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Effective electro-chemo-therapy for proliferation control of adult human mesenchymal stem cells

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ABSTRACT

Clinically chemo-resistant types of cancers do not respond well to conventional therapies. To treat and enhance the efficacy of drug delivery for these cancers, we have developed an in vitro model of a combination therapy using adult human mesenchymal stem cells, electrical pulses and chemo drug. Adult Mesenchymal stem cells were used because they are similar to cancer stem cells which cause the tumor to be chemo- and radiation resistant. These cells, derived from human adult bone marrow were subjected to low voltage, long duration (200 V/cm, 40 ms and 450 V/cm, 25 ms) and high voltage, short duration (1200 V/cm, 100 μs) pulses. The effect of these voltages on the viability and proliferation ability of these cells in the presence and absence of Bleomycin (chemodrug used for treating various cancers, FDA approved in US and other respective medical agencies in other countries,) indicate the potential of transfer of this technique to clinical practice for effective electro-targeted stem cell therapy.

1. Introduction

Cancers, kill about one American per minute, about 1500 deaths per day in US alone [1]. The deaths of 38,000 women due to breast cancer in the US, the pitiful cure rate of 5% for pancreatic cancers, and the mere one year median survival of glioblastoma cancer tell us that current treatments are inadequate [2,3]. The majority of cancer deaths are due to the metastasis and this is due to the non-elimination of the cancer stem cells. Cancer stem cells (CSCs) are a very small subset of all cancer cells and possess characteristics very similar to normal stem cells [2], in particular, the capacity for self-renewal, multipotency, relative quiescence, and cytoprotective mechanisms and expression of drug transporters (Fig. 1a and b [4,5]). Presence of cytoprotective mechanisms renders immunity to CSCs from cytotoxic therapies.

Various researchers have identified a small population of tumorigenic cells with stem cells like properties in bladder cancer, melanoma, ovarian cancer, head and neck squamous cell carcinoma, etc. It has also been reported that these CSCs are not only involved in tumor progression, but also in metastasis. CSCs are reported to be resistant to a wide variety of chemotherapeutic agents and possess remarkable ability of recovering from cytotoxic therapy. The Glioma stem cells (GSCs) not only play a crucial role in chemoresistance, but they are vital to the failure of radiation too as evidenced from the enriched CSCs in the tumors [2,3].

These chemo- and radiation resistant cells are responsible for maintaining tumor volume leading to therapy failure and recurrence. This necessitates alternate treatments to cancers and many novel treatment modalities are being investigated to specifically target this small group of cells. One effective, physical, treatment

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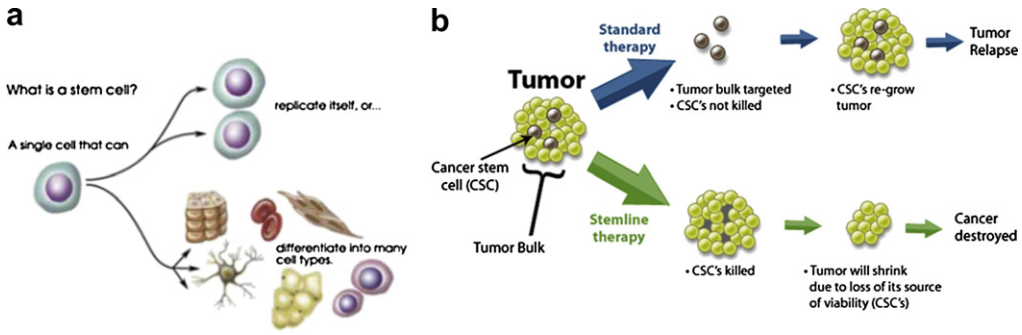

Fig. 1. Comparison of stem cell vs. cancer stem cell [4,5].

is electroporation or electropermeabilization (EP), where-in high intensity, short duration electrical voltage pulses are utilized to open up pores to upload drug molecules that are normally impermeable. Various clinical and preclinical trials attest to the efficacy of this technique to treat melanoma and other cancers using chemo drugs and genes [6–9]. In this research we utilize electrical pulses to impede the proliferation of mesenchymal stem cells, which are similar to cancer stem cells and investigate the various pulse parameters, especially low voltage ones that could be used to achieve this. In addition, the energy and charge applied are also studied as the electroporation process involves the accumulation of charges and their interaction with the cell plasma membrane.

The use of electrical pulses for active delivery of drug/gene molecules to the interior of cells, especially adult stem cells offers exciting prospects. The pulse parameters explored here rely on the cell's natural ability to heal itself after electroporation. This is because membrane disruption and resealing is common in vivo as cells normally heal smaller wounds naturally in day to day life. Using that inherent nature of cells has the advantage of uploading molecules of our choice as desired.

Towards this end, our study investigates the application of high and low intensity, short and long duration (micro and milli seconds) electrical voltage pulses for the proliferation control as well as the uploading of drug molecules into adult mesenchymal stem cells, as drug delivery is one of the most challenging issues of the medical community and the need for alternate treatments, especially non-chemical, non-viral, physical ones could not be overstated.

Many potential drugs that have been developed to treat cancer and other diseases have found limited success due to lack of safe and efficient delivery systems that allow molecules to cross the hydrophilic and hydrophobic lipid bilayers of cell plasma membranes which are normally impermeable. Use of external, physical means, such as electrical pulses to cause transient, electrostatic, dielectric breakdown of the bilayer of the cell membrane opens up transient pores (aqueous pathways) through the membranes and tissues allowing targeted delivery of therapeutic molecules including drugs, antibodies, and genes (DNA) [6–16]. The electroporation technique has been shown to offer 100–1000 fold improved therapeutic benefits compared to using drug alone, and is gaining acceptance as a viable technique for cancer treatments and is applicable to all histological types of tumors. The efficacy of EP could be attributed to the redistribution of charges [17] on the membrane surfaces and the variations in the membrane potential of cancer cells compared to normal ones. Electroporation-mediated chemotherapy or Electrochemotherapy (ECT) is a viable alternative to conventional cancer treatments as evidenced by successful Phase I and II clinical trials for various skin cancers, such as lymphomas, squamous cell carcinomas, testicular carcinomas, and malignant pleural effusions [14–16]. The treatment of 52 patients using ECT for melanoma and chest wall breast carcinoma by Campana et al. attest to its successes [8]. Clinical trials of delivering vaccines for prostate cancer using electroporation have been conducted at the University of Southampton Medical School [18]. Stanford Medical School used electroporation to study tumor oxygenation [19]. We also have previously shown that under appropriate conditions, electrical pulses can drive large number of molecules into breast cancer cells [20,21]. This study addresses the hypothesis that electrical pulse could effectively be used to control proliferation as well as enhance intracellular drug delivery and effects of chemotherapeutics in adult, human, mesenchymal stem cells and intends to develop an outpatient-based, efficient, economical, physical technique using short electrical pulses with minimal side effects. Since electroporation treatment uses reduced doses of the drug, it offers benefits to the patients in many ways, including physical, economical, and enhanced quality of life.

CSCs show marked similarity with normal stem cells in terms of cellular and genetic architecture. Hence, we chose adult stem cells, as stem cells being mulipotent, are capable of renewing themselves indefinitely like CSCs, while retaining the potential to differentiate into different lineages. These cells remain quiescent and become active when the necessity arises as in the case of tissue damage, disease or a need for regeneration of certain organs.

In this research, our goal is to assess the feasibility of controlling the proliferation of adult human mesenchymal stem cells (hMSCs) using electrical pulses and chemo drug. Mesenchymal stem cells are a phenotypically and functionally heterogeneous cell population [22,23]. In culture, they are defined as plastic-adherent, fibroblast-like cells which are able to self-renew and differentiate into bone, adipose and cartilage tissue. Since adult stem cells are more abundant and easier to isolate than cancer stem cells [22,24], we use adult hMSCs in this study. In addition, they share many of the molecular properties of cancer stem cells [2,5].

Another objective in delivering drug molecules into adult hMScs is to study the possibility of using these cells as carriers of therapeutic molecules to the tumor site, as, there have been recent reports of migration of human mesenchymal stem cells towards tumors [25] both in vivo and in vitro. Thus, adult mesenchymal stem cells possess the ability to home towards tumor cells, making the possibility to practice targeted tumor therapy more realistic [26]. Towards this end, we have developed an in vitro model of a combination therapy using adult human mesenchymal stem cells.