Poster

ANALYSIS OF GENE EXPRESSION PROFILING OF HUMAN CELLS BY X-RAY IRRADIATION AT HIGH DOSES FOR HIGH-RESOLUTION TOMOGRAPHY MEASUREMENT

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Introduction

Non-invasive inspection of biomaterials containing live cells has been increasingly demanded as the cell therapy rapidly advances in tissue engineering. In regenerative medicine, in particular, multipotent stem cells are often distributed in bio-compatible scaffolds and partially differentiated in vitro before grafted in patients. Mesenchymal stem cells generated in bone marrow have a capability to differentiate into osteoblast and ondrocyte with appropriate inducing factors. To date, a microfocus x-ray computed tomography has been used to visualize development of the bone network formed in pores of the scaffold. This measurement exposes the scaffold containing live cells to a considerable amount of x-ray dose. Because the mitogenic cells are sensitive to ionising radiation in general, it is expected that multipotent stem cells also may indicate sensitive response to x-ray radiation. In this study, we investigated the radiation response of the human cells exposed to a high x-ray dose comparable to micro-tomography measurement.

Materials and Methods

HeLa cells were cultured in DMEM medium supplemented with 10% fetal calf serum. Prior to x-ray exposure test, the cells in a 12 multi-well plates grew to a subconfluent state. Then the plates with a 2ml medium in each well were placed on a 37C thermoplate 0.18m away from the x-ray generator(KXO-ES, Toshiba, Japan) operated at a tube voltage of 100kV and tube current of 4mA for 30minutes, corresponding to 30Gy of absorbed dose at the well surface. Total RNA was isolated 2hrs and 24hrs after irradiation from unirradiated control and irradiated cells using RNeasy Mini Kit (Qiagen,Inc., Valencia, CA, USA). Whole Human Genome 60-mer Oligo Microarrays (G4112F, 4,1000 unique probes, Agilent Technologies) were used to for the analysis of radiation-induced gene expression patterns. To quantify the cell viability, a cell proliferation reagent WST-1 (Takara Bio Inc., Japan) was used as per protocol. Immediately after the irradiation, existing medium was removed and a 100mL of fresh medium was added in each well with a 10mL of WST-1.

Results

The transcriptional alteration profiles of irradiated cells were compared with the controls without irradiation. Out of 1441 genes statically significant ($p<0.05$) and with the fold change over 2.0, 556 genes were up-regulated, while 885 genes were down-regulated. It is known that radiologically altered genes belong functionally to the cell cycle pathways, DNA repair, oncogenes, mitochondrial and ribosomal proteins, transcription and translational regulators and genes encoding cytoskelton components[1]. Table 1 shows the list of genes altered by a high dose of x-ray irradiation (30Gy). The genes listed here are chosen on a basis that the 2hrs fold change is over 1.5 and 24hrs fold change is over 2.0 with t-test p-value <0.05. These include INHBA, CDKN1A, RRAD and SOCS1. Those related to well-known p53 dependent signaling pathways, such as TP53, GADD45A, MDM2, except CDKN1A, were not significantly altered even after 24hrs at the high dose of x-ray irradiation in this study[2]. Transcriptional alterations related to mitochondrial functions ($p>0.05$) are listed in Table 2. When the fold change is compared between 2hrs and 24hrs after 30Gy irradiation, ALDH1B1, UCP2, and SUOX were

up-regulated and TOMM40, MRPL30, PEMT, DUT, and TOMM40 were down-regulated. Functions of these sensitive genes in cellular response to high dose of x-ray may provide a clue for allowing us to use high-resolution x-ray tomograhy for live cell imaging.

References:

[1] Chaudhry MA, Chodosh LA, McKenna WG, Muschel RJ. Cancer Letters, 195, (2003) 221-233

[2] Kis E, Szatmari T, Keszei M, Farkas R, Esik O, Lumniczky K, Falus, Safrany G, Int. J. Radiation Oncology Biol. Phys., 66(5), (2006) 1506-14

Table 1 Gene expression profile induced by a high dose of x-ray (30Gy)

Genbank	GeneSymbol	2hrs, 30Gy		24hrs, 30Gy		Description
		Fold Change	t-test p-value	Fold Change	t-test p-value	
NM_002192	INHBA	2.2	0.04	3.0	0.01	inhibin, beta A (activin A, activin AB alpha polypeptide)
NM_000389	CDKN1A	1.7	0.01	2.9	0.00	cyclin-dependent kinase inhibitor 1A
ENST00000339441	ENST00000339441	2.9	0.00	2.9	0.00	hypothetical LOC387763
NM_145316	C6orf128	1.8	0.00	2.8	0.00	chromosome 6 open reading frame 128 (C6orf128)
NM_004165	RRAD	2.5	0.03	2.7	0.00	Ras-related associated with diabetes (RRAD)
NM_153690	FAM43A	1.9	0.05	2.3	0.03	family with sequence similarity 43, member A (FAM43A
BC087859	BC087859	2.3	0.00	2.2	0.02	cDNA clone IMAGE:3039810
NM_033513	C19orf20	1.6	0.03	2.1	0.00	chromosome 19 open reading frame 20 (C19orf20)
NM_025201	pp9099	1.7	0.01	2.1	0.00	PH domain-containing protein (pp9099)
NM_000201	ICAM1	1.7	0.01	2.1	0.00	intercellular adhesion molecule 1 (CD54)
NM_014931	KIAA1115	1.7	0.03	2.1	0.01	KIAA1115 (KIAA1115)
NM_004165	RRAD	2.6	0.02	2.0	0.01	Ras-related associated with diabetes (RRAD)
NM_003745	SOCS1	1.5	0.00	2.0	0.00	suppressor of cytokine signaling 1 (SOCS1)

Table 2 Gene expression alterations relating to mitochondrial functions

Genbank	GeneSymbol	2hrs, 30Gy		24hrs, 30Gy		Description
		Fold Change	t-test p-value	Fold Change	t-test p-value	
NM_000240	MAOA	0.7	0.01	0.7	0.02	monoamine oxidase A (MAOA), nuclear gene encoding mitochondrial protein
NM_033540	MFN1	0.8	0.03	0.7	0.01	mitofusin 1 (MFN1), nuclear gene encoding mitochondrial protein
NM_033540	MFN1	0.8	0.02	0.7	0.02	mitofusin 1 (MFN1), nuclear gene encoding mitochondrial protein
NM_000692	ALDH1B1	1.2	0.02	1.6	0.00	aldehyde dehydrogenase 1 family, member B1 (ALDH1B1), nuclear gene encoding mitochondrial protein
NM_006114	TOMM40	1.2	0.02	0.6	0.01	translocase of outer mitochondrial membrane 40 homolog (yeast) (TOMM40)
NM_016503	MRPL30	1.3	0.01	0.7	0.01	mitochondrial ribosomal protein L30 (MRPL30), nuclear gene encoding mitochondrial protein
NM_007169	PEMT	1.2	0.03	0.8	0.02	phosphatidylethanolamine N-methyltransferase (PEMT), nuclear gene encoding mitochondrial protein
NM_001948	DUT	1.2	0.02	0.5	0.05	dUTP pyrophosphatase (DUT), nuclear gene encoding mitochondrial protein
NM_004294	MTRF1	0.7	0.05	0.6	0.00	mitochondrial translational release factor 1 (MTRF1), nuclear gene encoding mitochondrial protein
NM_003355	UCP2	1.2	0.04	1.7	0.01	uncoupling protein 2 (mitochondrial, proton carrier) (UCP2), nuclear gene encoding mitochondrial protein
NM_003705	SLC25A12	0.8	0.05	0.7	0.01	solute carrier family 25 (mitochondrial carrier, Aralar), member 12 (SLC25A12)
NM_000456	SUOX	0.9	0.04	1.6	0.00	sulfite oxidase (SUOX), nuclear gene encoding mitochondrial protein
NM_006114	TOMM40	1.3	0.02	0.6	0.01	translocase of outer mitochondrial membrane 40 homolog (yeast) (TOMM40)