Identification of novel senescence-associated genes in ionizing radiation-induced senescent carcinoma cells

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1. Introduction

Cellular senescence is considered as a defense mechanism to prevent tumorigenesis. Ionizing radiation (IR) induces stress-induced premature senescence as well as apoptosis in various cancer cells. Senescent cells undergo functional and morphological changes including large and flattened cell shape, senescence-associated β-galactosidase (SA-βGal) activity, and altered gene expressions [1]. Even with the recent findings of several gene expression profiles and supporting functional data, it is obscure that mechanism of IR-induced premature senescence in cancer cells. We performed microarray analysis to identify the common regulated genes in ionizing radiation-induced prematurely senescent human carcinoma cell lines.

2. Materials and Methods

2.1 Cell culture and IR irradiation

MCF7, H460 and HCT116 cells were cultured in Dulbecco's modified Eagle's medium (DMEM), RPMI 1640, and McCoy medium, respectively. Cells were exposed to γ-ray with a $^{137}C_S$ gamma ray source (Atomic Energy of Canada, Mississauga, Canada) at a dose rate of 3.0 Gy/min.

2.2 RNA isolation and first cDNA strand synthesis

Total RNA was prepared using TRIzol reagent (Invitrogen, Calsbad, CA) followed by manufacturer's protocol. The extracted RNA was used as a template for cDNA synthesis using SuperScript™ III reverse transcrptase kits (Invitrogen, Calsbad, CA).

2.3 Microarray analysis and data analysis

Gene expression profiling was performed between control and IR-exposed cancer cell lines using Illumina Genome Analyzer Beadchips (Illmina Inc., San Diego, CA) according to the manufacturer's protocol. Fluorescence scanning was performed using a BeadArray Reader and BeadScan software (Illumina).

2.4 Senescence associated β-galactosidase staining

To determine the senescent phenotype, we performed senescence-associated β-galactosidase assay described in [2] with modifications.

2.5 Colony formation analysis

The cells were seeded in 60-mm dish and cultured for 10 days. The colonies were fixed and stained with crystal violet.

2.6 Reverse transcription-polymerase chain reaction

To validate the differential expression of genes screened by cDNA microarray, we performed RT-PCR of the selected genes that up- or down-regulated by radiation using the specific primers. The PCR products were examined by 1.2 % agarose gel electrophoresis.

2.7 Western blot analysis

Cells were lysed in RIPA buffer. Equal amounts of proteins were separated and transferred to nitrocellulose membranes. The membranes were incubated overnight at 4 °C with one of the specific antibodies. Proteins were visualized using enhanced chemiluminescence.

3. Results

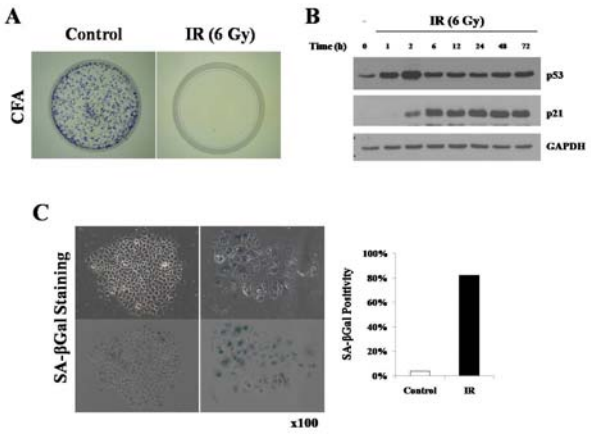

Figure 1. IR induces premature senescence. Effect of IR 6 Gy irradiation in MCF7 breast cancer cells on colony forming ability (A), p53 and p21 expression (B), and SA-βGal staining (C).

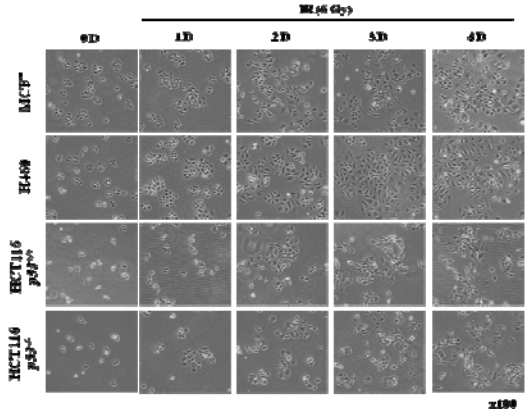

Figure 2. Morphologic changes of IR-exposed cancer cell lines that were used in experiments.

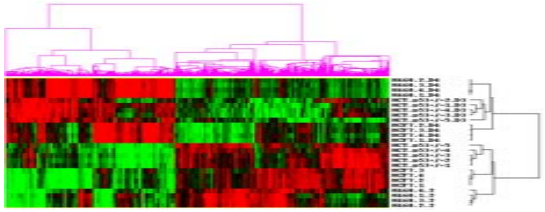

Figure 3. Hierarchical clustering data shows 463 genes that were commonly regulated by IR irradaiation in four cancer cell lines.

Table 1. Selected consensus radiation response genes during senescence induction

Gene	Gene ID	Fold change					
		FC(M) (C:D1)	FC(M) (C:D2)	FC(M) (C:D3)	FC(M) (C:D4)	FC(H4) (C:D4)	FC(+) (C:D3)
UP-REGULATED							
GDF15	NM_004864	5.1	13.4	17.5	21.8	11.8	2.1
NEU1	BC000722	1.6	1.8	1.9	2.8	3.2	1.8
PDGFRL	NM_006207	1.9	2.9	3	4.1	4.9	1.7
PINK1	NM_032409	1.3	1.4	1.7	1.9	1.9	1.8
PLA2G4C	NM_003706	1.4	2.1	3.7	5.4	1.9	1.9
PTGES	NM_004878	2.6	2.2	2.6	2.6	3.8	2.1
TP53INP1	NM_033285	4.1	5.1	5.7	4.3	4.9	2.7
WIG1	NM_022470	2	3.3	3.2	3.7	6.9	2.9
DOWN-REGULATED							
FoxM1	NM_202002	-1.5	-2.3	-2.3	-3	-4.7	-1.7
PBK	NM_018492	-2	-2.4	-2.1	-4.1	-6.3	-1.7
PKN3	NM_013355	-1.2	-1.6	-1.5	-1.8	-3.2	-1.5
TK1	NM_003258	-1.6	-2.4	-2.7	-3.2	-9.6	-1.8

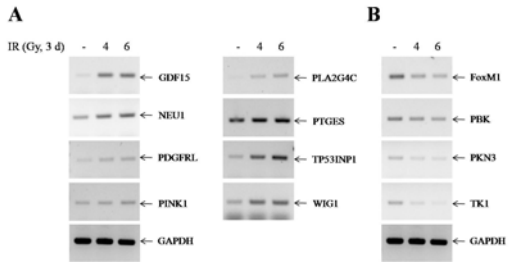

Figure 4. Dose-dependent transcriptional alterations of consensus response genes which were up-regulated (A) or down-regulated (B) during senescence induction. GAPDH was used as a normalization standard.

3. Conclusion

Gene expression profiles showed that 1642, 1912, and 924 genes responded to IR on the transcriptional levels in MCF7, H460, and HCT116 cells, respectively. And then, 463 putative senescence-related genes which commonly altered in three different cell types were identified. Finally, 12 common regulated genes (8 up- and 4 down-regulated) were selected for further study. Up-regulated genes were ontologically classified to signal transduction (*GDF15, PTGES, GRN*), metabolic process (*PLA2G4C, NEU1*), and DNA damage response (*WIG1, PINK1*) pathways. On the other hand, down-regulated genes were belonged to cell cycle (*PKMYT1*), transcriptional regulation (*FOXM1*) and DNA replication (*TK1*) pathways. The data presented here could help in the better understanding of stress-induced premature senescence in cancer cells, and suggest potential strategies for diagnostic and therapeutic purposes in cancer treatment.

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