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The activation of cancer cells by a nanosecond-pulsed magnetic field generator

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Abstract

Cancer treatment is a challenge in modern medicine. Several novel modalities have been invented to selectively treat cancer cells. The reactive species such as reactive oxygen species and reactive nitrogen species play important role in the anti-cancer capacity of several modalities, such as cold atmospheric plasma. In this study, we first demonstrated that a nanosecond-pulsed magnetic field (NMF) generator could sensitize the melanoma cell line B16-F10 to the cytotoxicity of an important ROS, H_2O_2. The magnetic field strength and the frequency were two key factors to control the activation level of NMF treatment. This study provided a novel strategy to use electromagnetic modality in cancer treatment particularly for the treatment on deep tumors such as brain, pancreatic, etc.

Keywords: nano-pulsed magnetic field, cancer treatment, activation, melanoma

(Some figures may appear in colour only in the online journal)

1. Introduction

Cancer is one of the most fatal diseases. Melanoma is a common skin and mucosa tumor, with a high mortality rate so far. Melanoma is also one of the fastest growing malignant tumors [1, 2]. Current therapy is mainly based on surgical treatment, radiotherapy and chemotherapy. Previous modalities tend to affect the growth of cancer cells via directly causing apoptosis or other cell deaths. For example, CAP is a potential method to treat melanoma based on its abundant reactive species component [3, 4]. CAP kills cancer cells through the direct transportation of these reactive species into the extracellular environment. We recently demonstrated the existence of an activation state on the CAP-treated cancer cells [5]. The CAP-treated pancreatic adenocarcinoma cells (PA-TU-8988T) could quickly enter into a specific state, in which cells were very sensitive to the cytotoxicity of reactive species [5]. Such activation might be due to physical effect such as the electromagnetic field.

Pulsed magnetic fields have been used as a medical modality for years. The low-intensity pulsed magnetic field was an effective treatment for osteoporosis [6]. Compared with the continuous magnetic field, the pulsed electromagnetic field owned a faster rising-edge, which caused a stronger induced and transient electric field and a wider spectrum without significant thermal effect [7–9]. Different from CAP treatment, the pulsed electromagnetic field only affected cancer cells via its physical effect. The previous studies tended to directly affect the growth of cancer cells via causing apoptosis or other

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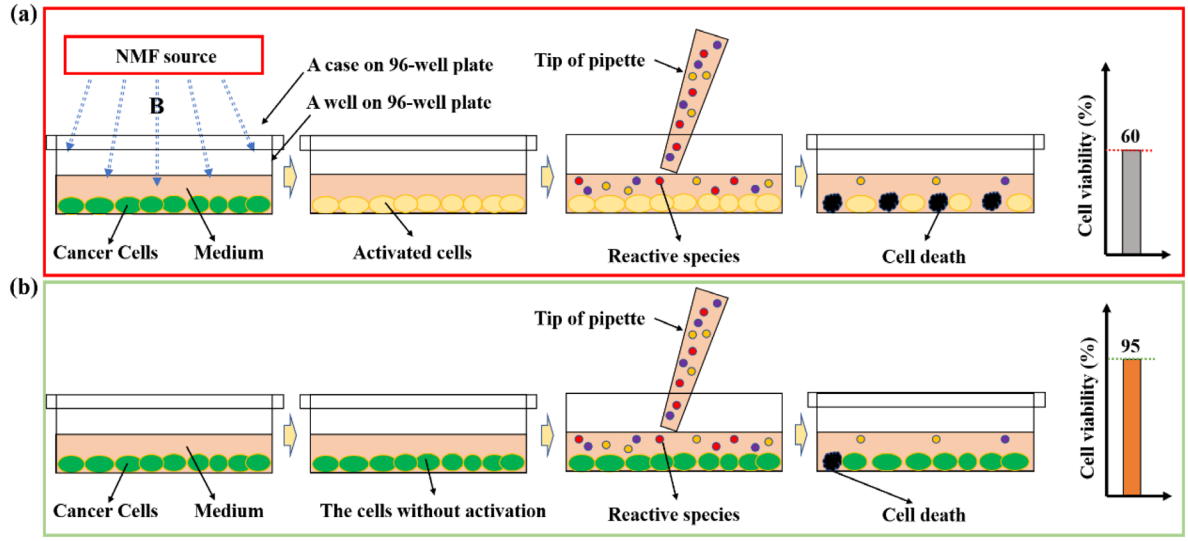

Figure 1. The schematic illustration of the NMF activation on melanoma cells. NMF source treats the melanoma cells through a barrier of plastic case on the 96-well plate. The cell viability ratio between (a) and (b) is used to describe the activation level by the NMF generator.

cell death pathways [10, 11]. In this study, we focused on the potential activation effect of NMF treatment on pancreatic cancer cells and melanoma cells (figure 1).

2. Materials and methods

The NMF generator was assembled at Prof Xueling Yao's lab, at Xi'an Jiaotong University, China. As shown in figure 2, it generated a NMF with a rise time less than 10 ns, a pulse width of 110 ns, and a magnetic intensity up to 6 mT. The schematic illustration and photos were shown in figures 2(a) and (b), respectively. The nanosecond-pulsed square wave current generating device worked at a coaxial cable energy storage mode. The main components included a DC source, a current limiting resistor, a coaxial cable, a switch, as well as a matching impedance. The matching resistor was 50 Ω, which was the same as the characteristic impedance. The outer part of coaxial cable was grounded. The main circuit diagram was illustrated in figure 2(c). The maximum output DC voltage was 12 kV. The waveform of the output magnetic field was shown in figure 2(d). As the distance between the coaxial cable and the cell varied, different magnetic field intensity was obtained. The distance from coaxial cable to cells was 13.5 mm, 20.6 mm, 28.8 mm, 37.4 mm, and 46.1 mm, respectively.

The magnetic field measurement system used in this study consisted of a detection coil and an oscilloscope. We used the electromagnetic induction method to measure the pulsed magnetic field intensity. The electromagnetic induction method was as follows: when the magnetic flux passed through a closed conductor loop changed, current and induced electromotive force ε appeared in the conductor loop. This electromotive force was proportional to the change rate of the magnetic flux φ passing through this loop (equation (1)). Accurate measurement of this electromotive force gave a differential form of magnetic induction. The induced electromotive force was then integrated. The result was linearly related to the measured magnetic induction intensity (equation (2)), where S was the cross-sectional area of the detection coil and N was the number of coil turns.

$$\varepsilon = N\frac{d\varphi}{dt} = N\frac{d(BS)}{dt} = NS\frac{dB}{dt} \tag{1}$$

$$B = \frac{1}{NS}\int \varepsilon dt \tag{2}$$

where ε is the induced electromotive force (V), N is the number of turns, φ is the magnetic flux passed through a closed loop, S is the square of the induction surface, B is the magnetic intensity, t is time.

The magnetic field measurement system used in this study consisted of a detection coil and an oscilloscope. The inner conductor of the coaxial cable was made into a detection coil. The detection coil was set to 1 turn and the diameter was 5 mm. The area S was 19.6×10^{-6} m^2.

When a pulsed-current passes the exposure cable, it will be exposed in a magnetic field. There's two kinds of electric fields co-exist due to magnetic field exposed. One is the electric field induced by the magnetic generator, and the other is the induced electric field caused by the magnetic field inside the cells. The maximum E-field induced by the generator is 20 V cm^{-1} according the charging voltage and distance between the coaxial cable and the cells. It is close to a DC waveform.

The human pancreatic adenocarcinoma cell line (PA-TU-8988T) was cultured using DMEM (11965-118, Life Technologies) supplemented with 1% (v/v) penicillin and streptomycin (15140122, Life Technologies) and 10% (v/v) fetal bovine serum (E17063, Atlanta Biologicals). Murine melanoma cells (B16-F10) were cultured using RPMI-1640 medium (80614171, ATCC) supplemented with 10% (v/v) fetal bovine serum and 1% (v/v) penicillin and streptomycin. In each experiment, 3×10^3 cells were seeded per well in a 96-well plate (62406-081, Falcon-Corning) and were cultured 24 h under the standard culture conditions prior to NFM

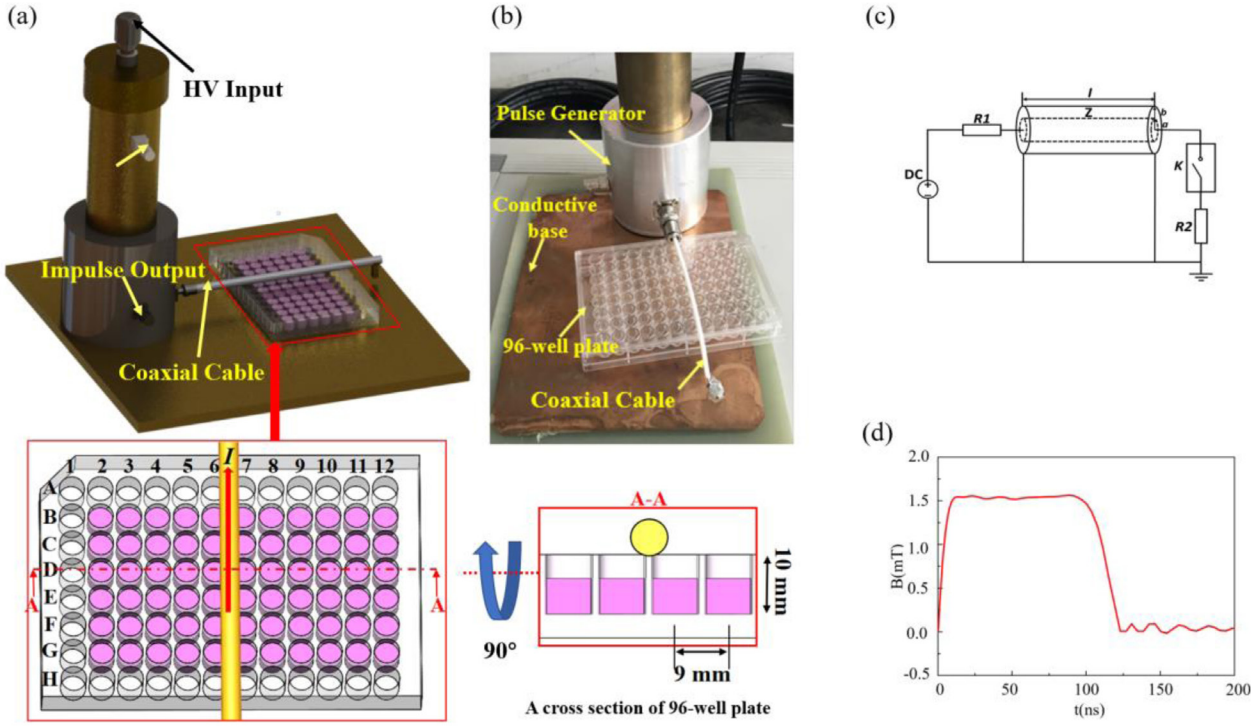

Figure 2. The nanosecond-pulsed square wave electromagnetic field generator. (a) The 3D model of setup. (b) The photo of the NMF device. (c) The circuit diagram of the NMF device. (d) Magnetic field waveform. R1: the charging resistor. R2: the coaxial cable matching resistor. Z: the coaxial cable impedance. K: the switch.

treatment. These initial media would be removed before the NMF treatment and were renewed by new media without FBS.

The activation phenomena in cancer cells was monitored by comparing the cytotoxicity of H_2O_2 on the activated cells to the cells without activation [5]. The H_2O_2-containing medium was transferred to affect the growth of cancer cells. The corresponding control was just cultured in the new DMEM or RPMI accordingly. The cancer cells were cultured 2 d after treatment. The cell viability was measured by using MTT assay according to the standard protocols (Sigma-Aldrich, M2128). The 96-well plates were read (570 nm) by a microplate reader (H1, Hybrid Technology). The measured absorbance at 570 nm was further processed to be relative cell viability via the division between the experimental group and the control. Throughout the whole paper, the relative cell viability was expressed as 'Cell Viability (%)'. All experiments were independently repeated for at least three times in sextuplicate.

To investigate the activation effect, we proposed a novel protocol. The whole strategy was based on three experimental cases. Case1: NMF treatment. In this case, the cancer cells were immersed in the 50 μl/well DMEM or RPMI during the treatment. After that, DMEM or RPMI was quickly (<20 s) removed and was renewed by the μl/well of new medium. The cancer cells were thought to be activated by NMF in this case. Case 2: the NMF treatment supplemented with H_2O_2 treatment. The cancer cells were first activated by NMF treatment in the 50 μl/well medium, followed by a quick remove (<20 s) of medium after the treatment. Then, a 50 μl/well H_2O_2-containing medium has been used to culture the NMF-treated cancer cells until the final cell viability assay. Case 3: H_2O_2 treatment. The cancer cells were just cultured in 50 μl of H_2O_2-containing medium before the final cell viability assay. In all cases, cancer cells were cultured for 3 d before the final cell viability assay. The cell viability was obtained using the methods mentioned in Materials and Methods. We defined that a noticeable activation occurred when the cell viability ratio between Case 2/Case 3 was less than 0.95.

3. Results and discussion

We first investigated the cytotoxicity of H_2O_2 on two cancer cell lines. The H_2O_2-containing DMEM or RPMI with different concentrations were prepared first and were immediately used to affect PA-TU-8988T cells and B16F10 cells, respectively. Based on the cytotoxicity (not shown here), we deliberately set the concentration of H_2O_2-containing medium to be 5 μM/10 μM and 20 μM/40 μM for PA-TU-8988T cells and B16F-F10 cells, respectively. Under these conditions, H_2O_2 did not cause noticeable cytotoxicity on two cell lines.

We investigated the potential activation effect on PA-TU-8988T cells. The strength of a 1 Hz magnetic field was increased from 0.119 mT to 1.176 mT. The NMF treatment lasted 2 h. As shown in figure 3, the NMF treatment alone did not cause the noticeable inhibition on PA-TU-8988T cells, except in one single experimental condition (0.119 mT, 10 μM H_2O_2). The NMF treatment also did not noticeably enhance the cytotoxicity of H_2O_2. Thus, the obvious activation effect has not been observed on the NMF-treated PA-TU-8988T cells.

We further investigated the potential activation effect on B16-F10 cells. The H_2O_2-containing RPMI (20 μM or

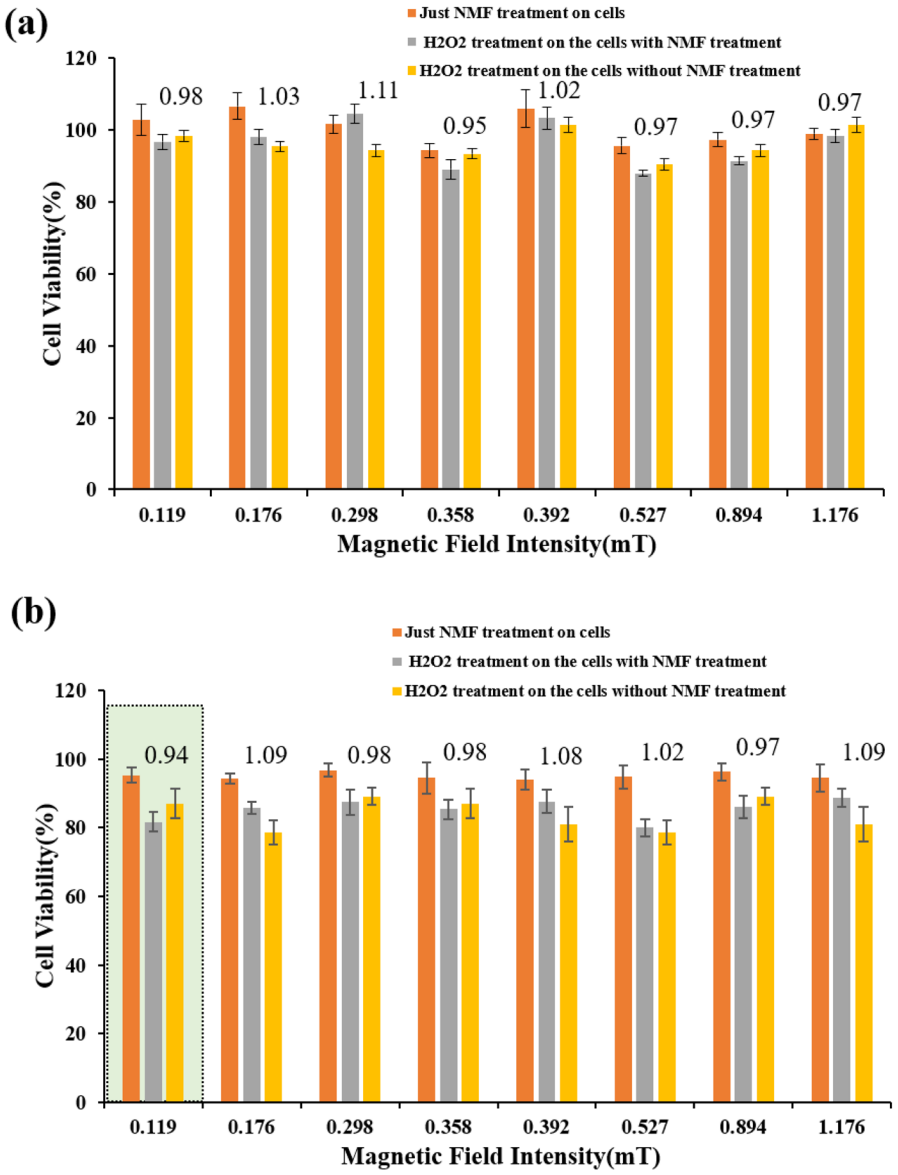

Figure 3. The effect of NMF treatment on the cytotoxicity of H_2O_2 on PA-TU-8988T cells. The cancer cells were treated by 1 Hz of NMF for 2 h. The H_2O_2 treatment with different concentration was performed. (a) 5 μM H_2O_2. (b) 10 μM H_2O_2. The results were shown as the mean $\pm$ standard deviation (s.d.). The cell viability ratios of grey bar/orange bar were marked in the figure, which was used to assess the activation level. The marked area in green corresponds to the case with noticeable activation effect (Case 2 (grey bar)/Case 3 (orange bar) < 0.95).

40 μM) were used to affect the growth of B16-F10 cells with or without the activation by NMF treatment (1 Hz) last 1 h or 2 h. The magnetic field strength increased from 0.119 mT to 1.176 mT. The NMF treatment alone just slightly inhibited the growth of B16-F10 cells when the magnetic field strength increased from 0.119 mT to 0.392 mT, which was 98%, 97%, 99%, 98%, 97% of the control group respectively (figure 4). However, the H_2O_2 treatment on the NMF-treated B16-F10 cells would cause noticeable stronger cytotoxicity compared with the B16-F10 cells without NMF treatment. For the H_2O_2 (20 μM) treatment, the ratio of Case 2 (grey bar)/Case 3 (orange bar) tended to decrease as the magnetic field strength increased (figures 4(a) and (b)). As I mentioned previously, the ratio of Case 2/Case 3 reflected the activation level. Thus, a low magnetic field facilitated the activation of B16-F10 cells. Just extending the NMF treatment time did not noticeably change activation level (figures 4(a) and (b)). The NMF treatment effectively enhanced the cytotoxicity of H_2O_2 on B16-F10 cells. A ratio of Case2/Case 3 of 0.68 could be achieved when the (20 μM) H_2O_2-treated B16-F10 cells were pre-treated by NMF generator for 1 h with a magnetic field strength of 0.392 mT or by NMF generator for 2 h with a magnetic field strength of 0.119 mT. In these two cases, the H_2O_2 inhibition rate of cells growth increased from 0% to 32% and from 3% to 34%, respectively (figure 4).

The frequency of NMF treatment is another important factor to affect the activation effect on melanoma cells. As shown in figure 5, a higher frequency of NMF treatment tended to cause a weaker activation effect. And, this trend was

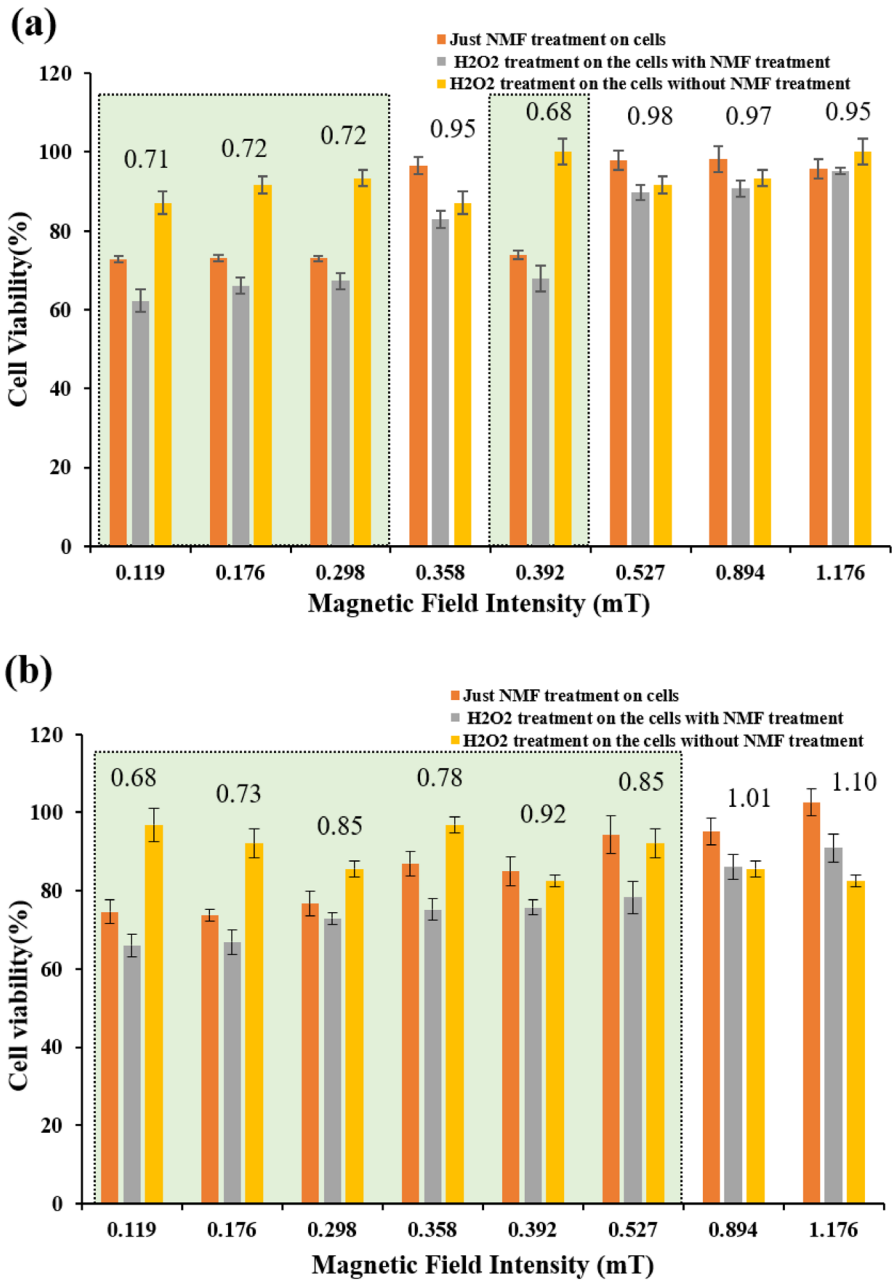

Figure 4. The activation effect of NMF treatment on B16-F10 cells. (a) 1Hz NMF treatment for 1 h. (b) 1 Hz NMF treatment for 2 h. The cell viability ratios of grey bar/orange bar were marked in the figure, which was used to assess the activation level. The marked area in green corresponded to the case with noticeable activation effect (Case 2 (grey bar)/ Case 3 (orange bar) < 0.95). The results are shown as the mean $\pm$ s.d.

more obvious when the magnetic field strength was increased from 0.119 mT to 0.358 mT. When the magnetic field was 0.119 mT, the obvious activation effect was observed in all four conditions, from 1 Hz to 20 Hz (figure 5(a)). As the magnetic field became stronger, the obvious activation state tended to be observed when the frequency was relatively low, such as 1 Hz or 5 Hz (figures 5(b) and (c)). When the magnetic field strength was 0.358 mT, only a weak activation effect was observed at a frequency as 1 Hz (figure 5(d)).

According to the law of electromagnetic induction, the changing magnetic field will induce electric field. In our experiment, the electric field and magnetic field cannot be separated also. The impact on the cell may be due to the co-effect of magnetic field and electric field. The activation by NMF generator may relate with the effect on the cellular functions, though this study did not reveal the mechanism. A magnetic field can affect the function of different channels on the cytoplasmic membrane, such as calcium (Ca^{2+}) channels [12, 13]. The change of pulse frequency affects the efficiency of plasma and magnetic field on drug delivery, the electrical field and particles could also play a significant role especially calcium channel or membrane depolarization [14–16]. The induced electric field inside the cell can be calculated according to the law of electromagnetic induction, a pulsed

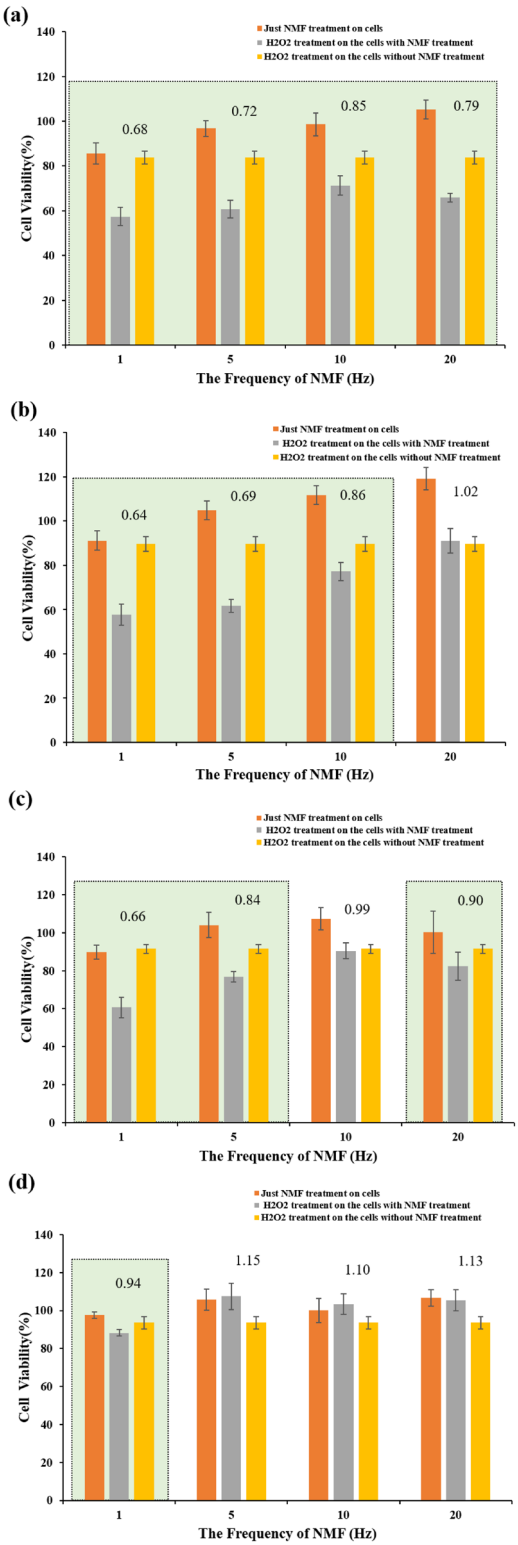

Figure 5. The effect of frequency on the activation effect of B16-F10 cells. The magnetic field strength of NMF treatment was 0.119mT (a), 0.176 mT (b), 0.298mT (c), and 0.358mT (d), respectively. The NMF treatment lasted 1 h in each experiment. The cell viability ratio of grey bar/orange bar was marked in the figure, which was used to assess the activation level. The marked area in green corresponds to the case with noticeable activation phenomenon (Case 2 (grey bar)/Case 3 (orange bar) < 0.95). The results are shown as the mean $\pm$ s.d.

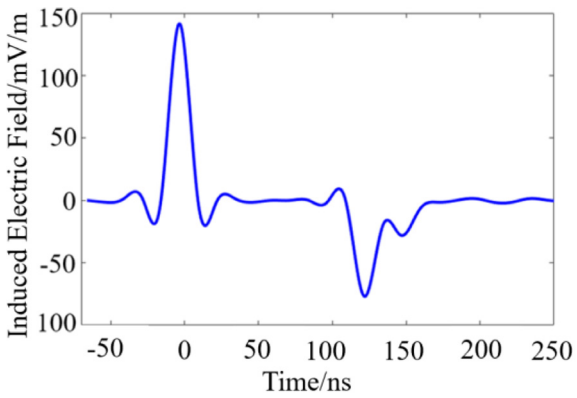

Figure 6. The induced electric field.

current induces a magnetic field that produces an induced electric field in the cell. Taking the magnetic field of 0.897mT as an example, the induced electric field was calculated based on the equation (1). The induced electric field waveform was shown in figure 6. The induced electric field was larger in the rising and falling sections of the square wave, and the induced electric field was zero in the flat section of the square wave. The maximum induced electric field generated by the square wave was 148.7 mV m^{-1}.

The change of electric field may cause change of cell membrane potential, ion concentration inside and outside the membrane and so on. It may also change the function of mitochondria and ion channels to adjust the cell status. The functional change of ion channels may facilitate the damage from ROS. The activation of melanoma cells by NMF generator is a new discovery, which may have wide application in plasma medicine and other related areas. ROS plays important role in the performance of many anti-cancer modalities. This study provides a novel strategy to enhance the efficacy of ROS-based modalities through a physical activation on the cancer cells. More important, the magnetic field can penetrate the skin and may deliver its activation effect on the deep tumors, which may enhance the efficacy of the chemotherapy.

4. Conclusions

An NMF generator activated melanoma cell line B16-F10 after a 1 h or 2 h of pretreatment with a frequency of 1–20 Hz and a magnetic field strength of 0.119–0.527 mT. The activated melanoma cells were much more vulnerable to the cytotoxicity of H_2O_2 than the melanoma cells without the treatment. The activation level could be modulated by controlling the magnetic field strength and the frequency of NMF. The NMF treatment with weaker magnetic field strength and a smaller magnetic frequency would generate a stronger activation effect on melanoma cells. In addition, the NMF treatment could not activate pancreatic cancer cell line PA-TU-8988T cells, which demonstrated that the activation effect was cellular specific.

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