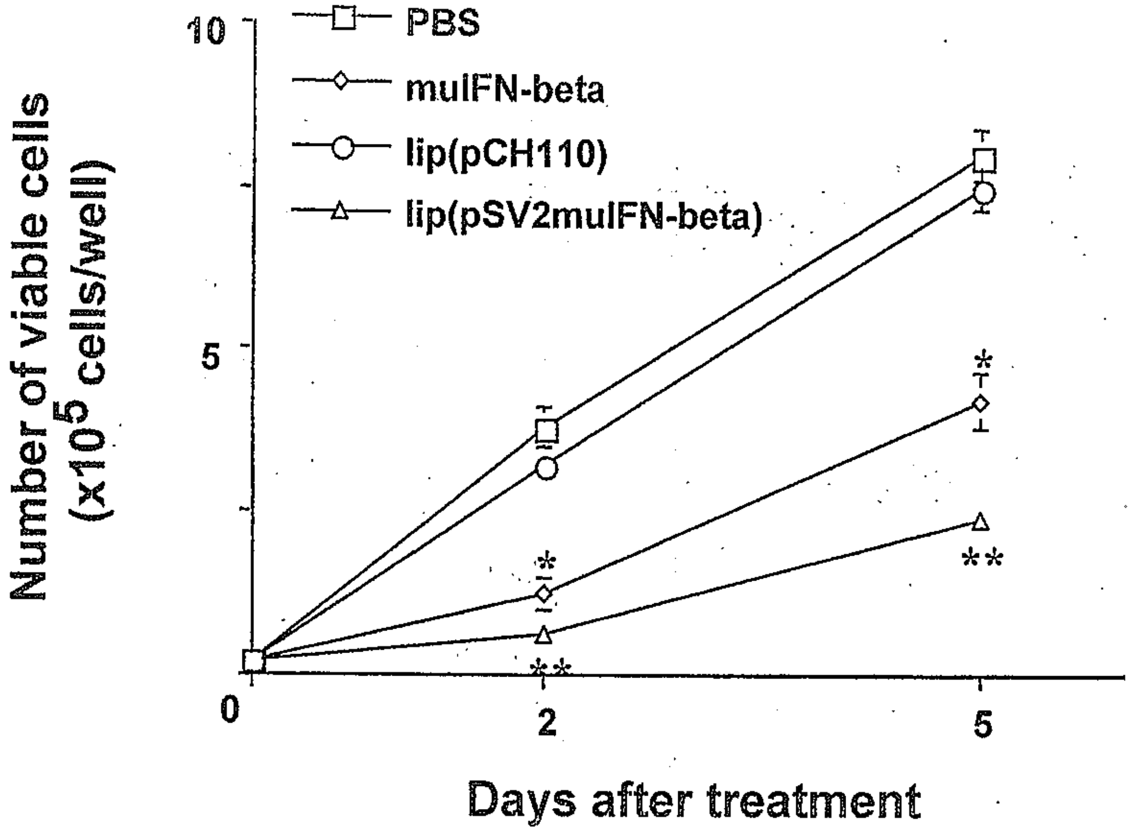

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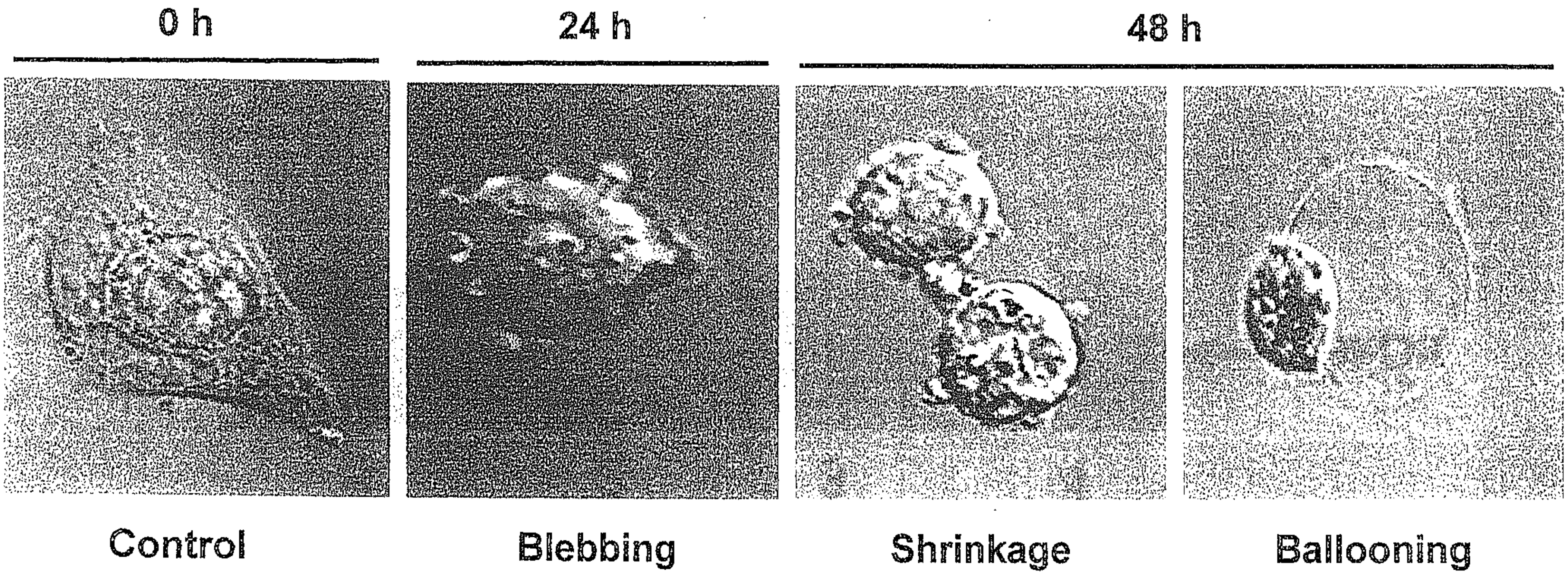

Fig. 3 Y. Ryuke et al.