Biochemical and Biophysical Research Communications 433 (2013) 36–39

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

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

Chen Zhijian [a,b], Li Xiaoxue [b], Zheng Wei [c], Lu Yezhen [b], Lou Jianlin [b], Lu Deqiang [b], Chen Shijie [b], Jin Lifen [b], He Jiliang [b,*]

[a] Department of Environmental and Occupational Health, Zhejiang Provincial Center for Disease Control and Prevention, Hangzhou 310051, Zhejiang, PR China
[b] Institute of Environmental Health, Medical College, Zhejiang University, Hangzhou 310058, Zhejiang, PR China
[c] Zhejiang International Travel Healthcare Center, 230 Zhonghezhong Road, Hangzhou 310003, PR China

ARTICLE INFO

Article history:
Received 4 February 2013
Available online 27 February 2013

Keywords:
Apoptosis
DNA repair
Radiofrequency radiation
Replication Protein A
p73
Protein microarray

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.

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.

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

* Corresponding author. Fax: +86 571 86996525.
E-mail addresses: he_jiliang@hotmail.com, hjl_zju@yahoo.com.cn (H. Jiliang).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.02.071

Table 1
List of differentially expressed proteins after RFR exposure, as compared with sham-exposure.

Proteins	Ratio	*
HIF-1α	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% CO_2 [4]. The exposure system designed by the Foundation for Information Technologies in Society (IT'IS, 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 wassample 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 temperature (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 μg proteins in each group was mixed with equal volume of 2× 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-1α (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 Detetion 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-1α) 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

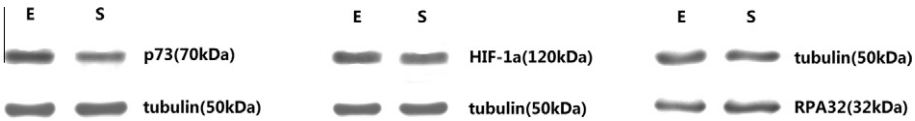

Fig. 1. The expression of RPA32, HIF-1α, p73 and loading control (tubulin) in Western blot assay. E: exposure group, S: sham-exposure group.

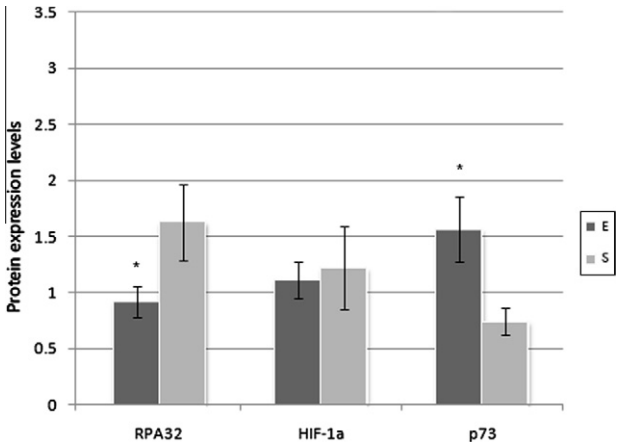

Fig. 2. The expression levels of three proteins (RPA32, HIF-1α, and p73) in human B-cell lymphoblastoid cells exposed to RFR. E: exposure group, S: sham-exposure group, $^{*}P < 0.05$, as compared with sham-exposure group.

difference of HIF-1α expression between exposure group and sham-exposure group was found.

4. Discussion

The real biological effects induced by RFR are still ambiguous. In present investigation, the protein microarray was employed to assess the impact of RFR on multiple cellular processes at protein level. According to our microarray data, 17 proteins with differential expression were found in lymphoblastoid cell after RFR exposure (table 1). Those proteins are mainly involved in DNA damage repair (e.g. p73, RPA32, ruv B, Thymidylate Synthase and $p19^{arf}$) [17–21], apoptosis (e.g. HIF-1α, p73, CIDE-A, $p19^{arf}$, IGF-1R, BIM, Bak, caspase 1, EGFR, THR-β, filamin and CD30) [17,21–31], oncogenesis (e.g. $p19^{arf}$, IGF-1R, BIM], S100, EGFR, CD30) [21,24,25,28,31,32], cell cycle and proliferation (e.g. CD16, CD46, CDw78, CDK4, GFAP, EGFR8, CD30) [28,31,33,34], which suggest the potential effects of RFR on the cellular biological processes.

Most of the apoptosis-related-proteins mentioned above are involved in the caspase-dependent pathway, HIF-1α, p73, $p19^{ARF}$ and filamin are key regulators [17,21,22,30], high expression of CIDE-A, BIM Bak or caspase 1 can activate the apoptosis pathway [23,25–27], while low expression of THR-β reduces its activity in apoptosis prevention [29]. Our results are consistent with those reported by Palumbo et.al. and Oral et.al. [9,10], and indicated that the increase of apoptosis induced by RFR may be due to the impact of RFR on the expression of those proteins involved in caspase-dependent pathway apoptosis.

The main DNA repair pathways include base excision repair (BER), NER, mismatch repair (MMR), non-homologous DNA end joining (NHEJ) and homologous recombination (HR) pathway [6], p73 and $p19^{ARF}$ are upstream regulators of p53 that activates BER, NER and MMR pathways [6,17,21], RPA and Thymidylate synthase are the single-stranded DNA-binding proteins in majority of DNA repair pathways including BER, NER, and MMR pathways [6]. In the present study, RFR exposure could up-regulate the expression of p73 and $p19^{ARF}$ and down-regulate the expression of RPA and Thymidylate synthase. Those changes may influence the BER, NER and MMR pathways, and explain the results reported previously, i.e. RFR could influence the DNA repair pattern in cells exposed to UVC [3], but could not alter the DNA repair pattern in cells exposed to X-rays [5], since the DNA damage induced by UVC is mainly repaired through NER pathway, while the DNA damage induced by X-rays is mainly repaired through NHEJ and HR pathway [6].

In our experiment, the differential expression of three proteins (RPA32, HIF-1α and p73) was more significant than that of other proteins and those proteins play key roles in DNA repair and cell apoptosis. So these three proteins were validated with Western blot assay. The results of validation for RPA32 and p73 were similar to the results of microarray. However, no difference of HIF-1α expression level between the exposure group and sham-exposure group was found. RPA is an indispensable player, not only in major DNA repair pathways, but also in DNA metabolic pathways (such as DNA replication, recombination, cell cycle, DNA damage checkpoints). Low RPA expression may lead to genomic instability [18]. p73, a transcription factor of p53 family, is responsible for guarding the genome, and overexpression of p73 in cells can induce apoptosis through different mechanisms, such as caspase-dependent pathway [17,35]. Hence, our data suggested that RFR may affect some DNA repair and apoptosis pathways by regulating key proteins, e.g. RPA, p73. However, further studies are needed to investigate those proteins in RFR-induced adverse effects.

Acknowledgments

This research work was supported by the National Natural Science Foundation of China (No. 30571547), the Natural Science Foundation of Zhejiang Province (No. 300434) and the International Cooperative Foundation of Science-Technique Bureau of Zhejiang Province (No. 012104). The authors would like to express their gratitude to Professor Jiang Huai and Professor Xu Zhengping of Zhejiang Provincial Key Bioelectromagnetics Laboratory in Zhejiang University for providing the 1.8-GHz RFR source.

References

[1] L. Verschaeve, Genetic damage in subjects exposed to radiofrequency radiation, Mut. Res. 681 (2009) 259–270.
[2] T.J. Vijayalaxmi, Prihoda, Genetic damage in human cells exposed to non-ionizing radiofrequency fields: a meta-analysis of the data from 88 publications (1990–2011), Mut. Res. 749 (2012) 1–16.
[3] W. Baohong, J. Lifen, L. Lanjuan, L. Jianlin, L. Deqiang, Z. Wei, H. Jiliang, Evaluating the combinative effects on human lymphocyte DNA damage induced by ultraviolet ray C plus 1.8 GHz microwaves using comet assay in vitro, Toxicology 232 (2007) 311–316.
[4] C. Zhijian, L. Xiaoxue, L. Yezhen, C. Shijie, J. Lifen, L. Jianlin, L. Deqiang, H. Jiliang, Impact of 1.8-GHz radiofrequency radiation (RFR) on DNA damage and repair induced by doxorubicin in human B-cell lymphoblastoid cells, Mut. Res. 695 (2010) 16–21.
[5] C. Zhijian, L. Xiaoxue, L. Yezhen, L. Deqiang, C. Shijie, J. Lifen, L. Jianlin, H. Jiliang, Influence of 1.8-GHz (GSM) radiofrequency radiation (RFR) on DNA damage and repair induced by X-rays in human leukocytes in vitro, Mutat. Res. 677 (2009) 100–104.
[6] F. Altieri, C. Grillo, M. Maceroni, S. Chichiarelli, DNA damage and repair: from molecular mechanisms to health implications, Antioxid. Redox Signal. 10 (2008) 891–937.
[7] Z.O. Merhi, Challenging cell phone impact on reproduction: a review, J. Assist. Reprod. Genet. 29 (2012) 293–297.
[8] C. Marino, I. Lagroye, M.R. Scarfi, Z. Sienkiewicz, Are the young more sensitive than adults to the effects of radiofrequency fields? an examination of relevant data from cellular and animal studies, Prog. Biophys. Mol. Biol. 107 (2011) 374–385.
[9] R. Palumbo, F. Brescia, D. Capasso, A. Sannino, M. Sarti, M. Capri, E. Grassilli, M.R. Scarfi, Exposure to 900 MHz radiofrequency radiation induces caspase 3 activation in proliferating human lymphocytes, Radiat. Res. 170 (2008) 327–334.
[10] B. Oral, M. Guney, F. Ozguner, N. Karahan, T. Mungan, S. Comlekci, G. Cesur, Endometrial apoptosis induced by a 900-MHz mobile phone: preventive effects of vitamins E and C, Adv. Ther. 23 (2006) 957–973.
[11] K. Freeman, From point B to point A: applying toxicogenomics to biological inference, Environ. Health Perspect. 113 (2005) A388–A393.
[12] J.P. McNamee, V. Chauhan, Radiofrequency radiation and gene/protein expression: a review, Radiat. Res. 172 (2009) 265–287.
[13] D. Leszczynski, R. Nylund, S. Joenvaara, J. Reivinen, Applicability of discovery science approach to determine biological effects of mobile phone radiation, Proteomics 4 (2004) 426–431.
[14] R. Nylund, D. Leszczynski, Proteomics analysis of human endothelial cell line EA.hy926 after exposure to GSM 900 radiation, Proteomics 4 (2004) 1359–1365.

[15] Q. Zeng, G. Chen, Y. Weng, L. Wang, H. Chiang, D. Lu, Z. Xu, Effects of global system for mobile communications 1800 MHz radiofrequency electromagnetic fields on gene and protein expression in MCF-7 cells, Proteomics 6 (2006) 4732–4738.
[16] Guidelines for limiting exposure to time-varying electric, magnetic, and electromagnetic fields (up to 300 GHz). International Commission on Non-Ionizing Radiation Protection, Health Physics, 74 (1998) 494–522.
[17] V. Dotsch, F. Bernassola, D. Coutandin, E. Candi, G. Melino, p63 and p73, the ancestors of p53, Cold Spring Harbor Perspect. Biol. 2 (2010) a004887.
[18] K. Sakaguchi, T. Ishibashi, Y. Uchiyama, K. Iwabata, The multi-replication protein A (RPA) system – a new perspective, FEBS J. 276 (2009) 943–963.
[19] J.R. Donaldson, C.T. Courcelle, J. Courcelle, RuvABC is required to resolve holiday junctions that accumulate following replication on damaged templates in *Escherichia coli*, J. Biol. Chem. 281 (2006) 28811–28821.
[20] L.S. Li, J.C. Morales, M. Veigl, D. Sedwick, S. Greer, M. Meyers, M. Wagner, R. Fishel, D.A. Boothman, DNA mismatch repair (MMR)-dependent 5-fluorouracil cytotoxicity and the potential for new therapeutic targets, Br. J. Pharmacol. 158 (2009) 679–692.
[21] T. Sperka, J. Wang, K.L. Rudolph, DNA damage checkpoints in stem cells, ageing and cancer, Nat. Rev. Mol. Cell Biol. 13 (2012) 579–590.
[22] X. Wang, S. Ma, G. Qi, Effect of hypoxia-inducible factor 1-alpha on hypoxia/reoxygenation-induced apoptosis in primary neonatal rat cardiomyocytes, Biochem. Biophys. Res. Commun. 417 (2012) 1227–1234.
[23] T. Yonezawa, R. Kurata, M. Kimura, H. Inoko, Which CIDE are you on? apoptosis and energy metabolism, Mol. Biosyst. 7 (2011) 91–100.
[24] J. Chaves, M.W. Saif, IGF system in cancer: from bench to clinic, Anticancer Drugs 22 (2011) 206–212.
[25] A.S. Gillings, K. Balmanno, C.M. Wiggins, M. Johnson, S.J. Cook, Apoptosis and autophagy: BIM as a mediator of tumour cell death in response to oncogene-targeted therapeutics, FEBS J. 276 (2009) 6050–6062.
[26] D. Westphal, G. Dewson, P.E. Czabotar, R.M. Kluck, Molecular biology of bax and bak activation and action, Biochim. Biophys. Acta 2011 (1813) 521–531.
[27] A. Denes, G. Lopez-Castejon, D. Brough, Caspase-1: is IL-1 just the tip of the ICEberg?, Cell Death Dis 3 (2012) e338.
[28] R.S. Herbst, Review of epidermal growth factor receptor biology, Int. J. Radiat. Oncol. Biol. Phys. 59 (2004) 21–26.
[29] R. Lopez-Fontal, M. Zeini, P.G. Traves, M. Gomez-Ferreria, A. Aranda, G.T. Saez, C. Cerda, P. Martin-Sanz, S. Hortelano, L. Bosca, Mice lacking thyroid hormone receptor beta show enhanced apoptosis and delayed liver commitment for proliferation after partial hepatectomy, PLoS ONE 5 (2010) e8710.
[30] Y. Shifrin, V.I. Pinto, A. Hassanali, P.D. Arora, C.A. McCulloch, Force-induced apoptosis mediated by the Rac/Pak/p38 signalling pathway is regulated by filamin A, Biochem. J. 445 (2012) 57–67.
[31] A.I. Berro, G.A. Perry, D.K. Agrawal, Increased expression and activation of CD30 induce apoptosis in human blood eosinophils, J. Immunol. 173 (2004) 2174–2183.
[32] I. Salama, P.S. Malone, F. Mihaimeed, J.L. Jones, A review of the S100 proteins in cancer, Eur. J. Surg. Oncol. 34 (2008) 357–364.
[33] S.W. Blain, Switching cyclin D-Cdk4 kinase activity on and off, Cell Cycle 7 (2008) 892–898.
[34] J. Middeldorp, E.M. Hol, GFAP in health and disease, Prog. Neurobiol. 93 (2011) 421–443.
[35] N. Jain, S. Gupta, C. Sudhakar, V. Radha, G. Swarup, Role of p73 in regulating human caspase-1 gene transcription induced by interferon-{gamma} and cisplatin, J Biochem. 280 (2005) 36664–36673.