



Studying the protein expression in human B lymphoblastoid cells exposed to 1.8-GHz (GSM) radiofrequency radiation (RFR) with protein microarray

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ABSTRACT

In the present study, the protein microarray was used to investigate the protein expression in human B-cell lymphoblastoid cells intermittently exposed to 1.8-GHz GSM radiofrequency radiation (RFR) at the specific absorption rate (SAR) of 2.0 W/kg for 24 h. The differential expression of 27 proteins was found, which were related to DNA damage repair, apoptosis, oncogenesis, cell cycle and proliferation (ratio >1.5-fold, $P < 0.05$). The results validated with Western blot assay indicated that the expression of RPA32 was significantly down-regulated ($P < 0.05$) while the expression of p73 was significantly up-regulated in RFR exposure group ($P < 0.05$). Because of the crucial roles of those proteins in DNA repair and cell apoptosis, the results of present investigation may explain the biological effects of RFR on DNA damage/repair and cell apoptosis.

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1. Introduction

To date, a number of investigations have been conducted on the bio-effects, especially the genetic and cytotoxic effects induced by radiofrequency radiation (RFR) emitted from mobile telephones. Evidences showed that RFR does not directly induce genetic damage at non-thermal exposure regimes, but the combinative effects of non-thermal RFR combining with the environmental (chemical or physical) mutagens are still controversial and inconclusive [1,2]. It was reported that the repair rate/pattern of the DNA damage induced by ultraviolet C (UVC) or doxorubicin (DOX) were altered in lymphocytes after RFR exposure as compared to the sham-exposure group [3,4], but the similar effects of RFR on DNA damage induced by X-rays were not found [5]. The difference in combinative effects may be related to DNA repair pathways when the different genetic damage is caused by various mutagens [4,6]. Moreover, the impact of RFR on cell apoptosis has not been clear. Although some studies reported that exposure to RFR fields could not increase the cellular apoptosis [7,8]. Palumbo et al. and Oral et al. [9,10] also found a significant increase of caspase-dependent apoptosis in human lymphocytes and rat endometrial surface epithelial cells exposed to RFR. It was suggested that the adverse effects of RFR may be due to the impact of RFR on certain important cell pathways, e.g. NER pathway, caspase-dependent apoptosis pathways and so on.

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Recently, high-throughput screening techniques (HTSTs) have been developed and widely applied in environmental health sciences to identify the exposure biomarkers [11]. So far, several studies on genome or proteome responses induced by RFR using HTSTs have been published [12], but only a few articles were associated with protein expression. Nylund and Leszczynski et al. used two-dimensional electrophoresis (2-DE) to detect the effects of RFR on human endothelial cell line EA.hy926, and found that RFR may affect the cytoskeleton and its physiological functions [13,14]. However, Zeng et al. did not find the effects of RFR on protein expression of human breast cancer cell line MCF-7 using 2-DE [15]. In present study, the protein microarray (Springbio, USA) was used to screen the protein differential expression in human B-cell lymphoblastoid cell line after exposure to 1.8-GHz Global System for Mobile Communications (GSM) radiofrequency radiation. Moreover, the proteins with differential expression were validated by Western blot assay.

2. Materials and methods

2.1. Cells and exposure

Human B-cell lymphoblastoid cell line HMy2.CIR from Cell Bank of Chinese Academy of Sciences was cultured in Petri dishes (Φ 35 mm) (NUNC, Denmark) with Iscove's Modified Dulbecco's Medium (IMDM) (HyClone, USA), supplemented with 10% fetal bovine serum (FBS) (HyClone, USA) in a fully humidified atmosphere

Table 1

List of differentially expressed proteins after RFR exposure, as compared with sham-exposure.

Proteins	Ratio	*
HIF-1 α	4.2713	↑
p73	3.4947	↑
CIDE-A	2.075974	↑
IGF-1R	1.936876	↑
BIM (BOD)	1.807956	↑
S100	1.806991	↑
FSH	1.800236	↑
CD16	1.712196	↑
Bak	1.686211	↑
Ruv B	1.669443	↑
b-2-Microglobulin	1.635216	↑
CD46	1.631091	↑
CDK4	1.62945	↑
Caspase 1	1.625344	↑
p19 ^{ARF}	1.620546	↑
CNPase	1.593065	↑
Thymidylate synthase	1.535565	↑
GFAP	1.532585	↑
MHC II (HLA-DP)	1.527896	↑
EGFR	1.524729	↑
RPA/p32 Replication Protein A	14.62671	↓
CDw78	1.933284	↓
THR- β	1.892991	↓
Filamin	1.64909	↓
CD30	1.646339	↓
Thyroglobulin	1.641968	↓
MHC II (HLA-DP and DR)	1.59616	↓

*Arrow-down indicates down-regulated proteins induced by RFR; arrow-up indicates up-regulated proteins induced by RFR.

at 37 °C with 5% CO₂ [4]. The exposure system designed by the Foundation for Information Technologies in Society (IT²S, Zurich, Switzerland) has been described in detail [4,15]. Briefly, Cells were intermittently (5 min fields on/10 min fields off) exposed/sham-exposed to 1.8-GHz GSM mobile phone radiation-like signal for 24 h at 37 °C and an average SAR of 2.0 W/kg which is defined as the safety limit of radiation emitted by mobile phones, according to the International Commission on Non-Ionizing Radiation Protection [16].

2.2. Protein microarray

After exposure, cells both in exposure and sham-exposure groups were immediately placed on ice, washed with ice-cold PBS. Total protein was extracted according to the manufacturer's protocol of Cell & Tissue Protein Extraction Reagent (Kangchen, Cat # KC-415, China). The protein concentrations were determined by BCA Protein Assay Kit (KangChen, KC-430, China). The protein sample from each group was divided into two parts: one was sample stored at –70 °C for Western blot assay, the other was used for microarray analysis. The protein microarray (the Spring's Master Antibody, Springbio, USA) contains about 700 antibodies which cover the proteins related to many important biological processes, including cancer markers, cell cycle, stress response, DNA damage repair, apoptosis, tumor metastasis and suppressors, oncogene, cell differentiation and so on. The protein samples were labeled with Biotination Reagent (Springbio, Cat. #AAP-100, USA) at room tem-

perature (23–25 °C) for 2 h with shaking, and Antibody Microarray was blocked by soaking in Blocking Buffer at room temperature for 30 min. Hybridization of Biotin Labeled Proteins with the Microarray was performed at room temperature for 2 h. Subsequently, Streptavidin Solution (Springbio, Cat. #AAP-100, USA) and the Antibody-Cy3 (Springbio, Cat. #AAP-100, USA) were added, respectively. Finally, the chips were scanned with Genepix 4000B (Axon, USA), and the data were analyzed with Genepix Pro 6.0 (Axon, USA).

2.3. Western blot assay

According to the manufacturer's protocol (Abcam, UK), 30 μ g proteins in each group was mixed with equal volume of 2 \times sample buffer, then boiled and subjected to 7–12% SDS-PAGE. After electrophoresis, the separated proteins were transferred electrophoretically from the gel to PVDF membranes (BIO-RAD, USA). Membranes were blocked for 2 h in phosphate buffered saline/Tween (PBS-T; 0.02% Tween) containing 3% albumin bovine fraction (BSA, Biotech, USA), then incubated with primary antibodies for RPA32 (Abcam, ab16855, UK), HIF-1 α (Abcam, ab1, UK), p73 (Abcam, ab22045, UK), and HRP-conjugated beta tubulin (loading control, Abcam, ab21058, UK) at 4 °C overnight. After washing and incubating with secondary antibodies, anti-Mouse IgG (H + L) (KangChen, KC-MM-035, China) for 2 h, the signal was detected via Chemiluminescence Detection Kit (Beit Haemek, ISRAEL). The autoradiograms were scanned and the proteins expression was quantified using Image-Pro Plus program 4.1 (Media Cybernetics, Inc. USA). The expression of target protein was normalized with respect to the expression of tubulin. The assay was repeated at least three times.

2.4. Statistical analysis

Independent sample *t*-test was utilized to compare the differences between the RFR exposure group and the RFR sham-exposure group using SPSS software V. 11.0 for Windows.

3. Results

3.1. Analysis of protein expression profile after RFR exposure

Table 1 shows the results of the protein microarray. A total of 20 proteins are significantly up-regulated in exposure group, and 7 are expressed in higher level in sham-exposure group (ratio >1.5-fold, *P* < 0.05).

3.2. Validation of certain protein expression by Western blot

Considering the importance of Replication Protein A 32 (RPA32), Hypoxia Inducible Factor 1- α (HIF-1 α) and p73 in DNA repair and apoptosis, as well as the significant differential expression (ratio >3-fold) in exposure group, we further validated their expression using Western blot. The results of validation indicated that the expression of RPA32 was significantly down-regulated (ratio >1.5-fold, *P* < 0.05), while the expression of p73 was significantly up-regulated (ratio >1.5-fold, *P* < 0.05) in exposure group when compared with sham-exposure group (Figs. 1 and 2). But no

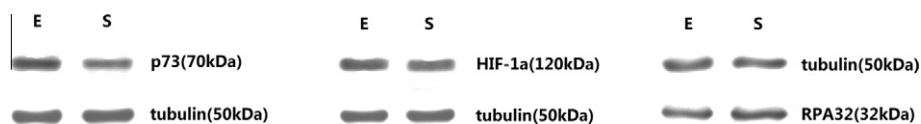


Fig. 1. The expression of RPA32, HIF-1 α , p73 and loading control (tubulin) in Western blot assay. E: exposure group, S: sham-exposure group.

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