P.O.Box 2345, Beijing 100023,China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

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• REVIEW •

Gene therapy for gastric cancer: A review

Chao Zhang, Zhan-Kui Liu

Chao Zhang, Zhan-Kui Liu, Department of General Surgery, Southwest Hospital, Third Military Medical University, Gaotan Yan, Chongqing 400038, China
Correspondence to: Dr. Chao Zhang, M.D. Department of General Surgery, Southwest Hospital, Third Military Medical University, Gaotan Yan, Chongqing 400038, China. meizhang6688@yahoo.com.cn
Telephone: +86-23-68773074
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Abstract

Gastric cancer is common in China, and its early diagnosis and treatment are difficult. In recent years great progress has been achieved in gene therapy, and a wide array of gene therapy systems for gastric cancer has been investigated. The present article deals with the general principles of gene therapy and then focuses on how these principles may be applied to gastric cancer.

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INTRODUCTION

Enormous progress has been seen in molecular genetics over the past few decades. It has given us insights at the molecular level, into vital progresses in living organisms, such as embryonic development, growth regulation, differentiation, pathogenesis and carcinogenesis. Insights into the mechanism of pathologic progresses such as developmental disorders and carcinogenesis, have stimulated efforts to develop therapeutic approaches to prevent or correct these processes. Techniques to directly change the genetic information of a cell have greatly improved expectations of the therapeutic potential of genetic manipulation. These developments have raised hopes that diseases appearing to be incurable can soon be cured. Especially for cancer such as gastric cancer, which is common in China. It is well established that most cancers result from a series of accumulated, acquired genetic lesions in somatic cells that are faithfully reproduced until a malignant clone is created, which is ultimately able to destroy the host. Gene therapy has emerged as a new method of therapeutic and possibly preventive intervention against cancer at the level of cellular gene expression[1-14]. Generally speaking, gene therapy can be defined as the introduction and expression of an exogenous gene into human cells for therapeutic benefit, and is conventionally restricted to human diseases associated with single gene defects[15].

In oncology, it can be defined as the introduction of DNA into cells (either neoplastic or normal) in order to shrink or eliminate a malignant tumor. This may be achieved by means of directly inducing malignant cell death, modulating immune response to tumors or reversing the malignant process by correcting genetic abnormalities. It may also be possible to enhance a tumor's responsiveness to conventional treatments such as chemotherapy and radiotherapy, and to protect normal tissue by introduction of genetic materials that confers resistance to the toxic effects of such treatment[16, 17]. A number of strategies have been developed to accomplish cancer gene therapy. These approaches included cytotoxic gene therapy, antisense therapy, and immunotherapy[18-22]. However, despite progress in the field, wide clinical applications and success have not been achieved[23,24]. As with all forms of gene therapy in cancer, the main problems to overcome will be optimizing delivery in order to maximize the proportion of successfully transduced cancer cells, In treatment of human malignant tumours, several obstacles explain the limitations of currently available treatments for achieving definitive cures in most cases of advanced disease. There are some problems in gene therapy. A combination of new chemotherapy drugs, higher doses of drugs, novel cytokines, improved regimens of radiotherapy, and more sophisticated surgery can achieve incremental improvements in cancer treatment. But these therapies do not address critical biological obstacles, and thus, probably will not bring about the much-needed radical advances in the implementation and results of cancer treatment. In contrast, gene therapy offers the potential for overcoming some of these fundamental barriers.

APPROACHES TO GENE THERAPY

Vector

One of the major problems in gene therapy is difficulty in delivering appropriate nucleic acid sequences to target cells. Various strategies have been evolved, which can be essentially divided into two types. One use viral vectors while the other uses non-viral vectors.

Viruses vector The main viruses which have been studied as potential vectors for transducing genes into cancer cells are retrovirus and adenovirus. Retroviruses are single-stranded RNA viruses, and after deletion of one or more structural genes, a foreign gene can be incorporated forming a "recombinant" retrovirus. This could then be used to infect a cell so that it integrates into the host cell's genome which then expresses viral genes as well as the "therapeutic" gene[25,26]. Adenoviruses consist of a core containing double-stranded DNA surrounded by a protein capsid. When an adenovirus vector is created, E1 genes are deleted in order to render the virus incapable of replication, thereby obviating the risk of transforming healthy host cells[27]. Adenoviruses have a greater potential than retroviruses in that they have a higher efficiency of infection and it is possible to incorporate larger segments of DNA. Weber *et al*[28] thought that oncoretrovirus-based vector was a safe and reliable vector system that could achieve permanent integration of delivered transgenes. Successful application of these vectors for gene therapy has proven difficult due to their relatively low transduction efficiency. However, cumulative improvements in methodology have recently yielded promising clinical results. Furthermore, significant improvements in basic retrovirus vector technology now can revitalize the field. But they do induce antiviral immune responses that may compromise the ability to treat an immunocompetent host on more than one occasions. Other viruses which have been used in this context include herpes virus, vaccinia virus and adenovirus-associated virus. Pieroni *et al*[29] found the use of baculovirus vectors for gene expression in mammalian cells was in continuous expansion. These vectors do not replicate in mammalian cells, do not cause a cytopathic effect upon

infection and are able to carry large DNA inserts. Baculovirus vectors have been shown to transduce various cell types *in vitro* and *in vivo* with significant efficiency leading to stable gene expression. This review focuses on recent the developments in baculovirus vector that highlight its potential use for new gene therapy strategies. Okada *et al*[30]. have done something about adeno-associated viral vector-mediated gene therapy for ischemia-induced neuronal death.

Non-viral vector The most widely studied non-viral vector is liposome. Liposome is a positively charged lipid membrane which can be complexed with DNA, and fusion of liposome-DNA complex with a negatively charged membrane leads to transfer of DNA into cells. Lasic *et al*[31] reviewed stabilized liposomes in cancer therapy and gene delivery. Unfortunately, the efficiency of gene transduction using liposomes was currently much lower than that achieved by viral vectors[32].

Other approach

Another approach is to use direct injection of plasmid DNA, but this technique can only transfect cells immediately adjacent to the injection site so that only a small number of cells can be treated[33].Vanden *et al*[34] found that oncoretroviral vectors and lentiviral vectors offered the potential for long-term gene expression by virtue of their stable chromosomal integration and lack of viral gene expression. Gomez *et al*[35]analyzed conditionally replicative adenoviral vectors. Liu *et al*[19] described that the successful transformation of C. sporogenes, a clostridial strain with the highest reported tumor colonization efficiency, with *E. coli* cytosine deaminase (CD) gene and showed that systemically injected spores of these bacteria expressed CD only in the tumor.

It is hoped, however, that by complexing adenovirus and plasmid DNA with protein ligands which bind to specific receptors, enhanced gene transfer into specific cellular targets might be achieved[36]. An example of this is the argenine-glycine-aspartic acid motif that targets integrin receptors[37]. Aside from different approaches to introducing genetic material into cells, gene therapy can be classified according to the different end results. The main aims in this respect are gene replacement, antisense therapy, cytotoxic gene therapy, immunotherapy and drug resistance transfer.

Cytotoxic gene therapy

One of the most promising strategies for gene therapy against various types of cancer is the introduction of a suicide gene, which is transduction of a gene that transforms a non- toxic "pro-drug" into a toxic substance. One approach to this general concept is the transfer of the gene for HSV thymidine kinase (HSV-tk), as this phosphorylates nucleoside analogues such as acyclovir and ganciclovir which are then incorporated into DNA as it replicates[38]. Floeth *et al*[39] analyzed the mechanisms of the "bystander effect" in VPC-mediated HSV-Tk/GCV gene therapy. Thus, these compounds are only toxic to cells expressing HSV-tk, although the bystander effect has also been noted in this type of gene therapy[40]. This is presumably due to release of toxic metabolites produced by the prodrug-activating enzymes which then kill surrounding non-transduced cells. A similar type of cytotoxic gene therapy involves an adenovirus carrying cDNA for cytosine deaminase enzyme of *E.coli* and prodrug 5-fluorocytosine. The prodrug is given orally and converted to 5-fluorouracil in the cells containing cytosine deaminase[41,42].

Antisense therapy

When oligonucleotides bind to their complementary RNA or DNA, they prevent translation or transcription, respectively. This process, known as "anti-sense", is a theoretically attractive method for inactivating oncogenes which are overexpressed in tumors[43,44]. Tang *et al*[45] amplified the 200 VEGF cDNA fragment and inserted it into human U6 gene cassette in the reverse orientation transcribing small antisense RNA which could specifically interact with VEGF165 and VEGF121 mRNA. Their conclusion was expression of antisense VEGF RNA in SMMC-7721 cells could decrease tumorigenicity and antisense-VEGF gene therapy might be an adjuvant treatment for hepatoma. Like gene replacement therapy, however, it would seem that all cells in tumors would have to be transduced, and oligonucleotides would have to last long enough to down-regulate the appropriate genes. Nonetheless, this approach did seem to be effective in certain animal models[46-48]. Kumai *et al*[49] investigated the effect of antisense oligodeoxynucleotides (AS ODN) against tyrosine hydroxylase (TH) on hypertension and sympathetic nervous system activity in spontaneously hypertensive rats (SHR). Systolic blood pressure (SBP) in SHR treated with TH AS ODN (50, 200 mg/rat, i.v.) was significantly lower than that in control SHR. Epinephrine and norepinephrine levels, TH activity, and TH protein levels in adrenal medulla of SHR were reduced concomitantly with TH AS ODN treatment-induced changes in SBP. In contrast, TH AS ODN (200 mg/rat) had no effect on SBP in Wistar-Kyoto rats (WKY), though catecholamine levels, TH activity, and TH protein levels were significantly decreased. These findings suggest that peripheral systemic injection of TH AS ODN may be effective as hypotensive therapy in SHR. Marchand *et al*[50] found the use of miniosmotic pumps, phosphate-buffered saline, VEGF, or VEGF combined with AS-Flk-1, AS-Flt-1, or AS-scrambled oligonucleotides were released in mouse testis for 14 days. VEGF (1, 2.5, and 5 mg) increased the formation of new capillary blood vessels by 236 %, 246 %, and 287 %, respectively. The combination of AS-Flk-1 or AS-Flt-1 (200 mg) to VEGF (2.5 mg) reduced by 87 % and 85 % of new blood vessel formation, respectively, and the expression of their corresponding proteins. These data demonstrate the therapeutical potential of AS-Flk-1 or AS-Flt-1 to prevent VEGF-mediated angiogenesis *in vivo*.

Immunotherapy

The main principle of genetic immunotherapy is to improve the host' s immune response to a particular tumor. One approach is to employ intramuscular injection of DNA which encodes a tumor-associated antigen such as CEA either directly or in form of a viral vaccine[51]. Cheng *et al*[52] have developed a new strategy to enhance nucleic acid vaccine potency by linking VP22, a herpes simplex virus type 1 (HSV-1) tegument protein, to a model antigen. This strategy facilitated the spread of linked E7 antigen to neighboring cells. In their study, they created a recombinant Sindbis virus (SIN)-based replicon particle encoding VP22 linked to a model tumor antigen, human papillomavirus type 16 (HPV-16) E7, using a stable SIN PCL. The linkage of VP22 to E7 in these SIN replicon particles resulted in a significant increase in the number of E7-specific CD8(+) T cell precursors and a strong antitumor effect against E7-expressing tumors in vaccinated C57BL/6 mice relative to wild-type E7 SIN replicon particles. Furthermore, a head-to-head comparison of VP22-E7-containing naked DNA, naked RNA replicons, or RNA replicon particle vaccines indicated that SINrep5-VP22/E7 replicon particles generated the most potent therapeutic antitumor effect. By leading to an active immune response, this was thought to be more effective than passive immunisation using specific antibodies against the antigen in question. Another way was to enhance immunity by using genes for cytokines such as interleukins (IL) which could recruit and stimulate appropriate effector cells[53,54]. Nishioka *et al*[55] have done something about genetic modification of dendritic cells and its application to cancer

immunotherapy. Although the results in the experimental systems were promising, the clinical application of gene-modified DCs had several problems such as the standardization of methods of manipulation and gene-transduction of DCs. Approaches to solve them require further studies. Takemura *et al*[56] have previously produced an anti-MUC1 x anti-CD3 diabody (Mx3 diabody) in an Escherichia coli (*E. coli*) expression system, other approaches have been found, for instance, Vonderheide *et al*[57] applied telomerase as a universal tumor-associated antigen. Schadendorf *et al*[58] reviewed the use of histamine in cancer immunotherapy.

APPLICATION OF GNEE THERAPY FOR GASTRIC CANCER

p53 gene

About 60 % of human gastric cancers carry point mutations of p53 gene, and because of its central role,this nuclear protein is believed to play a role in the regulation of cellular response to DNA damage. Wild-type p53 replacement therapy is an attractive concept in this disease. The responses of human gastric cancer cell lines to recombinant adenovirus encoding wild-type p53 gene have been analysed *in vitro* and *in vivo*[59]. In that study, growth inhibition was observed in cell lines expressing p53 mutations, but not in lines with wild-type p53. Furthermore, the mechanism of cell killing was found to be apoptosis. Thus, it seems that p53 replacement therapy has potential as a therapeutic strategy for human gastric cancer.

Antisense therapy

Antisense therapy has also been used in gastric cancer cell line. Proliferating cell nuclear antigen (PCNA) has been shown to stimulate DNA synthesis by DNA polymerase delta, and to be strongly expressed by gastric cancer cells with a high proliferative activity. Antisense oligonucleotides specific for PCNA mRNA have been shown to inhibit the growth of all gastric cancer cell lines tested, whereas random sequence oligonucleotides had no effect.

Cytotoxic gene therapy

Gastrointestinal cancer is the most important clinical target of gene therapy. Suicide gene therapy with herpes simplex virus type 1 thymidine kinase (HSV-TK) gene, has been shown to exert antitumor efficacy in various cancer models *in vitro*. A modificiation of this approach has been madee to insert carcinoembryonic antigen (CEA) promoter into the viral vector to increase the efficiency of transfection of HSV-tk into cells expressing CEA. When compared with transduction of HSV-tk with a ubiquitous promoter, the use of CEA promoter enhanced the killing effect of ganciclovir in CEA producing cells. While in colorectal cancer, about 40 % of gastric cancer expressed CEA, and CEA producing gastric cancer cell lines were susceptible to this treatment. Okino *et al*[60] described the sequential histopathological changes after suicide gene therapy of N-methyl-N' -nitro-N-nitrosoguanidine (MNNG)-induced gastric cancer in rats. Gastric tumors were induced by MNNG in 38/73 (52 %) of Wistar strain rats. The suicide gene therapy group (14 rats) was subjected to *in situ* gene transfer with a recombinant adenovirus vector carrying the HSV-TK gene driven by CAG promoter (Ad.CAGHSV-TK) in gastric tumor, followed by the antiviral drug ganciclovir (GCV). They observed the histopathological changes at various times after HSV-TK/GCV gene therapy, groups of animals were sacrificed at 3, 8, and 30 days after gene transfer. Apoptosis in gastric tumors was detected by the TUNEL method to assess the efficacy of HSV-TK/GCV gene therapy, and it was markable in the 8- and 30-day treatment groups compared to the sham operation controls (P<0.001). Various histopathological changes, degeneration of cancer tissue and fibrosis after necrosis and apoptosis were significantly greater in the 30-day treatment group. The HSV-TK gene was detectable in peripheral blood by PCR until 30 days after gene transfer. These results might be useful in devising a method of suicide gene therapy for humans.

Other forms of cytotoxic gene therapy which have been used with success in gastric cancer cell lines include transfection of E.coli phosphoribosyltransferase (UPRT) which could catalyse the synthesis of UMP from uracil and 5-phosphoribosyl-alpha-1-diphosphate, thereby sensitising the cell to 5-fluorouracil (5-FU). This has been shown to enhance the cell killing effect of 5-FU in gastric cell lines both *in vitro* and *in vivo*. Shimizu *et al*[61] have generated a recombinant adenovirus encoding the UP gene (AxCA.UP) which has been applied in gastric cancer gene therapy to sensitize cancer cells to lower concentrations of 5-FU.

Immunotherapy

Genetic immunotherapy is another area of active research, and work with severe combined immunodeficiency (SCID) mice given human peripheral lymphocytes and autologous human tumour cells from patients with gastric cancer has yielded interesting results. In one study, administration of an adenovirus vector expressing IL-6 cDNA-induced CD8+cytotoxic T-lymphocytes specific for tumour cells from the precursor human T-lymphocytes *in vivo*, inhibited growth and metastasis of autologous human tumours. In another study, SCID mice reconstituted with peripheral blood cells containing CD34+cells were inoculated with human gastric cancer cell lines transduced with cytokine genes including IL-2 and IL-6. It was found that the tumourigenicity of IL-2 producing tumour cells was significantly reduced in the CD34+ reconstituted but not in the non-reconstituted mice, whereas transduction of IL-6 did not affect tumourigenicity, irrespective of the reconstitution status of the mice. This system could provide a model for investigating the utility of transfecting tumours with individual cytokines. Yu *et al*[62] described the bioactivity of MG7 scFv for its application as a targeting mediator in gene therapy of gastric cancer. Two positive recombinant phage clones have been found to contain the exogenous scFv gene. ELISA showed that MG7 scFv had a strong antigen-binding affinity. Immunodotting assay showed that transfected E.coli HB2151 could successfully produce soluble MG7scFv with a high yield via induction by IPTG. The molecular mass of MG7 scFv was 30 kDa by Western blot. DNA sequencing demonstrated that VH and VL genes of MG7 scFv were 363 bp and 321 bp, respectively.

PROSPECT

With development of the genomic research, more and more individual patients have benefited from the revolution so far. Thus, despite a paucity of clinical information, gene therapy for gastric cancer is on the horizon. As with all forms of gene therapy in cancer, the main problems are to optimize delivery in order to maximize the proportion of successfully transduced cancer cells, and to choose the most appropriate targets for an individual tumor. There is no doubt that human cancers are heterogeneous in terms of genetic abnormality, and a better understanding of the mutational spectrum associated with a cancer type along with the ability to obtain mutation profiles for individual tumors is an important step to successful gene replacement and antisense therapy[63-68].

One factor critical to successful human gene therapy is the development of efficient gene delivery systems. Although numerous vector systems for gene transfer have been developed, a perfect vector system has not yet been constructed. Difficulties of *in vivo* gene transfer appear to be due to

resistance of living cells to invasion by foreign materials and interference of cellular functions. We should analyze what barriers in tissues affect *in vivo* gene transfection and focus on how to solve these problems for gene therapy[68-71].

REFERENCES

1 **Wadhwa PD**, Zielske SP, Roth JC, Ballas CB, Bowman JE, Gerson SL. Cancer gene therapy: scientific basis. *Annu Rev Med* 2002; **53**: 437-452
2 **Otsu M**, Wada T, Candotti F. Gene therapy for primary immune deficiencies. *Curr Opin Allergy Clin Immunol* 2001; **1**: 497-501
3 **Cupp CL**, Bloom DC. Gene therapy, electroporation, and the future of wound-healing therapies. *Facial Plast Surg* 2002; **18**: 53-57
4 **Yamaoka T**. Gene therapy for diabetes mellitus. *Curr Mol Med* 2001; **1**: 325-327
5 **Seto M**, Yamazaki T, Sonsda J, Matsumine A, Shinto Y, Uchida A. Suppression of tumor growth and pulmonary metastasis in murine osteosarcoma using gene therapy. *Oncol Rep* 2002; **9**: 337-340
6 **Englemann C**, Heslan JM, Fabre M, Lagarde JP, Klatzmann D, Panis Y. Importance, mechanisms and limitations of the distant bystander effect in cancer gene therapy of experimental liver tumors. *Cancer Lett* 2002; **179**: 59-69
7 **Okada Y**, Okada N, Nakagawa S, Mizuguchi H, Kanehira M, Nishino N, Takahashi K, Mizuno N, Hayakawa T, Mayumi T. Fiber-mutant technique can augment gene transduction efficacy and anti-tumor effects against established murine melanoma by cytokine-gene therapy using adenovirus vectors. *Cancer Lett* 2002; **177**: 57-63
8 **Ruiz J**, Mazzolini G, Sangro B, Qian C, Prieto J. Gene therapy of hepatocellular carcinoma. *Dig Dis* 2001; **19**: 324-332
9 **Li S**, Zhang X, Xia X. Regression of tumor growth and induction of long-term antitumor memory by interleukin 12 electro-gene therapy. *J Natl Cancer Inst* 2002; **94**: 762-768
10 **Manninen HI**, Makinen K. Gene therapy techniques for peripheral arterial disease. *Cardiovasc Intervent Radiol* 2002; **25**: 98-108
11 **Adachi O**, Nakano A, Sato O, Kawamoto S, Tahara H, Toyoda N, Yamato E, Matsumori A, Tabayashi K, Miyazaki J. Gene transfer of Fc-fusion cytokine by *in vivo* electroporation: application to gene therapy for viral myocarditis. *Gene Ther* 2002; **9**: 577-582
12 **Johnson-Saliba M**, Jans DA. Gene therapy: optimising DNA delivery to the nucleus. *Curr Drug Targets* 2001; **2**: 371-399
13 **Baum BJ**, Kok M, Tran SD, Yamano S. The impact of gene therapy on dentistry: a revisiting after six years. *J Am Dent Assoc* 2002; **133**: 35-44
14 **Havlik R**, Jiao LR, Nicholls J, Jensen SL, Habib NA. Gene therapy for liver metastases. *Semin Oncol* 2002; **29**: 202-208
15 **Li Y**, Okegawa T, Lombardi DP, Frenkel EP, Hsieh JT. Enhanced transgene expression in androgen independent prostate cancer gene therapy by taxane chemotherapeutic agents. *J Urol* 2002; **167**: 339-346
16 **Rieger PT**. The role of oncology nurses in gene therapy. *Lancet Oncol* 2001; **2**: 233-238
17 **Ohana P**, Bibi O, Matouk I, Levy C, Birman T, Ariel I, SchneiderY, Ayesh S, Giladi H, Laster M, De Groot N, Hochberg A. Use of H19 regulatory sequences for targeted gene therapy in cancer. *Int J Cancer* 2002; **98**: 645-650
18 **Huh WK**, Barnes MN, Kelley FJ, Alvarez RD. Gene therapy. *Cancer Treat Res* 2002; **107**: 133-157
19 **Liu SC**, Minton NP, Giaccia AJ, Brown JM. Anticancer efficacy of systemically delivered anaerobic bacteria as gene therapy vectors targeting tumor hypoxia / necrosis. *Gene Ther* 2002; **9**: 291-296
20 **Kaneko S**, Tamaoki T. Gene therapy vectors harboring AFP regulatory sequences. Preparation of an adenoviral vector. *Mol Biotechnol* 2001; **19**: 323-330
21 **Mc CF**. Cancer gene therapy: fringe or cutting edge? *Nature Rev Cancer* 2001; **1**: 130-141
22 **Chang LJ**, He J. Retroviral vectors for gene therapy of AIDS and cancer. *Curr Opin Mol Ther* 2001; **3**: 468-475
23 **Richardson PD**, Augustin LB, Kren BT, Steer CJ. Gene repair and transposon-mediated gene therapy. *Stem Cells* 2002; **20**: 105-118
24 **Xu AG**, Li SG, Liu JH, Gan AH. Function of apoptosis and expression of proteins Bcl-2, p53 and C-myc in the development of gastric cancer. *World J Gastroenterol* 2001; **7**: 403-406
25 **Roth JA**, Cristiano RJ. Gene therapy for cancer: what have we done and where are we going? *J Nat Cancer Inst* 1997; **89:** 21-39
26 **Brenner MK.** Genetic marking and manipulation of hematopoietic progenitor cells using retroviral vectors. *Immunomethod*s 1994; **5**: 204-210
27 **Mitani K,** Graham FL, Caskey CT. Transduction of human bone marrow by adenoviral vector. *Hum Gene Ther* 1994; **5**: 941-948
28 **Weber E**, Anderson WF, Kasahara N. Recent advances in retrovirus vector-mediated gene therapy: teaching an old vector new tricks. *Curr Opin Mol Ther* 2001; **3**: 439-453
29 **Pieroni L**, La Monica N. Towards the use of baculovirus as a gene therapy vector. *Curr Opin Mol Ther* 2001; **3**: 464-467
30 **Okada T**, Sakai T, Murata T, Kako K, Sakamoto K, Ohtomi M, Katsura T, Ishida N. Promoter analysis for daily expression of Drosophila timeless gene. *Biochem Biophys Res Commun* 2001; **283**: 577-582
31 **Lasic DD**, Vallner JJ, Working PK. Sterically stabilized liposomes in cancer therapy and gene delivery. *Curr Opin Mol Ther* 1999; **1**: 177-185
32 **Manthorpe M**, Cornefert-Jensen F, Hartikka J, Felgner J, Rundell A, Margalith M, Dwarki V. Gene therapy by intrmuscular injection of plasmid DNA: studies on firefly luciferase gene expression in mice. *Human Gene Therapy* 1993; **4**: 419-431
33 **Michael SI**, Curiel DT. Strategies to achieve targeted gene delivery via the receptor-mediated endocytosis pathway. *Gene Ther* 1994; **1**: 223-232
34 **Vanden Driessche T**, Naldini L, Collen D, Chuah MK. Oncoretroviral and lentiviral vector-mediated gene therapy. *Methods Enzymol* 2002; **346**: 573-579
35 **Gomez NJ**, Curiel DT. Conditionally replicative adenoviral vectors for cancer gene therapy. *Lancet Oncol* 2000; **1**: 148-158
36 **Simko V**, Michael S. Effect of ursodeoxycholic acid on *in vivo* and in vitro toxic liver injury in rats. *Aliment Pharmacol Ther* 1994; **8:** 315-322
37 **Hart SL**, Knight AM, Harbottle RP, Mistry A, Hunger HD, Cutler DF, Williamson R, Coutelle C. Cell binding and internalization by filamentous phage displaying a cyclic Arg-Gly-Asp-containing peptide. *J Bio Chem* 1994; **269**: 12468-12474
38 **Moolten FL**. Tumour chemosensitivity conferred by inserted herpes thymidine kinase genes: paradigm for a prospective cancer control strategy. *Cancer Research* 1986; **46**: 5276-5281
39 **Floeth FW**, Shand N, Bojar H, Prisack HB, Felsberg J, Neuen-Jacob E, Aulich A, Burger KJ, Bock WJ, Weber F. Local inflammation and devascularization-*in vivo* mechanisms of the "bystander effect" in VPC-mediated HSV-Tk / GCV gene therapy for human malignant glioma. *Cancer Gene Ther* 2001; **8**: 843-851
40 **Freeman SM**, Abboud CN, Whartenby KA, Packman CH, Koeplin DS, Moolten FL, Abraham GN. The "bystander effect": tumour regression when a fraction of the tumor mass is genetically modified. *Cancer Research* 1993; **53**: 5274-5283
41 **Huber BE**, Austin EA, Good SS, Knick VC, Tibbels S, Richards CA. *In vivo* antitumor activity of 5-fluorocytosine on human colorectal carcinoma cells genetically modified to express cytosine deaminase. *Cancer Research* 1993;**53**: 4619-4626
42 **Crystal RG,** Hirschowitz E, Lieberman M, Daly J, Kazam E, Henschke C, Yankelevitz D, Kemeny N< Silverstein R, Ohwada A, Russ T, Mastrangeli A, Sanders A, Cooke J, Harvey BG. Phase 1 study of direct administration of a replication deficient adenovirus vector containing the E. coli cytosine deaminase gene to metastatic colon carcinoma of the liver in association with the oral administration of the pro-drug 5-fluorocytosine. *Human Gene Therapy* 1997; **8**: 985-1001
43 **Milligan JF**, Matteucci MD, Martin JC. Current concepts in antisense drug design. *J Med Chem* 1993; **36**: 1923-1937
44 **Chen L**. Antibody gene therapy: old wine in a new bottle. *Nat Med* 2002; **8**: 333-334
45 **Tang YC**, Li Y, Qian GX. Reduction of tumorigenicity of SMMC-7721 hepatoma cells by vascular endothelial growth factor antisense gene therapy. *World J Gastroenterol* 2001; **7**: 22-27
46 **Sacco MG**, Barbieri O, Piccini D, Noviello E, Zoppe M, Zucchi I, Frattini A, Villa A, Vezzoni P. *In vitro* and *in vivo* antisense-me-

diated growth inhibition of a mammary adenocarcinoma from MMTV-neu transgenic mice. *Gene Therapy* 1998; **5**: 388-393

47 **Wagner RW.** Gene inhibition using antisense oligodeoxynucleotides. *Nature* 1994; **372**: 333-335

48 **Cotter FE**, Johnson P, Hall P, Pocock C, Al Mahdi N, Cowell JK, Morgan G. Antisense oligonucleotides suppress B-cell lymphoma growth in a SCID-hu mouse model. *Oncogene* 1994; **9**: 3049-3055

49 **Kumai T**, Tateishi T, Tanaka M, Watanabe M, Shimizu H, Kobayashi S. Tyrosine hydroxylase antisense gene therapy causes hypotensive effects in the spontaneously hypertensive rats. *J Hypertens* 2001; **19**: 1769-1773

50 **Marchand GS**, Noiseux N, Tanguay JF, Sirois MG. Blockade of *in vivo* VEGF-mediated angiogenesis by antisense gene therapy: role of Flk-1 and Flt-1 receptors. *Am J Physiol Heart Circ Physiol* 2002; **282**: H194-204

51 **Tsang KY**, Zaremba S, Nieroda CA, Zhu MZ, Hamilton JM, Scholm J. Generation of human cytotoxic T cells specific for human carcinoembryonic antigen epitopes from patients immunised with recombinant vaccinia-CEA vaccine. *J Nat Cancer Inst* 1995; **87**: 982-990

52 **Cheng WF**, Hung CF, Hsu KF, Chai CY, He L, Polo JM, Slater LA, Ling M, Wu TC. Cancer immunotherapy using Sindbis virus replicon particles encoding a VP22-antigen fusion. *Hum Gene Ther* 2002; **13**: 553-568

53 **Wei MX**, Tamiya T, Hurford JR, Boviatsis EJ, Tepper RI, Chiacca EA. Enhancement of interleukin-4-mediated tumour regression in athymic mice by in situ retroviral gene transfer. *Human Gene Therapy* 1995; **6**: 437-443

54 **Steele RJ**, Thompson AM, Hall PA, Lane DP. The p53 tumour suppressor gene. *British J Surgery* 1998; **85**: 1460-1467

55 **Nishioka Y**, Hua W, Nishimura N, Sone S. Genetic modification of dendritic cells and its application for cancer immunotherapy. *J Med Invest* 2002; **49**: 7-17

56 **Takemura S**, Kudo T, Asano R, Suzuki M, Tsumoto K, Sakurai N, Katayose Y, Kodama H, Yoshida H, Ebara S, Saeki H, Imai K, Matsuno S, Kumagai I. A mutated superantigen SEA D227A fusion diabody specific to MUC1 and CD3 in targeted cancer immunotherapy for bile duct carcinoma. *Cancee Immunol Immunother* 2002; **51**: 33-44

57 **Vonderheide RH**. Telomerase as a universal tumor-associated antigen for cancer immunotherapy. *Oncogene* 2002; **21**: 674-679

58 **Schadendorf D**. The use of histamine in cancer immunotherapy. *J Invest Dermatol* 2002; **118**: 560-561

59 **Ohashi M**, Kanai F, Ueno H, Tanaka T, Tateishi K, Kawakami T, Koike Y, Ikenoue T, Shiratori Y, Hamada H, Omata M. Adenovirus mediated p53 tumour suppressor gene therapy for human gastric cancer cells *in vitro* and *in vivo*. *Gut* 1999; **44**: 366-371

60 **Okino T**, Onda M, Matsukura N, Inada KI, Tatematsu M, Suzuki S, Shimada T. Sequential histopathological changes *in vivo* after suicide gene therapy of gastric cancer induced by N-methyl-N'-nitro-N-nitrosoguanidine in rats. *Jpn J Cancer Res* 2001; **92**: 673-679

61 **Shimizu T**, Shimada H, Ochiai T, Hamada H. Enhanced growth suppression in esophageal carcinoma cells using adenovirus-mediated fusion gene transfer (uracil phosphoribosyl transferase and herpes simplex virus thymidine kinase). *Cancer Gene Ther* 2001; **8**: 512-521

62 **Yu ZC**, Ding J, Pan BR, Fan DM, Zhang XY. Expression and bioactivity identification of soluble MG7 scFv. *World J Gastroenterol* 2002; **8**: 99-102

63 **Robbins PD**, Evans CH, Chernajovsky Y. Gene therapy for arthritis. *Gene Ther* 2003; **10**: 902-911

64 **Baker D**, Hankey DJ. Gene therapy in autoimmune, demyelinating disease of the central nervous system. *Gene Ther* 2003; **10**: 844-853

65 **Thomas CE**, Ehrhardt A, Kay MA. Progress and problems with the use of viral vectors for gene therapy. *Nat Rev Genet* 2003; **4**: 346-358

66 **Bagley J**, Iacomini J. Gene Therapy Progress and Prospects: Gene therapy in organ transplantation. *Gene Ther* 2003; **10**: 605-711

67 **Guarini S.** New gene therapy for the treatment of burn wounds. *Crit Care Med* 2003; **31**: 1280-1281

68 **Kaneda Y**. Gene therapy: a battle against biological barriers. *Curr Mol Med* 2001; **1**: 493-499

69 **Denny WA**. Prodrugs for gene-firected rnzyme-prodrug therapy (suicide gene therapy). *J Biomed Biotechnol* 2003; **2003**:48-70

70 **Scholl SM**, Michaelis S, McDermott R. Gene Therapy Applications to Cancer Treatment. *J Biomed Biotechnol* 2003; **2003**: 35-47

71 **Lundstrom K**. Latest development in viral vectors for gene therapy. *Trends Biotechnol* 2003; **21**: 117-122

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