ELECTROTRANSFER OF CYTOKINE GENES FOR CANCER TREATMENT

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Abstract: Gene electrotransfer, which designates the combination of gene transfer and electroporation, is a physical method for transfecting genes into cells and tissues. Many reports for the utilization of this techniques in animals confirmed that gene electrotransfer is a safe and efficient method. One of the major advantages of electrogene therapy is that it does not result in systemic toxicity. Gene electrotransfer (GET) of plasmids encoding cytokines has been shown to generate a potent anti-tumor effect. Delivery of plasmids encoding cytokines induces not only a local immune response but a systemic one as well. Cytokines can be used to stimulate host inflammatory responses and immunity to cancers. This review aims to summarize preclinically tested cytokine genes with the help of electroporation for cancer treatment.

UDC Classification: 577; **DOI:** http://dx.doi.org/10.12955/cbup.v6.1291

Keywords: gene electrotransfer, electroporation, cytokine, cancer,

Introduction

The mechanism between the immune system and tumor cells is complex and dynamic. For the elimination of foreign particle, the interaction process is required between the adaptive and innate immunity of the immune system. These counter and protective mechanism are also effective to fight cancer cells (Kalb et al., 2012; Baginska et al., 2013). Tumor cells elude immune responsea by suppressing major histocompatibility complex (MHC) class I expression which strongly reduces the antigenicity of tumor cells, therefore preventing an immune response. The host defense mechanism is used by the immune system to avoid and prevent tumor growth. The major contributing immune cells that show a significant antitumor response are natural killer (NK) cells, T lymphocytes, macrophages, and B lymphocytes. Cytokine protein is used as a chemical mediator to enhance the efficiency of these immune cells to kill the malignant cells (Verbik & Joshi, 1995). Cytokines are the cell signaling molecules, that play a vital role in body's immunological functions. Cytokines are supposed to be a major component of the immune system as they regulate all biological functions of the immune system such as development, maturation and localization of cellular components of the immune system. They are also responsible for initiation, execution, and extinction of innate and adaptive immunity against foreign particle as well as against tumors as they elicit autocrine, paracrine or endocrine signaling pathways (Vacchelli et al., 2015). Shirley (2017) suggested that further progression of a disease can be prevented by cytokine therapy either by blunting specific cytokine expression or their signaling cascades to modulate immune function. In response to cellular stress such as infection, inflammation etc. cytokines work by minimizing cellular damage and controlling cellular stress (Dranoff, 2004) and hence for human cancer immunotherapy, cytokine treatment has been established as one of the main pillars (Floros & Tarhini, 2015). Cytokines should provide enough stimulus to excite host immunity, which once established will initiate a long-lasting antitumor response. It is a practical approach to target certain inflammatory cytokines which are associated with disease progression. In cancer pathogenesis, the cytokines that are produced in the tumor microenvironment have an important role (Floros & Tarhini, 2015; Kortylewski et al., 2009). Cytokines consist of interleukins, interferons and growth factors. Showalter et al. (2017) found that IFN-γ or TNF cytokines are very useful to prevent metastatic cancer by employing the immunogenic type microenvironment. Several therapeutic options are available for tumor treatment such as surgery, radiotherapy, radiofrequency, cryosurgery and electrochemotherapy. Out of these, electrochemotherapy was brought into use for the treatment of different cutaneous and subcutaneous tumors because of its effectiveness and safety (Miklavcic et al., 2014). But in addition to electrochemotherapy, another biomedical application based on electroporation is gene electrotransfer. Gene electrotransfer has been already entered into several clinical trials (Heller & Heller, 2015). Hence, electroporation is one of the most promising and efficient approaches to delivering cytokines (Shirley, 2017). The duration of desired cytokine expression and level can be selected by selecting the appropriate delivery parameters. Cellular and humoral responses are more likely to overcome intrinsic immune evasive and apoptotic defects of cancer cells. For cancer cell apoptosis, synchronized cellular and humoral responses are needed as shown in Figure 1.The aim of this paper is to provide an understanding of how electroporation has been used to deliver plasmid-encoded cytokines for cancer treatment. It

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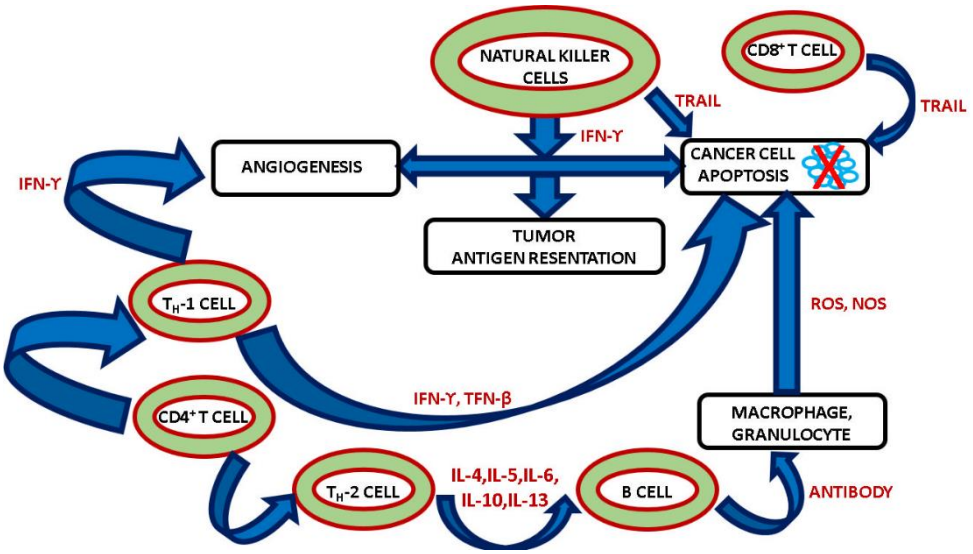
provides a brief overview of preclinical studies that are either currently underway or have been completed.

Figure 1: Synchronized cellular and humoral responses for cancer cell apoptosis. After stimulation, natural killer cells lyse cancer cells through TRAIL (tumor necrosis factor related apoptosis-inducing ligand). Natural killer cells secrete (IFN-γ), which inhibits angiogenesis, enhance tumor antigen presentation and cancer cell apoptosis. CD8+ and CD4+ production by NKT cells. CD8+T cells lyse the cancer cell through TRAIL, whereas CD4+ T cells differentiate TH1 or TH2 cells. TH1 cell release IFN-γ and TNF-β and TH2 releases interleukins which increase the production of antibodies by B cells. The role of antibodies is to inhibit oncogenic signaling and stimulate cancer cell apoptosis by macrophages and granulocytes. Macrophages lyse cancer cells by the production of reactive oxygen species (ROS) and nitric oxide (NOS). Lymphocytes also attack tumor blood vessels.



Source: Authors

Gene Electrotransfer

To deliver molecules into the inside of cells, many methods have been developed for in vitro and in vivo applications. One of the efficient methods for gene delivery is gene electrotransfer which is also referred as electroporation (EP). Electroporation is a technique in which an external electric field is applied in order to alter the membrane's permeability. When transmembrane potential exceeds the threshold value, it induces transient pores on the cell membrane (Neumann et al., 1982; Yarmush et al., 2014). Depending on the pore size, this allows the delivery of both small and large molecules (Kanduser, Miklavcic, & Pavlin, 2009). As described by (Rols, 2008), for successful gene electrotransfer multiple steps are involved: (i) complex formation between DNA and cell membrane; (ii) DNA translocation across the membrane; (iii) DNA transfer from the cytoplasm into the nucleus; and (iv) gene expression. In vivo delivery of pDNA were first performed on brain tumors (Nishi et al., 1996). Since then, the technique has been applied to many tumor types (Heller & Heller, 2006; Favard et al., 2007). Gene electrotransfer, uses electric pulses to deliver the molecules into cells. Delivery of DNA offers several medical applications, among the most promising is gene therapy for cancer treatment. It is a safe and effective physical method which was introduced in the mid of 80s. The concept of gene therapy is based on the delivery of genetic material into the nucleus, so that it is expressed and produces a therapeutic effect (Burgain-Chain & Scherman, 2013). Electroporation is a versatile method which is used to deliver the molecules to a wide variety of tissues such as skin, heart, lung, liver, tumor, and muscle. Gene transfer by electroporation increases the expression up to 1000-fold as compared to DNA injection alone (Mir et al., 1998). Electrotransfer of a gene depends on different parameters such as pulse duration, orientation of pulses, polarity and field strength. But in many cases, parameters for gene transfer depends on the tissue type. The most common ways by which electrotransfer is used to deliver the cytokines are to the skin, to the muscle or to the tumor itself. Some of the studies with electrotransfer of cytokine genes for cancer treatment are listed in Table 1.

Table 1: Cytokines as cancer therapy

Cytokine	Cell sources	Cell targets	Role in tumor formation	Therapeutic Action	Cancer type
IL-1	Macrophages, Lymphocytes Keratinocytes, Dendritic cell,	Epithelial and endothelial cells	Tumor invasion Angiogenesis	Induction of proinflammatory proteins	Fibrosarcoma
IL-6	Endothelial cells, fibroblasts, macrophages	Hepatocytes, leukocytes, T cells, B cells,	Required for chemically induced lymphomas	Enhances T-cell and B-cell function	Melanoma
IL-12	Macrophages, dendritic cells, neutrophils	T cells (Th1 cells), NK cells	Inhibits chemical carcinogenesis	Enhances TH1 immunity and cytotoxicity; inhibits angiogenesis	Colon, breast, Merkel cell carcinoma, malignant melanoma
IL-15	Monocytes, activated CD4+ T cells, keratinocytes, skeletal muscle cells	Natural killer cells	Promotes natural killer T cell leukemias	Enhances Cytotoxicity	Melanoma
IFN-γ	Natural killer cells, natural killer T cells, B cells, macrophages	Macrophage	Inhibits lymphomas, Stat1 and Rag2	Enhances tum our antigen presentation cytotoxicity	Prostrate
Gm-CSF	T cells, natural killer cells, macrophages, eosinophils, endothelial cells,	macrophage, neutrophils, and eosinophils	Inhibits lymphomas and carcinomas	Enhances Tumor antigen presentation	Colorectal
TNF-α	Macrophages, natural killer cells, B cells, T cells, neutrophils, fibroblasts, keratinocytes	Activation of MAPK pathway	Required for chemically-induced skin cancers	Induces Tumor-cell apoptosis;	Bladder

Source: Authors

Electrotransfer of cytokine gene for cancer immunotherapy

For cytokine gene therapy, electroporation is considered as an efficient technique for the delivery of genes directly to tissues of interest and results in increasing host immunity to tumor antigens. This technique avoids the involvement of nearby tissues. There are many diseases in which Cytokine gene therapy is being used as an effective treatment. Out of those diseases, cancer treatment is of interest. Cytokine gene delivery can either prevent the progression of tumor cells or kill them completely. By eliciting strong inflammatory reactions in the host, cytokine therapy prevents the progression of tumor cells. Cytokine gene therapy has a potential of generating long-lasting immune memory, which helps in tumor elimination. For cancer treatment, studies have been done on various therapeutic cytokines such as interferons (IFN), interleukins (IL), growth factors and tumor necrosis factor family members. In vitro T cell function and proliferation is enhanced through the delivery of cytokine mRNA by electroporation (Weinstein-Marom et al., 2016). For adoptive cell cancer therapy, these findings may have potential. Other studies showed the production of tumor-specific CD8 T cells when mRNA associated with a tumor antigen was electroporated (Bonehill et al., 2008). With many potential candidate genes, preclinical studies have been performed for cancer gene therapy using electroporation. Candidate genes including inhibitors of cell growth, tumor suppressors, and tumor antigens. On a variety of tumor models, immunotherapy approaches have been studied (Bodles-Brakhop et al., 2009). In 1950 discoveries related to interleukins started. But, it took 2 decades for their structure and function to be identified. IL-1 was the first discovered interleukin. About 35 interleukins have been identified. For cancer treatment clinical use of IL-2 was started by Rosenberg et al. in 1985 (Rosenberg et al., 1985). Local administration of IL-2 inhibits the growth of human tumors (Pizza et al., 1984). The growth of

killer cells such as lymphokine activated killer cells (LAK), cytotoxic T lymphocytes, tumor infiltrating lymphocytes and natural killer cells are stimulated by IL-2. Without destroying normal cells, it is able to recognize and kill tumor cells. An increased dose of IL-2 is a limitation for systemic delivery due to its toxicity. But the alternative method that reduces systemic toxicity is an Il-2 plasmid DNA (pDNA) intratumoral injection with electroporation. Decreased tumor size shows the systemic and local activity of IL-2 plasmid DNA with electroporation (Richards et al., 2007). It has been shown that electrotransfer of IL-2 is safe and well tolerated in patients. Schmidt-Wolf et al. (1999) showed high levels of IFNγ, GM-CSF, and TGF-β as well as CD3 lymphocytes during treatment with IL-2 for metastatic renal cancer and colorectal cancer. Combination use of IL-2 and IFN alpha (IFN-α) for melanoma and renal cell carcinoma were the mainstay of treatment. For recurring metastatic melanoma patients, a phase I study investigated the electrotransfer of VCL-IM01, a plasmid-encoded IL-2. To date, the results of this study have not been published. IL-2 has no direct cytostatic and cytolytic effects on tumor cells (Antony & Dudek, 2010). It has been shown that delivery of plasmid-encoded IL-2 via electroporation is safe and effectively tolerated in patients. IL-2 is in clinical trials for the treatment of metastatic melanoma.

The most studied cytokine for cancer therapy delivered by electroporation either as DNA or RNA is IL-12. In clinical studies, it is used as a single agent or in combination for the treatment of lung cancer, triple negative breast cancer, head and neck carcinoma, melanoma and pancreatic cancer. IL-12 electrotransfer has shown effective results during phase I studies with metastatic melanoma (Daud et al., 2008). Later, some studies have been performed for the treatment of other types of cancers such as Merkle cell carcinoma, cutaneous lymphoma, and carcinoma of the head and neck. Phase II studies for the effectiveness of IL-12 electrotransfer have started for metastatic melanoma (A. Daud et al., 2014). With IL-12 delivery, researchers showed an increased frequency of circulating Natural killer (NK) cells. Co-delivery of human catalytic reverse transcriptase subunit of telomerase (hTERT) and IL-12 using electrotransfer will produce an immune response that will reduce the risk of recurring in pancreatic, breast and lung cancers (Vonderheide et al., 2015). Some studies show only inhibition of tumor growth induced by IL-12 rather than complete regression (Kishida et al., 2003). After IL-12 delivery, long-term tumor eradication of up to 83% of solid melanomas have been observed (Lucas, Heller, Coppola, & Heller, 2002).

IL-18 is a multifunctional cytokine. IL-12 and IL-18 share many biological activities. Several studies have been done based on its effects on T and NK cells. IL-18 stimulates Th1 cells differentiation. Th1 cells secrete cytokines which are necessary for the defense against tumors by activating cell-mediated immune responses (Jiang et al., 2001). It also induces IFN gamma production in T cells (Okamura et al., 1995). GM-CSF is delivered in combination via electroporation for the treatment of advanced metastatic carcinoma. Cancer cells are harvested from the patient for irradiation and then transferred back. The researcher hypothesizes that GM-CSF increases antigen presentation as well as dendritic cell recruitment. Similar studies have been done for the treatment of colorectal cancer (Simons et al., 1999). Interferons have an antitumor effect. With IFN α more than 14 types of cancer were targeted. IFNα makes cancer cell unprotected for killing by cytotoxic T cells. It was shown in a study that transfer of Interferon-gamma inhibits tumor cell growth (Ferrantini & Belardelli, 2000). But other studies of interferon gamma showed some cases with increased metastases in a murine model. The study showed tumor regression after delivery of IL-15 via electroporation in C57BL/6J mice induced with B16 and F10 melanoma tumors. Chances of tumor recurrence were also negligible (Marrero, Shirley, & Heller, 2014) .IL-33 is also currently in studies.

Conclusion

Cytokines are able to stimulate the immune system in different ways as shown in Table 1. This paper has outlined several studies for cancer treatment based on the electrotransfer of cytokine genes. As per the studies, the electroporation method has no adverse effect and is well tolerated. Delivery of pDNA using electric pulses has greatly increased protein expression as compared to DNA injection alone. But further optimization of electrical parameters and DNA concentration must be done for different grades of tumors. Over the past several years, studies have been published justifying the efficacy of this delivery method. Even clinical trials have been initiated using the electrotransfer of IL-2, GM-CSF, and IL-12 and soon there will be several new electrogene transfer clinical trials for cancer treatment.

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