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Identification of cellular senescence-related proteins in gamma-ray irradiated MCF7 cells

Na-Kyung Han, [a, b] Hae-Ok Byun, [a] Je-Jung Lee, [a,b] Sung-Gil Chi, [b] Young-Gyu Ko, [b] Jae-Seon Lee [a]
[a] *Laboratory of Radiation Molecular Oncology, Korea Institute of Radiological and Medical Sciences, Seoul 139-706, Korea*
[b] *School of Life Sciences and Biotechnology, Korea University, Seoul 136-701, Korea*

1. Introduction

Cellular senescence, has been originally defined as proliferative arrest that occurs in normal cells after a limited number of cell divisions. It has now become regarded more broadly as a general biological program of terminal growth arrest. Senescence was originally described in normal human cells undergoing a finite number of divisions before permanent growth arrest [1, 2]. However, variety of stresses also induce rapid and permanent cell growth arrest. The accelerated senescene does not involve telomere shortening. Cellular senescence is belived to be essential anticarcinogenic program in normal cells. Tumor cells must avoid cellular senescence through various mechanisms [3]. In other words, induction of cellular senescence is promising way of tumor treatment. Thus, the elucidation of the biological aspects of tumor cell senescence offers plausible approaches to the development of novel therapeutic targets to stop the growth of tumor cells. In this study, we monitored the changes of protein expression profile in MCF7 human breast cancer cells exposed to gamma-ray radiation, using two-dimensional electrophoresis. We identified biomarkers which evidently changed their expression levels in ionizing radiation-induced cellular senescence

2. Methods and Results

2.1 Preparation of protein lysates

Protein lysates were prepared in Lysis buffer (9M Urea, 2M thio Urea, 100mM DTT, 2% CHAPS (w/v), 60 mM *n*-octyl-D-glucopyranoside, 2% IPG buffer (pH 3-10, Amersham biosciences, Piscataway, NJ) containing protease inhibitor). After centrifugation at 14,000 rpm, 10min, 4 °C, the supernatant was taken for the subsequent 2-dimensional gel electrophoresis.

2.2 Two dimensional electrophoresis

The isoelectric focusing of each sample containing equal amount of protein (120 μg) was conducted on a linear wide-range immobilized pH gradient (pH 3-10) with a total focusing time of 81,780Vhr, at 20°C, using the IPGphor system in accordance with the manufacturer's instructions (Amersham Biosciences, Piscataway, NJ). The second dimension was then conducted on SDS-PAGE gels (11% polyacrylamide, 0.26% 1,4-Bis (acryloyl) piperazine (PDA) / 25.5 cm × 19.6 cm × 1 mm), under constant current, in three steps (Step 1: 5 w/gel; Step2: 10 w/gel; step 3: 15 w/gel) at 20°C, using an Ettandalt 6 system (Amersham Biosciences). The analytical gels were stained using the PlusOne™ silver staining kit (Amersham Biosciences) in accordance with the manufacturer's instructions, with the exception of the glutaraldehyde treatment [4]

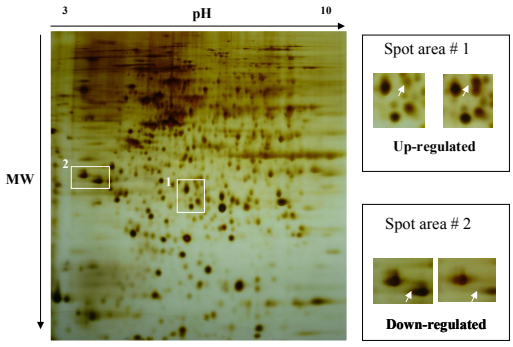

Figure 1. Two-dimensional gel electrophoresis image of proteins extracted from MCF7 human breast cancer cells.

2.3 In-gel protein digestion and electrospray ionization tandem mass spectrometry (ESI-MS/MS)

Proteins were subjected to in-gel trypsin digestion. The excised gel spots were then destained using 100 $\mu\ell$ of destaining solution (1:1=30 mM potassium ferricyanide :100 mM sodium thiosulfate, v/v). Then, the gel spots were incubated using 200 mM ammonium bicarbonate for 20 min. These gel pieces were dehydrated and dried, and then rehydrated with 20 $\mu\ell$ of 50 mM ammonium bicarbonate containing 0.2 μg modified trypsin (Promega). After solution was removed, 30 $\mu\ell$ of 50 mM ammonium bicarbonate was added and incubated overnight at 37℃. The peptide solution was desalted with a C_{18} nano column (IN2GEN Co., Ltd.).

The MS/MS of peptides generated via in-gel digestion was conducted by nano-ESI on a Q-TOF2 mass spectrometer (Micromass, Manchester, UK). The product ions were analyzed with an orthogonal TOF analyzer, and were fitted with a reflector, a micro-channel plate detector, and a time-to-digital converter. These data were processed with a Mass Lynx Windows NT PC system.

In order to identify the proteins, all MS/MS spectra recorded on tryptic peptides derived from the spots were searched against protein sequences from the NCBInr databases, using the MASCOT search program (www.matrixscience.com).

Table 1. Identified Proteins from MCF7 Cells by LC-MS/MS

spot no.	protein	mass	Mascot score
1	UR071	24919	98
2	DR072	26457	60

2.4 Western Blotting and Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

For Western Blot assay, Cells were collected in extraction buffer (25 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1% Triton X-100, 100 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, 10 mM sodium azide, 1 mM orthovanadate, 20 μg/ml aprotinin, and 3 mM dithiothreitol). The collected cells were incubated on ice for 30 min. The lysate was centrifuged and quantitated with a Bradford Assay Reagents (Bio-Rad, Hercules, CA). Equal amount of proteins was loaded onto an 8 or 10% SDS-PAGE gel. After separation of proteins depending on their molecular weights, it was transferred to a nitrocellulose membrane. The membrane blot was incubated with antibody at 4°C overnight, and washed three times in TBST. Protein bands were detected by sequential treatment with an HRP-conjugated secondary antibody (Santa Cruz, CA), and an enhanced chemiluminescence substrate kit (Amersham Bioscience, NJ).

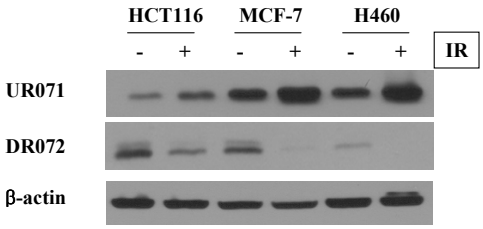

Figure 2. Western blot analysis of IR-induced senescence-related genes in human colon cancer HCT116, breast cancer MCF7, and non-small cell lung cancer H460 cells.

For RT-PCR, total RNA was prepared using TRIzol reagent (Invitrogen). Total RNA was used as template for cDNA synthesis with M-MLV RTusing SuperScript^{TM} III reverse transcrptase kits (Invitrogen) Subsequently, PCR cycles were performed by 28 cycles. The final RT-PCR products were electrophoresed on 1.5% agarose gel, stained with 0.5 g/ml ethidium bromide solution, and visualized on a UV transilluminator (Gel Doc 2000; Bio-Rad).

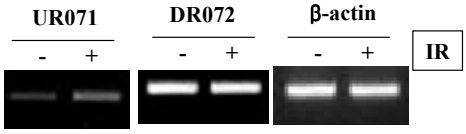

Figure 3. Reverse Transcriptase-Polymerase Chain Reaction with specific probes in MCF7 cells.

3. Conclusion

We conducted comparative proteomic analysis to identify differentially expressed proteins in IR-induced senescence MCF7 cells. UR072 was up-regulated in the transcriptional level, and DR072 was down-regulated in the posttranslational level during the process of stress-induced premature senescence pathway. Altered expressions of UR071 and DR072 were confirmed in three different cancer cell types (human colon cancer HCT116, breast cancer MCF7, and non-small cell lung cancer H460 cells). These proteins will be useful as prognostic biomarkers .for the radiotherapy.

REFERENCES

[1] I. B. Roninson, Tumor cell senescence in Cancer treatment. Cancer Research 63:2705-2715, 2003.
[2] M. Narita, S.W. Lowe, Senescence comes of age. Nature Medicine 11:920-922, 2005.
[3] B.-D. Chang, E. V. Broude, M. Dokmanovic, H. Zhy, A. Ruth, Y. Xuan, E. S. Dande., E. Lausch, K. Christov, and I. B. Roninson, A senescence-like phenotype Distinguishes tumor cells that undergo terminal proliferation arrest after exposure to anticancer agents. Cancer Research 59:3761-3767, 1999.
[4] K.-B. Kim, J.-W Lee, C.S. Lee, B.-W. Kim, H.-J. Choo, S.-Y. Jung, S.-G. Chi, Y.-S. Yoon, G Yoon and Y.-G. Ko. Oxidation-reduction respiratory chains and ATP synthase complex are localized in detergent-resistant lipid rafts. Proteomics 6; 2444-2453, 2006.