On Advances in Cancer Suicide-genes Therapy

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Abstract

Chemotherapy, radiotherapy and surgery are still the main cancer treatments. However, they carry considerable toxicity that often causes other adverse symptoms. The gene therapy intends to eliminate the tumor without damaging the normal host tissue. The classical suicide gene therapy needed the conversion of non-toxic prodrug into toxic metabolite and the bioavailability of active drugs. The discovery of genes that encode cytotoxic proteins overcomes these needs. There are many patents which describe the direct action of these new genes, and their uses as antitumor treatments. Some of the drawbacks of the tumor gene therapy are the low efficiency of in vivo gene delivery and the low tumor specificity. Advances on this field suggest the use of a combined gene therapy and chemotherapy or radiotherapy to increase the effectiveness of the antitumor treatment.

Keywords: Cancer; Suicide gene therapy; Combined therapy

Introduction

The aim of the conventional cancer therapy is to eliminate the tumor without damaging the normal host tissue. Chemotherapy, radiotherapy and surgery still remain as the main cancer treatments. However, they carry considerable toxicity that, often, causes other adverse symptoms. Nausea and emesis are some of the effects that the cisplatin-based chemotherapy may cause. An important problem after radiotherapy is the damage caused to the bone marrow. [1] Gene therapy is presented as a possible way to modify or damage the tumor cell from within. In the last years, the gene therapy have been investigated in several fields, such as: immunomodulatory gene therapy, antiangiogenic gene therapy, RNA interference (mi) therapy, genetic modulation of resistance or sensitivity, corrective and pro-apoptotic gene therapy and suicide gene therapy. [1] In spite of those advances, the tumor gene therapy shows low efficiency of in vivo gene delivery and low tumor specificity. A combined gene therapy and chemotherapy [3, 4] and or radiotherapy [5, 6] has been proposed to increase the effectiveness of the antitumor treatment.

In this review, we show the current state of the cancer suicide gene therapy and its great potential in combination with chemotherapy and radiotherapy.

Suicide gene therapy

Suicide gene therapy is considered as a potential way to treat cancer. It is based on the use of genes from bacterial or viral origin, whose encoding enzymes are responsible for converting a nontoxic prodrug into toxic metabolites. [7, 8] The suicide gene therapy has been investigated in many cancer types: breast, glioblastoma, lung, hepatoma and bladder cancers. [9-12]

In this review, we will outline some of the suicide genes systems proposed for cancer therapy.

Cytosine deaminase: Cytosine deaminase (CD) catalyzes the deamination of the prodrug 5-fluorocytosine (5-FC), to lead to the toxic drugs 5-fluorouracil (5-FU), 5-FU inhibits the DNA and RNA synthesis, resulting in cell death. [9] The CD/5-FC therapy has a potential clinical utility for lung [10], gliomas [11], neck squamous [12], cervical [13] and breast [14] cancers. Mouse lung cancer models were used by Zarogoulidis et al. [10], to study the antitumor efficacy of an adenovirus (Ad) vector-mediated CD followed by systemic 5-FC. Three groups, of 20 mice each, were studied: a control group without therapy, another group with intratumoral administration of 5-FC for 10 days; and another one with intratumoral administration of 5-FC for 10 days plus Ad-CD on the day 2. The group treated with the Ad-CD plus 5-FC exhibited a greater decrease in the tumour volumes than either of the other two.

Also, the CD/5-FC system enhances the effects of the radiotherapy. [16, 17] HeLa tumor xenografts were randomly divided into five groups: group A (control), group B (intratumorally injected with and Ad-CD/5-FC), group C (radiation only) and group D (intratumoral injected with and Ad-CD/5-FC and radiation). The groups C and D received a single (12.5 Gy) or fractionated (3 Gy x 5 days) radiation respectively. The group D showed a greater decrease in the tumor growth by up to 2.4-fold (P<0.01) and 2.5-fold (P<0.05), for 12.5 Gy or 3 Gy x 5 days respectively as compared to the group C. [17]

Thymidine kinase: The Herpes Simplex Virus type 1 (HSV-1) thymidine kinase (TK) converts non-toxic ganciclovir (GCV) into the toxic triphosphate ganciclovir (GCV-TP). GCV-TP is incorporated into DNA, stopping the replication and causing cell death. [11] This mechanism of DNA damage is p53-dependent,
and other genes such as ATM and p27 are also activated. The HSV-TK/GCV system exhibited a promising approach for cancer suicide-gene therapy in lung, breast, hepatoma, and bladder cancers. Current studies, with a Bifidobacterium infantis (BI) carrying HSV-TK plus GCV administration, showed inhibition of the bladder tumour in rat models. The rat models were randomly divided into three groups: injected with normal saline (group A), BI only (group B) and BI-TK (group C). The group C evidenced the highest level of apoptosis, which was correlated with higher expression levels of Fas, fasL, Cyt-C and caspase-9.

Several studies use both systems, HSV-TK/GCV and CD/5-FC, and have been shown to support a synergistic interaction. Huang et al. designed a double suicide gene system with CD/TK to study their bystander effects. Colon cancer cells were transfected with this double system and, after transfected, GCV or 5-FU prodrugs or both of them were administered. The results revealed a greater inhibition rate and a stronger bystander effect when both prodrugs were administered, in contrast with the use of a single prodrug.

**Carboxypeptidase**

Carboxypeptidase G2 (CPG2) activates carboxy, phenol, or aniline mustard prodrugs, in which the DNA alkylating chain has been synthetically deactivated through N-substitution of l-glutamate, causing DNA-DNA interstrand cross-links and cell death. CPG2 has been used as an antitumor therapy in breast, colon, hepatocellular and colon carcinomas. The ZD2767P prodrug is a substrate of CPG2, which activates this prodrug as a bifunctional DNA interstrand cross-linking alkylating agent. This system has been used in hepatocellular carcinoma cells and xenografts exhibiting inhibition of tumour growth and extending the lifespan of the mice. Similar results have been shown in human colon carcinoma xenografts.

According to this reaction, other prodrugs have been studied. Oncolytic Salmonella typhimurium-derived bacterium VNP2009 was designed to deliver the CPG2. Human breast carcinoma, human colon carcinoma cells and B16-F10 mouse melanoma xenografts were injected with VNP2009/CPG2 and treated with several derivatives of L-glutamic prodrugs. CPG2 activated the prodrug and a significant decrease in the tumor growth of xenografts was observed. Two novel benzoic acid derivatives of CPG2, being cytotoxic for human breast carcinoma cells, while the tetra fluorinated prodrug is a competitive inhibitor of CPG2.

**Nitroreductase**

The nitroreductase gene (NTR) from E. Coli, encodes an enzyme which is frequently used for transforming prodrugs into active drugs. NTR attaches a 4-nitrobenzyl group to a leaving group such as a phosphor amide or a carboxylate. 5-[aziridin-1-yl]-2,4-dinitrobenzamide (CB1954) is a prodrug of NTR, whose antitumor effect has been observed in human hepatocellular carcinoma and squamous carcinoma xenografts. A phase I/II clinical trial in 19 patients with prostate cancer analyzes the effectiveness of an adenovirus vector encoding NTR plus the systemic prodrug CB1954. Changes in the kinetic of the prostate-specific antigen (PSA) were used as indicators of tumor progression. >10% PSA was decreased in five patients, and two patients showed >50% decrease in 1 month, implying a reduction in the tumor size. Furthermore, the NTR-CB1954 system has an enhancer effect on radiotherapy. Ovarian cancer cells were stably transduced with the NTR gene. When they were treated with CB1954 and a single-fraction irradiation, the effect of radiotherapy was enhanced. These results were similar in human colon cancer xenografts.

Other prodrugs for NTR have been tested as antitumor treatment for colon and lung carcinomas. 1-(2-methylperidin-1-yl) diazen-1-ium-1,2-diolate is a nitric oxide prodrug, which is converted to its active form in the presence of NTR. Sharma et al added exogenously NTR to DLD-1 human adenocarcinoma cells, which were exposed to 1-(2-methylperidin-1-yl) diazen-1-ium-1,2-diolate. NTR transformed this prodrug into an active form and caused a high inhibition of the growth of the DLD-1 cells.

The previous systems have two fundamental limitations: the conversion of non-toxic prodrug into toxic metabolite and the bioavailability of active drug. These limitations are overcome by the use of genes that encode cytotoxic proteins, which have a direct antitumor action. The traditional therapy is targeted to cells undergoing division; however, this new therapy could be effective both in tumours with rapid development, and in tumours with slow growth.

**Gef**

The gef gene encodes a membrane protein composed of 50 amino acids, which is anchored in the cytoplasmic membrane by its N-terminal. In human tumour cells, gef induces apoptosis and cell cycle arrest, so it could be a complement to classical strategies such as chemotherapy. Prado's et al. have transfected the gef gene into MCF-7, a human breast cancer cell line, to determine their combined effect with some cytotoxics. Four groups were established: a control group, a group transfected with gef, a group treated with paclitaxel (Pac), docetaxel (Doc) or doxorubicin (Dox) at different concentrations, and a group treated with a combination of the gef gene and the cytotoxins. Reduction in cell growth was produced by all the cytotoxins, and the gef gene was able to induce a continuous decrease in the MCF-7 cell viability in the absence of any drug. Nevertheless, the combined therapy showed the largest growth inhibition supporting a strong synergistic effect. The combined therapy gef/Dox (10 µM) had approximately 15% more effect than the sum of both treatments. This effect may allow to reduce the concentration of drug in the treatment of breast cancer.

Similar findings have been noted in colon and lung cancer. In both cancers, gef produced a significant decrease in cell growth, and its combination with some drugs of choice in the clinical treatment enhanced notably their effect. A 50% reduction of the cell growth was observed 24 h after transfection of the gef gene, and showed a 15-20% enhancement of the antiproliferative effect when combined with the drugs. A study in lung multicellular tumour spheroids (MTS) revealed a decrease of MTS volume up to 87.4% when MTS was treated with gef/Pac versus untreated control MTS.
E: is a phiX174 bacteriophage gene that encodes a 91-amino acid membrane protein with lytic function and a C-terminal domain that is involved in oligomerization. The E protein is a non-competitive inhibitor of the mRay translocase of E.coli, which catalyzes the formation of lipid I, the first lipid intermediate in the synthesis of the cell wall. Several studies demonstrated the antitumoral activity of the E gene in lung, colon, melanoma and breast cancers. The expression of E induced a decrease in cell growth producing an irregular distribution of cells in the monolayer cultures. Dilated mitochondrias with disrupted cristae, a significant activation of caspase 3, and the release of cytochrome C into the cytoplasm 48 h after transfection, supported these results [Figure 1].

The combined therapy with the E gene and some cytotoxic drugs showed a greater inhibition of cell proliferation than the treatment with either the E gene or the drugs alone, showing a synergistic effect as described for the gef gene. The growth of A-549 and T-B4 cultured cells decreased a 19.8±1.3% and 19.2±1.7% respectively after E gene transfection, and the combination with some cytotoxins increased the inhibition of proliferation. E/Pac therapy induces up to 85% of growth inhibition in A-549 lung cells. This level of growth inhibition is only reached with the highest concentration of Pac alone. Studies with MCF-7 MTS corroborated this enhancement of proliferation inhibition. The volume of spheroids decreased an 18.9 ± 2.3% after E gene transfection. The E/Dox combination caused greater percentage of volume decrease (63.9 ± 2.7%) than Dox (35.1 ± 1.5%) or the gene of volume decrease (18.9 ± 1.0%) treatments alone. Thus, in this case, it is also possible to reduce the dose of the drugs to achieve a safe and effective treatment, reducing side-effects.

Apoptin gene: The Chicken Anemia Virus VP3 protein, also known as Apoptin, has a nuclear localization in tumor cells but not in "normal" cells. This allows a specific induction of apoptosis in cancer cells. This ability to kill only tumor cells has been reported in breast, head and neck, prostate, lung cancer, osteosarcoma, hepatocellular and nasopharyngeal carcinomas. A study analyzed the ability to induce apoptosis of five truncated apoptin proteins in human breast adenocarcinoma MCF-7 cells and human liver Chang cells as control. Three out of the five truncated proteins targeted the nucleus of the MCF-7 cells initiating apoptosis and showing no effect on normal cells.

Dacarbazine is an agent used in the treatment against malignant melanoma. When combined with apoptin they have demonstrated a synergistic antitumor effect in human and mouse B16-F1 melanoma cells. Similar findings were reported by Olijslagers et al., which indicated that the cytotoxicity of paclitaxel was enhanced by the combination with apoptin in several cancer cells. The combination of apoptin with photodynamic therapy or radiotherapy has a better response than the therapies alone.

E1A: The early region 1A (E1A) gene of the human adenovirus type 5 is a tumor suppressor and induces apoptosis in tumor cells. E1A has four conserved domains (CR1-CR4). CR1 domain is needed for its angiogenesis-inhibition, and CR2 domain is needed for the induced cell apoptosis and its chemosensitization activity. The antitumor activity of E1A has been tested in patients with breast, lung, prostate, bladder, ovarian and pancreatic cancers. E1A enhances the sensitivity of the cancer cells to some chemotherapeutic agents like paclitaxel, etoposide (VP-16), cisplatin, 5-FU or gemcitabine. Bhattacharya et al. studied the combination of a mutated E1A CR2 domain (dl922–947), with a defective pRb binding, with gemcitabine or 5-FU agents, in human pancreatic cancer cell. The combination of dl922–947 with either agent demonstrated to kill more cells than when administered alone. The greatest synergistic effect was observed with the dl922–947/5-FU combination.

E1A has also been proved to enhance the radiosensitivity of human nasopharyngeal cancer in nude mice. Mice tumors treated with the combination E1A/radiotherapy were 4.7-fold smaller than those treated only with radiotherapy and 5.3-fold smaller than those treated only with E1A. Similar findings have been demonstrated in human cervical, hepatocellular carcinoma and glioblastoma.

Patents
The current studies about cancer suicide-genes therapy clearly show its enhancing effect. Some advances in this field have been patented. An example is "Cancer gene therapy based..."
on translational control of a suicide gene"[65] in which TK mRNA was joined to the 5’ UTR of the basic fibroblast growth factor. This gene is translationally regulated by eIF4E, which shows high levels in most solid tumors, causing the translation of TK mRNAs in cancer cells, but not in normal cells, suggesting an enhanced sensitivity to GCV.

Another patent is entitled “Polypeptide having an improved cytosine deaminase activity”[66] A native CD was modified by addition of an amino acid sequence. This new CD showed an improved CDase activity in LoVo human cells (adenocarcinoma of colon) transfected with the new-CD/5-FC system as compared to the original CD/5-FC system.

The use of the E gene as a suicide gene for cancer gene therapy was registered in the patent “E gene for antitumor therapy”. [67] Tumors induced by B16-F10 (melanoma cells) in mice and treated with the E gene exhibited a decrease of the initial tumor volume and a significant reduction as compared to the tumor control.

A research group has two patents about the use of the apoptin. “Apoptin-associating protein” describes the apoptotic activity of the apoptin when p53 is completely or partially non-functional. [68] A year later, they registered the other one: “Modifications of apoptin”. [69] This suggests the use of the phosphorylation state of apoptin as a marker of cancer cells.

Conclusion

As shown in this review, suicide gene therapy has proved to be an effective treatment against cancer. The classic suicide gene therapy consists of the conversion of non-toxic prodrugs into toxic metabolites. The TK/GCV and the CD/5-FC systems are the most extensively studied, and other suicide genes, as CPG or NTR, are on the way. The discovery of genes that do not need the use of a prodrug, but can kill directly has been a breakthrough in this therapy. Good examples are: the Gf protein, which induces apoptosis and cell cycle arrest; the E protein, which performs its antitumoral activity through mitochondrial apoptosis. Apoptin protein, that only induces apoptosis in cancer cells, and does not affect normal cells; and the E1A protein, whose CR1 domain inhibits angiogenesis, while its CR2 domain induces cell apoptosis and chemosensitization. Promoting research on these genes may provide a great advance on future cancer gene therapy.

Currently, the research on suicide gene therapy is focused on their combination with commonly used anticancer drugs, allowing the development of new drugs and suicide-genes-drugs combinations to decrease their toxicity and side effects and enhance their antitumoral activity.

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