Available online at www.ijpsdr.com

**International Journal of Pharmaceutical Sciences and Drug Research 2015; 7(6): 444-450**

Review Article

**ISSN: 0975-248X**
**CODEN (USA): IJPSPP**

# Gene Therapy of Cancerous Diseases

**Valenčáková, A.*, Dziaková, A., Hatalová, E.**

*University of Veterinary Medicine and Pharmacy, Komenského 73, Košice, Slovak Republic*

**ABSTRACT**

Gene therapy of cancerous diseases provides new means of curing patients with oncologic illnesses. There are several approaches in treating cancer by gene therapy. Most commonly used methods are: cancer immunogene therapy, suicide gene therapy, application of tumor-suppressor genes, antiangiogenic therapy, mesenchymal stem cells used as vectors, gene directed enzyme/prodrug therapy and bacteria used as anti-cancer agents. Cancer gene immunotherapy uses several immunologic agents for the purpose of explaining effective anti-tumor immune response. Another method is suicide gene therapy, based on introducing viral or bacterial agents to tumor cells, allowing the conversion of a non-toxic compound to a lethal medication. The application of intact suppressor genes to cancer cells will avert their neoplastic behavior and will induce tumor regression. Inhibition of angiogenesis is also a promising strategy for treating oncologic patients. Mesenchymal stem cells can also be used as vectors in targeted gene therapy. An increasing list of experimental evidence shows, that therapeutically modified mesenchymal stem cells in "gene directed enzyme/prodrug therapy" can attack cancer tissue can kill tumor cells, cancer stem cells included. Bacteria are used as anti-cancer agents independently of in combination with conventional therapeutic methods.

**Keywords:** Cancer, gene therapy, tumor-suppressor genes, antiangiogenic therapy, mesenchymal stem cells, gene directed enzyme/prodrug therapy.

## INTRODUCTION

Chemotherapy, radiotherapy and surgery represent the conventional means of treating cancerous diseases, from which complete surgical resection is the most effective method for treating oncologic patients. [1] Current conventional therapy, for deficient tumor selectivity, can lead to the destruction of healthy tissue and has serious side effects in the patient; beside this it is limited in effectiveness and the quantity of the used dose. For these reasons it is urgent to seek alternative methods for the treatment of cancerous diseases, which localize the therapeutic agent to the tumor localization and bypass healthy tissue. [2] Gene therapy presents an adequate alternative and it is a very promising method for treating various types of diseases, including cancer, as many recently published studies shown. [3]

### Cancer Immunogene Therapy

The first study of cancer immunotherapy is dated to the 19th century, when William Coley used a preparation from *Streptococcus* cultures for tumor treatment. In some of his patients the difficulties connected to cancer reduced or even disappeared. Molecular biology and genetic engineering created a new era in cancer immunotherapy. In the last two decades, a number of genes coding immunostimulating factors, MHC molecules and co-stimulatory molecules were

***Corresponding author: Dr. DVM A. Valenčáková,**
University of Veterinary Medicine and Pharmacy; Department of Biology, Zoology and Radiobiology; Komenského 73, 04181 Košice, Slovak Republic;
**E-mail:** alexandra.valencakova@uvlf.sk
**Received:** 10 November, 2015; **Accepted:** 23 November, 2015

synthesized and are now being used in various clinical trials of gene therapy. [4]

Tumor cells can be eliminated by cellular or humoral mechanisms of the immune system. [5] It is presumed that cell cytotoxicity plays a main role in anti-cancer immunity. Specific cytotoxicity is mediated by a subpopulation of T-cells, which have the T-cell receptor (TCR) composed of alpha-beta chains. Alpha-beta cells include two subtypes: $CD4^+$ and $CD8^+$ cells, which can identify, in the association with MHC II and MHC I molecules, tumor cell antigens. Non-specific cytotoxicity is mediated by NK cells, capable of identifying tumor cells not expressing MHC molecules. This type of immune response is not antigen-specific and does not require previous antigen contact of the effector cells with the targeted cells. [4]

Humoral antitumor immunity is mediated by antibodies produced by activated B-cells. Antibodies can eliminate tumor cells by several means – by activation of the complement, opsonisation and antibody-dependent cellular cytotoxicity mediated by Fc receptors on effector cells. The key role played in inducing an antigen-specific immune response is by professional antigen-presenting cells (APCs) – dendritic cells (DC). Dendritic cells pose as sentinels of the immune system. Immature DCs are localized in peripheral tissues and are characteristic with a high phagocytic activity, a low MHC and co-stimulatory molecule expression and a low cytokine secretion. [6] They intercept and process different antigens, such as tumor antigens from apoptotic cell remains or antigens released from tumor cells destructed by the antigen-specific way. After receiving the threat signal DCs start to mature. They form MHC I and II molecules, co-stimulatory molecules (CD40, CD54, CD80, CD86) on their surface, and produce cytokines. Mature DCs migrate to regional lymph nodes, where they present tumor antigens in complex with MHC I or II molecules to the $CD4^+$ or $CD8^+$. Co-stimulatory molecules are necessary for the activation of the so called second signal, critical for the activation of naïve T-lymphocytes. IL-12, produced by dendritic cells affects the $CD4^+$ cells and converts them to interferon gamma (IFN-γ) producing Th-1 cells. This way divided Th-1 cell population demonstrated the ability of mediating a strong and long-lasting anti-tumor immune response. Dendritic cells are very effective in T-cell stimulation; one DC can convert 100 – 3000 T-cells. [6]

Cancer gene immunotherapy uses several immunologic agents for the purpose of explaining effective anti-tumor immune response, for example, the introduction of genes coding various cytokines *ex vivo* or *in vivo* (intratumorally) to cancer cells. Cancer cells secrete proteins coded by genes, which were induced to the environment of the tumor. These proteins, as immunostimulating factors, modify the environment inside the tumors and dispatch a signal about the danger, attracting professional APCs. It was assumed in the past that some cytokines (IL-2, IL-6, IL-12, IFN-γ) directly activate killer cells ($CD8^+$, NK). However, it appears, in the light of current research, they pose mainly as signal transmitters of the danger to the dendritic cells. [7] Tumor cells expressing cytokines, such as INF-γ or IL-12 not only strongly activate dendritic cells, but also induce a strong bearing of the immune response of functional Th-1 cells. [8]

Genetically modified tumor vaccine (GMTV) was tested since 1996 in polish patients with malignant melanoma. GMTV was composed of alogenous melanoma cells modified with genes coding IL-6 and of an agonistic soluble receptor (sIL-6R). [9]

Another approach in cancer gene immunotherapy alters the tumor cells to antigen-presenting cells. Tumor antigens are presented in the association of MHC I molecules in most tumor cells. However, in tumor cells missing the co-stimulation molecules creating the second signal necessary for the correct lymphocyte activation, an antigen-specific tolerance is induced against effector cells. By introducing genes coding co-stimulatory molecules (such as B7.1 or B7.2) aversion of tumor induced tolerance was achieved and a strong anti-tumor immune response mediated by $DC8^+$ cells was explained. [10]

Dendritic cells, as key elements of inducing anti-tumor immune response are promising means of gene therapy. The introduction of genes coding tumor antigens to dendritic cells clarified the strong antigen-specific immune response mediated by $CD4^+$ and $CD8^+$ cells. [11] But this procedure has some disadvantages, for example the modification of the dendritic cells *in vivo* is extremely complicated and the isolation and propagation of these cells *in vitro* is a lengthy process. Beside this, dendritic cells, which are genetically modified and activated cells *ex vivo*, migrate after subcutaneous application intensively to regional lymph nodes and by doing this have much lesser effectiveness in anti-tumor response than was anticipated. [4]

**Suicide Gene Therapy in Cancer**

Suicide gene therapy is based on introducing viral or bacterial genes to tumor cells, which allows the conversion of a non-toxic compound to a lethal medication. Between the great many suicide systems described, the most explored is the *Herpes simplex virus* carrying the gene for thymidine-kinase (HSV-tk) with ganciclovir (GCV) as a precursor, and a gene for cytosine-deamidase (CD) carried by *Escherichia coli*, which converts the non toxic antifungal 5-fluorocytosine (5-FC) to 5-fluorouracil (5-FU). [1]

The ganciclovir-nucleoside analog is used in the treatment of patients infected with herpes viruses (HSV, VZV). Cells infected with herpes virus produce thymidine-kinase (tk) coded by viral genome and after ganciclovir application they are incapable of division, dying subsequently. [12] For purposes of treating oncologic patients, cDNA coding tk can be supplemented to tumor cells via adenoviruses,

lentiviruses, liposomes or by physical methods. After several days, ganciclovir is administered intravenously to the patients. Ganciclovir is transported by blood to every organ and tissue, tumors included. After entering the cell, this precursor tranduces with the vector coding tk, phosphorylates to its toxic metabolite (ganciclovir-triphosphate), which induces cellular death by inhibiting DNA synthesis. [4]

In the tumor mass, the tumor cells are connected with bridges in the intercellular compartment ("gap junctions"). In the process called "bystander effect", the phosphorylated form of ganciclovir can expand from one genetically modified cell to others, where it accomplishes the same effect. [13] Thanks to "bystander effect" not all cancer cells have to be modified with the tk gene for achieving complete elimination of the tumor. There is an effort of combining suicide gene therapy with immunotherapy for the purpose of amplifying the effectiveness of suicide gene therapy. It is proved IL-6 and GM-CSF increase the suicide effect of tk in mice models of malignant melanoma. [14]

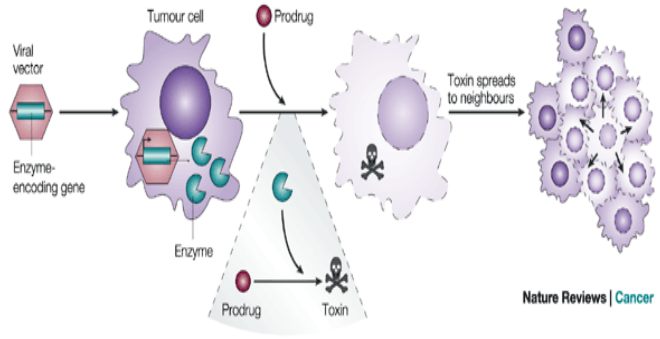


**Fig. 1: Schematic illustration of suicide gene therapy mechanism [15]**

**Application of Tumor-Suppressor Genes**

The basis of neoplastic transformation of healthy cells is the mutation in two gene classes: proto-oncogenes and and tumor-suppressor genes. Proteins coded by mutated proto-oncogenes carry a signal to the nucleus and subsequently induce cell division. On the other hand, proteins coded by mutated tumor-suppressor genes are not capable of inhibiting of the proliferation induced by proto-oncogenes. The transformed cells proliferate dynamically and create a tumor. Several scientists proved applying intact suppressor genes to cancer cells will avert their neoplastic behavior and will induce tumor regression.

However, the outcomes of clinical therapies are not satisfactory, mostly for the low effectiveness of transduction achieved by currently available distribution systems. For the purpose of eliminating all cancer cells, each and every one of them must be modified by an intact suppressor gene. If there is one unmodified cell remaining, it can later cause proliferation and the forming of new tumors. [4]

However, Xu *et al.* (1997) proved, that liposome mediated p53 gene transfer into mammary gland tumors in mice led to less than 5% transfection of tumor cells, but was connected with strong tumor regression. It has shown the relatively low p53 gene expression inside tumor mass induced a significant reduction of blood-vessels in treated tumors [16]. Another approach in cancer suppressor gene therapy is the application of a mutated adenovirus. The initial sequence E1b is responsible for shutting down p53-mediated apoptosis at the time of the adenovirus entering the cell. The apoptotic death of infected cells prevents viral replication. E1b adenovirus applied to oncologic patients can replicate only in cancer cells lacking the functional p53 gene. Viruses can replicate in them, cause cytophatic effect and subsequently infect and destroy cancer cells. [17]

**Antiangiogenic Gene Therapy**

Tumors require effective blood supply for their growth. The inhibition of angiogenesis is a promising strategy for treating oncologic patients. Despite of many endogenous inhibitors of angiogenesis being found, clinical evaluation were prevented by the need of high doses, production limits and the relative instability of the proper recombinant proteins. [4] Antiangiogenic therapy is specifically directed against microvascular endothelial cells created in the tumor location. Specific antiangiogenic therapy has low or none toxicity at all, does not demand the entrance of therapeutic agents into tumor cells and does not pass the hematoencephalic barrier. It controls the tumor growth, independent of the cell type of the tumor and does not induce acquired drug resistance. [18] The supplementation of genes coding antiangiogenic proteins is a promising procedure avoiding obstacles connected to systemic application of medicaments. Therapeutic genes coding antiangiogenic substances can be distributed to patients by numerous carrier systems, for example by recombinant adenoviruses or liposomes. [19] Antiangiogenic gene therapy can be carried out as a systemic or local treatment. Scientists still cannot agree on the best means of application. Local (intratumor) application is joined with a strong "bystander effect", increasing the antagonistic activity of introduced genes and should not be connected with potential side effects of systemic therapy. [16] On the other hand, systemic application of genes coding antiangiogenic factors enables a long-lasting elevation of endostatins in blood. [20]

**Mesenchymal Stem Cells as Vectors in Targeted Gene Therapy**

In spite of the progress accomplished by cytotoxicity, cytostatics and targeted chemotherapy in malignant diseases, a lack of specificity and growing drug resistance in cell subpopulations often lower the effectiveness of systemic therapies with medications. Beside metastases, many difficulties are connected with solitary tumor therapy. [21] The aiming of pharmaceutics to cancer cells in the tumor mass is mostly limited by uneven vascularisation and necrotic regions. [22] Other main disadvantages are several severe side effects of toxic chemotherapy, frequently limiting this therapeutic procedure. These factors lead

to the conclusion, that other systems enabling local tumor elimination with the absence of non-target toxicity, connected with systemic chemotherapy, should be created. Precursor gene therapy is based on stem cells, and when targeted on tumors and/or metastases, it can help to overcome these disadvantages. [21]

Mesenchymal stem cells (MSCs) have the unique ability of selective proliferation in tumors and contribute in the creation of stroma connected to the tumor. This ability designates them to become vectors in targeted gene therapy of cancer of the basis of stem cells. [21] Experimental evidence shows, beside stromal fibroblasts introduced to tumor mass from local tissue, other stromal cells of MSC origin exist. Several studies show mesenchymal stem cells isolated from bone marrow are capable of migrating to tumors of various origins. Experiments with human xenoimplants on mice shown the systemically applied of MSCs have a natural migration capability, preferably to breast cancer cells, lung metastases, melanoma cells, intracranial glioma, and colon cancer cells. Besides this, exogenously applied MSCs are capable of forming a significant fraction of the tumor mass. [23] It was shown that MSCs isolated from bone marrow can also be present in cancer metastases forming a pre-metastatic niche. [24] These findings support the concept of mesenchymal stem cells being produced for therapeutic purposes can be used for metastasis treatment. Therefore, the introduction of transgenes to own stem cells presents an attractive distribution strategy on a cellular basis. [25]

**MSC Developed For Therapeutic Purposes**

Mesenchymal stem cells can be relatively easily transduced by a vector on the basis of adenoviruses, retroviruses ant lentiviruses [26], without changing the minimal criteria described in defining human MSCs. [27] Mesenchymal stem cells isolated from bone marrow were successfully tested as distribution systems for oncolytic adenoviruses in a model of ovarian cancer metastases. [28] MSCs were successfully used as helper vectors for conditionally replicating adenoviruses for the purpose of targeting breast cancer metastases *in vivo.* [29] MSCs producing therapeutic cytokines, such as INF-β and IL-2, shown anti-tumor effect. [23]

Mesenchymal stem cells are generally isolated from bone marrow, but they are also isolated from a number of other tissues, such as muscle, synovia, the umbilical cord and adipose tissue. [30-31] The advantages of using MSCs isolated from adipose tissue (AT-MSCs) are higher against the ones from bone tissue. [26, 32] The easy and repeatable access to subcutaneous adipose tissue and a simple method of isolation presents a clear advantage against other MSC sources. [33] For this reason, AT-MSCs can be considered as a convenient source of own stem cells for the personalized cell-based therapy with minimal risks to the donor and without ethical limits. [31, 33]

**Gene Directed Enzyme/Prodrug Therapy by MSCS**

An increasing list of experimental evidence, that therapeutically modified MSCs in GDEPT system, thanks to their migration capability, can attack cancer tissues, and by combined mechanisms, including "bystander effect", apoptosis induction and reduction of the mutual contact between cells, kill tumor cells, cancer stem cells included [34]. Experimental studies with glioblastomas, in which cancer initiating cells are studied thoroughly, support this claim. Human glioblastoma cells, like cancer stem cells, were identified as cells expressing CD133 surface markers. [35-36] Gene expression and chemoresistance analysis of CD133-possitive stem cells in glioblastoma confirms the connection between this chemoresistance and the markers of cancer stem cells on the surface of these cells. [37]

Therapy based on human neural stem cells using cytosine-deaminase/5-fluorcytosine precursor system was experimentally tested on human medulloblastoma, a malignant pediatric brain tumor. The stem cell line of cloned perpetual human neural cells, designed for the purpose of precursor-activating cytosine-deaminase secretion, maintain their ability of migration to tumor cells. Therapeutic studies on mice *in vivo* intracranial medulloblastoma models with the use of neural stem cells transduced by cytosine-deaminase, after subsequent systemic therapy with 5-fluorcytosine shown 76% reduction of the tumor mass in the sample of treated animals. [38] In a similar experimental model, the neural progenitor cells, transuced by retrovirus cytosine-deaminase, stroke numerous metastasis areas. [39] Beside this, MSCs effectively distribute oncolytic adenoviruses to intracranial gliomas. [40]

As a result of these experiments, the MSCs of various origins are distribution vectors on a cellular basis and are used in place of specific enzyme/prodrug conversion in targeted chemotherapy. MSCs are easily isolated; they can expand quickly in *in vivo* environment and can be developed with contents of converting genes. They possess a specific resistance and migration capability into tumors; this is why they should be considered as valuable, mature stem cells for autologous use in cancer therapy. [21]

**Bacteria in Cancer Gene Therapy**

Bacteria were used as anti-cancer agents for the first time more than a century ago. Nowadays, this field has reemerged and is proceeding in development at a quick pace. Bacteria are used as anti-cancer agent independently or in combination with conventional therapeutic methods and are being armed with a supplement of therapeutic genes elevating their effectiveness. [2] The finding that bacteria can infect and attack tumors is dated 150 years ago. It was observed for the first time in Europe and America, some types of cancer retreated after accidental *Streptococcus pyogenes* infection in hospitalized patients. William Coley was an American doctor starting a study in this area and he dedicated his whole career to the research of the use of

bacteria as an alternative method of treating cancer. [41] Despite of his success, he was never able to create a perfect system, and that is why all interest in bacteria as anti-cancer agents decreased in time. Nevertheless, William Coleys findings are the template for two different modern science areas: immunotherapy and bacterial cancer therapy. [2]

The selectivity of bacterial growth in tumors refers to tissue phenotype, differentiating tumor tissue from the healthy one. It is this microenvironment inside the tumor, protecting it from most anti-cancer treatments, is the weak spot in this therapy, sensitizing the tumor to bacterial anti-cancer agents. It is well documented that different bacteria can accumulate in preference to different types of experimental tumors. For example, *Salmonella* VPN 20009 shown a rate of 30 – 25 000:1 tumor tissue to healthy one. [41] Many theories were proposed for the purpose of explaining these observations. [42] The primary factors denying this specificity are direct or indirect outcomes of the tumor growth process arising from strictly necrotic tissues. For their growth and evolution, tumors require the creation of many blood vessels in a process called neoangiogenesis. A feature and necessity of cancer is the continual oxygen and nutrient supplementation of the tumor. [43]

When the tumor diameter reaches critical size, oxygen cannot access the internal layers of the tumor adequately and cells become hypoxic. In the hypoxic zone, the low oxygen partial pressure induces new angiogenesis. But these newly created blood vessels have abnormal structure and create physiological barriers for therapeutic agent and immune cell supplementation. [43] One of the applicable attribute of their abnormality is the content of pores of size varying from 200nm to 2μm (depending on the tumor). [44] This potentially enables microorganisms, such as bacteria, to enter the bloodstream and to colonize the tumor mass. Necrotic regions are areas containing dead cells, mostly localized in the tumor mass. These areas are appropriate for bacterial growth; they offer protection from the immune system and adequate nutrition (*i.e.* purines) from dead tumor cells. [2]

The precise localization of bacterial proliferation inside the tumor can be different between species. Recent 3D imaging studies showed the growth of anaerobic bifidobacteria in the form of multiple aggregations in lifeless tumor regions. [45] The evidence presented by Forbes *et al.* (2003), shows Salmonellae proliferating inside the necrotic tumor models. [46] These observations imply their use is limited to large tumors. However, this denies the earlier published statements and recent data showing the proliferation of *Salmonella* in normoxic as well as hypoxic regions. [47] This capability is preferred in clinical context. The ideal anti-cancer agent on bacterial basis should target and proliferate inside micrometastatic tumors, which have no natural necrotic regions. For example, it was observed *Escherichia coli* K12 MG1655 and HJ1020, marked with genes emitting light, targets very small tumors, as well as large ones [48], and furthermore, anaerobic *Bifidobacterium breve* demonstrated the same capability. [49] This means that several bacterial types can proliferate specifically inside tumors; for example *Magnetospirillum magneticum*, *E. coli* CFT073, *E. coli* Top10 and *Salmonella flexneri* 2a SC602. [50-51]

Current conventional therapy, for deficient tumor selectivity, can lead to the destruction of healthy tissue and has serious side effects in the patient; beside this it is limited in effectiveness and the quantity of the used dose. Gene therapy presents an adequate alternative and it is very promising method for treating various types of disease, including cancer. Dendritic cells, as key elements of inducing anti-tumor immune response are promising means of gene therapy. There is an effort of combining immunotherapy with suicide gene therapy for the purpose of amplifying the effectiveness of suicide gene therapy. Tumor-suppressor genes avert the neoplastic behavior and induce tumor regression, however, the outcomes of clinical therapies are not satisfactory, mostly for the low effectiveness of transduction achieved by currently available distribution systems. The supplementation of genes coding antiangiogenic proteins is a promising procedure, avoiding the obstacles connected with systemic application of medicaments. The ability of mesenchymal stem cells to selectively proliferate in tumors designates them to become vectors in targeted gene therapy of cancer on the basis of stem cells. The introduction of transgenes to stem cells presents an attractive distribution strategy. Mesenchymal cells can be used in enzyme/prodrug conversion in targeted chemotherapy; they possess a specific resistance and migration capability into tumors and therefore should be considered as valuable stem cells for use in cancer therapy. Bacteria used as anti-cancer agents have the ability to infect and proliferate inside the tumor mass, enabling the use of bacteria as an alternative method of treating cancer.

## REFERENCES


1. Duarte S, Carle G, Faneca H, de Lima MC, Pierrefite-Carle V. Suicide gene therapy in cancer: where do we stand now? Cancer Lett. 2012; 324(2):160-70.
2. Lehouritis P, Springer C, Tangney M. Bacterial-directed enzyme prodrug therapy. J Control Release. 2013; 170(1):120-31.
3. Guan YS, Liu Y, He Q, Li X, Yang L, Hu Y, La Z. P53 gene therapy in combination with transcatheter arterial chemoembolization for HCC: one-year follow-up. World J. Gastroenterol. 2011; 17:2143-2149.
4. Wysocki PJ, Grabarczyk P, Mackiewicz-Wysocka M, Kowalczyk DW, Mackiewicz A. Genetically modified dendritic cells--a new, promising cancer treatment strategy? Expert Opin Biol Ther. 2002; 2(8):835-45.
5. Habal N, Gupta RK, Bilchik AJ, Yee R, Leopoldo Z, Ye W, Elashoff RM, Morton DL. CancerVax, an allogeneic tumor cell vaccine, induces specific humoral and cellular immune responses in advanced colon cancer. Ann Surg Oncol. 2001; 8(5):389-401.

6. Van Gool SW, Zhang Y, Kasran A, de Boer M, Ceuppens JL. T helper-independent activation of human CD8+ cells: the role of CD28 costimulation. Scand J Immunol. 1996; 44(1):21-9.
7. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature. 1998; 392(6673):245-52.
8. Fuchs, EJ, Matzinger, P. Is cancer dangerous to the immune system? Semin Immunol. 1996; 8(5):271-80.
9. Nawrocki S, Murawa P, Malicki J, Kapcinska M, Gryska K, Izycki D, Kaczmarek A,Laciak M, Czapczyk A, Karczewska A, Rose-John S, Mackiewicz A. Genetically modified tumour vaccines (GMTV) in melanoma clinical trials. Immunol Lett. 2000; 74(1):81-6.
10. Tasaki K, Yoshida Y, Maeda T, Miyauchi M, Kawamura K, Takenaga K, Yamamoto H, Kouzu T, Asano T, Ochiai T, Sakiyama S, Tagawa M. Protective immunity is induced in murine colon carcinoma cells by the expression of interleukin-12 or interleukin-18, which activate type 1 helper T cells. Cancer Gene Ther. 2000; 7(2):247-54.
11. Nishiyama T, Tachibana M, Horiguchi Y, Nakamura K, Ikeda Y, Takesako K, Murai M. Immunotherapy of bladder cancer using autologous dendritic cells pulsed with human lymphocyte antigen-A24-specific MAGE-3 peptide. Clin Cancer Res. 2001; 7(1):23-31.
12. Suzuki S, Tadakuma T, Kunitomi M, Takayama E, Sato M, Asano T, Nakamura H, Hayakawa M. Liposome-mediated gene therapy using HSV-TK/ganciclovir under the control of human PSA promoter in prostate cancer cells. Urol Int. 2001; 67(3):216-23.
13. Carrió M, Mazo A, López-Iglesias C, Estivill X, Fillat C. Retrovirus-mediated transfer of the herpes simplex virus thymidine kinase and connexin26 genes in pancreatic cells results in variable efficiency on the bystander killing: implications for gene therapy. Int J Cancer. 2001; 94(1):81-8
14. Mackiewicz J, Karczewska-Dzionk A, Laciak M, Kapcinska M, Wiznerowicz M, Burzykowski T, Zakowska M, Rose-John S, Mackiewicz A. Whole Cell Therapeutic Vaccine Modified With Hyper-IL6 for Combinational Treatment of Nonresected Advanced Melanoma. Medicine (Baltimore). 2015; 94(21): e853
15. McCormick F. Cancer gene therapy: fringe or cutting edge? Nat Rev Cancer. 2001; 1(2):130-41.
16. Xu M, Kumar D, Srinivas S, Detolla LJ, Yu SF, Stass SA, Mixson AJ. Parenteral gene therapy with p53 inhibits human breast tumors in vivo through a bystander mechanism without evidence of toxicity. Hum Gene Ther. 1997; 8(2):177-85.
17. Heise C, Hermiston T, Johnson L, Brooks G, Sampson-Johannes A, Williams A, Hawkins L, Kirn D. An adenovirus E1A mutant that demonstrates potent and selective systemic anti-tumoral efficacy. Nat Med. 2000; 6(10):1134-9.
18. Boehm T, Folkman J, Browder T, O'Reilly MS. Antiangiogenic therapy of experimental cancer does not induce acquired drug resistance. Nature. 1997; 390(6658):404-7.
19. Gorrin-Rivas MJ, Arii S, Mori A, Kaneda Y, Imamura M. Mouse macrophage metalloelastase gene delivery by HVJ-cationic liposomes in experimental antiangiogenic gene therapy for murine CT-26 colon cancer. Int J Cancer. 2001; 93(5):731-5.
20. Goldman CK, Kendall RL, Cabrera G, Soroceanu L, Heike Y, Gillespie GY, Siegal GP, Mao X, Bett AJ, Huckle WR, Thomas KA, Curiel DT. Paracrine expression of a native soluble vascular endothelial growth factor receptor inhibits tumor growth, metastasis, and mortality rate. In the Proceedings of the 1998 National Academy of Science of the United States of America. 1998, pp. 8795-800.
21. Altaner C. Prodrug cancer gene therapy. Cancer Lett. 2008; 270(2):191-201.
22. Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science. 2005; 307(5706):58-62,
23. Studeny M, Marini FC, Dembinski JL, Zompetta C, Cabreira-Hansen M, Bekele BN, Champlin RE, Andreeff M. Mesenchymal stem cells: potential precursors for tumor stroma and targeted-delivery vehicles for anticancer agents. J Natl Cancer Inst. 2004; 96(21):1593-603.
24. Kaplan RN, Psaila B, Lyden D. Bone marrow cells in the 'pre-metastatic niche': within bone and beyond. Cancer Metastasis Rev. 2006; 25(4):521-9.
25. Hamada H, Kobune M, Nakamura K, Kawano Y, Kato K, Honmou O, Houkin K, Matsunaga T, Niitsu Y. Mesenchymal stem cells (MSC) as therapeutic cytoreagents for gene therapy. Cancer Sci. 2005; 96(3):149-56.
26. Kucerova L, Altanerova V, Matuskova M, Tyciakova S, Altaner C. Adipose tissue-derived human mesenchymal stem cells mediated prodrug cancer gene therapy. Cancer Res. 2007; 67(13):6304-13.
27. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop Dj, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006; 8(4):315-7.
28. Komarova S, Kawakami Y, Stoff-Khalili MA, Curiel DT, Pereboeva L. Mesenchymal progenitor cells as cellular vehicles for delivery of oncolytic adenoviruses. Mol Cancer Ther. 2006; 5(3):755-66.
29. Stoff-Khalili MA, Rivera AA, Mathis JM, Banerjee NS, Moon AS, Hess A, Rocconi RP, Numnum TM, Everts M, Chow LT, Douglas JT, Siegal GP, Zhu ZB, Bender HG, Dall P, Stoff A, Pereboeva L, Curiel DT. Mesenchymal stem cells as a vehicle for targeted delivery of CRAds to lung metastases of breast carcinoma. Breast Cancer Res Treat. 2007; 105(2):157-67.
30. Gronthos S, Zannettino AC, Hay SJ, Shi S, Graves SE, Kortesidis A, Simmons PJ. Molecular and cellular characterisation of highly purified stromal stem cells derived from human bone marrow. J Cell Sci. 2003; 116(9):1827-35.
31. Strem BM, Hicok KC, Zhu M, Wulur I, Alfonso Z, Schreiber RE, Fraser JK, Hedrick MH. Multipotential differentiation of adipose tissue-derived stem cells. Keio J Med. 2005; 54(3):132-41.
32. Kern S, Eichler H, Stoeve J, Klüter H, Bieback K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem Cells. 2006; 24(5):1294-301.
33. Schäffler A, Büchler C. Concise review: adipose tissue-derived stromal cells--basic and clinical implications for novel cell-based therapies. Stem Cells. 2007; 25(4):818-27.
34. Iannolo G, Conticello C, Memeo L, De Maria R. Apoptosis in normal and cancer stem cells. Crit Rev Oncol Hematol. 2008; 66(1):42-51.
35. Kang MK, Kang SK. Tumorigenesis of chemotherapeutic drug-resistant cancer stem-like cells in brain glioma. Stem Cells Dev. 2007; 16(5):837-47.
36. Kang MK, Hur BI, Ko MH, Kim CH, Cha SH, Kang SK. Potential identity of multi-potential cancer stem-like subpopulation after radiation of cultured brain glioma. BMC Neurosci. 2008; 9:15.
37. Liu G, Yuan X, Zeng Z, Tunici P, Ng H, Abdulkadir IR, Lu L, Irvin D, Black KL, Yu JS. Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma. Mol Cancer. 2006; 5:67.
38. Kim SK, Kim SU, Park IH, Bang JH, Aboody KS, Wang KC, Cho BK, Kim M, Menon LG, Black PM, Carroll RS. Human neural stem cells target experimental intracranial medulloblastoma and deliver a therapeutic gene leading to tumor regression. Clin Cancer Res. 2006; 12(18):5550-6
39. Danks MK, Yoon KJ, Bush RA, Remack JS, Wierdl M, Tsurkan L, Kim SU, Garcia E, Metz MZ, Najbauer J, Potter PM, Aboody KS. Tumor-targeted enzyme/prodrug therapy mediates long-term disease-free survival of mice bearing disseminated neuroblastoma. Cancer Res. 2007; 67(1):22-5.
40. Sonabend AM, Ulasov IV, Tyler MA, Rivera AA, Mathis JM, Lesniak MS. Mesenchymal stem cells effectively deliver an oncolytic adenovirus to intracranial glioma. Stem Cells. 2008; 26(3):831-41.

41. Pawelek JM, Low KB, Bermudes D. Tumor-targeted Salmonella as a novel anticancer vector. Cancer Res. 1997; 57(20):4537-44.
42. Sznol M, Lin SL, Bermudes D, Zheng LM, King I. Use of preferentially replicating bacteria for the treatment of cancer. J Clin Invest. 2000; 105(8):1027-30.
43. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. Nature. 2000; 407(6801):249-57.
44. Hobbs SK, Monsky WL, Yuan F, Roberts WG, Griffith L, Torchilin VP, Jain RK. Regulation of transport pathways in tumor vessels: role of tumor type and microenvironment. In the Proceedings of the 1998 National Academy of Sciences of the United States of America, 1998; 95(8):4607-12.
45. Cronin M, Akin AR, Collins SA, Meganck J, Kim JB, Baban CK, Joyce SA, van Dam GM, Zhang N, van Sinderen D, O'Sullivan GC, Kasahara N, Gahan CG, Francis KP, Tangney M. High resolution in vivo bioluminescent imaging for the study of bacterial tumour targeting. PLoS One. 2012; 7(1):e30940.
46. Forbes NS, Munn LL, Fukumura D, Jain RK. Sparse initial entrapment of systemically injected Salmonella typhimurium leads to heterogeneous accumulation within tumors. Cancer Res. 2003; 63(17):5188-93
47. Ganai S, Arenas RB, Sauer JP, Bentley B, Forbes NS. In tumors Salmonella migrate away from vasculature toward the transition zone and induce apoptosis. Cancer Gene Ther. 2011; 18(7):457-66.
48. Min JJ, Nguyen VH, Kim HJ, Hong Y, Choy HE. Quantitative bioluminescence imaging of tumor-targeting bacteria in living animals. Nat Protoc. 2008; 3(4):629-36.
49. Cronin M, Morrissey D, Rajendran S, El Mashad SM, van Sinderen D, O'Sullivan GC, Tangney M. Orally administered bifidobacteria as vehicles for delivery of agents to systemic tumors. Mol Ther. 2010; 18(7):1397-407.
50. Benoit MR, Mayer D, Barak Y, Chen IY, Hu W, Cheng Z, Wang SX, Spielman DM, Gambhir SS, Matin A. Visualizing implanted tumors in mice with magnetic resonance imaging using magnetotactic bacteria. Clin Cancer Res. 2009; 15(16):5170-7.
51. Stritzker J, Weibel S, Hill PJ, Oelschlaeger TA, Goebel W, Szalay AA. Tumor-specific colonization, tissue distribution, and gene induction by probiotic Escherichia coli Nissle 1917 in live mice. Int J Med Microbiol. 2007; 297(3):151-62.

**Source of Support: Nil, Conflict of Interest: None declared.**