

## Summary

Genes for two cytokines, one chemokine and gene coding for one angiostatic factor were used in the present work for transfection of mouse HPV-16- transformed tumor cells. Main characteristics of transduced cells were tested *in vitro* and *in vivo* and compared with the parental tumor cells.

In the first part I used a thymidine-kinase deficient (cTK<sup>-</sup>) cell line designated 123IA, which had been derived from HPV16 transformed mouse (C57BL/6) cells MK16. To obtain genetically modified cells, 123IA cells were transfected with bicistronic plasmids carrying the herpes simplex type 1 thymidine kinase (*HSV-TK*) gene and either the gene for the mouse *B7-1* (*CD80*) co-stimulatory molecule or the gene for the monocyte-chemoattractant protein 1 (*MCP-1*). For control purposes, a plasmid vector carrying only the HSV-TK gene was used. For comparative purposes I also used B9 cells, previously isolated in our laboratory, which express the mouse granulocyte-macrophage colony stimulation factor (GM-CSF) and HSV-TK gene. All the cell lines tested were found to be sensitive to minute amounts of ganciclovir, revealing the production of functional HSV-TK. When inoculated into syngeneic mice, cells expressing either GM-CSF or B7-1 were non-oncogenic. Nearly all mice inoculated with MCP-1-producing cells developed tumors. Animals injected with GM-CSF or B7-1-producing cells were protected against challenge with the parental MK16 cells. When another mouse (C57BL/6) HPV16-transformed oncogenic cell line, TC-1, which differs from the MK16 cells in a number of properties, was used for the challenge, the protective effect was much less pronounced.

In the second part, MK16 cells and TC-1 cells, which had been also derived from HPV16 transformed mouse (C57BL/6) cells, were transduced with the gene for angiostatic mouse endostatin. Two clones constitutively expressing endostatin were isolated from each cell line. They were denoted ME3 and ME9 (derived from MK16 cells), and TE2 and TE5 (derived from TC-1 cells), respectively. When inoculated into mice, ME3 cells were non-oncogenic. Nearly all mice inoculated with ME9 cells developed tumors, but metastasis formation was strongly reduced in these animals. TE2 and TE5 cells displayed oncogenic potential similar to that of the parental TC-1 cells. Animals immunized with ME3 cells were protected against challenge with the parental MK16 cells. Cell lysates of all six lines studied were tested for the content of 25 factors known to be involved in angiogenesis. The MK16 cells differed from the TC-1 cells and also from all endostatin producing sublines by a markedly higher production of interleukin-1 $\alpha$  (IL-1 $\alpha$ ). Additional experiments indicated that the suppression of the production of IL-1 $\alpha$  by the parental MK16 caused by endostatin was due to an autocrine mechanism.