Role of the p53-p21 pathway in the cancer cell invasion induced by ionizing radiation

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1. Introduction

While ionizing radiation (IR) is widely used as an effective therapeutic method for cancers, increasing evidence suggests that IR can enhance the migratory and invasive potentials of such cancer cells that resist to the treatment(1). To date, however, the mechanism underlying this potentially detrimental effect of IR has been poorly understood.

p53 is known as tumor suppressor and IR responsible molecule. Past studies reveals p53 is involved in several important cellular regulatory systems like cell cycle, apoptosis, DNA repair and angiogenesis(2). p21 is a transcriptional target molecule of p53 and member of cyclin-dependent kinase inhibitor(CKIs) family, which regulates cell cycle progression(3).

Recent studies focus on the relationship between members of cell cycle regulatory pathway molecule and metastatic progression (4-6). Here we show some evidence that p53 and its downstream protein, p21, is closely related in lung cancer cell invasiveness and IR induced invasion. Mutant study suggests p53 might be involved more than two alternative invasion pathways which require transcriptional activity or not.

2. Materials and Methods

2.1 Cell culture, Transfection and IR Irradiation

NSCLC cell lines were used in this study. A549 and H460 express wild type p53(p53wt), NCI-H1299 and Calu-1 is p53 null type. These cell lines were cultured at 37 ℃, 5% CO_2 atmosphere, 10% FBS in RPMI 1640 culture media were used for A549, H460 and NCI-H1299, same percentage of FBS in McCoy's 5A media were used for Calu-1. A day before experiment, cells were trypsinized for 2 minutes and were suspended in culture media, 2 x 10^5 cell /35mm cell culture dish or 5 x 10^5 cell/60mm dish were seeded. For maintenance, 2 x 10^6 cell/100mm dish were plated and media were changed every 2-3 days. Plasmid DNA transfection was performed with lipofectamin 2000 reagent according to manufacturer's protocol. Protein expression levels form transfected DNA were detected by western blot methods.

γ -ray from Cs^{137} were irradiated with GammaCell 3000 Elan irradiator(Best Theratronics Ltd., Canada).
All experiments in this study were preformed 24hour after treatment.

2.2 Transfilter invasion assay

To investigate NSCLC cell invasiveness *in vitro*, modified transfilter cell invasion assay were used. Briefly, 15ul of 1:15 diluents of matrigel(BD Bioscience Co., U.S.A.) in serum free media were layered on 8um pore size polycarbonate membrane, transwell(Corning Life Sciences Inc., U.S.A.) and dried at room temperature for over 2h. Coated transwell were inserted on 24well culture plate. Cultured cells were trypsinized and harvested at 1500rpm for 3min.; cells were suspended in serum free media containing 0.1% BSA. 200ul of each sample, 2.5 x 10^5 cells/ml in suspension media, were loaded into upper chamber of coated transwell, lower chamber of culture wells were filled with 600ul of complete growth media(10% FBS) and allowed at 37℃, 5% CO_2 culture condition for 24hour. Inside of transwell were carefully brushed with wet cotton swab to remove non-invaded cells; invaded cells on outside of transwell were stained. Stained cells were photographed under microscope and counted.

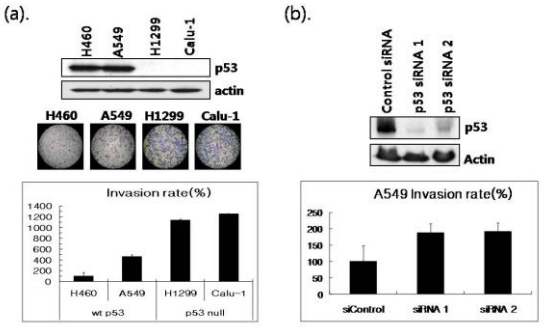

Fig. 1. p53 suppress NSCLC cell invasiveness. (a). Wild type p53 NSCLC cells exhibits lower invasiveness than p53 null type. (b). Down regulation of p53 expression increase A549 invasion rate.

2.3 Western blot analysis

Protein expression levels were determined by using SDS-PAGE/western blot analysis. Separated proteins in 12% PAGE gel were transferred on nitrocellulose membrane by using semidried electro-transfer system.

To detect expression levels of target proteins, immunospecific primary antibodies were used. Membrane were blocked with 5% skim milk in PBS/Triton X-100 and primary antibodies were treated at 4℃ overnight. Binding amounts of primary antibody

were detected by HRP conjugated secondary antibodies, which were risen from the host of primary antibodies, and ECL system. Chemiluminescence was detected by X-ray film.

2.4 Immuno-fluorescence laser scanning confocal microscopy

Laser scanning confocal microscopy techniques were used to study subcellular localization and expression changes in intact cellular structure. Cells were grown on 12mm diameter poly-L-lysine coated glass coverslip and fixed in 3.7% prarformaldehyde solution for 20minutes at room temperature. Fixed cells were permiablized in PBS solution containing 0.1% Triton X-100, 1% normal goat serum and were blocked with 1% normal goat serum in PBS for 1 hour. Primary antibodies were treated for 1hour at room temperature or overnight at 4℃. Fluorescence tagged secondary antibodies were treated for 1hour at room temperature. Samples were washed 3 times for 5minutes with PBS solution between every step.

Fluorescence tagged samples were mounted on slid glass and examined under LSM710 (Carl ZEISS, Germany) confocal microscope.

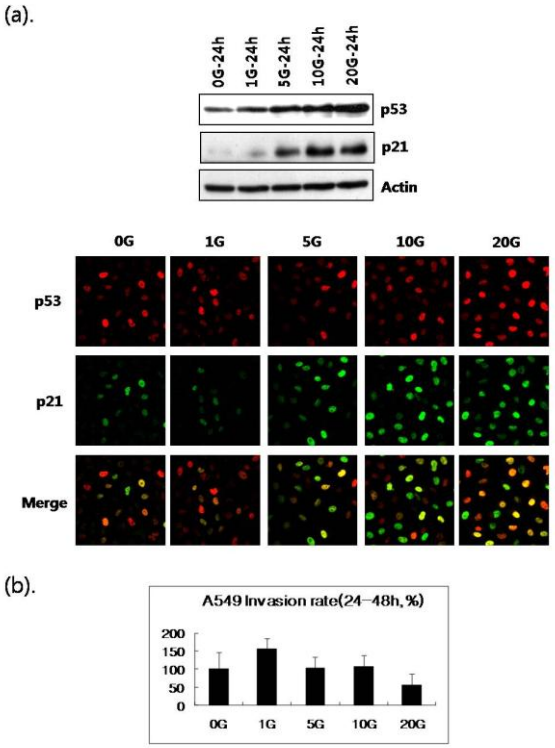

Fig. 2. Ionizing radiation increase A549 invasiveness.
(a). Ionizing radiation increase p53 and p21 level in dose dependent manner. (b). Invasion rate of 1Gy irradiated A549 cell, at low level of p53 and p21, was increased.

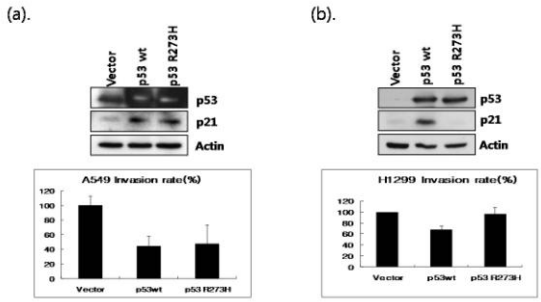

Fig. 3. p53 transcription inactive mutant differently act in A549 and NCI-H1299. (a). overexpression of wild type p53(p53 wt) and transcription inactive mutant(p53 R273H) inhibits A549 invasiveness. But, (b), inhibitory function of p53 requires transcriptional activity in NCI-H1299 cell.

3. Results and Discussion

Basal invasiveness of wild type p53 cell line is lower than p53 null type. Ectopic expression of wild type p53 and p53 small interfering RNA experiments (Fig. 1.) showed wild type p53 plays inhibitory role in NSCLC cell invasiveness. In A549, 1Gy irradiation enhanced invasion, there were no significant change in p53 and p21 protein level. On the contrary, higher gray irradiation (over 1Gy) increased p53 and p21 protein level and invasiveness were not changed or reduced (Fig. 2.). Interestingly, transcription inactive mutant of p53 (p53R273H) differently act in A549 and NCI-H1299 cell line (Fig. 3.).

These data suggest p53 and p21 plays inhibitory role in cancer cell invasiveness including ionizing radiation induced invasion. Different responsibility for p53R273H between A549 and NCI-H1299 reflect inhibitory function of p53 should mediate more than two different pathways which require transcriptional activity of p53 or not.

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