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Adult human dermal fibroblasts exposed to nanosecond electrical pulses exhibit genetic biomarkers of mechanical stress

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ABSTRACT

Background: Exposure of cells to very short (< 1 μs) electric pulses in the megavolt/meter range have been shown to cause a multitude of effects, both physical and molecular in nature. Physically, nanosecond electrical pulses (nsEP) can cause disruption of the plasma membrane, cellular swelling, shrinking and blebbing. Molecularly, nsEP have been shown to activate signaling pathways, produce oxidative stress, stimulate hormone secretion and induce both apoptotic and necrotic death. We hypothesize that studying the genetic response of primary human dermal fibroblasts exposed to nsEP, will gain insight into the molecular mechanism(s) either activated directly by nsEP, or indirectly through electrophysiology interactions.
Methods: Microarray analysis in conjunction with quantitative real time polymerase chain reaction (qRT-PCR) was used to screen and validate genes selectively upregulated in response to nsEP exposure.
Results: Expression profiles of 486 genes were found to be significantly changed by nsEP exposure. 50% of the top 20 responding genes coded for proteins located in two distinct cellular locations, the plasma membrane and the nucleus. Further analysis of five of the top 20 upregulated genes indicated that the HDFa cells' response to nsEP exposure included many elements of a mechanical stress response.
Conclusions: We found that several genes, some of which are mechanosensitive, were selectively upregulated due to nsEP exposure. This genetic response appears to be a primary response to the stimuli and not a secondary response to cellular swelling.
General significance: This work provides strong evidence that cells exposed to nsEP interpret the insult as a mechanical stress.

1. Introduction

Pulsed electrical discharges, in an aqueous medium, can cause a multitude of physical events to occur that, in turn, affect the biology of living things near the exposure area. Physical events such as: thermal-elastic expansion [1,2], electrostriction [3], electrochemistry [4] and plasma formation [3,5] can occur, if the correct exposure conditions are met. Thermal-elastic expansion depends on pulse duration, electrostriction occurs with high electric fields, electrochemistry is driven by high current and plasma formation dominates when there is break down within the exposure medium. Nanosecond electrical pulses (nsEP), a type of pulsed electrical discharge, are used in a variety of applications ranging from cancer therapy to food preservation [6,7]. The nsEP are too short to elicit a thermal response and typically do not form plasmas (at the voltages used for biological research). Research undertaken by our group suggests that electrostriction and electrochemistry may occur following nsEP and thus may be responsible in part for the biological effects associated with these exposures [unpublished personal communication].

Nanosecond electrical pulses have been shown to cause a wide variety of biological effects to cells, both morphological and biochemical in nature, These effects include swelling [8,9], blebbing [8,9],

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Table 1
nsEP parameters used for pulse ramp experiment.

Electric field	50 kV/cm	100 kV/cm	150 kV/cm
Number of pulses applied	10	10	10
	30	30	30
	100	100	100
	300	300	300
	1000	1000	1000

phospholipid translocation [9–12] and membrane permeabilization [11,13–15]. These morphological changes are closely associated with cell death and have been identified as markers of both apoptosis [8,16] or necrosis [8,16]. In addition to these phenomena, molecular/biochemical events such as the influx of calcium [17–20], the activation of the inositol triphosphate (IP_3) pathway [21–23], the creation of reactive oxygen species (ROS) [24–26] and the induction of autophagy [27] have also been reported as being associated with nsEP exposure.

Despite this wealth of biological evidence, very little is known about how nsEP affect gene expression in primary cells. Understanding how a cell interprets a stress can give great insight in the nature of the stress itself. Therefore, to better understand the nature of nsEP we performed a microarray analysis of a primary cell type exposed to nsEP. Human dermal fibroblasts (HDFa) are a primary cell, isolated from adult skin. These cells are considered to be "normal" with little genomic instability. These cells have also been used extensively in studies involving mechanical stress. Fibroblasts are often subjected to many different kinds of mechanical force, such as tension, compression and shear forces [28–30]. It has been reported that fibroblasts in a human tendon respond to stretching forces in a stretch-magnitude-dependent manner, where gene expression increases with stretch magnitude [30]. Given the ability of HDFa cells to not only regulate gene transcription based on specific types of mechanical stress, but also on the amplitude of that stress, we selected these cells to further investigate the mechanical stress imparted on cells by nsEP exposure. We exposed these cells to 100 nsEPs at a duration of 10-ns and an electric field of 150 kV/cm and assayed with microarray for global changes in gene expression 4 h post exposure. Quantitative Real-Time PCR was used to confirm the microarray data. The genomics data presented in this paper provide further genetic evidence necessary to characterize the nature of the stress endured by these cells when exposed to nsEP. This study represents the first global genetic analysis of normal human primary cells exposed to nsEP.

2. Materials and methods

2.1. Cell culture/exposure

Primary adult human fibroblast were acquired from Cascade Biologics (Carlsbad, CA), sub-cultured and maintained according to the supplier's protocol. Cells were grown in Medium 106 supplemented with a low serum growth supplement (LSGS) Kit, both purchased from Gibco (Carlsbad, CA). All cells were maintained at 37 °C/5% CO_2/95% humidity. The HDFa were prepared for exposure in accordance with

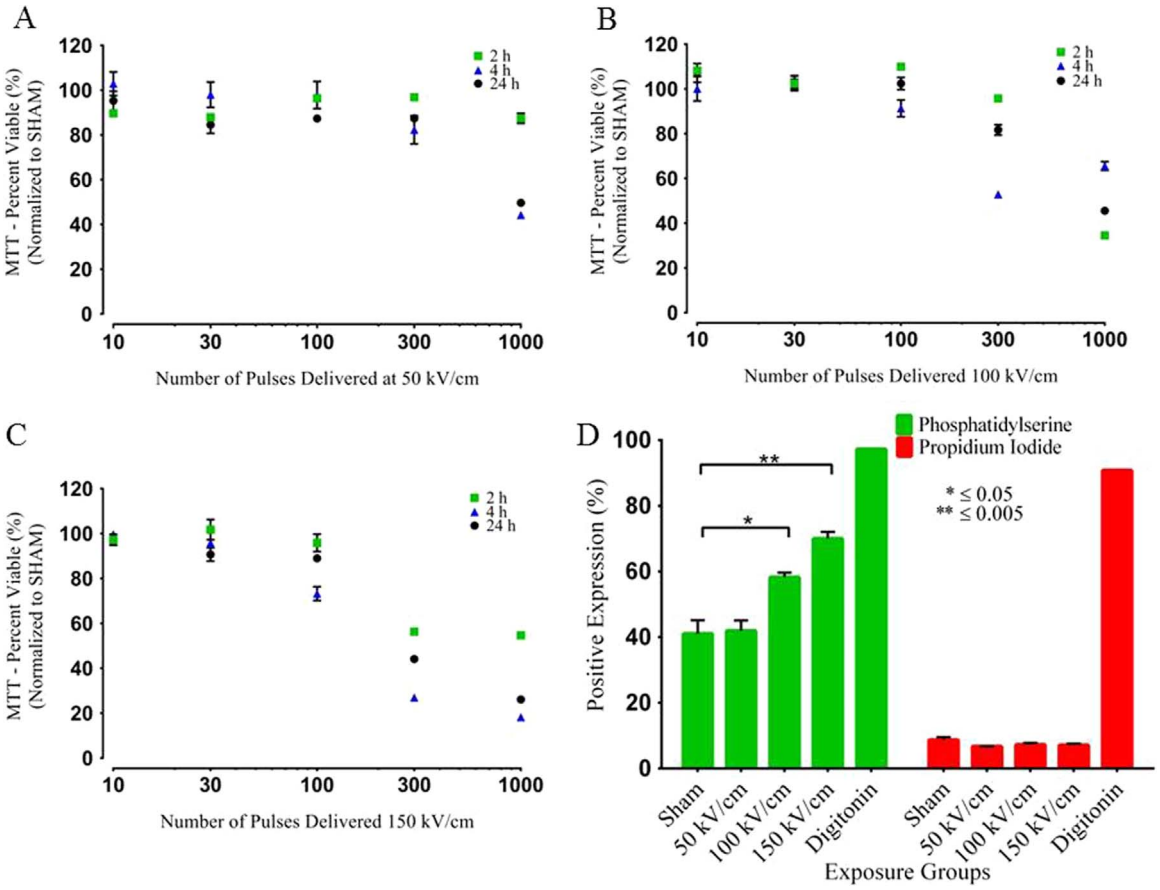

Fig. 1. Viability of HDFa after exposure to nsEP and normalized to SHAM. A) HDFa cells exposed to 10 ns duration pulses at an applied voltage of 50 kV at 1, 10, 30, 100, 300, or 1000 pulses at 1 Hz and at 2, 4 or 24 h post exposure. B) HDFa cells exposed to an applied voltage of 100 kV at 1, 10, 30, 100, 300, or 1000 pulses at 1 Hz and at 2, 4 or 24 h after exposure. C) HDFa cells exposed to an applied voltage of 150 kV at 1, 10, 30, 100, 300, or 1000 pulses at 1 Hz and at 2, 4 or 24 h after exposure. At 150 kV and at 100 pulses we achieved the desired level of viability. D) Flow cytometry data for phosphatidylserine (PS) and Propidium Iodide (PI) in cells exposed to 100 pulses at 50, 100, and 150 kV/cm electric fields. Cells exposed at 150 kV/cm and 100 pulses showed the highest level of membrane disruption (PS) with minimal death (PI). All experiments (flow cytometry and MTT) were performed in triplicate (3 independent nsEP exposures which were then divided into triplicate in each well plate for a total of 9 samples). A two-tailed unpaired t-test was performed using GraphPad Prism.