8

Modulation of Gene Expression After Exposure to Ionizing Radiation

Mitsuru Nenoi and Vares Guillaume
National Institute of Radiological Sciences, Japan

1. Introduction

Regulation of gene expression is one of the fundamental mechanisms by which cells utilize the information in their DNA to obtain diverse characteristics, such as ability of differentiated cells to play specific roles and ability to respond to extracellular stresses including ionizing radiation (IR). The research on transcriptional regulation of genes after exposure to IR has a long history tracing back to early studies in late 1980's. In those studies, expression of individual genes was separately measured by a classical hybridization method. Recently, however, functional genomics approaches, such as microarray profiling, enable us to simultaneously monitor the expression of thousands of genes, and are now recognized as a firmly established methodology in radiation biosciences.

The porpose of investigation of transcriptional gene regulation by IR is primarily to gain an insight into how the human bodies respond to IR and eventually how radiation hazards develop. IR-induced hematopoietic death is an example where proapoptotic gene expression is enhanced in radiosensitive hematopoietic stem cells after exposure to high doses of IR, which causes untolerable loss of peripheral blood cells, ultimately resulting in the individual death. Secondary, the purpose of the reserach on transcriptional gene regulation is to search for biomarkers which indicate the quantitative IR-exposure records. Such biomarkers may help us estimate the exposed dose of public peolpe in the emergency cases. For these purposes, studies have been extensively carried out by lots of researchers, and plenty of insights were obtained.

In this review article, some of these studies, including those carried out in our laboratory, will be overviewed with an emphasis on the important roles of a tumor supressor p53 in transcriptional regulation after IR exposure and organ-dependence of transcriptional regulation *in vivo*. Methodology for the research on transcriptional regulation is also briefly touched. And the future perspectives of the research on transcriptional responses to IR will be discussed.

2. Studies with bacteria and yeasts

The SOS response is a well known bacterial response to DNA damage (d'Ari, 1985). There is a group of bacterial genes called SOS genes which are induced by severe DNA damages causing stoppage of DNA synthesis. The SOS genes play roles in DNA repair, cell cycle arrest and mutagenesis. Under normal growth conditions, the SOS genes are negatively regulated by

LexA repressor protein by binding to a 20 bp consensus sequence (the SOS box) in the operator region for the SOS genes. RecA, one of the SOS gene products, is expressed at certain levels even in the repressed state, because of the low affinity of LexA to its SOS box. When DNA damages are produced, DNA polymerase is blocked at the damages, generating single stranded (ssDNA) regions at replication forks. RecA forms a filament around the ssDNA regions, and becomes activated. The activated RecA facilitates dissociation of the LexA repressor from the operator. Then the SOS genes are transcriptionarily activated (Figure 1). First, the genes having SOS box with a lower affinity to LexA repressor protein (such as lexA, recA, uvrA, uvrB, and uvrD) are fully induced in response to mild DNA damages. Thus the first SOS repair mechanism to be induced is nucleotide excision repair (NER). However, if NER is not capable of fixing the damage, the LexA is further inactivated, so the transcription of genes containing SOS box with a stronger affinity to LexA (such as sulA, umuD, umuC - these are expressed late) is induced. SulA binds to FtsZ causing aborted cell division, which results in filamentation and the induction of UmuDC-dependent mutagenic repair.

Regulation of the SOS genes is utilized to detect the environmental mutagen and carcinogens (Oda et al., 1985). The umu-test is based on the ability of the DNA-damaging agents, most of which are potential carcinogens, to induce the umu operon. A bacterial strain containing a fusion gene of umuC and lacZ can be used to monitor the levels of umu operon expression by measuring the β-galactosidase activity. Using this strain, a wide range of environmental mutagens and carcinogens were shown to be inexpensively and sensitively screened.

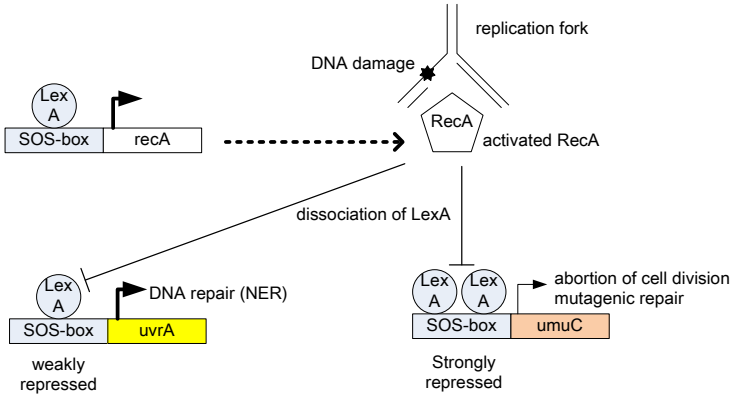


Fig. 1. Illustration of SOS response in bacterial cells.

3. Methodology in research on transcriptional regulation in mammalian cells

Transcriptional regulation of eukaryotic cells is usually mediated by binding of transcription factors (TF) to a DNA region (promoter) neighboring to the protein coding region of a gene. The activity of transcription is determined by a combination of basal TFs, such as RNA polymerase II, TATA binding protein (TBP), TBP-associated factor (TAF), and inducible TFs which act to either enhance or repress initiation of transcription by RNA polymerase II. Regulation of specific gene transcription is attributable to the inducible TFs. Therefore, identification of the inducible TF for IR-responsive genes provides important insight into

how the cells respond to IR. First, the methodologies in research on transcriptional regulation is briefly introduced below.

3.1 Nuclear run-on analysis

Transcriptional activity of a gene is usually measured, as an amount transcription products (mRNA), by Northern blot analysis. However, mRNA level does not necessarily represent the transcription rate accurately. Instead, a nuclear run-on assay is performed to measure the transcription rate at a certain time point, which often differ from steady state mRNA levels revealed by Northern blot analysis. It is believed that the nuclear run-on assay is the most reliable method to measure transcription rates directly.

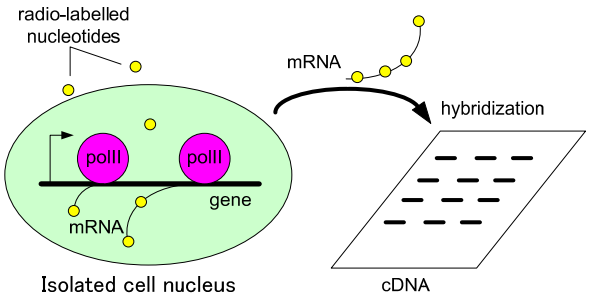


Fig. 2. Schematic illustration of the nuclear run-on assay.

As illustrated in Figure 2, the cell nuclei are isolated rapidly, and incubated in a short period of time in the presence of radio-labelled nucleotides, and the transcribed products are hybridized to a slot-blotted cDNA of interested genes.

3.2 Reporter gene analysis

Reporter genes are the genes that confer the cells characteristics easily identified and measured. In order to investigate the promoter activity, the researchers often attach reporter genes to a DNA fragment containing regulatory elements, and introduce them into cultured cells. The promoter activity can be measured by signal intensity produced by reporter genes as shown in Figure 3.

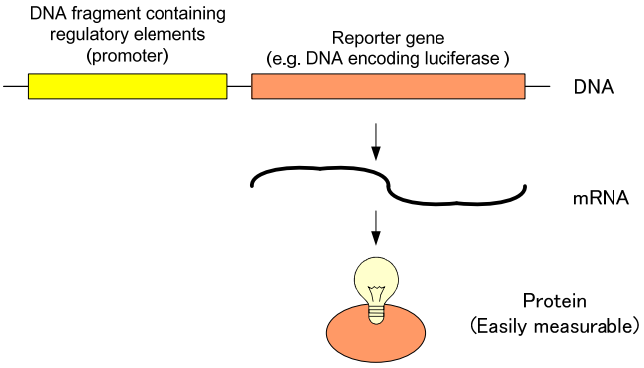


Fig. 3. Schematic illustration of reporter gene analysis.

3.3 Electrophoretic mobility shift assay

An electrophoretic mobility shift assay (EMSA) is an affinity electrophoresis technique used to determine if a protein is capable of binding to a given DNA sequence, as well as to identify the protein molecule. It is often performed to study binding of TFs to promoter region of interested genes. Electrophoresis of a DNA-protein mixture on a polyacrylamide gel can separate the protein-bound DNA from unbound DNA fragments, producing an additional band on the gel that represents the less mobile complex of DNA bound to protein (Figure 4). The DNA fragment is usually radio-labeled to be visible. An antibody that recognizes the protein is often added to the DNA-protein mixture to form an even larger complex with a greater shift. This method, called as a supershift assay, is used to unambiguously identify a protein present in the DNA-protein complex.

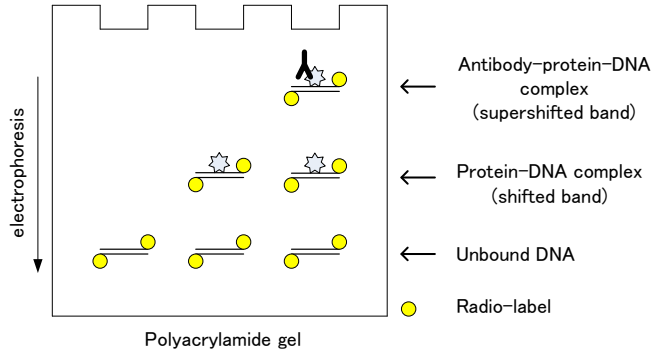


Fig. 4. Scematic illustration of electrophoretic mobility shift assay (EMSA)

3.4 DNase I footprinting assay

A DNase I footprinting assay is a molecular biological technique by which DNA-protein interaction can be detected and located using the fact that a DNA region bound to proteins is often protected from enzymatic cleavage (Figure 5). The method uses an enzyme, deoxyribonuclease I(DNnase I, for short) to cut the radioactively end-labeled DNA, followed by gel electrophoresis to detect the resulting cleavage pattern.

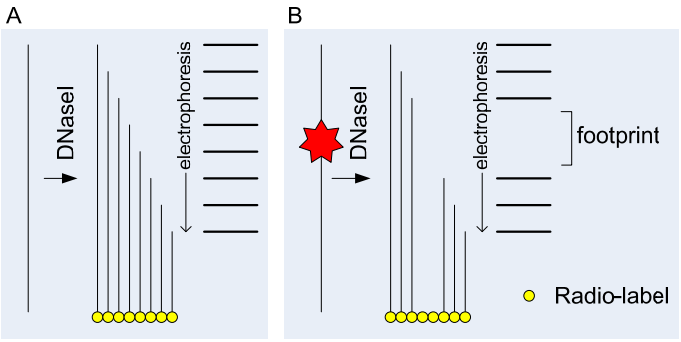


Fig. 5. Schematic illustration of DNase I footprinting assay

3.5 Chromatin immunoprecipitation assay

Chromatin Immunoprecipitation (ChIP) is a technique used to investigate the interaction between proteins and DNA in the cell, especially to determine whether specific proteins are interacted with specific genomic regions. It is applicable to investigate interaction of TFs with promoters *in vivo*. Protein and genomic DNA are temporarily crosslinked *in vivo*, and the DNA-protein complexes are fragmented by shear stresses as illustrated in Figure 6. Then the DNA fragments associated with the proteins of interest are selectively immunoprecipitated by using antibodies specific to those proteins. The associated DNA fragments are purified and their sequence is determined.

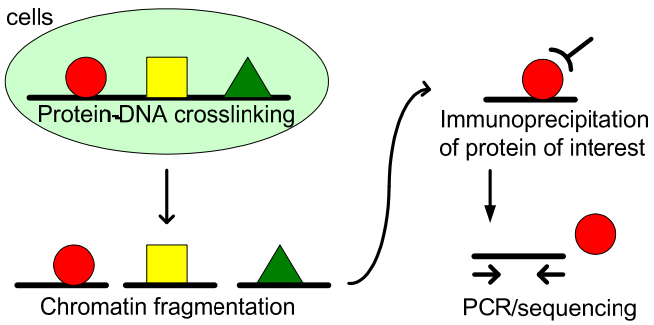


Fig. 6. Schematic illustration of Chromatin Immunoprecipitation assay

4. p53-mediated regulation of gene transcription after IR in cultured cells

Systematic studies on transcriptional regulation of mammalian genes have started in late 1980's. The representative studies were performed by Fornace and his colleagues (Fornace et al., 1988, Fornace et al., 1989). They screened a cDNA library for UV-induced genes by use of a hybridization subtraction method to obtain 20 differentially expressed genes including unidentified at that time. Some of these genes were co-ordinately regulated by DNA damaging agents and growth arrest signals, and were therefore called as gadd (**g**rowth **a**rrest and **D**NA **d**amage responsive) genes. The most widely characterized of these genes is *GADD45A*. The increase in *GADD45A* protein levels is correlated with a block in S phase progression as well as a stimulation of DNA repair (*Smith, et al., 1994, Chan, et al., 1995, Zhan, et al., 1994*). *GADD45A* gene was soon shown to respond to IR through a tumor supressor p53. The function of p53 in DNA damage responses was vigorously investigated at that time, and its pivotal roles as the guardian of the genome was established (Fei & El-Deiry, 2003). P53 functions primarily as a TF, and has been shown to be involved in multiple cellular pathways including cell cycle arrest, apoptosis, DNA repair, and so on, by controling many other genes. *CDKN1A* ($p21^{CIP1/WAF1}$) gene is another member of gadd genes which was identified among 20 clones originally isolated by cDNA subtraction. *CDKN1A* gene is induced after exposure to IR also through p53, and inhibits the cycln dependent kinase activity resulting in arrest of cell cycle progression at G1/S border (Hartwell & Weinert, 1989). Although both of *GADD45A* and *CDKN1A* were clearly demonstrated to be regulated through p53, involvement of other TFs has not been investigated. Elucidation of a set of TFs involved in gene regulation after IR should be pivotal for understanding of delicate mechanisms for biological responses to IR which include variety of cellular

pathways. In the following sections, researches performed in our laboratory on regulation mechanisms for the *GADD45A* and *CDKN1A* genes are reviewed.

4.1 Regulation *GADD45A* gene

In human cells, *GADD45A* is up-regulated in response to IR. Figure 7 is a Northern blot analysis for induction of the *GADD45A* gene expression in human breast adenocarcinoma cell line MCF7 after exposure to IR. The induction was transient with the maximum induction at 2 h after irradiation.

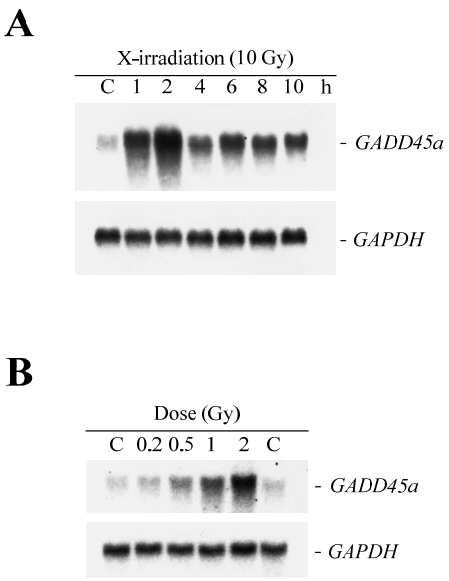


Fig. 7. Northern analysis for induction of the *GADD45A* gene expression after exposure to IR. (A) Total RNA isolated from the MCF-7 cells at various time intervals after irradiation with 10 Gy of X-rays was hybridized with a *GADD45A* gene-specific probe. The *GAPDH* gene was used as a control. (B) Induction of the *GADD45A* gene after irradiation with various doses of X-rays was analyzed. (Reproduced with permission of Elsevier B. V. from Ref (Daino et al., 2006))

To search for the DNA element functioning in transcriptional regulation of the *GADD45A* gene, a series of reporter gene constructs were designed. As the p53 recognition sequence was known to reside in the third intron, the third intron was fused to the 3′ end of the luciferase gene (*luc* +) (Figure 8A). MCF-7 cells were transiently transfected with these constructs, and luciferase activity was measured after treatment with or without X-rays. As shown in Figure 8B, constructs P/I through P6/I gave rise to a similar level of the basal luciferase activity, suggesting that there was no major DNA element functioning in transcriptional activity in the -2244 bp/-107 bp region. However, when the 5′ flanking region was truncated to -62 bp in P7/I, the luciferase activity was drastically decreased, suggesting that a regulatory element required for the basal expression of the *GADD45A*

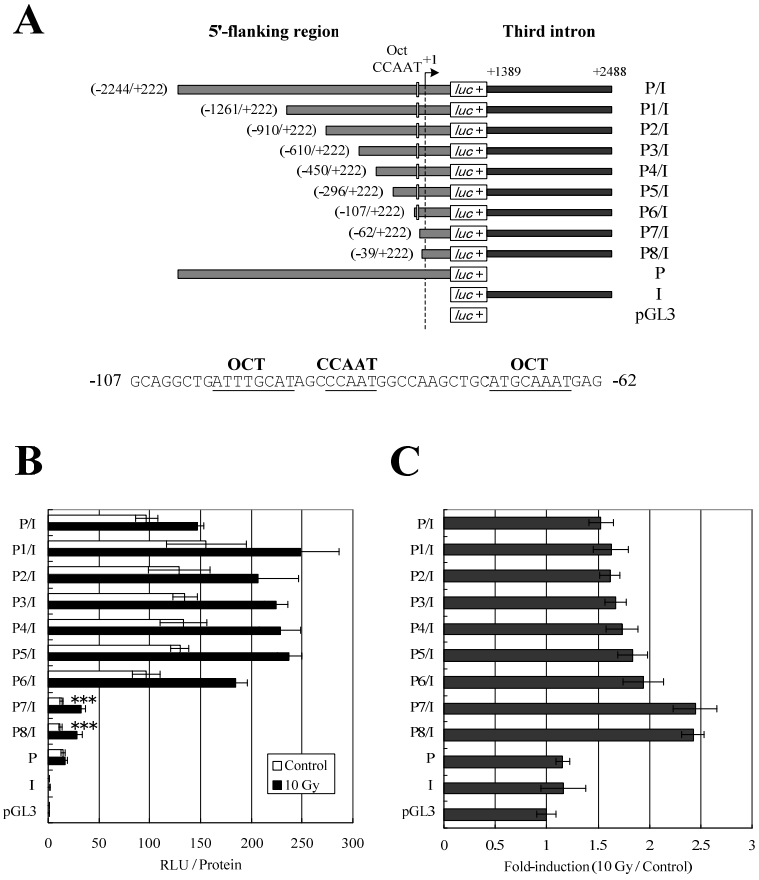


Fig. 8. Reporter gene analysis for the promoter activity of the *GADD45A* gene. (A) Various *GADD45A*-luciferase constructs are schematically illustrated. The light gray bars and the dark gray bars represent the 5′-flanking region and the third intron region of the *GADD45A* gene, respectively. The transcription start site is indicated by an arrow. The nucleotide sequence of the region, which reduced the basal promoter activity significantly as shown in (B), is presented at the bottom. Two OCT sites and a CCAAT box are underlined. (B) MCF-7 cells transiently transfected with reporter constructs were either irradiated with 10 Gy of X-rays (closed bars) or mock-treated as a control (open bars), and the luciferase activity in the cell lysates prepared 5 h after irradiation was measured. The relative light unit (RLU) per protein concentration is plotted. (C), Fold-induction of the luciferase activity after irradiation with 10 Gy of X-rays is plotted. Results are the mean of at least three independent experiments. Error bars represent the S.D. (*** $p < 0.001$ versus P/I, student's t-test). (Reproduced with permission of Elsevier B. V. from Ref (Daino et al., 2006))

gene was present in the region between -107 bp and -62 bp. There were two OCT sites and one CCAAT box in this region (Figure 8A). It was thought that these OCT and CCAAT elements functioned as the core promoter because a canonical TATA sequences could not be found. It can be seen, in Figure 8C, that fold-induction of luciferase expression after irradiation with 10 Gy of X-rays was not reduced even when the 5'-flanking region was deleted down to -39 bp. This result indicated the absence of major regulatory elements functioning in response to IR in the upstream region of the *GADD45A* gene.

When the region +1747 bp/+2488 bp in the third intron was deleted, the luciferase activity was not so much reduced. However, when the region +1625 bp/+2488 bp was deleted, the luciferase activity was decreased dramatically (data not shown), suggesting the presence of a DNA element, in +1625/+1746, that plays a role in the basal expression of the *GADD45A* gene. As AP-1 recognition element existed at the locus +1625 bp/+1631 bp, we constructed another reporter gene experiment with a plasmid (P6/I AP-1m) in which the AP-1 recognition sequence was mutated (Figure 9A). As shown in Figure 9B, the basal luciferase expression from P6/I AP-1m was drastically reduced compared with P6/I which contains wild type AP1 sequence. This result demonstrate the important role of AP-1. Deletion of the p53 site (P6/I p53Δ) also resulted in reduction of basal luciferase activity compared to that of the P6/I construct. Then it was suggested that both the AP-1 and p53 sites in the third intron of the *GADD45A* gene are critical for the basal expression. Concerning IR responsiveness, Figure 9C shows that deletion of the p53 site (P6/I p53Δ) diminished IR-response, while fold induction for the construct containing the mutated AP-1 site (P6/I AP-1m) was reduced, suggesting that the AP-1 site contributes to a part of p53-dependent transcriptional activation of the *GADD45A* gene after exposure to IR.

It was speculated that transiently transfected plasmid DNAs may not form tight chromatin complexes. Then, we used the recombinant adeno-associated virus (rAAV) vectors, which can stably integrate reporter genes into the genome of host cells. As a result, a remarkably higher fold-induction of luciferase expression (~3.4-fold, rAAV-P6/I) could be observed after IR irradiation as shown in Figure 9D. With this vector system, rAAV-P6/I AP-1m containing mutation at the AP-1 site gave rise to significantly decreased fold induction compared to the rAAV-P6/I construct (Figure 9E). Taken together, these results indicate that the AP-1 recognition site plays a significant role in the p53-dependent transcriptional activation of the *GADD45A* gene by IR.

When EMSA was performed with a DNA probe containing the AP-1 recognition sequence and nuclear extracts from MCF-7 cells, DNA-protein complexes were created. And these DNA-protein complexes disappeared when an anti-JunD antibody was added in the DNA-protein mixture. This result indicated the presence of JunD in the DNA-protein complexes.

When ChIP assay was carried out (Figure 10A), the binding of p53 and JunD to the third intron of the *GADD45A* gene was confirmed. However they were detected even in unirradiated cells. While the affinity of p53 to its site in the intron 3 was enhanced in X-ray-irradiated cells (Figure 10C), the bining of JunD to its recognition site was not increased in the X-ray irradiated cells (Figures. 10B and 10C). The binding of phospho-p53 (Ser20) to the p53 site was detected only in X-ray-irradiated cells (Figure 10C). These results indicated that JunD constitutively binds to the third intron of the *GADD45A* gene even in unstressed cells *in vivo*. JunD is considered to play an important role both in basal expression of the *GADD45A* gene and in transcriptional enhancement after IR irradiation via binding to the AP-1 site in the third intron.

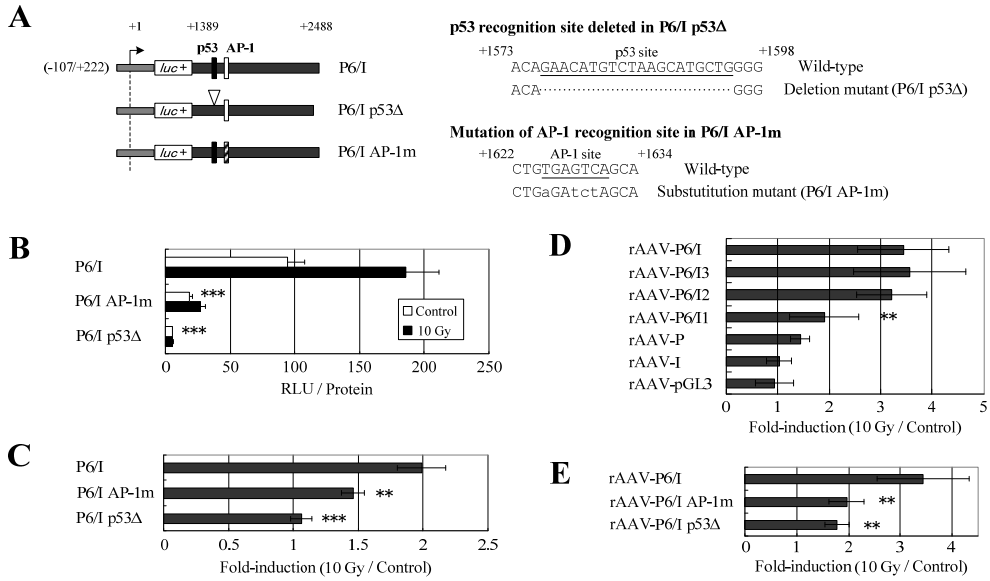


Fig. 9. Requirement of p53 and AP-1 recognition sites for both basal expression and transcriptional enhancement of the *GADD45A* gene after exposure to IR. (A) Mutant *GADD45A*-luciferase constructs are schematically illustrated. The light gray bars and the dark gray bars represent the 5′-flanking region and the third intron region of the *GADD45A* gene, respectively. The nucleotide sequences at the p53-deletion in P6/I p53Δ and the AP-1 mutation in P6/I AP-1m are shown on the right of the panel. The small letters represent the substituted nucleotides. The closed box and open box represent the p53 site and the AP-1 site, respectively. The arrowhead represents the site of p53-deletion, and the hatched box represents the mutated AP-1 site. (B) MCF-7 cells transiently transfected with reporter constructs were either irradiated with 10 Gy of X-rays (closed bars) or mock-treated as a control (open bars), and luciferase activity in the cell lysates prepared 5 h after irradiation was measured. The relative light unit (RLU) per protein concentration is plotted. (C) Fold-induction of the luciferase activity after irradiation with 10 Gy of X-rays is plotted. Results are the mean of at least three independent experiments. Error bars represent the S.D. (** $p < 0.01$, *** $p < 0.001$ versus P6/I, student's t-test). (D) and (E) MCF-7 cells transduced with reporter constructs by use of rAAV were either irradiated with 10 Gy of X-rays or mock-treated, and luciferase activity in the cell lysates prepared 5 h after irradiation was measured. Fold-induction of luciferase activity after irradiation with 10 Gy of X-rays is plotted. Results are the mean of at least three independent experiments. Error bars represent the S.D. (** $p < 0.01$ versus P6/I, student's t-test). (Reproduced with permission of Elsevier B. V. from Ref (Daino et al., 2006))

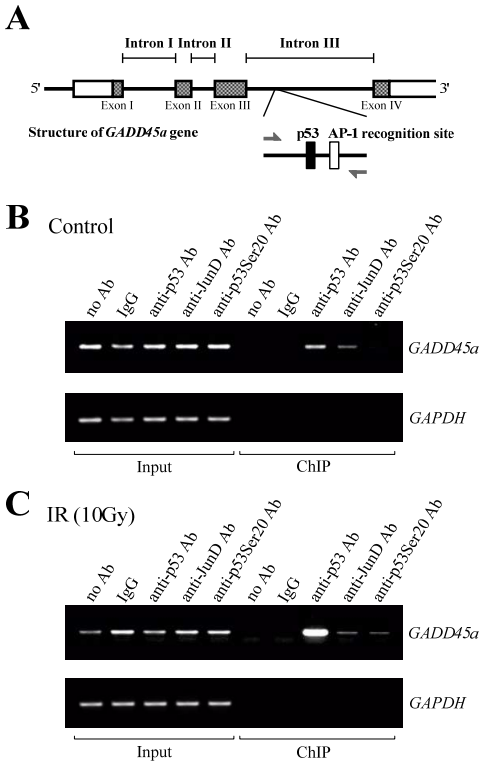


Fig. 10. Chromatin immunoprecipitation (ChIP) analysis for the in vivo binding of JunD at the third intron. (A) Schematic illustration of genomic structure of the *GADD45A* gene. PCR primers were designed to amplify the region containing both the p53 site and the AP-1 site, as represented by arrows. (B) ChIP analysis was performed with MCF-7 cells, unirradiated (B) or exposed to 10 Gy of X-rays with 1.5 h postincubated (C). Sheared chromatin was incubated without antibodies (no Ab) or with the IgG, anti-p53 antibody, anti-JunD antibody, and anti-phospho-p53 (Ser20) antibody. Immunoprecipitants were analyzed by PCR. The *GAPDH* promoter containing neither an AP-1 nor p53 site was analyzed as a negative control. PCR was performed for 32 cycles, which was in the linear range of amplification, except for the case using the sample prepared from X-ray-treated cells with the anti-p53 antibody. (Reproduced with permission of Elsevier B. V. from Ref (Daino et al., 2006))

4.2 Regulation *CDKN1A* gene

Regulation of *CDKN1A* gene after exposure to IR occurs in transcription level as shown by a nuclear run-on analysis (Figure 11). While the induction kinetics of *CDKN1A* is similar to that of *GADD45A*, the fold-increase of *CDKN1A* is much higher, and is thought to be one of the most responsive genes to IR.

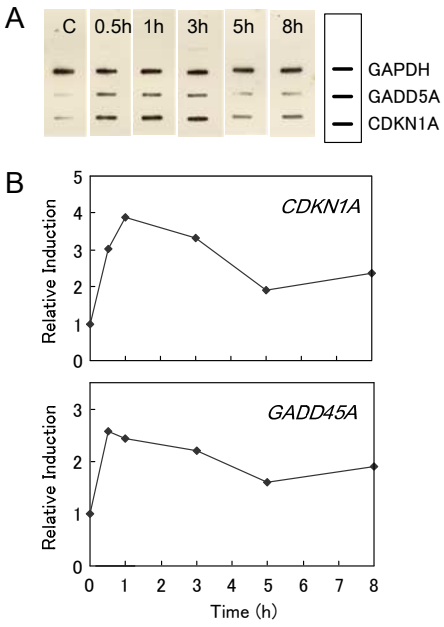


Fig. 11. Nuclear run-on analysis for transcription rates of the *CDKN1A* and *GADD45* genes in human myeloblastic leukemia cell line ML-1 after irradiation with 0.5 Gy of X-rays. (A) The resulting hybridization images. cDNA probes were blotted as shown in the right side panel. (B) The signal intensity was analyzed and plotted. (Reproduced with permission of Radiation Research Society of the USA from Ref (Daino et al., 2002))

The functional DNA region involved in the p53-mediated IR response was investigated by reporter gene analysis using AAV vectors, which can give rise to remarkably high responsiveness to IR as shown in Figure 12A. A series of reporter gene constructs with or without deletions in the 5′ flanking region of the *CDKN1A* gene was prepared as shown in Figure 12B, and were transduced into MCF-7 cells. The p53 recognition site at –2.2 kb was preserved in every construct. It can be seen, in Figure 12C, that the fold induction of luciferase activity increased linearly with radiation dose for every constructs. However, IR responsiveness of the constructs rAAV-del2, rAAV-del4 and rAAV-del5 was much reduced compared to that of rAAV-PLS which contains an intact 5′ flanking region of the *CDKN1A* gene, suggesting the presence of pivotal DNA elements for IR responsiveness in the regions –1962 bp/–1679 bp (rAAV-del2), –1398 bp/–1119 bp (rAAV-del4), and –1118 bp/–839 bp (rAAV-del5).

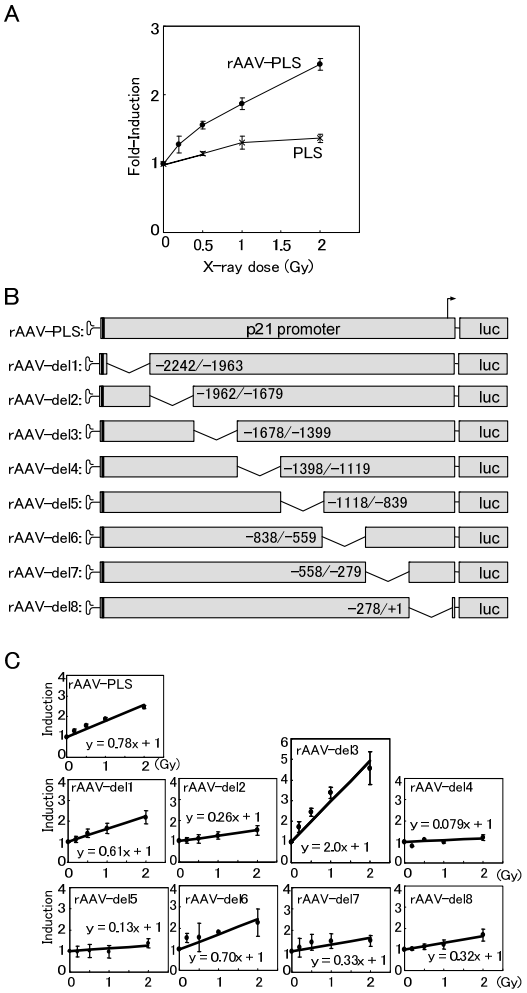


Fig. 12. DNA regions functioning in the IR response of the *CDKN1A* gene. A: Reporter gene constructs containing the luciferase gene under the control of the *CDKN1A* gene promoter were either transfected by electroporation (PLS) or transduced by rAAV vectors (rAAV-PLS) into MCF-7 cells. The cells were irradiated with X-rays and assayed for luciferase activity 5 h after irradiation or mock treatment. Results are the means of three independent experiments. Error bars represent the S.D. B: The reporter gene constructs with or without deletions in the 5′ flanking region are schematically represented. The TP53 site at –2.2 kb is indicated by a heavy line. These constructs were transduced into MCF-7 cells using rAAV vectors. C: X-ray response of each construct was assayed in the dose range of 0.2-2.0 Gy. Since the fold induction increased linearly with dose, the slope was used as an index of IR responsiveness. Results are the mean of three independent experiments. Error bars represent the S.D. (Reproduced with permission of Elsevier B. V. from Ref (Nenoi et al., 2009))

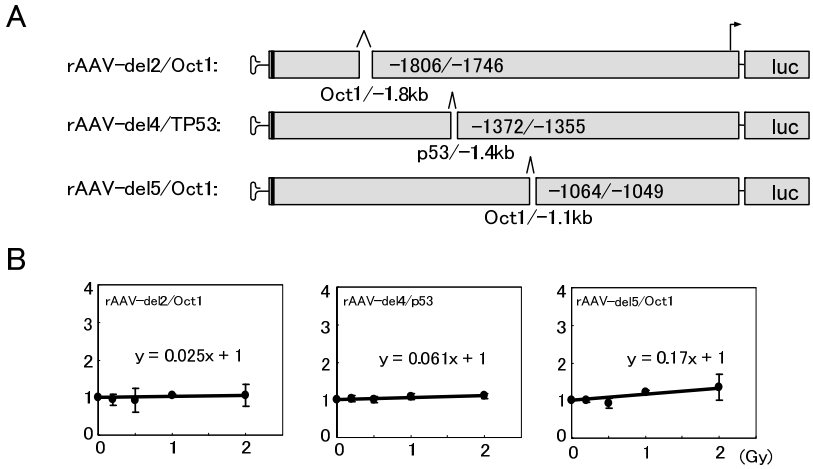


Fig. 13. Reporter vectors were constructed in which the Oct-1 site at –1.8 kb, TP53 site at –1.4 kb, and Oct-1 site at –1.1 kb were specifically deleted, resulting in rAAV-del2/Oct1, rAAV-del4/TP53, and rAAV-del5/Oct1, respectively (D). X-ray response of each construct was assayed at a dose range of 0.2-2.0 Gy (E). (Reproduced with permission of Elsevier B. V. from Ref (Nenoi et al., 2009))

The potential recognition sequences for TFs were searched in these regions, and Oct-1 recognition sequences were found at –1.8 kb and –1.1 kb. It was interesting to note that Oct-1 is the TF involved in regulation of IR-responsive genes *GADD45A*, *Prx1*, *Notch1*, and endothelial lipase, suggesting that Oct-1 may play a role in regulation of variety of IR-responsive genes by cooperating with p53. Then, mutant reporter vectors rAAV-del2/Oct1, rAAV-del4/TP53, and rAAV-del5/Oct1 were constructed in which the Oct-1 site at –1.8 kb, the p53 site at –1.4 kb, and the Oct-1 site at –1.1 kb, respectively, were specifically deleted (Figure 13A). Figure 13B shows that the IR responsiveness of these reporter constructs was mostly diminished, indicating that the recognition sequences for these TFs play pivotal roles in the IR response of the *CDKN1A* gene promoter.

EMSA analysis revealed that the DNA probes Oct-1/–1.8kb (Figure 14A) and Oct-1/–1.1kb (Figure 14B) could form complexes with nuclear proteins as indicated by arrows. These bands were supershifted by addition of anti-Oct-1 antibodies and competed away by addition of an excess amount of unlabeled oligonucleotides containing the Oct-1 consensus sequence. These results demonstrate Oct-1 could bind to its recognition sequences at -1.8 kb and -1.1 kb. However, binding of Oct-1 to these sites was not X ray-dependent. In accordance with the EMSA analysis, constitutive binding of Oct-1 to the sites at –1.8 kb and –1.1 kb was observed by ChIP analysis as shown in Figures 14C and 14E. In contrast, binding of p53 to the site at –1.4 kb was not constitutive but inducible after irradiation (Figure 14D).

Overall, it was considered that Oct-1 is constitutively bound to the sites at –1.8 kb and –1.1 kb and plays a cooperative role with p53 in induction of the *CDKN1A* gene promoter in response to IR.

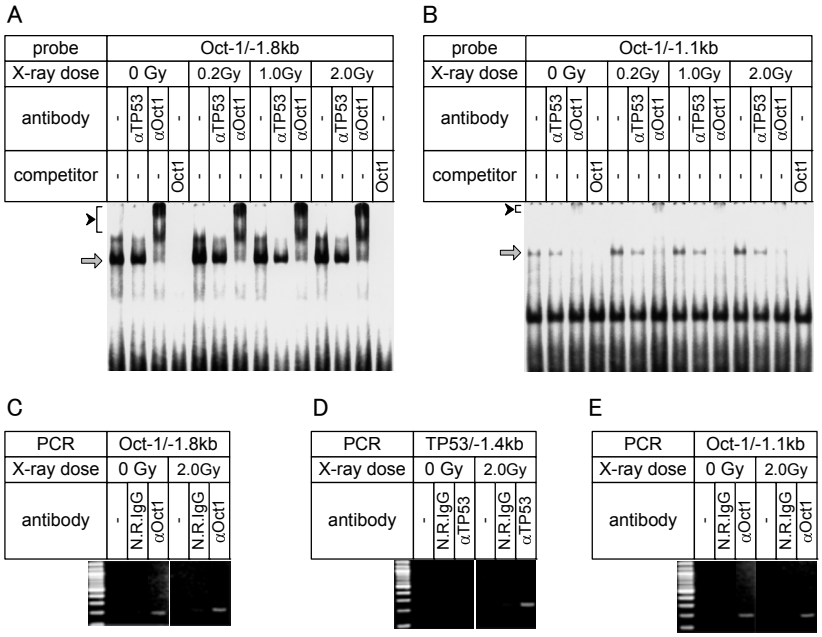


Fig. 14. DNA/chromatin binding of Oct-1. *In vitro* DNA binding of Oct-1 to the sites at –1.8 kb (A) and –1.1 kb (B) was analyzed by EMSA. Sequences of the probe and competitor oligonucleotides for Oct-1 are described in the text. Arrows indicate the DNA-protein complex containing Oct-1. Arrowheads indicate the supershifts caused by addition of anti-Oct-1 antibodies. *In vivo* chromatin binding of Oct-1 and TP53 was analyzed by ChIP. MCF-7 cells were either not irradiated or irradiated with 2.0 Gy of X-rays. After a period of 1.5 h, the chromatin was immunoprecipitated with anti-Oct-1 antibodies (C and E) or anti-p53 antibodies (D). Normal rabbit IgG was used as a control. Binding of the factors to chromatin was assessed using site-specific PCR. (Reproduced with permission of Elsevier B. V. from Ref (Nenoi et al., 2009))

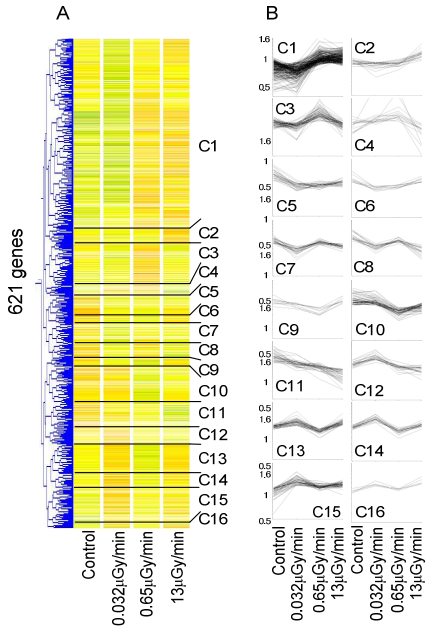
5. *In vivo* studies

Regulated initiation of DNA replication (entry of S-phase) is one of the important cellular responses to IR. When DNA damages are produced by IR, multiple factors involved in cell cycle regulation such as CDKN1A which causes cell cycle arrest at G1/S are modulated. The so-called checkpoint regulation is thought to be necessary for sufficient repair of damaged DNA before DNA replication. However, the checkpoint regulation is absent in some of fully differentiated cells such as those *in vivo* organs. Thus, the cellular response to IR *in vivo* is much different from that in cultured cells, and it should have wide variety. In order to understand the difference in transcriptional responses to IR between tissues of mammals and also to reveal the underlying mechanisms for those differences, a microarray analysis of the global gene expression profile is a promising approach. There have been lots of *in vitro* studies investigating gene expression modulation after exposure to IR, where evident modulation of genes associated with DNA repair, stress response, cell cycle and apoptosis

and the pivotal role played by p53 have been elucidated. However, few studies investigated the *in vivo* modulation of gene expression profiles after exposure to IR and its underlying mechanisms. Especially importance of studies with low dose and low dose-rate IR which people may encounter in social life should be emphasized.

5.1 Modulation of gene expression in the kidneys and testes of mice after exposure to IR

C57BL/6J mice were continuously irradiated with γ-rays for 485 days at the dose-rates in the range of 0.032 – 13 μGy/min. The lowest dose rates were only about 10 times higher than the normal background level. Gene expression profiles in the kidney and testis from irradiated and unirradiated mice were analyzed, and differentially expressed genes were identified after the standard procedure of quality control of the data. By hierarchical cluster analysis of differentially expressed genes (Figure 15), it was suggested that expression of the



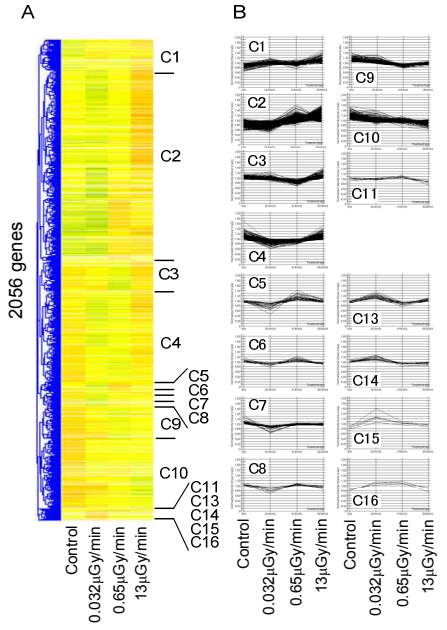
C

	Category	p-Value
C1	cytoplasm	2.8E-07
	mitochondrion	5.7E-04
	mitochondrion organization and biogenesis	0.0013
	energy pathways	0.0055
	organelle organization and biogenesis	0.0071
	cell organization and biogenesis	0.0071
	cytoplasm organization and biogenesis	0.0071
	transferase	0.031
C10	cell organization and biogenesis	1.4E-05
	cytoplasm organization and biogenesis	1.4E-05
	organelle organization and biogenesis	1.4E-05
	cytoplasm	0.0016
	mitochondrion	0.0061
	nucleosome	0.012
	mitochondrion organization and biogenesis	0.032
	GO SLIMS Cellular Component	0.040
C13	cytochrome-c oxidase activity	1.2E-05
	organelle inner membrane	6.5E-05
	mitochondrion organization and biogenesis	3.3E-04
	mitochondrion	3.3E-04
	energy pathways	7.1E-04
	cytoplasm	0.0086

Fig. 15. Hierarchical cluster analysis of genes whose expression was modulated after irradiation with a statistical significance in the kidney. A: Expression levels of 621 of significantly modulated genes are indicated by color. Green, yellow, and red colors represent low, medium, and high expression, respectively. On the basis of the expression pattern, genes were classified into 16 clusters. B: Expression profiles of genes in each of the 16 clusters are collectively shown. C: Gene Ontology categories significantly overlapped with the gene clusters are shown. No Gene Ontology categories significantly similar to the clusters other than C1, C10 and C13 were found. (Reproduced with permission of the Japanese Radiation Research Society from Ref (Taki et al., 2009))

genes involved in mitochondrial oxidative phosphorylation was elevated in the kidney after irradiation at the dose-rates of 0.65 μGy/min (the level of dose rate comparable to that in the spaceshuttle) and 13 μGy/min. A particular mitochondrial response may have occurred after low dose rate irradiation. It was reported that several cell cycle-related genes are up-regulated in the kidney after acute whole-body irradiation with 10 Gy of γ-rays (Zhao et al., 2006). However, we did not observe such gene modulation after irradiation at 13μGy/min We did not either extract clusters of significantly modulated genes that overlapped with the Gene Ontology category "cell cycle" (Figure 15C). These results suggested that modulation of the cell cycle-related genes *in vivo* following exposure to radiation may have a dose-rate threshold.

It was also demonstrated that alteration of the gene expression profile in the testis was largely different from that in the kidney. Gene Ontology categories "DNA metabolism", "response to DNA damage" and "DNA replication" were significantly overlapped to up-regulated genes in the testis in a dose rate-dependent manner (Figure 16). These results provide fundamental insight into the organ-specific responses to IR.



	Category	p-Value
C1	response to temperature	0.0013
C2	physiological process	0.0012
	protein biosynthesis	0.0088
	Tricarboxylic acid cycle intermediate metabolism	0.0077
	Chaperone cofactor dependent protein folding	0.0060
	aldehyde metabolism	0.0014
	glutathione metabolism	0.0077
	coenzyme metabolism	0.0058
	regulation of endocytosis	0.0095
	cellular physiological process	0.0003
	cytoskeleton-dependent intracellular transport	0.0049
	microtubule-based movement	0.0046
	organelle organization biogenesis	0.0078
	microtubule-based process	0.0015
	response to heat	0.0095
	response to hormone stimulus	0.0095
C9	DNA metabolism	0.0086
	DNA repair	0.0019
	response to DNA damage	0.0040
	response to endogenous	0.0049
C10	DNA replication	0.0024
	mitotic cell cycle	0.0056

Fig. 16. Hierarchical cluster analysis of genes whose expression was significantly modulated after irradiation in the testis. A: Expression levels of 2056 of significantly modulated genes are indicated by color. On the basis of the expression pattern, genes were classified into 16 clusters. The cluster 12 contained no gene. B: Expression profiles of genes in each of the 16 clusters are collectively shown. C: Gene Ontology categories significantly overlapped with the gene clusters C1, C2, C9 and C10 are shown. (Reproduced with permission of the Japanese Radiation Research Society from Ref (Taki et al., 2009))

5.2 Transcription factors

By use of microarray data, it is possible to pick up TFs whose recognition sequences are preferentially identified in the promoter of genes modulated by IR. These TFs are suggested

	E47	AP-4	Elk-1	NF-kappaB (p65)	Evi-1 (AGAY · ·)	Evi-1 (NGAT · ·)	CDP	S8	HFH-1	Brn-2	HSF1
unchanged	0.538	0.032	0.591	0.430	0.505	0.280	0.086	0.935	0.624	0.656	0.742
down-regulated	0.595	0.119	0.571	0.643	0.595	0.119	0.000	0.976	0.571	0.500	0.738
na	93.000	93.000	93.000	93.000	93.000	93.000	93.000	93.000	93.000	93.000	93.000
nb	42.000	42.000	42.000	42.000	42.000	42.000	42.000	42.000	42.000	42.000	42.000
P	0.556	0.059	0.585	0.496	0.533	0.230	0.059	0.948	0.607	0.607	0.741
Z	0.624	1.977	0.218	2.289	0.969	2.053	1.960	0.988	0.575	1.717	0.047
unchanged	0.538	0.032	0.591	0.430	0.505	0.280	0.086	0.935	0.624	0.656	0.742
up-regulated	0.346	0.077	0.346	0.692	0.308	0.192	0.000	0.808	0.308	0.423	0.538
na	93.000	93.000	93.000	93.000	93.000	93.000	93.000	93.000	93.000	93.000	93.000
nb	26.000	26.000	26.000	26.000	26.000	26.000	26.000	26.000	26.000	26.000	26.000
P	0.496	0.042	0.538	0.487	0.462	0.261	0.067	0.908	0.555	0.605	0.697
Z	1.726	1.004	2.217	2.365	1.787	0.896	1.548	1.989	2.866	2.147	1.997
down-regulated	0.595	0.119	0.571	0.643	0.595	0.119	0.000	0.976	0.571	0.500	0.738
up-regulated	0.346	0.077	0.346	0.692	0.308	0.192	0.000	0.808	0.308	0.423	0.538
na	42.000	42.000	42.000	42.000	42.000	42.000	42.000	42.000	42.000	42.000	42.000
nb	26.000	26.000	26.000	26.000	26.000	26.000	26.000	26.000	26.000	26.000	26.000
P	0.500	0.103	0.485	0.662	0.485	0.147	0.000	0.912	0.471	0.471	0.662
Z	1.996	0.556	1.806	0.419	2.306	0.829	#DIV/0!	2.381	2.117	0.618	1.691

	COUP-T	GR (NNNN · ·)	AP-1	GR (GGTAT · ·)	HNF-1	SREBP-1	AhR/Ar	Egr-3	Egr-2	Olf-1
unchanged	0.280	0.086	0.946	0.075	0.032	0.301	0.129	0.065	0.108	0.011
down-regulated	0.119	0.333	0.857	0.214	0.119	0.190	0.143	0.167	0.119	0.000
na	93.000	93.000	93.000	93.000	93.000	93.000	93.000	93.000	93.000	93.000
nb	42.000	42.000	42.000	42.000	42.000	42.000	42.000	42.000	42.000	42.000
P	0.230	0.163	0.919	0.119	0.059	0.267	0.133	0.096	0.111	0.007
Z	2.053	3.602	1.752	2.313	1.977	1.345	0.219	1.863	0.197	0.675
unchanged	0.280	0.086	0.946	0.075	0.032	0.301	0.129	0.065	0.108	0.011
up-regulated	0.192	0.269	0.769	0.077	0.038	0.500	0.346	0.192	0.115	0.115
na	93.000	93.000	93.000	93.000	93.000	93.000	93.000	93.000	93.000	93.000
nb	26.000	26.000	26.000	26.000	26.000	26.000	26.000	26.000	26.000	26.000
P	0.261	0.126	0.908	0.076	0.034	0.345	0.176	0.092	0.109	0.034
Z	0.896	2.488	2.755	0.028	0.155	1.887	2.567	1.989	0.114	2.617
down-regulated	0.119	0.333	0.857	0.214	0.119	0.190	0.143	0.167	0.119	0.000
up-regulated	0.192	0.269	0.769	0.077	0.038	0.500	0.346	0.192	0.115	0.115
na	42.000	42.000	42.000	42.000	42.000	42.000	42.000	42.000	42.000	42.000
nb	26.000	26.000	26.000	26.000	26.000	26.000	26.000	26.000	26.000	26.000
P	0.147	0.309	0.824	0.162	0.088	0.309	0.221	0.176	0.118	0.044
Z	0.829	0.556	0.924	1.495	1.139	2.685	1.965	0.270	0.046	2.252

p=0.01: Z=2.576
p=0.05: Z=1.960

[a] "Pro unaff.", "Pro down" and "Pro up" are the proportion of genes containing recognition sequence for the TF.
[b] "No unaff.", "No down" and "No up" are the number of genes contained in the unaffected, down-regulated and up-regulated gene groups, respectively.
[c] Po is the population proportion defined by Po=(Noxx•Proxx+Novv•Provv)/(Noxx+Novv), where xx and vv is replaced either by "unaff.", "down" or "up".
[d] Z is the random variable defined by Z=|Proxx-Provv|/sqrt(Po•(1-Po)•(1/Noxx+1/Novv)), where xx and vv is replaced either by "unaff.", "down" or "up". If Z>1.960 then p<0.05, and if Z>2.576 then p<0.01.
[e] Evi-1 recognition sequence, AGAYAAGATAA
[e] Evi-1 recognition sequence, NGATANGANWAGATA
[g] GR recognition sequence, NNNNNNCNNTNTGTNCTNN
[h] GR recognition sequence, GGTACAANNTGTYCTK

Table 1. Statistical analysis of difference in the proportion of genes containing recognition sequences for TFs between unchanged, up-regulated and down-regulated gene groups. Boxes of yellow and red indicate that the difference in populationproportion is significant with $p < 0.05$ and $p < 0.01$, respectively. (Reproduced with permission of the Japanese Radiation Research Society from Ref (Vares et al., 2011))

to be functionally involved in gene modulations. Then, nucleotide sequences in the neighboring region of the up-regulated, down-regulated, and unaffected genes were retrieved from the Entrez Gene database, and recognition sequences for TFs were searched on the database using the TFSEARCH. As a result, 21 potential TF-binding sites with significantly different incidence between the three gene groups (up-regulated, down-regulated and unaffected gene groups) could be identified (Table 1).

The binding sites for sterol regulatory element-binding protein 1 (SREBP-1), aryl hydrocarbon receptor (AhR/Ar) and olfactory 1 (Olf-1) were suggested to be involved in up-regulation, while the binding sites for glucocorticoid receptor (GR) and hepatocyte nuclear factor 1 (HNF-1) were suggested to be involved in down-regulation of the genes. In addition, the binding sites for activating enhancer-binding protein 4 (AP-4), nuclear factor-kappaB (NFκB), and early growth response 3 (Egr-3) were correlated with modulation of gene expression regardless of the direction of modulation. Among them, GR and/or SREBP-1 are interesting TFs in association with the recent findings of a trend of elevated body weight in irradiated mice. GR is known to be associated to the metabolic syndrome. Similarly, SREBP-1 is also involved in metabolic process by mediating the final regulatory step in LDL metabolism. Alteration of TF activity of GR and/or SREBP-1 as well as the related signaling pathways after exposure to IR may be the underlying mechanisms for this metabolic process.

6. Conclusion

Studies of modulated gene expression after exposure to IR have historically played an important role in elucidating the molecular mechanisms underlying cellular IR response. Especially finding of transcriptional network derived from p53 made a deep impact on understanding of biological defence system as well as development of radiation hazards such as radiation carcinogenesis. Recent technologies enable us simultaneously monitor modulation of gene expression across essentially the entire genome. It was revealed that so many TFs are involved in gene regulation *in vivo* that gene expression is finely modulated depending on tissues. However no single consistent picture of IR response has not been obtained because variety of experimental models, experimental conditions and data analyses have been employed. As a future perspective in this research area, carefully worked out methods for data analysis, data mining and construction of signalling network are strongly need. In addition, protein researches, especially a comprehensive analysis of protein expression, modification, sub-cellular localization is required, because these data cannot be obtained from the researches on transcriptional regulation.

7. Acknowledgment

This article was prepared with a support by the Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan (21510060), the Budget for Nuclear Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan based on screening and counseling by the Atomic Energy Commission, and under contract with the Aomori Prefectural Government, Japan.

8. References

Chen, I. T., Smith, M. L., O'Connor, P. M. & Fornace, A. J. Jr. (1995). Direct interaction of Gadd45 with PCNA and evidence for competitive interaction of Gadd45 and p21Waf1/Cip1 with PCNA. *Oncogene*. Vo.11, No.10, pp.1931-1937.

Daino,K., Ichimura,S. & Nenoi,M. (2002) Early induction of CDKN1A (p21) and GADD45 mRNA by a low dose of ionizing radiation is due to their dose-dependent post-transcriptional regulation. *Radiation Research*. Vol.157, No.4, pp.478-482.

Daino, K., Ichimura, S. & Nenoi, M. (2006). Both the basal transcriptional activity of the GADD45A gene and its enhancement after ionizing irradiation are mediated by AP-1 element. *Biochim Biophys Acta*. Vol.1759, No.10, pp.458-469.

d'Ari, R. (1985). The SOS system. *Biochimie.* Vol.67, No.3-4, pp.343-347.

Fei, P. & El-Deiry, W. S. (2003). P53 and radiation responses. *Oncogene*. Vol.22, No.37, pp.5774-5783.

Fornace, A. J. Jr, Alamo, I. Jr & Hollander, M. C. (1988). DNA damage-inducible transcripts in mammalian cells. *Proceedings of the National Academy of Sciences*. 8Vol.5, No.23, pp.8800-8804.

Fornace, A. J. Jr, Nebert, D. W., Hollander, M. C., Luethy, J. D., Papathanasiou, M., Fargnoli, J. & Holbrook, N.J. (1989). Mammalian genes coordinately regulated by growth arrest signals and DNA-damaging agents. *Molecular and Cellular Biology*. Vo.9, No.10, pp.4196-4203.

Hartwell, L. H. & Weinert, T. A. (1989). Checkpoints: controls that ensure the order of cell cycle events. *Science*. Vol.246, No.4930, pp.629-634.

Nenoi, M., Daino, K., Nakajima, T., Wang, B., Taki, K. & Kakimoto, A. (2009). Involvement of Oct-1 in the regulation of CDKN1A in response to clinically relevant doses of ionizing radiation. *Biochim Biophys Acta*. Vol.1789, No.3, pp.225-223.

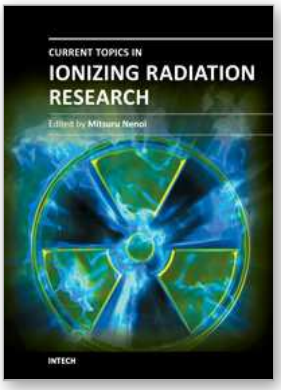
Oda, Y., Nakamura, S., Oki, I., Kato, T. & Shinagawa, H. (1985). Evaluation of the new system (umu-test) for the detection of environmental mutagens and carcinogens. *Mutatation Research*. Vol.147, No.5, pp.219-229.

Smith, M. L., Chen, I. T., Zhan, Q., Bae, I., Chen, C. Y., Gilmer, T. M., Kastan, M. B., O'Connor, P. M. & Fornace, Jr A. J. (1994). Interaction of the p53-regulated protein Gadd45 with proliferating cell nuclear antigen. *Science*. Vol.266, No.5189, pp.1376-1380.

Taki, K., Wang, B., Nakajima, T., Wu, J., Ono, T., Uehara, Y., Matsumoto, T., Oghiso, Y., Tanaka, K., Ichinohe, K., Nakamura, S., Tanaka, S., Magae, J., Kakimoto, A. & Nenoi, M. (2009), Microarray analysis of differentially expressed genes in the kidneys and testes of mice after long-term irradiation with low-dose-rate γ-rays. *Journal of Radiation Research*. Vol.50, No.3, pp.241-252.

Vares, G., Uehara, Y., Ono, T., Nakajima, T., Wang, B., Taki, K., Matsumoto, T., Oghiso, Y., Tanaka, K., Ichinohe, K., Nakamura, S., Tanaka, S. & Nenoi M. (2011). Transcription Factor-recognition Sequences Potentially Involved in Modulation of Gene Expression after Exposure to Low-dose-rate γ-rays in the Mouse Liver. *Journal of Radiation Research*. Vol.52, No.2, pp.249-256.

Zhan, Q., Bae, I., Kastan, M. B. & Fornace, A. J. Jr. (1994). The p53-dependent gamma-ray response of GADD45. *Cancer Research*. Vol.54, No.10, pp.2755-2760.



Current Topics in Ionizing Radiation Research

Edited by Dr. Mitsuru Nenoi

ISBN 978-953-51-0196-3
Hard cover, 840 pages
Publisher InTech
Published online 12, February, 2012
Published in print edition February, 2012

Since the discovery of X rays by Roentgen in 1895, the ionizing radiation has been extensively utilized in a variety of medical and industrial applications. However people have shortly recognized its harmful aspects through inadvertent uses. Subsequently people experienced nuclear power plant accidents in Chernobyl and Fukushima, which taught us that the risk of ionizing radiation is closely and seriously involved in the modern society. In this circumstance, it becomes increasingly important that more scientists, engineers and students get familiar with ionizing radiation research regardless of the research field they are working. Based on this idea, the book "Current Topics in Ionizing Radiation Research" was designed to overview the recent achievements in ionizing radiation research including biological effects, medical uses and principles of radiation measurement.

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821