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Cancer Letters 246 (2007) 149–156
CANCER Letters
www.elsevier.com/locate/canlet

# Identification of genes responsive to low intensity pulsed ultrasound in a human leukemia cell line Molt-4

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Received 20 December 2005; received in revised form 15 February 2006; accepted 15 February 2006

**Abstract**

We examined the gene expression of human leukemia Molt-4 cells treated with non-thermal low intensity pulsed ultrasound. Six hours after 0.3 W/cm$^2$ pulsed ultrasound treatment, apoptosis ($24 \pm 3.3\%$, mean $\pm$ SD) with minimal cell lysis was observed. Of approximately 16,600 genes analyzed, BCL2-associated athanogene 3 (*BAG3*), DnaJ (Hsp40) homolog, subfamily B, member 1 (*DNAJB1*), heat shock 70 kDa protein 1B (*HSPA1B*), and heat shock 70 kDa protein 6 (*HSPA6*) showed increased levels of expression while isopentenyl-diphosphate delta isomerase (*IDI1*) and 3-hydroxy-3-methylglutaryl–coenzyme A synthase 1 (*HMGCS1*) showed decreased levels in the cells 3 h after the ultrasound treatment. The expression levels of these six genes were confirmed by a real-time quantitative polymerase chain reaction. To our knowledge, this is the first report of DNA microarray analysis of genes that are differentially expressed in response to apoptosis induced by non-thermal low intensity pulsed ultrasound in human leukemia cells. The present results will provide a basis for further understanding of the molecular mechanisms of effects of not only low intensity pulsed ultrasound but also that of mechanical shear stress in the cells.


*Keywords:* DNA microarray; Gene expression; Low intensity pulsed ultrasound

## 1. Introduction

In many medical fields, ultrasound (US) has been widely used for diagnosis and therapy. Biophysical actions of US are divided into three modes, thermal, cavitational and non-thermal non-cavitational effects. Cavitation is known to lead to both mechanical shear stress and free radical formation arising from the oscillation and collapse of cavitation bubbles [1–3]. In general, these two effects (mechanical shear stress and free radicals) of cavitation have been inferred to act simultaneously on all of biological materials. It is well known that fairly intense US induces cell killing, cell lysis, loss of viability and loss of clonogenicity [2]. Currently, of particular interest is its ability to induce apoptosis in human leukemia cell lines [4–12]. Different factors that can influence apoptosis were cited. Among them were, sonochemical mechanism [12], cavitation-mediated enhancement of intracellular calcium ion concentration, intracellular free radical

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doi:10.1016/j.canlet.2006.02.011

formation due to mitochondrial membrane damage and free radical formation [10,11], and sonomechanical mechanism [9].

It has been indicated that DNA microarrays are one of the most powerful technologies for functional genomics as it can simultaneously analyze the expression levels of many 100s or many 1000s of genes [13,14]. Recently, we applied DNA microarray technologies to analyze gene expression in cellular differentiation [15] and in a variety of biological responses to physical and chemical stresses, such as sodium butyrate [16], bisphenol A [17,18] and hyperthermia [19]. In our previous study, using UniGEMV Ver2.0 human gene expression microarrays to detect approximately 9200 genes, five up-regulated genes and two down-regulated genes were identified in the human lymphoma U937 cells at 6 h after exposure of fairly intense continuous waves (1 MHz, 4.9 W/cm$^2$, for 1 min), where not only free radical formation, but also cell lysis and apoptosis were significantly observed [20]. Moreover, under nearly same condition in U937 cells, another microarray system and real-time quantitative polymerase chain reaction (PCR) confirmed up-regulation of heme oxygenase-1 (HO-1) and revealed that HO-1 is the most sensitive gene for US in U937 cells [21]. More recently, following a report by Lagneaux et al. [12], we also reported that non-thermal low intensity pulsed US treatment (1 MHz, 0.3 W/cm$^2$, 1 min) induced apoptosis in human leukemia cell lines such as U937, Jurkat and Molt-4, and it was revealed that the up-regulation of HO-1 was observed above apoptosis-inducing intensities [9,22]. However, the knowledge of the details of molecular signaling in response to mechanical pressure or pressure waves such as US, particularly low intensity pulsed waves, remains elusive.

In the present study, the gene expression of human leukemia Molt-4 cells treated with low intensity pulsed US were examined by using high-density IntelliGene HS human Expression microarrays to detect approximately 16,600 genes.

## 2. Materials and methods

### 2.1. Cell culture

Molt-4 human leukemia cells were obtained from Japanese Cancer Research Resource Bank (Tokyo, Japan). The cells were grown in RPMI 1640 medium (Invitrogen Co., Tokyo, Japan) supplemented with 10% fetal bovine serum (Invitrogen Co.) at 37 °C in humidified air with 5% $CO_2$.

### 2.2. US apparatus and intensity measurement

The ultrasonic apparatus (Sonicmaster ES-2, OG Giken Co. Ltd, Okayama, Japan) with a resonant frequency of 1 MHz with 100 Hz pulse repetition frequency (PRF) was used in all the sonication experiments. This device is equipped with a built-in digital timer, intensity regulator, and duty factor (DF) controller. For the sonication procedure, the transducer with a diameter of 5 cm was fixed with a clamp attached to a metal stand to keep the transducer facing directly upward (Fig. 1). We used such near acoustic field produced by the liquid–air interface and did not reduce the standing waves because of the effective occurrence of cavitation.

The spatial-average–temporal-average intensities (ISATA) at 10% DF corresponding to the reading output intensity were measured using an ultrasonic power meter (PM-DT-10E, Ohmic instrument Co., Easton, MD). The peak acoustic amplitude in degassed water was also measured at the distance of 5 cm from the transducer with a calibrated poly-(vinyledene difluoride–trifluoroethylene) needle-type hydrophone, 0.5 mm in diameter (Toray techno Co., Ltd, Shiga, Japan) connected to a PC/AT compatible computer and a digitizing oscilloscope (TDS3034, Tektronix Japan, Ltd, Tokyo, Japan). The ISATA and the peak acoustic pressures corresponding to the reading output of 0.1, 0.2, 0.3, 0.4 and 0.5 W/cm$^2$ (device-indicated) were 0.048, 0.072, 0.081, 0.092 and 0.105 W/cm$^2$, and 0.061, 0.105, 0.132, 0.144 and 0.146 MPa, respectively. In this paper we used device-indicated intensities to refer to these values.

For most of the experiments, 0.3 W/cm$^2$ was used. During such an ultrasonic exposure experiment the change in absorbance of 2 ml of aqueous air-saturated ferrous (Fricke) dosimeter solution after 5 min exposure at 304 nm was $0.0082 \pm 0.0015$ (mean $\pm$ SD, $n = 4$) in a 1 cm path length quartz cell. This dosimeter monitors the extent of the cavitational activity induced by ultrasound by measuring the sum of $H_2O_2$, OH radicals, and H atoms available to react with ferrous ions.

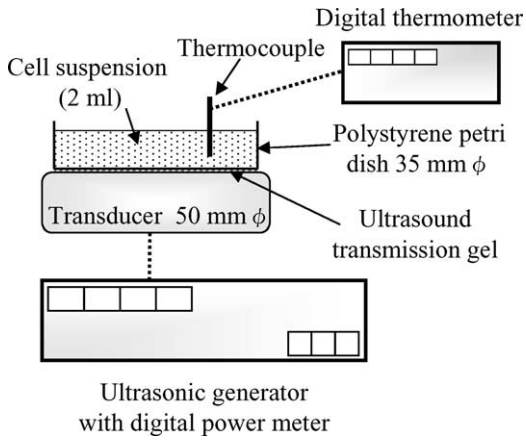


Fig. 1. A set-up for ultrasonic exposure.