CHANGING OF RGS TRANSCRIPTS LEVELS BY LOW-DOSE-RATE IONIZING RADIATION IN MOUSE TESTIS

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Deleterious effects of high dose radiation exposure with high-dose-rate are unarguable, but they are still controversial in low-dose-rate. The regulator of G-protein signaling (RGS) is a negative regulator of G protein-coupled receptor (GPCR) signaling. In addition, it is reported that irradiation stress led to GPCR-mediated mitogen-activated protein kinase (MAPK) and phosphotidylinositol 3-kinase (PI3-k) signaling. The RGS mRNA expression profiles by whole body radiation with low-dose-rate has not yet been explored. In the present study, we, therefore, examined which RGS was modulated by the whole body radiation with low-dose-rate (3.49 $mGy \cdot h^{-1}$). Among 16 RGS expression tested, RGS6, RGS13 and RGS16 mRNA were down-regulated by low-dose-rate irradiation. This is the first report that whole body radiation with low-dose-rate can modulate the different RGS expression levels. These results are expected to reveal the potential target and/or the biomarker proteins associated with male testis toxicity induced by low-dose-rate irradiation, which might contribute to understanding the mechanism beyond the testis toxicity.

Keywords : Gamma radiation, Low-dose-rate, RGS, Testis

1. INTRODUCTION

Recently, emerging evidences suggest that Regulator of G protein signaling (RGS) levels were changed in response to various cellular stress conditions, and in some cases, the control of cell apoptosis or survival by RGS proteins has been demonstrated[1-3]. Regulator of G protein signaling proteins negatively regulates the G protein coupled receptor (GPCR) signaling through their GTPase-activating protein (GAP) activity on Gα subunit of G proteins. Due to the pathophysiological significance of GPCR signalings, fine tuning of their signaling by RGS is crucial. Up to now, more than 30 RGS and RGS-like members have been reported[4,5]. Based on the amino acid sequences, structures of the proteins, and functions within the subfamilies, RGS proteins have been classified into 6 distinct subfamilies[6,7]. Those RGS proteins have a shared domain (120- to 130-amino acids) that binds to the subunits of Gα to activate their intrinsic GTPase activity[6-8]. Recently emerging evidences showed that the traditional roles of RGS have been expanded such as G protein-independent functions including microtubule polymerization, protein synthesis, and ion channel currents[4,9-11]. In this regard, variety of cellular stress modulate RGS gene expression[1,12,13], and some of RGS proteins are regarded as a biomarker for cellular damage[14] or prognosis for cancer therapy such as

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RGS16 in colorectal[15], and pancreatic cancer[16], RGS5 in tumor angiogenesis [17], and RGS1 in melanoma[18].

Deleterious effects of whole body radiation (WBR) with high doses and high-dose-rate are widely studied and well established[19,20]. But the information on the effects of WBR with low-dose-rate is limited. In the previous study, we evaluated the overall deleterious effects of low-dose-rate radiation (0, 0.2, 2 Gy of total dose, 3.49 $mGy \cdot h^{-1}$ of dose rate) in balb/c mouse, and there were no significant clinical symptoms except testis tissue for the 2 Gy exposed group by the reduced organ weight [21]. The present study is to determine whether RGS transcripts levels were changed by WBR of gamma rays with low-dose-rate. We found that RGS 6, 13, and 16 levels were down-regulated in a dose-dependent manner.

2. MATERIALS AND METHODS

2.1 Animals and ethics

Male Balb/c mice were used after acclimatization for 7 days. Animals were randomized five per cage, in the temperature of 20~24℃ and the humidity of 40~60%, at specific pathogen free (SPF) conditions. Light to darkness cycle was 12 hours to 12 hours, and food (Purina, Korea) and water were supplied as free of access. The animals used in this study were reviewed and approved, based on the ethical and scientific care procedures of the Dongnam Institute of Radiological and Medical Science (DIRAMS) and the Institutional Animal Care and Use Committee (IACUC). Animals were marked by picric acid.

2.2 Irradiation

Animals were irradiated with γ rays from a low-dose-rate irradiator (^{137}Cs source, 1.85 GBq, Gamma-irradiator, Chiyoda Technol Co., Japan) at a dose rate of 3.49 $mGy \cdot h^{-1}$ at SPF conditions (n=five per group). After 24 hours of whole body radiation with the low-dose-rate, animals were sacrificed in accordance with 'Guide for the Care and Use of Laboratory Animals'.

2.3 Analysis of mouse testis mRNA levels by quantitative real-time polymerase chain reaction (PCR)

To determine RGS mRNA levels, total RNA was isolated from mice testis tissues using Trizol reagent (Qiagen,USA, Valencia, CA) according to the standardized protocol. The extracted total RNA was then used for quantification, by using real time-PCR and SYBR Premix Ex Taq (Takara, Japan) applied according to the manufacturer's instructions, and by using a

Table 1. Primers of the Investigated Genes in an RT-PCR Analysis.

Gene		Primer sequences
RGS1	F	5'- GAC CAT GAG AGC GGC AGC CAT- 3'
	R	5'- GAC CTG TCT GGT TGG CAA GGA GT- 3'
RGS2	F	5'- ACC GCT GAC GGC TCT CCT GAA - 3'
	R	5'- GCA AAT GGA AGG CGG CCA GCA - 3'
RGS3	F	5'- CTG TCA CCC GGG GCT TTG GGA - 3'
	R	5'- TTC CAC GGC TGG CAG CAG ATT - 3'
RGS4	F	5'- TCC CTG GTC TCC CAG TGT GCC - 3'
	R	5'- TGG AAG TGA CTC ACA CGC GGC - 3'
RGS5	F	5'- CCC AAG GAG TGA ACC GGC TGT - 3'
	R	5'- GCA CTG CCC TTG AGG CAC CC- 3'
RGS6	F	5'- ACT GAG ACG CCG GAG CGA GT- 3'
	R	5'- TGG GCA CAC CCC CTG TCT TGT- 3'
RGS7	F	5'- TGA AAG GCT GCT CCA CGC CG- 3'
	R	5'- GCG ATG GCT CCG TTC AGC ACT- 3'
RGS8	F	5'- GGG CCT CGC CAG GTT TTG GG-3'
	R	5'- GGA TTC GTC GGC CAA GCG GT-3'
GAPDH	F	5'- GTG GGC CGC CCT AGG CAC CAG -3'
	R	5'- GGA GGA AGA GGA TGC GGC AGT -3'
RGS11	F	5'-TGA ATG CGC CCA ACG TGG CT- 3'
	R	5'- CCT GGG CCT GTC CGC CAA AG- 3'
RGS12	F	5'- GAC GGC GGT GAC CGT TGA TGC- 3'
	R	5'-GCC ACT TCG ACG CTC CGC AC - 3'
RGS13	F	5'- TGC AAT GCC ACA GCA CTA CTT TCC - 3'
	R	5'- TAT GGC ACT CCG GGT CCC TGT - 3'
RGS14	F	5'- AGC CCG GAC ACA GCG AGG AA- 3'
	R	5'- ACT CTC GTC GCA GGG CCG AA- 3'
RGS16	F	5'- CCG GCC TGT GAG CCC CTT TC- 3'
	R	5'- GGA GCG CAG GGC CCA GTA AG- 3'
RGS17	F	5'- TCT CGA GCA CCC TCG GAC CC- 3'
	R	5'- TGC TTC AGA ACC CAA CTG CCA TC- 3'
RGS18	F	5'- AAT TTT GGG TCG CCT GTG AAG ACT- 3'
	R	5'- GTG GGC TGG GCG ATG CTC TT- 3'
RGS20	F	5'- CAG ACT CAG ACA TGG GCC AGC A- 3'
	R	5'- GGC AGC AAC TGT GCC TGT GC- 3'

Abbreviations: F, forward; GAPDH, glyceraldehydes-3-phosphate dehydrogenase; R, reverse; RGS, regulator of G protein signaling; RT-PCR, real time-polymerase chain reaction

real-time thermal cycler (Bio-Rad, Hercules, CA, USA). The results are expressed as the ratio of optimal density to glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The RGS-subtype specific primers are listed in Table 1.

2.4 Statistical analysis

Data were represented as the mean ± SEM of three independent experiments. Student's *t*-test was carried out to analyze the statistical significance between the groups using SPSS ver. 18.0 (SPSS Inc., Chicago, IL, USA). *p* values less than 0.05 were considered statistically significant.

3. RESULTS AND DISCUSSION

DNA sequences of various RGS proteins show close similarities to each other. Hence, isotype-specific primers were designed for a gene-specific real-time PCR reaction, and were confirmed by NCBI nucleotide blast search service. The gene specific primers used in this study are listed in Table 1. As shown in Fig. 1, among the 16 subtypes of RGSs, the following RGS transcript levels in mouse testis were down-regulated on radiation dose-dependent manner: RGS6, RGS13, and RGS16.

As shown in Fig. 1F, RGS6 mRNA expression levels were significantly down-regulated by both 0.2 and 2 Gy of TBI exposed group. RGS6 is a member of the R7 subfamily of RGS that contains RGS, GGL (G protein γ subunit-like), and DEP (dishevelled, Egl-10, pleckstrin) domains[22], and it activates GTPase of $G_{\alpha i}$ family Gproteins[23]. Besides its classical roles as a GPCR signaling regulator, several studies showed the correlation between the RGS6 and cellular stress or cancer initiation. Under cellular stress conditions, such as proteasome-mediated stress or mild heat stress, RGS6 is translocated into the nucleoli[24]. RGS6 also modulates doxorubicin-mediated ATM and p53 activation[25] and suppressed breast cancer initiation and progression[26]. Furthermore, the indirect interaction of RGS6 and DNA methyltransferase (Dnmt1), which is considered as a novel oncogene that suppresses the transcription of pro-apoptotic genes, have also been reported [27]. Taken together, RGS6 has both pro-apoptotic and anti-apoptotic actions depending on the cellular stress type.

We observed that the RGS13 levels were also down-regulated in a radiation dose dependent manner in Fig. 1 K. The RGS13 is one of the smallest RGS family members, and it is mainly expressed in immune tissues such as tonsil, spleen, lymph node, and thymus[28]. As a negative regulator of GPCR signaling, their classical roles are mainly limited to transcriptional regulations. For instance, it is reported that RGS13 is a nuclear suppressor of CREB, independent of its classical GAP activity[29]. The cAMP analog, which activates the cAMP response element-binding (CREB) protein pathway, reduces RGS13 mRNA expression[30]. However, the exact role of RGS13 in mouse testis is still unknown, and there are only a limited number of reports on RGS13 expression pattern in respond to cellular stress. RGS13 expression levels were regulated by p53, which takes a major part in DNA repair-related gene regulation in immune cells[31]. Up to now, none of the reports were available about the effects of WBR with low-dose-rate on RGS13 expressions.

Lastly, RGS16 levels in mouse testis tissue (Fig. 1 M) were also down-regulated by WBR with low-dose-rate in a radiation dose-dependent manner. RGS16 was first identified as a retinal specific RGS, which binds an intermediate conformation of transducin and enhances recycling[32]. Later, binding and inhibitory effects of RGS16 on Gi- and Gq- mediated signaling pathways were reported[33]. RGS16 also has a potent inhibitory effect on Gq-dependent Ca^{2+} signaling[34]. Like other RGS subfamily members, recent reports suggest that RGS16 also has a GTPase-independent activity, and is considered as a biomarker for cellular stress or cancer prognosis. RGS16 was significantly down-regulated in the metastatic pancreatic cancer patient group, and is associated with poor survival rate[16]. On the other hand, RGS16 was higher in colorectal cancer tissues than in the corresponding normal tissues[35]. Like RGS6 and RGS13, there are no reports regarding the correlation of RGS16 and radiation until now.

Changing patterns of RGS by WBR with low-dose-rate are major novel findings in this study. Due to the lack of background information on the correlation between WBR and RGS, interpretation of our results is limited, but the differential expression of certain RGS transcripts in mouse testis may result in the biosynthesis of the corresponding RGS proteins. Further studies are needed to determine the extent and the regulation of the RGS protein synthesis and its patho-physiological roles.

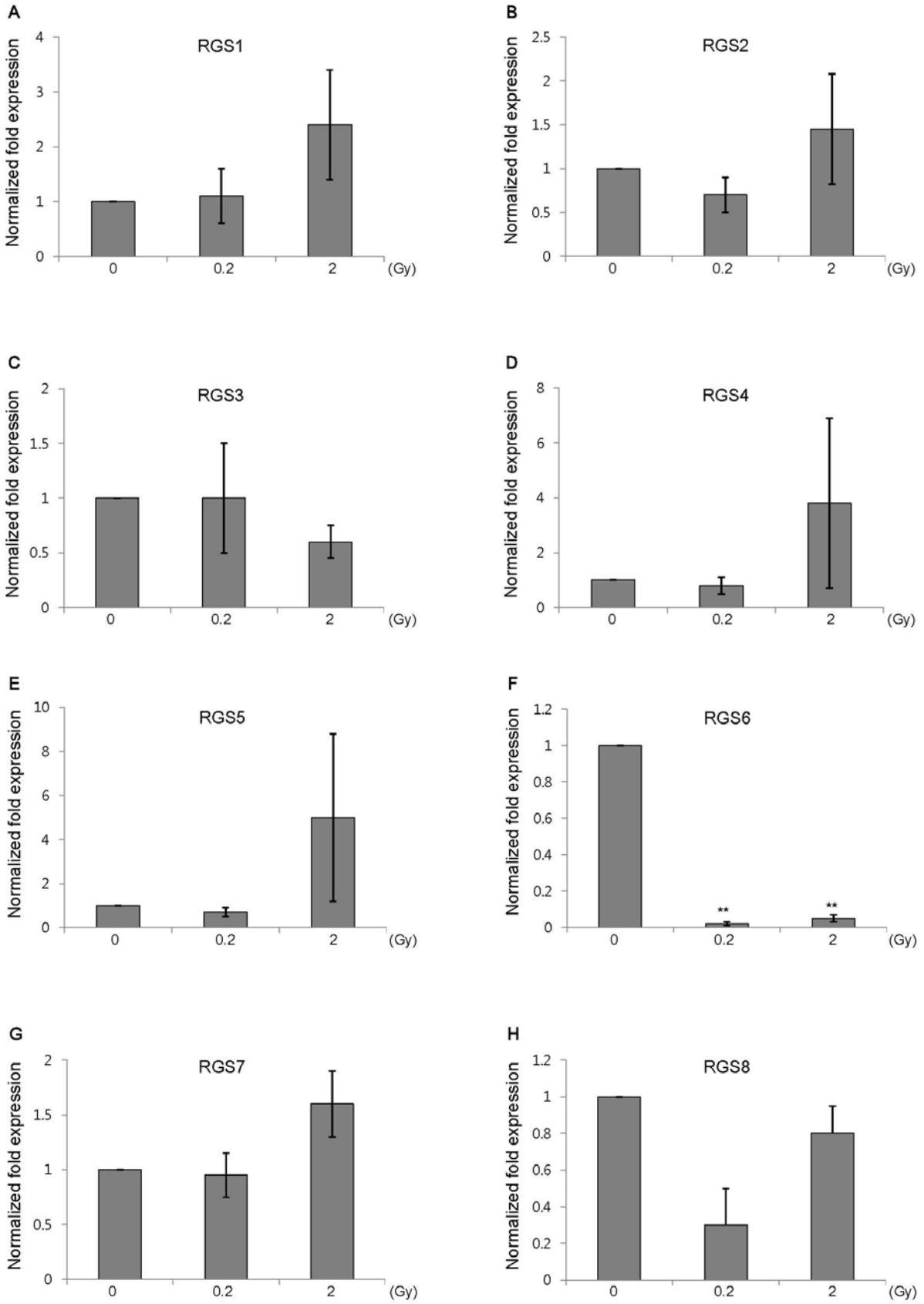
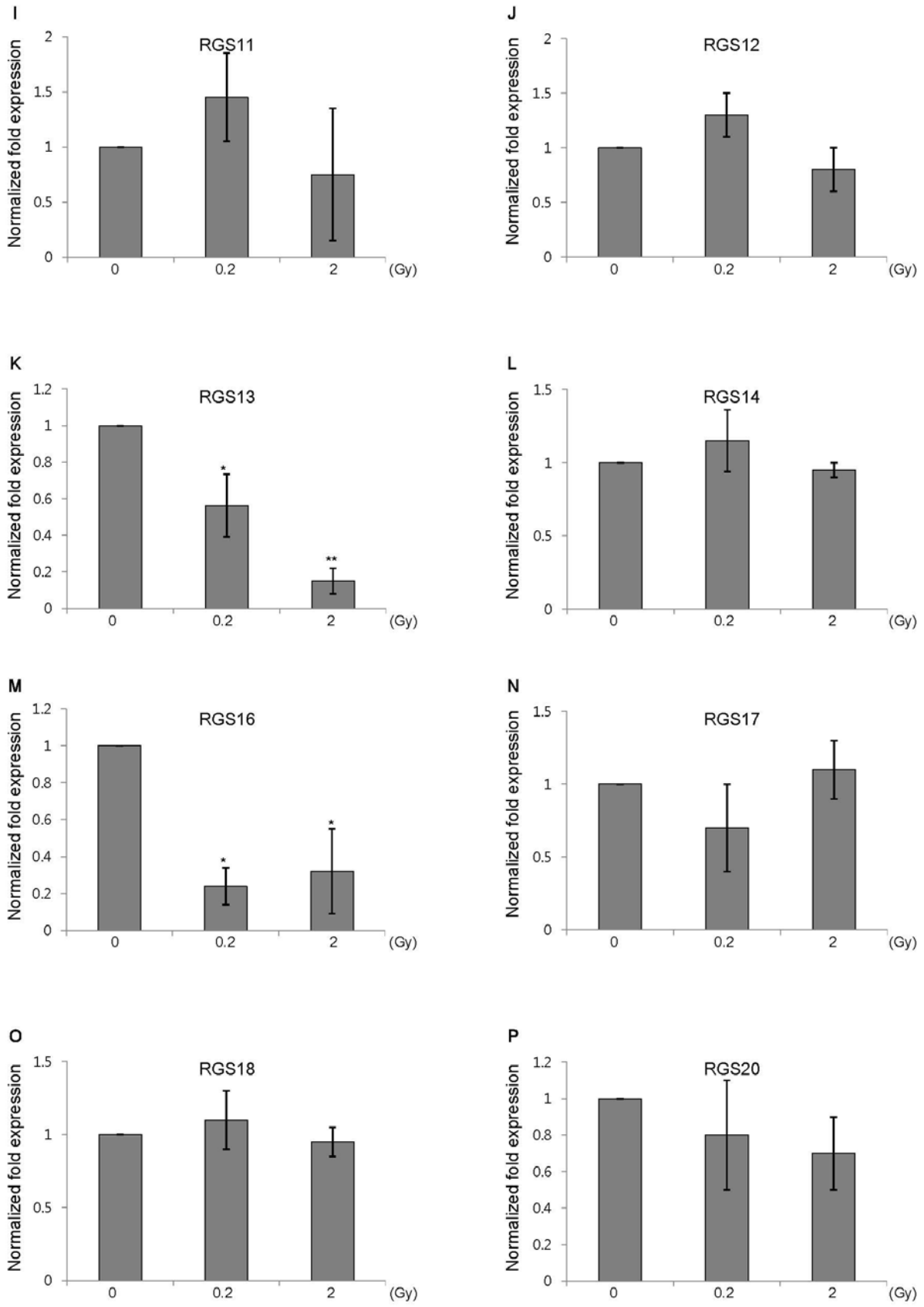


Fig. 1. Expression patterns of RGS induced by low-dose-rate irradiation. Randomized balb/c mice were irradiated with low-dose rate irradiator at a dose rate of 3.49mGy/h at SPF conditions (n = five per group). After 24 hours of total body irradiation with low-dose-rate, animals were executed, and the levels of RGS in mouse testis were analyzed using quantatitive Real-Time PCR. Each bar graph represents mean ± standard error of the mean. *p<0.05, **p<0.01 versus the control groups. (To be continued)



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4. CONCLUSION

We showed for the first time that certain subtypes of RGS expression levels were affected by WBR with low-dose-rate. WBR with low-dose-rate down-regulated 3 members of RGS protein (RGS6, RGS13, and RGS16), and mRNA expression levels indicate that those RGS proteins may play a role in the regulation of the low-dose-rate WBR –induced deleterious effect of signaling in mouse testis.

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