Microarray Analysis of Gene Expression in MC3T3-E1 after Specific Far-infrared Radiation

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Abstract: We developed the CO_2 incubator (Fig.1) which can emit specific wavelength (7 to 12 υm) of far-infrared ray (FIR), which range was regarded to have strong effect to living body, and had been analyzing the effect of specific FIR in radiated osteoblasts. The results suggested that specific FIR inhibited the proliferation and promoted the differentiation of osteoblastic MC3T3-E1 cells. In the present study, the gene expression in MC3T3-E1 cell affected by specific FIR was analyzed. MC3T3-E1 cells were exposed to full time specific FIR and total mRNA were analyzed by using Mouse oligo microarray (Agilent technologies) on day 3 of differentiation. Data filtering and cluster analysis were done by Genespring (Silicon Genetics). The results suggested that most important genes belonged to the control systems for transcription and, cell-cell signaling and growth affected by specific FIR.
Still more, it was found that the DEAD related system and Homeobox related system were important for transcription, and interferon related system, thyroid hormone related system, and fibroblast growth hormone related system, platelet-derived growth factor related system and interleukin related system were important for cell-cell signaling and growth by our developed profiling system of genes in specific FIR radiation.

Key words: Far-infrared ray (FIR), FIR-CO_2 incubator, MC3T3-E1 cell, osteoblast, oligo microarray analysis,

Introduction

Far infrared ray (FIR) is invisible range of light, wavelength from 4 υm to 1mm and they can be perceived as heat by specialized nerve endings known as thermo receptors in the skin. The benefit of biological effect of FIR with heat to animal and human body has known, but non-heat effect to cultured cell is not studied strictly. The previous study was to examine an effect of specific FIR on the proliferation and differentiation of cultured cell, osteoblastic MC3T3-E1 by using FIR CO_2 incubator. These results suggested that specific FIR suppressed significantly the proliferation and activated differentiation of MC3T3-E1 cell. But, the gene expression in MC3T3-E1 cell affected by specific FIR was not comprehensively analyzed using by oligo-microarray yet. Then, we analyzed comprehensively the gene expression in MC3T3-E1 cell cultured in FIR CO_2 incubator using our profiling system.

Materials and Methods

MC3T3-E1 cells purchased from Riken Cell Bank (Tsukuba, Japan) were cultured in α-MEM medium (Sigma) containing 10%FBS, 50 υg/ml AA(maintenance medium). After confluent on day 5, 1mM Dexamethazone and 0.62mg/ml β-glycerophosphate were added for differentiation. Cells were maintained at 37 °C, humidified 5% CO_2 in air in normal and FIR CO_2 incubator for 3 days.
Total RNA was extracted with RNeasy Mini Kit (Qiagen, Maryland, USA). All RNA samples showed A260/280 ratios over 2.0. RNA integrity was determined using an Agilent 2100 Bioanalyzer (Agilent Technologies, CA, USA) and only high quality RNA (28S/18S>1.7) was used for further analysis.
Microarray analysis were performed by 22k Mouse Oligo Microarray (Agilent Technologies, CA, USA) according to the manufacturer's protocol. The hybridized microarray was visualized using Agilent Technologies Microarray Scanner. Fluorescence intensity was imported into GeneSpring 7 software (Silicon Genetics, CA, USA) for normalization and filtering. Data were normalized per spot and per chip by intensity-dependent LOWESS (locally weighted least squares). Genes with expression ratios greater than backgrownd +2.6*SD, t-test P-value <0.05, were considered differentially expressed. The cut off value for induction or repression was 1.3.

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Results and Discussion

Fig.1 FIR CO_2 incubator

[cell-cell signaling (GO:0007267)

Gene Name	Fold change	Acc. No.	Discription
Ifi205	1.597	NM_17264	Interferon activated gene 205
Pdgfd	1.555	AK003359	Platelet-derived growth factor, D polypeptide
Tgfbi	1.505	NM_00936	Transforming growth factor, beta induced
Trip13	1.424	AK010336	Thyroid hormone receptor interactor 13
Il15ra	1.422	NM_00835	Interleukin 15 receptor, alpha chain
Ifi27	1.388	AK010014	Interferon, alpha-inducible protein 27
Map3k7	1.365	BC006665	Mitogen activated protein kinase kinase kinase 7
Tdgf1-ps2	1.345	NG_00146	Tdgf3teratocarcinoma-derived g.f., pseudogene 2
Ifi203	1.328	NM_00832	Interferon activated gene 203
Olfr56	1.323	NM_00833	Olfactory receptor 56
Fgf8	-1.323	D12483	Fibroblast growth factor 8
Fgf21	-1.332	NM_02001	Fibroblast growth factor 21
Rhbdf1	-1.337	M99623	Rhomboid family 1 (Drosophila)
Fgf2	-1.342	NM_00800	Fibroblast growth factor 2
Il1f6	-1.348	NM_01945	Interleukin 1 family, member 6
Fgfr3-ps	-1.364	U52463	fibroblast growth factor receptor-3 pseudogene
Il17d	-1.393	NM_14583	Expressed sequence AU020094
Dtr	-1.395	NM_01041	Heparin-binding EGF-like growth factor
Thrap3	-1.443	AK046010	Thyroid hormone receptor associated protein 3
Il3	-1.471	NM_01055	Interleukin 3
Il17rc	-1.481	NM_13415	Interleukin 17 receptor C
Il17b	-1.631	NM_01950	Interleukin 17B
Gab1	-1.667	NM_02135	RIKEN cDNA 1700019H22 gene
Pdgfb	-2.304	NM_01105	Platelet derived growth factor, B polypeptide

Table 1 gene list for cell-cell signaling

The number of upregulated gene was 613, downregulated gene was 719, of total 20916 genes. Human all genes were separated three groups “Biological process, Cellular component, Molecular function”. Biological process contains 6 groups, (1) biological process unknown (no branch), (2) cell communication (7 branches), (3) cell growth and-or maintenance (22 branches), (4) death (1 branch), (5) development (6 branches), (6) viral life cycle (no branch). Cellular component contains 7 groups. Molecular function contains 14 groups. On biological process, the strongly upregulated genes belong to (2) cell communication, (3) cell growth, metabolism, (5) development. The strongly downregulated genes belong to (2) cell communication, (3) cell growth, metabolism, (4) death, (5) development. These genes showed four activities. (A) Chromosome- DNA-RNA synthesis, (B) Cell proliferation, (C) Signal transduction, (D) Energy metabolism. In this study, the analysis of genes on biological process was concentrated. The strong three groups which contained 5 over changed genes and showed high change rate over 4 % (Bp up), 5% (Mf up, down and Cc down), 6% (Bp down) and 10%(Cc up) that calculated by (Up gene/ whole gene)×(FIR/Cont) were selected. The three groups of genes in MC3T3-E1 cells affected by specific FIR were selected and listed in Table 1 for cell-cell signaling. The related genes were picked up and showed control system for transcription, cell-cell signaling and growth affected by FIR.

It was found that the DEAD related system and Homeobox related system were important for transcription, and interferon related system, thyroid hormone related system, and fibroblast growth hormone related system, platelet-derived growth factor related system and interleukin related system were important for cell-cell signaling and growth by our developed profiling system of genes in specific FIR condition.

It was suggested that our profiling system was available for finding unknown control system on effect of specific FIR from the data of microarray analysis.