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Steep pulsed electric fields modulate cell apoptosis through the change of intracellular calcium concentration

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Abstract

A steep electric pulsed field with low intensity (150–250 V/cm) and relative long time (10 min) was applied to adherent liver cancer cell line SMMC-7721 and the liver cell line HL-7702. Results showed that the electric field with intensity of 200 and 250 V/cm could trigger cell apoptosis, whereas the SMMC-7721 cell was more sensitive to the electric stimulation than the HL-7702 cell. Laser Scanning Confocal Microscope (LSCM) was used to measuring the real-time change of cytosolic free Ca^{2+} concentration. When cells were exposed electric pulses with 100 V/cm intensity for 10 min, there was no significant change of intracellular calcium concentration. With the intensity increased to 200 and 250 V/cm, intracellular calcium concentration decreased significantly. Results demonstrated the relationship between the apoptosis and change of intracellular calcium concentration. And the steep electric pulsed field can be used to the cancer therapy.

Keywords: Steep electric pulsed field; Cancer cell; Apoptosis; Intracellular calcium concentration

1. Introduction

Electric fields have been used in cancer treatment for many years. Hoffmanm et al. [1] suggested a electroporation therapy (EPT) for the treatment of head and neck cancer, in which the pulsed electric fields can improve the effects of chemical therapy medicine such as bleomycin (BLM). It was found that the electric stimulation resulted in the electroporation of cell membrane. However, the chemical therapy medicine can damage human bodies as well as kill cancer cells. Therefore, a new approach is proposed that a short pulsed electric fields could be used independently to kill cancer cells [2–4].

In most studies, electric fields were applied on the suspensions of cells [5–7]. We previously determined the effect of steep pulsed electric fields on ovarian cancer cells [8]. The results showed that the electric fields can inhibit the growth of cancer cells. However, many kinds of cancer cells are adherent in vivo. Furthermore, adhesive cells and suspending cells have different responses to electric fields [9]. Therefore, in this paper a new device was developed to apply the electric pulses on adherent cells.

The steep electric pulse fields have been found to trigger physiological responses in cells, in which the release of intracellular free calcium was paid a great attention to [10–13]. Intracellular calcium is stored in endoplasmic reticulum compartments and mitochondria, and calcium release from mitochondria is considered to initiate apoptosis [14].

For the purpose of cancer treatment, a steep electric pulsed field with low intensity and relative long time (10 min) was applied to adherent liver cancer cell line SMMC-7721 and the liver cell line HL-7702. An important goal of this contribution is to evaluate the different responses of cancer cells and normal cells triggered by electrical signals. And the real-time imaging method was used to investigate the intracellular calcium concentration in the whole course of electric stimulation. The different sensitivity of two cell types suggests the feasibility of the therapy with steep pulsed electric fields independently.

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Fig. 1. Pulse generator used in these experiments.

Understanding the intracellular effects in real-time will help elucidate how steep pulsed electric fields induce cell apoptosis through the change of intracellular calcium concentration and possibly lead to new therapeutic and diagnostic applications.

2. Materials and methods

2.1. Cell culture

Experiments were performed using SMMC-7721 cells, a human liver cancer cell line and HL-7702, a human liver cell line (Shanghai Biochemical Institution, Shanghai, China). Cells were cultured with RPMI-1640 medium containing 2% glutamine supplemented with 10% fetal bovine serum and 2% penicillin and streptomycin in a 37 °C, 5% CO_2 incubator. Before experiment, cells were digested with 0.25% trypsin and seeded in the chamber of the electric stimulation apparatus.

2.2. Electric pulse treatment

Energy-controllable pulse device is developed in the key lab of high voltage engineering and electrical new technology of Chongqing University [8]. Pulse curve is in form of exponent attenuation with the utmost voltage peak value 250 V, rise time 90–180 ns, pulse duration 1–20 μs, and the frequency 10 Hz–1 kHz. The energy-controllable pulse device and the typical pulse curve are showed as Figs. 1 and 2, respectively.

The results of Sun et al. [8] demonstrated that this device can kill cancers if appropriate parameters are selected. The parameters used in this paper are shown in Table 1.

Table 1
The parameters of applied steep pulsed electric fields

Intensity (V/cm)	Frequency (Hz)	Capacitance (μF)	Duration (μs)	Time (min)
150	50	0.033	1.8	10
200	50	0.033	1.8	10
250	50	0.033	1.8	10

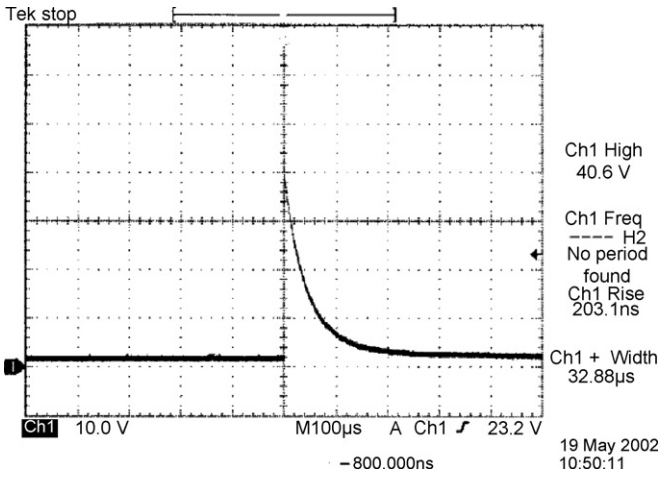

Fig. 2. Typical pulse generated by pulse generator.

2.3. Determination of cell apoptosis

2.3.1. Flow cytometry

Flow cytometry provides a rapid and quantitative analysis of multiple features of individual cells. Cells were exposed to the steep pulsed electric fields with different intensity. After digested with 0.25% trypsin, cells in suspension were incubated with propidium iodide (PI). Then cell suspensions were subjected to flow cytometry using a flow cytometer (FACSC alibur, Becton Dickinoson Cop, USA).

2.3.2. Fluorescent markers for apoptosis

Annexin-V-FITC (fluorescein isothiocyanate) was used as the fluorescent marker. Annexin-V-FITC specifically binds to the cell membrane lipid phosphatidylserine (PS), which is on the outside of intact outer plasma membrane of apoptotic. Cells were exposed to the steep pulse and digested. The cells in suspensions were incubated with Annexin-V-FITC for 10 min prior to analysis by flow cytometry.

2.3.3. Real-time intracelluar free calcium ion concentration

Cells were loaded with the calcium sensitive dye Fluo3-AM (4 μmol/L, Sigma) for 30 min at 37 °C. Laser Scanning Confocal Microscope (LSCM) was used to measuring the real-time change of cytosolic free Ca^{2+} concentration. $[Ca^{2+}]_i$ measurements were obtained by recording the intensities of the fluorescence every 2 s. All experiments were performed at room temperature with two solutions. The one is the culture medium, the other is the medium without Ca^{2+}.

3. Results

3.1. The effect of pulses on the apoptosis of cells

Electrical fields with different intensity were applied to liver cancer cells and liver cells. The effect of steep pulses on cells apoptosis was studied using flow cytometry and Annexin-V-FITC. As showed in Fig. 3, electric field at relatively low intensity (150 V/cm) did not trigger cell apoptosis, whereas liver