Investigation of P53, HIPK-2 and EIF2S1 Genes Expression Changes Induced by in Vitro Gamma Radiation

İn Vitro Olarak Gama Radyasyon ile İndüklenen *P53*, *HIPK-2* ve *EIF2S1* Genlerinin Ekspresyon Değişikliklerinin Araştırılması

Research Article

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ABSTRACT

In this study, we have investigated induction of *P53*, *HIPK-2* and *EIF2S1* genes after exposure to low doses gamma radiation by using the peripheral human lymphocytes. Gene expression changes were analyzed in quantitative real time polymerase chain reaction (QRT PCR) by using B2M as a reference gene. Whole blood samples of ten young, healthy and non-smoking donors were taken. All samples were divided in to five specimens and four of them were irradiated using constant gamma irradiation source (60Co) giving the dose rate of 0.0327 kGy/h while one sample used as control. Blood samples were exposed to 0.1 Gy, 0.5 Gy, 1 Gy and 3 Gy γ-rays. Results were expressed in terms of the threshold cycle value (Ct) in REST 2009 (Relative Expression Software Tool V. 2.0.13) program and the difference between the CT values of the target gene and the reference gene was calculated. We found a linear dose–response relationship for *P53*, *HIPK-2* and *EIF2S1* genes. Results of the QRT PCR showed that three of the genes were up-regulated after radiation exposure. We concluded that *P53*, *HIPK-2* and *EIF2S1* genes can be promising for the assessment of gene expression changes in radiation exposure induced DNA damage.

Key Words
Gene expression; Ionizing radiation, QRT PCR.

ÖZET

Bu çalışmada periferal insan lenfositlerinde düşük doz gama radyasyon maruziyeti sonrası *P53*, *HIPK-2* ve *EIF2S1* genlerinin indüksiyonunu araştırılmıştır. Gen ekspresyon değişiklikleri, B2M referans gen olarak kullanılarak kantitafif gerçek zamanlı polimeraz zincir reaksiyonu ile (KRT PZR) değerlendirilmiştir. Bu amaçla genç, sağlıklı ve sigara içmeyen on bireye ait kan örnekleri toplanmıştır. Her birey için beş tüpe bölünen örneklerden ilki kontrol olarak ayrılmış diğer dördü sabit gama kaynağında (Co60) 0,0327 kGy/sa doz hızına göre hesaplanmış 0,1 Gy, 0.5 Gy, 1 Gy ve 3 Gy gama radyasyonuna maruz bırakılmıştır. Sonuçlar eşik değerinin aşıldığı döngü değerlerine (CT) göre REST 2009 (Relative Expression Software Tool V. 2.0.13) programında değerlendirilmiş, hedef genin ve referans genin CT değerleri arasındaki farklılıklara göre hesaplanmıştır. *P53*, HIPK2 ve *EIF2S1* genleri için linear bir doz-cevap ilişkisi bulunmuştur. KRT PZR sonuçları bu üç geninin maruz kalınan dozlarda up-regüle olduğunu göstermiştir. Elde edilen sonuçlar *P53*, HIPK2 ve *EIF2S1* genlerinin radyasyon maruziyeti sonrasında indüklenen DNA hasarında ki gen ekspresyon değişikliklerinin değerlendirilmesinde kullanılabileceğini göstermiştir.

Anahtar Kelimeler
Gene ekspresyonu, iyonize radyasyon, QRT PCR.

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INTRODUCTION

Radiation induced DNA damage may result in genomic instability. These damages stimulate signal transduction pathways inside the cell and can trigger a series of events for an appropriate DNA damage response, such as apoptosis and autophagy [1]. The effects of high dose radiations are well defined but the cell responses to low dose exposure remain to be determined [2]. It is known that ionizing radiation causes cellular responses and damages to cellular organelles, membranes and biomolecules, and as well exposure of DNA to ionizing radiation results in single-strand breaks, double-strand breaks, alkali-labile sites and oxidized bases [3,4].

One of the pathways in the nucleus is ataxia-telangiectasia mutated *(ATM)/P53* tumor suppressor pathway which is a major cellular mechanism in the cellular response to alterations in genomic integrity and in the activation of cell cycle checkpoints [5-7]. ATM is a damage-activated protein kinase and phosphorylates *P53* after irradiation. This phosphorylation causes transcriptional activation of *P53* and many genes are activated by *P53*-dependent transcription [5,8]. Another pathway is the PERK-eIF2alpha signaling pathway, which mediates the first response to endoplasmic reticulum stress [9]. The pattern of the genes in these pathways may be up or down regulated after irradiation. It was shown that genes such as the tumor suppressor *P53*, Homeodomain-interacting protein kinase-2 (*HIPK-2*) and Eukaryotic translation initiation factor 2 subunit 1 (*EIF2S1*) contribute to stress responses to ionizing radiation [6,10,11]. In this study we aimed to evaluate the effects of low-dose gamma radiation on *P53*, *HIPK-2* and *EIF2S1* genes. In dose response studies while microarray studies are perfect tools for large-scale screening, an accurate relationship between dose and the level of expression of various genes can only be validated with QRT-PCR [12]. We have used human lymphocytes and evaluated the genes expressions with quantitative real-time polymerase chain reaction (QRT PCR), which is a reliable and sensitive method.

MATERIALS AND METHODS

In Vitro Whole Blood Sample Irradiation

The whole blood samples of young, healthy and non-smoking donors were taken by venipuncture to five different tubes with lithium heparin. First tube of the samples was used as their own control and not irradiated. Other four tubes were irradiated using constant gamma irradiation source (^{60}Co). According to 373 kGy/hr dose rate of the constant gamma irradiation source, 0.1 Gy, 0.5 Gy, 1 Gy and 3 Gy were calculated and were given the four different tubes of the samples.

RNA Extraction and cDNA Synthesis

Lymphocytes were isolated from whole peripheral blood on a density gradient by using the Histopaque-1077 (Sigma). Total RNA was isolated from lymphocytes using an RNeasy plus mini kit (Qiagen) by following the manufacturer's protocol. RNA samples were quantified by GeneQuant 1300 spectrophotometer (GE Healthcare) at 260 and 280 nm and stored at −80°C until further analysis.

cDNA was synthesized by using the QuantiTect® Reverse Transcription Kit (Qiagen) according to the manufacturer's protocol and the cDNA product was stored at -20°C. Primer and probe sets used for quantitative real-time PCR are listed in the Table 1. Reactions were performed in a 10 µl reaction volume containing 1 µl of cDNA, 5 µl 2x QuantiFast Probe RT-PCR master mix (Qiagen), 1µl of each primer and 1 µl of probe. Real-time PCR was performed in a RotorGene 6000 real-time PCR machine (Corbett Research, Sydney, Australia) with the 72-well rotor. Reaction conditions were as follows: 5 min 94°C initial denaturation, followed by 45 cycles of 10s at 94°C, 20s at 60°C -a combined annealing and extension step.

Statistical Analysis

The comparative threshold cycle (CT) method for the relative quantification of gene expression was used. Results were expressed in terms of CT value in REST 2009 (Relative Exp ression Software Tool V. 2.0.13) program and the difference (ΔCT)

Table 1. Real-time primer and probe sequences.

Gene	Primer/Probe Sequences
P53	Forward primer 5′- CATGAGCGCTGCTCAGATAG-3′
	Reverse primer 5′- CCAGTGTGATGATGGTGAGG-3′
	Probe 5′-FAM- CCCCTCCTCAGCATCTTATCCGAGTGG -BHQ1-3′
HIPK2	Forward primer 5′- CCGGGACAAAGACAACTAGG -3′
	Reverse primer 5′- CCCTTCCAAATCTGTCGTC -3′
	Probe 5′-FAM- CGTGACACGGACTCACCATATCCTTTG -BHQ1-3′
EIF2S1	Forward primer 5′- GAACAGGCTTGGCAAAGAAG-3′
	Reverse primer 5′- AGGCTTTGATGGGAGATTCA-3′
	Probe 5′-HEX- GATCACTCCAGTGCACTCGA -BHQ1-3′

between the CT values of the target gene and the reference gene was calculated.

RESULTS

Peripheral human lymphocytes, which were proved to be sensitive to ionizing radiation and previously established as suitable biodosimeters, demonstrate a DNA damage response following the low dose ionizing radiation [13,14]. Real time PCR analysis showed that, *P53*, *HIPK-2* and *EIF2S1* genes expressions were increased after exposure to ionizing radiation in vitro. According to QRT- PCR results, a linear and reproducible up-regulation was detected in the expression of *P53*, *HIPK-2* and *EIF2S1* genes, which were detailed graphically in Figure 1. C_T values were obtained and according to the Ratio = $(E_{target})^{\Delta CP}$target(control-sample)/$(E_{ref})^{\Delta CP}$ref(control-sample),gene expression changes were calculated using REST 2009 software program and relative expressions of target genes were determined.

DISCUSSION

Irradiation of human cells to low dose ionizing radiation alters the gene expression profiles. In this study we aimed to demonstrate the transcriptional changes of *P53*, *HIPK-2* and *EIF2S1* genes caused by ionizing radiation in vitro and we observed a linear and reproducible up-regulation of *P53*, *HIPK-2* and *EIF2S1* genes. Our results suggest that following the irradiation, human lymphocyte cells responded to radiation. It is clear that some nuclear pathways are activated by low dose ionizing radiation [1]. ATM protein, the product of ATM gene mutated in ataxia-telangiectasia, is activated by DNA damage and may initiate cell cycle arrest by activating the *P53* tumor suppressor protein, the product of a tumor suppressor gene. *P53*, which is known for its role as a transcription factor that regulates the expression of a wide range of stress response genes, is stabilized and activated in response to cellular stress and extremely sensitive to DNA damage [15,16]. The levels of *P53* increases and bounds its specific DNA sites in the genome after irradiation. By this binding some growth arrest genes are activated and on the other hand cell cycle progression genes are repressed. Blocking the progression of the cells from G1 to S phase and from G2 to mitosis after DNA damage may provide time to cell for repair [15]. These may explain the linear up-regulation of *P53* gene. *HIPK-2* also participates in cell cycle arrest and DNA repair/damage pathways. After DNA damage *HIPK-2* phosphorylates *P53* [17]. *HIPK-2*, which is an unstable protein in unstressed cells because it is constantly degraded through the ubiquitin-proteasome system, is a protein kinase that interacts with numerous transcription factors as well as transcriptional regulators and stabilizes after ionizing radiation [18,19]. It is reported that ionizing radiation provokes *HIPK-2* accumulation and activation [11]. HIPK2 is regulated after this activation by the DNA damage checkpoint kinase

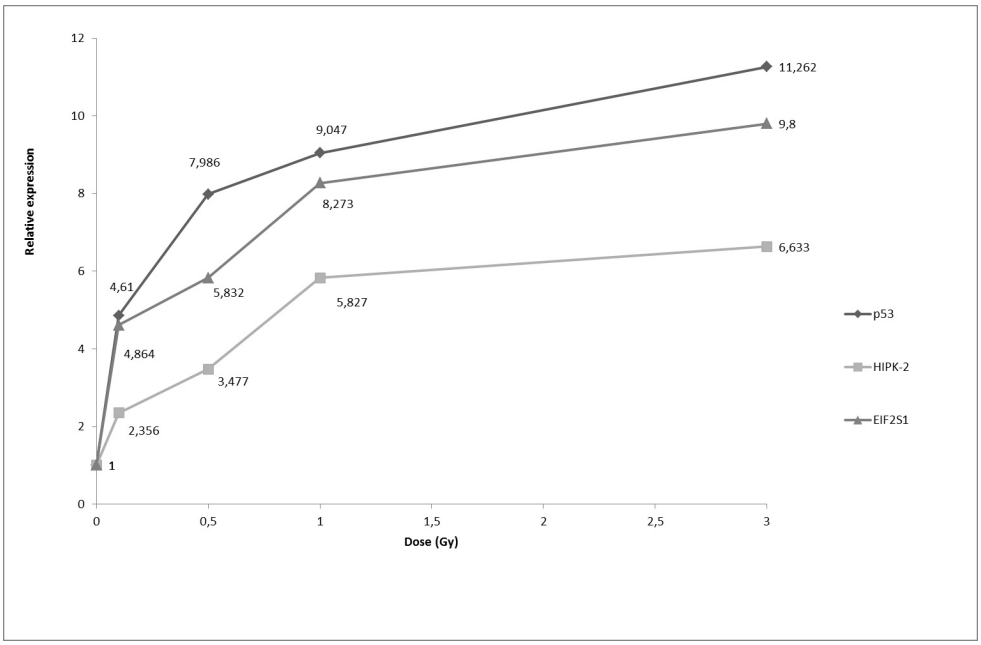

Figure 1. Relative expression ratios of *P53*, *HIPK-2* and *EIF2S1* genes according to increasing ionizing radiation doses. Error bars represent 95% confidence intervals.

ATM. Up-regulation of HIPK2 may explain with *P53* serine-46 phosphorylation after ionizing radiation exposure. The PERK-eIF2 alpha signaling pathway is also activated following the DNA damage. PERK belongs to a family of eukaryotic translation initiation factor 2α (eIF2) kinases and is a type I endoplasmic reticulum resident transmembrane protein that reside in the endoplasmic reticulum [20]. Zhang et al. were reported that PERK pathway has a stress sensor and involved in ER stress induced by radiation. They also have found that phosphorylated eIF2alpha level increased in epithelial cell line [21]. It was shown that following the ionizing radiation exposure, PERK kinase is activated and after this activation it phosphorylates serine-51 of eIF2alpha, which is its only identified target [22].

Gene expression studies on radiation induced DNA damage have led to insights into DNA repair and cell survival mechanisms. Our QRT PCR results suggest that relative levels of gene expression can be used efficiently to detect radiation-induced DNA damage related gene expression changes.

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