

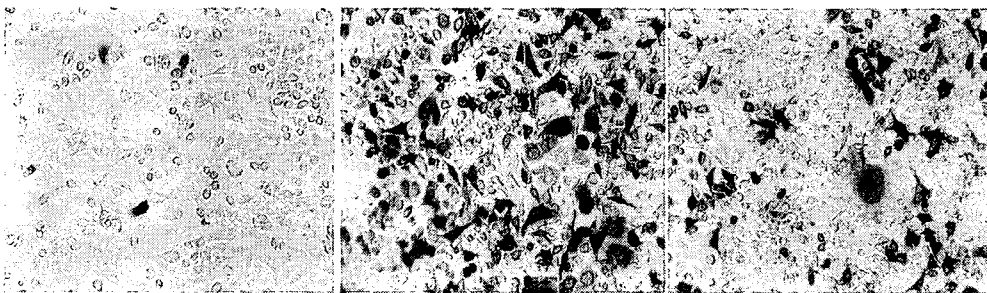
[SV-5]

Development and Application of Retroviral Vector System for Gene Transfer into Mammalian Cells

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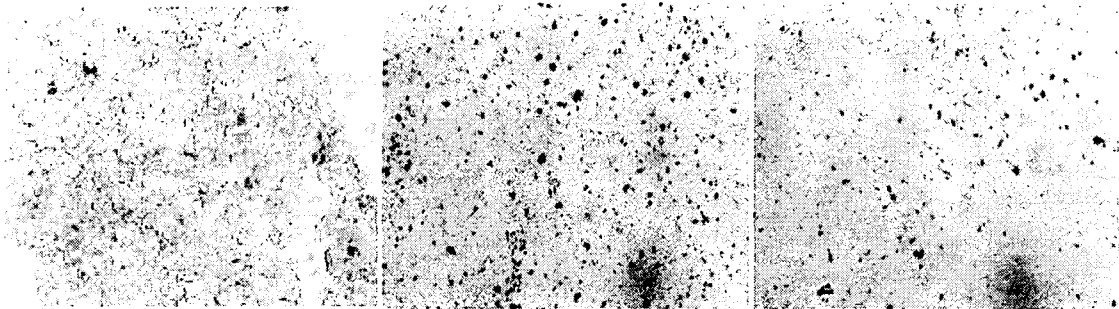
Efficient *in vivo* gene transfer is an important goal for cancer gene therapy. Although adenoviral vectors have proven effective for this purpose in animal models, the ability to achieve comparable gene transfer with retroviral vectors would provide potentially desirable safety and toxicity features for clinical studies. A potential strategy to improve gene transfer efficiency is the utilization of alternative retroviral envelopes. Therefore, we compared the transduction efficiency of human cancer cells with retroviruses bearing the envelope of amphotropic murine leukemia virus (MuLV) and gibbon ape leukemia virus (GaLV). Transduction efficiency of the human cancer cell lines with retroviruses bearing GaLV envelope was 5- to 30-fold higher than that with retroviruses bearing MuLV envelope. We also tested cationic lipids for enhancement of transduction efficiency. In the presence of polybrene, 30% of the cells were transduced by the retroviruses bearing GaLV envelope at a multiplicity of infection (MOI) of 10 as demonstrated by X-Gal staining. However, 80% of the human cancer cells were transduced in the presence of a cationic liposome at same MOI. Since cationic lipids are approved for human use by the U.S. Food and Drug Administration, these results suggest a new, more effective strategy of human gene therapy for malignant disease using combination of a cationic liposome and GaLV envelope-bearing retrovirus-mediated gene transfer.



Control Lipofectamine Polybrene

In addition, intratumoral retrovirus-mediated transduction efficiency was examined with human breast tumor tissues established in nude mouse. Surprisingly, single intratumoral injection with 1×10^6 virus producer cells resulted in a large number of infected tumor cells nearly all over the tumor tissues. These data demonstrate that direct inoculation of retrovirus producer cells into solid tumors can mediate sufficient transduction of the tumor for gene therapy.

Control Lipofectamine Polybrene



In the last decade scientific and technical advances in molecular biology have enabled the scientists to identify the genetic origins of human diseases, especially in the area of oncology. A variety of tumor suppressor genes and more than 100 oncogenes have been identified. In addition, the concept that the immune response may be manipulated to eliminate established neoplasms made tumor immunologists to figure out the function of many genes encoding cytokines and co-stimulatory factors in the tumor-specific immunity. These advances have revolutionized our ability to treat patients with cancer. However, successful gene therapy for cancer has been elusive, although several genes have been used for the cancer gene therapy. Recently, studies on the mechanisms of angiogenesis and metastasis provided an alternative to conventional approaches for the treatment of cancer.

During tumor invasion and metastasis, the balance between MMPs (matrix-degrading metalloproteinases) and TIMPs (tissue inhibitor of metalloproteinases) is often shifted in favor of the proteases, resulting in an excessive proteolytic degradation of the extracellular matrix. This observation indicates that genetic manipulation of the protease-protease inhibitor balance in tumors in favor of the inhibitors may have a significant cytostatic effect in cancer. Thus, in this aspect, protease inhibitor genes may represent attractive candidates in a gene therapy approach to cancer it may not be necessary to deliver and express these genes in every single tumor cell as long as the level of expression in a limited number of transduced cells is sufficient to prevent the excessive breakdown of the extracellular matrix.

Family of tissue inhibitors of metalloproteinases (TIMPs)

TIMP-1	TIMP-2	TIMP-3	TIMP-4
30-kDa	21-kDa	21-kDa	22-kDa
Highly induced at transcript level by cytokine and MMP-9	inhibits the active forms of almost all the MMPs, in particular MMP-2 and MMP-9	Extracellular matrix blindness disease regulated during development, mitogenic stimulation, normal cell cycle progression.	no transcripts : the liver, brain, lung, thymus, spleen tissue specific manner transcripts : kidney, placenta, colon, heart and testes

In this study, we used retroviral-mediated TIMP-2 overexpression by intratumoral injection for suppression of metastasis in human breast carcinoma established in mammary fat pad of nude mouse. In order to improve transduction efficiency of human cancer cells the recombinant retroviruses were produced from a packaging cell line expressing envelope glycoprotein of gibbon ape leukemia virus.

Effect of Infection with Retroviruses Encoding TIMP-2 cDNA on Distant Pulmonary Metastases of MDA-MB435 Cells



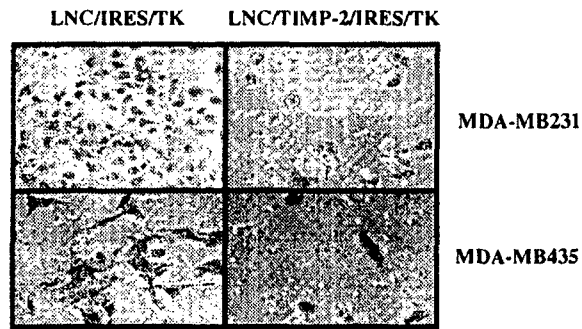
LNC/IRES/TK



LNC/TIMP-2/IRES/TK

In vitro invasion assay of human breast cancer cells transduced with the retrovirus vector encoding the TIMP-2 cDNA revealed the decrease of invasiveness on metrigel coated with collagen. Also, single intratumoral injection of the TIMP-2 retrovirus into human breast tumor tissue established in mammary fat pad of nude mice showed dramatic decrease in size and number of lung metastatic tumor.

Matrigel Assay with Human Breast Cancer Cell Lines Overexpressing TIMP-2



In Vivo Tumor Growth of MDA-MB435/TK and MDA-MB435/TIMP-2 Cells in the Mammary Fat Pads of Nude Mice

