Bioelectrochemistry 126 (2019) 163–171

Contents lists available at ScienceDirect

Bioelectrochemistry

journal homepage: www.elsevier.com/locate/bioelechem

Cytochrome P450 genes play central roles in transcriptional response by keratinocytes to a high-voltage alternating current electric field

Masayo Aoki *, Noriko M. Matsumoto, Yuri Okubo, Rei Ogawa

Department of Plastic, Reconstructive, and Aesthetic Surgery, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113-8602, Japan

ARTICLE INFO

Article history:
Received 6 September 2018
Received in revised form 29 November 2018
Accepted 30 November 2018
Available online 11 December 2018

Keywords:
Electric field
Keratinocytes
Cytochrome P450
Wound healing
Anti-aging

ABSTRACT

The endogenous electric field (EF) of skin wounds plays an important role in the biological processes that underlie wound healing. Treatments that modulate wound-EFs promote healing. However, the mechanism(s) that underlie this effect remain unclear. Agilent-based microarrays were used to determine the transcriptomes of the keratinocyte line HaCaT, normal human dermal fibroblasts, and the human dermal endothelial cell line HMEC-1 before and after high-voltage alternating current (AC)-EF (14,000 V, 90 Hz) treatment. The keratinocytes had the most genes whose transcription was altered by EF. They included the cytochrome P450 (CYP) genes CYP1A1 and CYP1B1, HMOX1, EREG, DUSP5, and SLC7A11 (all upregulated), and DOCK8, ABCC6, and CYP26A1 (all downregulated). As shown by transcriptional-network analysis, all three CYP genes played central roles in the EF-induced changes in keratinocyte transcriptome. To the best of our knowledge, this is the first study that demonstrates that CYP genes play a key role in the transcriptional responses of human keratinocytes to EF treatment. Further investigations into the effects of EF on wound healing, aging, and regenerative medicine are likely to yield promising results.

1. Introduction

Electric field (EF) therapy has evolved as a traditional complementary and alternative medicine in Japan, and was approved for therapeutic use in Japan by the Ministry of Health, Labor and Welfare in 1972. It is believed to modulate the nervous, endocrine, and other health systems and is used to treat headaches, shoulder stiffness, chronic constipation, and insomnia, among many other diseases [1]. Several studies have shown formally that EF has therapeutic effects. Thus, high-voltage static EF reduces the subjective pain in active rheumatoid arthritis [1]. Moreover, when a non-healing bone defect in rats was subjected to a 100 Hz pulsing electromagnetic field, the defective bone exhibited increased alkaline phosphatase activity, calcium uptake, and bridging bone production [2].

Currently, the mainstream EF therapy in Japan is a high-voltage alternating EF of 9000–14,000 V and 50–90 Hz. The EFs used in these therapies do not have a deleterious effect on human health [3]. However, the precise molecular mechanisms that underlie the therapeutic effects of EF remain unclear. Electroporation and electrofusion, achieved *via* pulsed EF, are particularly important in cell biology and biotechnology [4]. However, even in the case of electroporation, which is commonly used to deliver molecules such as drugs, proteins, and DNA into cells, the mechanism remains unclear [5]. EF pulses induce neural electrostimulation, Ca^{2+} signaling activation, and tissue ablation [6]. Low frequency EF induces heat-inducible heat shock protein 70 (HSP70) expression in rat primary fibroblasts [7].

Multiple lines of evidence suggest that EF therapy may promote wound healing. In particular, the human body bears an endogenous EF: while it plays important physiological roles in the electrical activation of the nervous system and muscles, it also appears to participate in the dynamic and well-ordered biological processes that underlie wound healing and scarring [8,9]. For example, Yan et al. showed that endogenous EF plays a key role in the re-epithelialization of wounds [9]. The putative beneficial role of EF in wound healing is also supported by Graebert et al., who showed that when the ischemic skin wounds in preclinical *in vivo* model rats were subjected to exogenous electrical stimulation, they exhibited improved wound healing [10].

The mechanisms that underlie the beneficial effects of EF on wound healing remain unclear. However, multiple *in vitro* and *in vivo* studies show that exogenous EFs shape the activities of the cells that play key roles in wound healing. Wound healing is a dynamic and complex process that consists of several overlapping phases, namely, the hemostasis, inflammatory, proliferative, and remodeling phases [11]. Many cell types are involved in these phases, including keratinocytes, endothelial cells, and fibroblasts [12–14]. Dubey et al. showed that exogenous pulse EF stimulation affects the metabolism of and multiple biochemical mechanisms in both keratinocytes and endothelial cells [15]. Moreover, electrical stimulation enhances epithelial cell proliferation [16], promotes dermal fibroblast activity, increases the differentiation of dermal

* Corresponding author.
E-mail address: masayok@nms.ac.jp (M. Aoki).

https://doi.org/10.1016/j.bioelechem.2018.11.014
1567-5394/

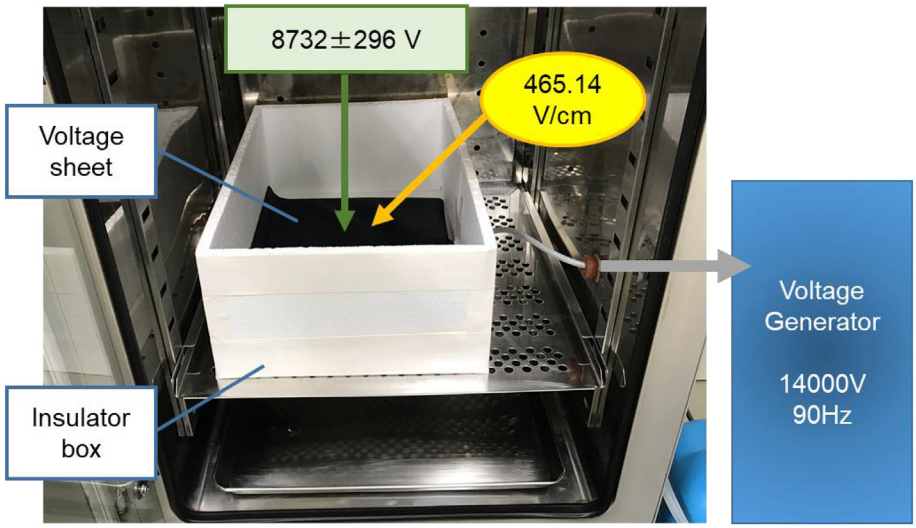

Fig. 1. Photograph of the electric field treatment setup that was used to stimulate the cultured cells. The voltage sheet was placed in an insulator box so that it efficiently produced an electric field. The cell culture dishes were placed cell-side down onto the sheet so that an electric field would be established inside the dish.

the gene expression profiles of all three cell types. However, they differed markedly in terms of their sensitivity to EF exposure. Specifically, HaCaT cells had the largest number of genes whose expression was significantly changed by EF exposure. Thus, keratinocytes appear to be particularly sensitive to EF. By contrast, the HMEC-1 cells were relatively resistant (Fig. 2). NHDF cells had an intermediate number of genes that were differentially expressed after EF exposure. The genes that exhibited the most marked up or downregulation are listed on Supplementary Tables 1–3.

The genes whose transcription was significantly altered by EF in the three types were categorized according to their gene ontology (GO) into three categories (Biological Process, Molecular Function, and Cellular Component). The three cell types differed markedly in terms of the distribution of their EF-upregulated genes in the three GO categories. In HaCaT cells, EF treatment significantly upregulated 155 genes in the Biological Process GO category; it also significantly upregulated 11 genes in the Molecular Function GO category. By contrast, in HMEC-1 cells, EF treatment only significantly upregulated nine, three, and two genes in the Biological Process, Molecular Function, and Cellular Component GO categories; it also only downregulated seven genes in the Biological Process GO category. In NHDF cells, EF treatment significantly upregulated 14 and eight genes in the Biological Process and Molecular Function GO categories, respectively (Fig. 3).

Fig. 4 shows the pathways in each cell type that demonstrated significant enrichment in terms of EF-induced component-gene up or downregulation. HaCaT cells had the largest number of pathways that were influenced by EF treatment, followed by NHDF cells. The HMEC-1 cells had the lowest number of affected pathways. A pathway that was significantly affected by EF in all three cell types was not detected. In HaCaT cells, CYP and T cell receptor signaling pathways were significantly influenced in terms of both up and downregulation.

The validity of the microarray results in the three cell types was confirmed by quantitative RT-PCR of the genes that exhibited extremely strong responsiveness to EF (defined as fold change >2 or >−2). Thus, the effect of EF on the expression of 15, 3, and 7 genes in HaCaT, NHDF, and HMEC-1 cells, respectively, was tested by RT-PCR. In HaCaT cells, the expression of CYP1A1 and CYP1B1 were significantly increased by 20.3- and 5.9-fold, respectively. Moreover, HMOX1, EREG, DUSP5, and SLC7A11 expression was increased by 3.8-, 2.6-, 1.4-, and 2.0-fold, respectively, while DOCK8, ABCC6, and CYP26A1 were significantly downregulated by 0.34-, 0.70-, and 0.44-fold, respectively (Fig. 5).

By contrast, in NHDF, none of the 3 tested genes exhibited significant EF-induced changes on RT-PCR. Furthermore, in HMEC-1 cells, only MGA expression was confirmed to be slightly upregulated by EF exposure (by 1.2-fold) (Fig. 6).

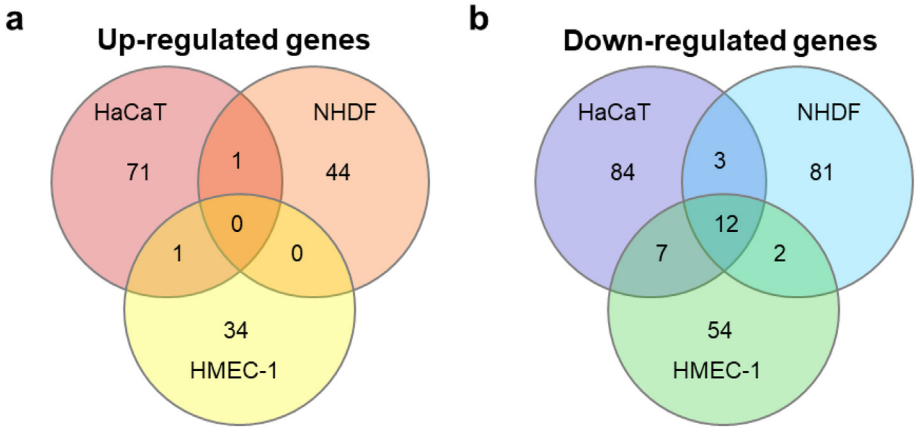

Fig. 2. Changes in the transcriptomes of the three cell types after high-voltage electric field treatment. The Venn diagram shows the number of genes in the HaCaT, normal human dermal fibroblast (NHDF), and HMEC-1 cells that were (a) upregulated or (b) downregulated after high-voltage electric field treatment, and how many of these genes showed the same transcriptional changes in the three cell types.