Journal of BUON **10:** 167-173, 2005

Gene therapy of cancer

T. Mitrović[1], S. Radulović[2]

[1]Faculty of Sciences and Mathematics, Department of Biology and Ecology, Niš; [2]National Cancer Research Center, Laboratory of Experimental Oncology, Beograd, Serbia and Montenegro

Tatjana Mitrović, PhD
Faculty of Sciences and Mathematics, Department of Biology and Ecology, Niš, Serbia and Montenegro

Summary

The original concept of gene therapy was the introduction of a healthy copy of gene into an ill human cell in order to correct gene defects in monogenic hereditary diseases. Since then the idea of gene therapy was expanded to cure or slow down the progression of numerous inherited and acquired diseases. Presently, there are 918 ongoing gene therapy clinical trials worldwide. The major indication in these trials is cancer (608 trials or 66% of the total number).

Gene therapy of cancer can be defined as transfer of nucleic acids into tumor or normal cells aiming at eradicating or reducing the tumor mass by direct killing of cells, immunomodulation or correction of genetic errors and reversion of the malignant status. Initially started with lots of optimism and enthusiasm, cancer gene therapy has shown limited success in the treatment of patients. This lesson highlights current limitations and almost endless possibilities of cancer gene therapy.

The major difficulty in advancing gene therapy technology from the lab bench to clinical practice is the problem with gene delivery vehicles (so-called vectors) needed to ferry genetic material into a cell. Despite few reports of therapeutic responses in some patients, there is still no proof of clinical efficacy of most cancer gene therapy approaches, primarily due to very low transduction and expression efficacy in vivo of available vectors. An "ideal" gene therapy vector should: be administered through a noninvasive route; target not only the primary tumor mass but disseminated tumor cells and micrometastases at distant and unreachable sites as well; carry a therapeutic gene with tumor-restricted and time-regulated and sustained expression.

Current strategies for combating cancer with gene therapy can be subdivided into 4 basic concepts: 1) replacement of missing tumor suppressor gene and/or blocking of oncogenes or proinflammatory genes; 2) suicide gene strategies; 3) induction of immune-mediated destruction; and 4) inhibition of tumor angiogenesis. Clinical advance will probably come first from cooperation with standard cancer treatment such as chemotherapy, radiotherapy and immunotherapy.

Key words: cancer, cancer gene therapy, gene, targeted gene therapy

Introduction

Cancer is a major life-threatening disease. The latest data for the European population reveals that almost 6 million people are currently living with

cancer. During this year more than 3 million new cases will be diagnosed and 2 million people will die because of ineffective traditional treatment (surgery, chemotherapy or radiation therapy) [1]. A promising alternative has been found in gene therapy. The idea of gene therapy dates back in the 1960's and at first it was meant to treat monogenic hereditary diseases by intentional insertion of a healthy copy of gene into affected human cells and correction of the defected gene [2,3]. Later this concept was expanded to cure or slow down the progression of numerous inherited and acquired diseases. Presently, cancer is the major indication in gene therapy clinical trials (608 out of 918 gene therapy trials or 66%) [2].

Gene therapy is a rational approach to the direct attack of cancer cells based on their molecular characteristics and defects. Two tools constitute cancer gene therapy approach – the vector for transfer of therapeutic gene(s) into the cancer cell and the therapeutic gene(s). Potentials of current gene therapy vectors and problems with efficient and targeted expression of therapeutic gene(s) into cancer cells are major topics of this lesson.

Molecular therapeutic strategies such as gene replacement/mutation correction, "suicide" gene therapy, immune modulation and antiangiogenesis may offer unique and novel ways of fighting cancer.

Vectors

The major difficulty in advancing gene therapy technology from the lab bench to patients is the very low transduction and expression efficacy *in vivo* of the available vectors.

One of the most promising areas of vector development are the non-viral vectors, which consist of liposomes, molecular conjugates and naked DNA delivered by mechanical methods [4]. Non-viral systems tend to be comparatively less efficient than viral systems, but they have the inherent advantage of flexibility and safety.

On the other hand, viral vectors are the most frequently used at the moment. Three main classes of clinically applicable viral vectors (replication-incompetent, hybrid, and replication-competent) are available [5,6].

The first class of vectors (replication- incompetent or replication- defective vectors) are genetically altered viruses that function simply like a shuttle to the cells with a single round of infection either integrating or transiently expressing the transgene without subsequent viral replication. The most advanced vectors ready for clinical use are retrovirus- and adenovirus-derived vectors (Table 1).

Retroviral vectors derived from murine C- oncoretroviruses were the first vectors in gene therapy and still remain the most frequently applied [2]. They have the ability to integrate into the cell genome providing long-term expression of the therapeutic gene. However, their random integration could be associated with risk of insertional activation of cellular oncogenes (2 leukemia cases in the French gene therapy trial of X-linked severe combined immunodeficiency (SCID-X1) [7]. Also, there is a problem with low titre production and limited capacity for therapeutic gene (8 kb) [5].

For clinical application, retroviral vectors showed remarkable success in the treatment of monogenic hereditary disorders which require lifetime production of the affected gene. Fischer and co-workers in Necker Hospital for Children in Paris used retroviral vector to transfer a healthy gene encoding γ chain into the bone marrow of children with a rare, lethal immune disorder (SCID-X1) and achieved effective and life-saving immune reconstitution in 10 out of 11 patients enabling them to leave a protective bubble for

Table 1. Comparative analysis of retroviral and adenoviral vectors

Features	*Retrovirus*	*Adenovirus*
Number of gene therapy trials (%)	254 (28)	240 (26)
Maximum capacity for therapeutic gene	8 kb	36 kb
Integration	Yes	No
Drawbacks	Inability to infect non-dividing cells, random integration of its genome with associated risk of insertional mutagenesis, problems with low titre production, limited capacity for therapeutic gene and possibility of generation of new recombinant replication-competent retrovirus (RCR)	Short-term expression of therapeutic gene due to inability to integrate into host genome, high immunogenicity

the first time [8]. Follow-up observation showed that sustained production of the transgenic protein lasted up to 30 months [9]. Unfortunately, a serious adverse event (T-cell leukemia and T-cell lymphoproliferative disorder) developed in 2 out of 10 cured children due to abnormal expression of LMO-2 gene, triggered by the insertion of the retroviral vector, which darkened the primary success of this pioneering gene therapy treatment [7,10].

Application of retroviral vectors in the treatment of cancer showed extremely encouraging preclinical results, but their clinical utility has not been proved yet. For instance, a phase III clinical trial of retroviral delivery of the herpes simplex virus thymidine kinase (HSV-TK) gene to 248 patients with glioblastoma failed due to the low tumor cell transduction efficiency [11,12].

Adenoviral vectors are the second most commonly used vectors in gene therapy trials [2]. They originate from adenoviruses known for their low pathogenicity in humans, causing only mild symptoms associated with the common cold. Adenoviral vectors are able to infect both dividing and non-dividing cells and can be produced at high viral titres. They can accommodate relatively large segments of foreign DNA (up to 36 kb-long in "gutless" vectors) [13]. However, transgene is transported to the host nucleus, but not inserted into the host genome, a fact that makes its expression temporary. In addition, adenoviral particles stimulate strong immune reactions that clear the vector from the body, making long-term therapy impossible. Therefore, high doses and repeated administration of the adenoviral vector are required for continued transgene expression. Unfortunately, a phase I gene therapy clinical trial of ornithine transcarbamylase (OTC) deficiency at the University of Pennsylvania (USA) with intrahepatic administration of the highest dose ever received by human of recombinant adenoviral vector (6×10^{11} particles/kg) containing OTC, caused systemic inflammatory response syndrome, multiple-organ system failure and death of a 18-year-old boy, Jesse Gelsinger, on September 17, 1999 [14, 15]. This is the only death in the 15-year history of gene therapy trials [16].

Efficient readministration of adenoviral vectors was demonstrated in several studies. In a phase I/II trial for recurrent ovarian cancer with intraperitoneal readministration, transgene expression was measurable in 17 of 20 samples obtained after 2 or 3 cycles [17].

The second class of gene delivery vehicles (hybrid or chimeric vectors), combine the favorable properties of established viral vector systems. For example, hybrids between adenoviruses and retroviruses with the best features of both components are being developed [18]. The area of synthetic, hybrid systems is the most challenging.

The third class of viral vectors (replication-competent, replication-selective, conditionally replicating, oncotropic or oncolytic viruses) relies on a property of the tumor cell (such as loss of tumor suppressor function) which makes it uniquely susceptible to productive infection with the virus [19]. The best known oncolytic virus and first successfully tested in humans is ONYX-015, also known as CI-1042 and dl1520 (Onyx Pharmaceuticals, Pfizer Corp, USA) [20,21]. This oncolytic adenovirus with an E1B-55kD gene deletion selectively targets, replicates within and destroys p53-negative tumor cells by oncolysis, sparing the surrounding normal tissue. The fact that p53 is deleted or mutated in >50 % of all human cancers makes this system an adequate anticancer agent [22]. Phase I and II clinical trials with ONYX-015 as single anticancer agent in patients with recurrent or refractory squamous cell carcinoma of the head and neck have shown durable responses and clinical benefit in 14-21% of these end-stage patients [23]. In combination with chemotherapy (cisplatin and 5-fluorouracil), however, encouraging antitumor activity has been demonstrated. Objective response (i.e. at least a 50% reduction in tumor size) was detected in 19 cases (63%), with 8 (27%) complete responses (i.e. complete disappearance of measurable tumor) and 11 (36%) partial responses (i.e. decrease in cross-sectional tumor area of 50-99%) [24]. Nowadays, ONYX-015 is under evaluation in a phase III study in patients with head and neck cancer performed by Onyx Pharmaceuticals and Pfizer. In the last 3 years, ONYX-015 was tested as monotherapy and in combination with chemotherapy in phase I and II trials in the treatment of colorectal, hepatobiliary, hepatocellular, ovarian and pancreatic carcinomas, liver metastasis from gastrointestinal malignancies and lung metastasis from adrenals, colon, head and neck and thyroid carcinomas [25].

No perfect vector system has yet been created. In terms of clinical application, the "ideal" vector should be safely administered through a noninvasive route, transducing only the desired cells within the target tissue. This vector should be loaded with tumor-restricted and time-regulated expressed therapeutic gene(s). Another major restriction in treating cancer with gene therapy is the limitation in specifically targeting tumor cells, especially cells that have entered systemic circulation.

There are two major strategies of targeting vectors to cancer cells only. The first one, targeted delivery, cellular targeting or transductional targeting, is achieved

by modification of viral envelopes and capsids (chimeric envelopes, pseudotyping, molecular conjugation with specific antibodies or ligands, etc.) which restrain their interaction with specific antigens overexpressed on tumor cells [26, 27]. The second principle, so-called targeted expression or transcriptional targeting, restricts the expression of the therapeutic gene to tumor cells by placing this gene under specific internal or external control. Internal control of therapeutic gene expression is achieved by application of tissue-specific promoters and enhancers (human mammaglobin-1 (SCGB2A2) promoter/enhancer and midkine (MK) and c-erbB-2 promoters for breast carcinomas; prostate-specific antigen (PSA), human glandular kallikrein (KLK2) and rat probasin (rPB) promoters and/or enhancers for prostate cancer; secretory leukoprotease inhibitor (SLPI) promoter and L-plastin promoter for ovarian cancer, etc) [28-31]. External control of therapeutic gene expression means its fine modulation by small molecules in pharmacologically regulated systems (tetracycline in Tet-ON system), physiological signals in physiologically regulated systems (glucose deprivation - promoter of glucose-regulated proteins (GRP78), chronic hypoxia - hypoxia response element (HRE)/ hypoxia-inducible factor (HIF) system, etc) or radiation in radiation-inducible systems (early growth response 1 (Egr-1) and WAF-1 promoter radiation-inducible promoters) [32-35].

Therapeutic genes

A broad spectrum of therapeutic genes is currently available. Selection of an appropriate therapeutic gene, i.e. therapeutic strategy, is a crucial step in designing vectors in which some of the above mentioned limitations can be compensated. We could choose among the following approaches: gene replacement of mutated tumor suppressor genes, introduction of prodrug genes for suicide induction, immunomodulation by addition of cytokine genes or dendritic cells and destruction of tumor vasculature (Table 2).

Expression of tumor suppressor genes in tumor cells causes cell cycle arrest and/or apoptosis, even though such cells harbor many other genetic changes. Since the first clinical trial in 1996, at least 20 clinical trials of p53 gene replacement, either alone or in combination with chemotherapy, were performed with limited success [25]. The most promising are: INGN 201 or ADVEXIN (Ad5CMV-p53 vector, Introgen Therapeutics, USA) and SCH 58500 (rAd-p53, Canji) currently in phase III in patients with refractory head and neck cancer and stage III ovarian cancer, respectively [36-38]. Other tumor suppressor genes, such as retinoblastoma (Rb), PTEN (phosphatase, tenesin homologue), mda-7 (melanoma differentiation associated gene -7) and OPCML (opioid binding protein/cell adhesion molecule-like gene) are under evaluation, too [25].

Table 2. Various cancer gene therapy strategies and examples of therapeutic genes

Therapeutic strategy	*Therapeutic gene*	*Examples*
Gene replacement therapy	Tumor-suppressor gene	p53, PTEN, mda-7, OPCML, etc
Suicide gene therapy		
Toxin gene therapy	Toxin gene	Pseudomonas gene for exotoxin, diphtheria toxin A (DTA) gene, etc.
GDEPT therapy*	Suicide gene	Herpes simplex virus thymidine kinase/ganciclovir (HSV-TK/GCV), E. coli cytosine deaminase (CD), E. coli uracil phosphoribosyltransferase (URPT), E. coli xanthine-guanine phosphoribosyltransferase (XGPRT), rabbit cytohrome P450 4B1 (CYP4B1), etc.
Immunogene therapy		
Cytokine gene therapy	Cytokine gene	Interleukins (IL-2, IL-4, IL-6, IL-7, IL-12), tumor necrosis factor α (TNF-α), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon γ (INFγ), etc.
Anti-angiogenic gene therapy	Extracellular matrix remodelators	TIMPs
	Angiogenic growth factors	VEGF, basic fibroblast growth factor (bFGF), etc.
	Angiogenesis inhibitors	Angiostatin, endostatin, vasostatin, thrombospondin-1, etc.

*gene-directed enzyme/prodrug therapy

The self-renewal potential of malignant tumors dictates that tumor cells should be eradicated as efficiently as possible rather than genetically corrected. The most frequently used strategies are induction of suicide of tumor cells (suicide gene therapies) and stimulation of the patients' immune system to destroy tumor cells (immunogene therapies).

Suicide gene therapies can be subdivided into two types. The first one, toxin gene therapy, kills tumor cells directly by cytotoxic protein product. For example, the gene for *Pseudomonas* exotoxin causes regression of established human tumors in xenografted models [39]. The second type of suicide gene therapies, gene-directed enzyme / prodrug therapy (GDEPT), is a two-step approach. In the first step, the therapeutic gene is delivered into the tumor and expressed. The second step implies systemic administration of a harmless prodrug and its selective conversion into a potent cytotoxic drug by an enzyme, product of the therapeutic gene. Only cells bearing the suicide gene will be killed upon the subsequent prodrug treatment. The most widely used enzyme / prodrug system is herpes simplex virus thymidine kinase/ganciclovir (HSV-TK/GCV) [40].

Indirect killing of tumor cells by induction of antitumor immune response (immunogene therapy) can be achieved in different ways. These include transferring genes encoding proinflammatory proteins to tumor cells, suppressing immunosuppressive gene expression, and transferring proinflammatory genes and/or tumor antigen genes to professional antigen-presenting cells [41]. Syngeneic bone marrow-derived dendritic cells (DCs) are often used for induction of specific T-cell responses, alone or in combination with vectors [42]. An example for this is the combination therapy of glioma with interferon β (INF β) gene vector and DCs, reported as superior to treatment with INF β gene vector and DCs solely [43].

Blocking the process of tumor vasculature formation (neoangiogenesis) is an alternative approach to prevent tumor progression and metastasis (antiangiogenic gene therapy). It involves various targets at the interface between the malignant population, supporting stroma and new capillaries in formation from pre-existing blood vessels [44]. For instance, the migration of tumor endothelium can be inhibited by interfering with matrix metalloproteinases (MMPs) and their unique ability to degrade extracellular matrix (EMC). Another target can be a critical mediator of tumor vascularization known as vascular endothelial growth factor (VEGF). VEGF is also a key factor produced by solid tumors to inhibit recognition and destruction of tumor cells by the immune system. In addition, genes for naturally circulating factors capable of suppressing angiogenesis, such as thrombospondin-1, endostatin, angiostatin and vasostatin, can be delivered and overexpressed in tumor cells.

Also, different transductional and transcriptional approaches have been applied to destroy tumor vasculature, e.g. endothelial cells (EC) targeting. For example EC specific promoters, such as endoglin, endothelin and von Willebrand factor promoters were preclinically tested [44].

Conclusions

Gene therapy is a rapidly evolving concept of molecular treatment of different forms of cancer. Although preclinical results have been extremely encouraging, many practical obstacles need to be overcome before gene therapy fulfil its goal in the clinic. Future research should be focused on increasing the transduction efficiency of non-viral vectors, modifying viral vectors to reduce toxicity and immunogenicity, enhancing vector targeting and specificity, regulating gene expression and identifying synergies between gene-based agents and other cancer therapeutics.

Clinical trials have nevertheless produced a substantial amount of data and have contributed to the continuous improvement of vector systems, delivery methods and clinical protocols. The death of Jessie Gelsinger and the Paris leukemia cases at first meant a temporary hold on research to allow time to gather and evaluate new data, to make improvements in the design and safety of the vectors, to re-evaluate the ethical acceptability of the research, but soon it proceeded with trials with caution. In both cases, limited alternatives for these dying patients motivated efforts to continue with gene transfer research.Direct injection of vectors containing therapeutic genes resulted in regression of the tumor at the injection site but did not affect tumor cells at distant sites. Future wide clinical application demands safe systematic administration of vectors with selective and effective gene therapeutic action not only against the primary tumor mass, but also towards distant sites of disseminated tumor cells and micrometastases.

Conditionally replicating viruses (i.e. virotherapy) offer a powerful weapon in the clinical armamentarium against cancer. Therapy with oncolytic viruses seems to hold more promise in early clinical trials than gene therapy with non-replicating virus vectors. Some of them, such as ONYX-015, enter phase III clinical trials. However, further major advancements in virus designs, application modalities and understanding of

the host's immune system with the virus are clearly needed before oncolytic virus therapy can be introduced into clinical practice.

Development of noninvasive imaging technologies (positron emission tomography-PET, single-photon emission computed tomography-SPECT, magnetic resonance imaging-MRI etc.) for the determination of the efficiency of vector delivery and therapeutic gene expression will shorten the evaluation procedures for cancer gene therapy in patients [45]. Also, the latest microarray technologies for molecular profiling of cancer cells will reveal potential targets for cancer gene therapy and allow patient-tailored therapy in the molecular medicine of the future.

Combinations of non-replicating and replicating viruses, different gene therapies (toxin and immunogene therapy, suicide and immunogene therapy, immunogene and antiangiogenic therapy, etc) and gene therapies with standard antitumor therapies-chemotherapy and radiotherapy (virotherapy and chemotherapy, antiangiogenic therapy and chemotherapy, suicide and radiotherapy, etc) give signs of enhanced effectiveness in the treatment of cancer [24,39,46-50].

References

1. Sancho-Garnier H, Levi F, Pisani P, Lynge E. Evidence-based cancer prevention: strategies for NGOs. In: Sancho-Garnier H, Anderson A, Biedermann A, Lynge E, Slama K, de Sabata M, Rajeswaran L (eds): Europe's cancer burden. John Wiley & Sons, Indianapolis, USA, 2004, pp 16-33.
2. Edelstein ML, Abedi MR, Wixon J, Edelstein RM. Gene therapy clinical trials worldwide 1989-2004 - an overview. J Gene Med 2004; 6: 597-602.
3. Mountain A. Gene therapy: the first decade. TIBTECH 2000; 18: 119-128.
4. Niidome T, Huang L. Gene therapy progress and prospects: Nonviral vectors. Gene Ther 2002; 9: 1647-1652.
5. Kay MA, Glorioso JC, Naldini L. Viral vectors for gene therapy: the art of turning infectious agents into vehicles of therapeutics. Nat Med 2001; 7: 33-40.
6. Nemunaitis J, Lin E. Oncolytic viral therapies. Cancer Gene Ther 2004; 11: 643-664.
7. Hacein-Bey-Abina S, Von Kalle C, Schmidt M et al. *LMO2*-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. Science 2003; 302: 415-419.
8. Cavazzana-Calvo M, Hacein-Bey S, de Saint Basile G et al. Gene therapy of human severe combined immunodeficiency (SCID) – X1 disease. Science 2000; 288: 669-672.
9. Hacein-Bey-Abina S, Le Deist F, Carlier F et al. Sustained correction of X-linked severe combined immunodeficiency by ex vivo gene therapy. N Engl J Med 2002; 346: 1185-1193.
10. Hacein-Bey-Abina S, Von Kalle C, Schmidt M et al. A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency. N Engl J Med 2003; 348: 255-256.
11. Rainov NG. A phase III clinical evaluation of herpes simplex virus type 1 thymidine kinase and ganciclovir gene therapy as an adjuvant to surgical resection and radiation in adults with previously untreated glioblastoma multiforme. Hum Gene Ther 2000; 11: 2389-2401.
12. Rainov NG, Ren H. Clinical trials with retrovirus mediated gene therapy – what have we learned? J Neurooncol 2003; 65: 227-236.
13. St George JA. Gene therapy progress and prospects: adenoviral vectors. Gene Ther 2003; 10: 1135-1141.
14. Raper SE, Yudkoff M, Chirmule N et al. A pilot study of in vivo liver-directed gene transfer with an adenoviral vector in partial ornithine transcarbamylase deficiency. Hum Gene Ther 2002; 13: 163-175.
15. Raper SE, Chirmule N, Lee FS et al. Fatal systemic inflammatory response syndrome in a ornithine transcarbamylase deficient patient following adenoviral gene transfer. Mol Genet Metab 2003; 80: 148-158.
16. Marshall E. Gene therapy on trial. Science 2000; 288: 951-957.
17. Buller RE, Runnebaum IB, Karlan BY et al. A phase I/II trial of rAd/p53 (SCH 58500) gene replacement in recurrent ovarian cancer. Cancer Gene Ther 2002; 9: 553-566.
18. Jevremovic D, Gulati R, Hennig I et al. Use of blood outgrowth endothelial cells as virus-producing vectors for gene delivery to tumors. Am J Physiol Heart Circ Physiol 2004; 287: 494-500.
19. Connolly JB. Conditionally replicating viruses in cancer therapy. Gene Ther 2003; 10: 712-715.
20. Heise C, Sampson-Johannes A, Williams A et al. ONYX-015, an E1B gene-attenuated adenovirus, causes tumor-specific cytolysis and antitumoral efficacy that can be augmented by standard chemotherapeutic agents. Nat Med 1997; 3: 639-645.
21. Cohen EE, Rudin CM. ONYX-015. Onyx Pharmaceuticals. Curr Opin Invest Drugs 2001; 2: 1770-1775.
22. Zhu J, Gao B, Zhao J, Balmain A. Targeting gene expression to tumor cells with loss of wild-type p53 function. Cancer Gene Ther 2000; 7: 4-12.
23. Kirn D. Oncolytic virotherapy for cancer with the adenovirus dl1520 (Onyx-015): results of phase I and phase II trials. Expert Opin Biol Ther 2001; 1: 525-538.
24. Khuri FR, Nemunaitis J, Ganly I et al. A controlled trial of intratumoral ONYX-015, a selectively replicating adenovirus, in combination with cisplatin and 5-fluorouracil in patients with recurrent head and neck cancer. Nat Med 2000; 6: 879-885.
25. McNeish IA, Bell SJ, Lemoine NR. Gene therapy progress and prospects: cancer gene therapy using tumor suppressor genes. Gene Ther 2004; 11:497-503.
26. Haynes C, Erlwein O, Schnierle BS. Modified envelope glycoproteins to retarget retroviral vectors. Curr Gene Ther 2003; 3: 405-410.
27. Mizuguchi H, Hayakawa T. Targeted adenovirus vectors. Hum Gene Ther 2004; 15: 1034-1044.
28. Shi CX, Long MA, Liu L, Graham FL, Gauldie J, Hitt MM. The human SCGB2A2 (mammaglobin-1) promoter/enhancer in a helper-dependent adenovirus vector directs high levels of transgene expression in mammary carcinoma cells but not in

normal nonmammary cells. Mol Ther 2004;10: 758-767.
29. Yu L, Yamamoto N, Kadomatsu K et al. Midkine promoter can mediate transcriptional activation of a fused suicide gene in a broader range of human breast cancer compared with c-erbB-2 promoter. Oncology 2004; 66:143-149.
30. Tsui KH, Wu L, Chang PL, Hsieh ML, Juang HH. Identifying the combination of the transcriptional regulatory sequences on prostate specific antigen and human glandular kallikrein genes. J Urol 2004; 172: 2029-2034.
31. Casado E, Gomez-Navarro J, Yamamoto M et al. Strategies to accomplish targeted expression of transgenes in ovarian cancer for molecular therapeutic applications. Clin Cancer Res 2001; 7: 2496-2504.
32. Toniatti C, Bujard H, Cortese R, Ciliberto G. Gene therapy progress and prospects: transcription regulatory systems. Gene Ther 2004; 11: 649-665.
33. Gazit G, Hung G, Chen X, Anderson WF, Lee AS. Use of the glucose starvation inducible glucose-regulated protein 78 promoter in suicide gene therapy of murine fibrosarcoma. Cancer Res 1999; 59: 3100-3106.
34. Binley K, Askham Z, Martin L et al. Hypoxia-mediated tumor targeting. Gene Ther 2003; 10: 540-549.
35. Scott SD, Joiner MC, Marples B, Optimizing radiation-responsive gene promoters for radiogenic cancer therapy. Gene Ther 2002; 9: 1396-1402.
36. Zhang JY. Apoptosis-based anticancer drugs. Nat Rev 2002; 1: 101-102.
37. Brukner I. INGN-201. Introgen Therapeutics. Curr Opin Investig Drugs 2001; 2: 1776-1785.
38. Barnard DL. Technology evaluation: Sch-58500, Canji. Curr Opin Mol Ther 2000; 2: 586-592.
39. Kawakami K, Kawakami M, Puri RK. Specifically targeted killing of interleukin-13 (IL-13) receptor-expressing breast cancer by IL-13 fusion cytotoxin in animal model of human disease. Mol Cancer Ther 2004; 3:137-147.
40. Fillat C, Carrio M, Cascante A, Sangro B. Suicide gene therapy mediated by the herpes simplex virus thymidine kinase gene/ganciclovir system: fifteen years of application. Gene Ther 2003; 3: 13-26.
41. Simpson E. Immunotherapy and gene therapy. IDrugs 2004; 7: 105-108.
42. Lemoli RM, Curti A, Fogli M, Ferri E, Baccaranni M. The therapeutic role of dendritic cells in cancer immunotherapy. Hematologica 2002; 87: 62-66.
43. Nakahara N, Pollack IF, Storkus WJ, Wakabayashi T, Yoshida J, Okada H. Effective induction of antiglioma cytotoxic T cells by coadministration of interferon-beta gene vector and dendritic cells. Cancer Gene Ther 2003; 10: 549-558.
44. Tandle A, Blazer DG 3rd, Libutti SK. Antiangiogenic gene therapy of cancer: recent developments. Transl Med 2004; 2: 2-22.
45. Min JJ, Gambhir SS. Gene therapy progress and prospects: noninvasive imaging of gene therapy in living subjects. Gene Ther 2004; 11: 115-125.
46. Nagayama Y, Nakao K, Mizuguchi H, Hayakawa T, Niwa M. Enhanced antitumor effect of combined adenovirus and nonreplicative adenovirus expressing interleukin-12 in an immunocompetent mouse model. Gene Ther 2003; 1: 1400-1403.
47. Morini M, Albini A, Lorusso G et al. Prevention of angiogenesis by naked DNA IL-12 gene transfer: angioprevention by immunogene therapy. Gene Ther 2004; 11: 284-291.
48. Quist SR, Wang-Ghorke S, Kohler T, Kreienberg R, Runnebaum IB. Cooperative effect of adenoviral p53 gene therapy and standard chemotherapy in ovarian cancer cells independent of the endogenous p53 status. Cancer Gene Ther 2004; 11: 547-554.
49. Mavria G, Harrington KJ, Marshall CJ, Porter CD. In vivo efficacy of HSV-TK transcriptionally targeted to the tumour vasculature is augmented by combination with cytotoxic chemotherapy. Gene Med 2004; [E-pub ahead of print].
50. Kanazava T, Mizukami H, Okada T et al. Suicide gene therapy using AAV-HSVtk/ganciclovir in combination with irradiation results in regression of human head and neck cancer xenografts in nude mice. Gene Ther 2003; 10: 51-58.

Correspondence to:

Tatjana Mitrović, PhD
Faculty of Sciences and Mathematics
Department of Biology and Ecology
33, Višegradska,
18000 Niš
Serbia and Montenegro
Tel: +381 18 533 015, ext. 23
Fax: +381 18 533 014
E-mail: tatjanamitrovic@hotmail.com