# Differential Sensitivity of Cells to Radiation Mediated by p53 Pulses

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## 1. Introduction

One of the most important biological endpoints for assessment of cell's sensitivity to ionizing radiation is cell death. Exposure of cells to ionizing radiation activates protein genes related cell cycle arrest and cell death (apoptosis or autophagy). The tumor suppressor p53 participates not only in regulation of apoptosis, but also in autophagy mechanism. Apoptosis (type I cell death) is characterized by the activation of caspases and the formation of apoptotic bodies, and plays essential roles in all multicellular organisms. On the other hand, autophagy (type II cell death) is characterized by the presence of cytoplasmic engulfing vesicles, alias autophagosomes, and is a major intracellular pathway for degradation and recycling of proteins, ribosomes and entire organelles.

The purpose of this study was to determine whether ionizing radiation treatment induces autophagy depending on the p53 expression levels. RKO (wild-type p53) and RKO E6 (null-type p53) cells were used to evaluate the effects of p53 on the sensitivity of cells to ionizing radiation. In the RKO E6 cells, the function of p53 was disabled with human papillomavirus E6 oncoprotein.

## 2. Materials and Methods

### *2.1 Cell Culture*

RKO and RKO E6 cells were used to evaluate cellular responses after irradiation. Cells were grown in MEM supplemented with 10% Fetal Bovine Serum (FBS), antibiotic-antimycotic at appropriate concentration (all from GIBCO, USA) and 5 $\mu$g/ml plasmocin (InvivoGen, CA, USA) at 37 ℃ with 5% $CO_2$ in a fully humidified atmosphere.

### *2.2 Irradiation*

The cell samples were treated with 1, 5 and 10 Gy of gamma rays from an isotopic $^{60}$Co source (42.6 TBq, AECL, Canada at Korea Atomic Energy Research Institute) for 5 min at room temperature, respectively.

### *2.3 Analyses of Apoptosis and Autophagy*

Cells were lysed following irradiation with various doses of gamma rays, and immunoblotted against proteins related to p53-mediated apoptosis. Cell cycle was analyzed using flow cytometry. Caspase-3 activity was measured with an caspase-3/CPP32 colorimetric assay kit (BioVision, USA) and apoptotic features were visualized using DAPI staining under fluorescence microscope. Appearance and accumulation of Acidic Vesicular Organelles (AVO) were detected by cell staining with acridine orange.

## 3. Results

### *3.1 Proteins Related Cell Cycle and Apoptosis*

After irradiation of the cells, cell lysates were isolated at each incubation time. The expression of p53 protein in RKO and RKO E6 cells increased after irradiation in a dose-dependent manner. At 48h post-irradiation, p21 protein levels were up-regulated according to p53 expression in RKO cells. However, increased levels of p21 in RKO E6 cells were not significant. The p53 death signals lead to caspase activation and cleavage of caspase-7 and cleavage of the caspase substrates poly (ADP-ribose) polymerase (PARP, m.w. 89 kDa) were demonstrated in RKO E6, which was on the contrary to expectation. RKO cells did not show drastic increase in apoptosis related proteins.

### *3.2 Cell Cycle Arrest in sub-G1 stage*

An accumulation in G1 phase after treatment of RKO cells with IR was observed. Cells were arrested in G1 accompanied by up-regulation of p21. On the other hand, an increase in sub-G1 population was observed in RKO E6 cells. Any sub-G1 populations were counted as apoptotic cells.

### *3.3 Caspase Activities*

The induction of p53 was dependent on caspase activity. Caspase-3, effector caspase, cleaves various cellular death substrates involved in the regulation and execution of apoptosis. Caspase-3 activity leading to

cleavage of DEVD was higher in RKO E6 cells obtained at 48h after irradiation than RKO cells.

*3.4 Apoptotic Signals*

The induction of cellular apoptosis as measured by DAPI staining showed RKO cells were more resistant to IR than RKO E6 cells. Apoptotic features were significant in RKO E6 cells.

*3.5 Acidic Vesicular Organelles*

As a marker of autophagy, the volume of the cellular acidic compartment was visualized by acridine orange staining. Acridine orange-positive cells with higher bright red fluorescence were frequently detected in RKO cells 48hr after 10 Gy irradiation compared to RKO E6 cells.

## 4. Discussions and Conclusions

Ionizing radiation-induced DNA damage activates "p53→p21 pathway" leading to elevated p53 and p21 and cell cycle arrest, thereby allowing time for DNA repair. These results indicated that p53 and p21 were required to block apoptosis and induce autophagy in RKO cells. The expression of p21 by a p53-dependent mechanism is required to develop autophagic properties after DNA damage.

Results in this study suggest that the radioresistance of the RKO cells was associated with the increased p21 expression, resulting in autophagy induction.

The tumor suppressor p53 could regulate radiosensitivity by inhibiting autophagy and activating apoptosis; the ionizing radiation-induced expression of p53 in the RKO cells regulated autophagy, suggesting the significance of the level of p53 in determining the radiosensitivity by regulating autophagy and apoptosis.

It was found that the role of p53 in the regulation of the radiosensitivity was dependent on different p53 phenotypes, which would read to different outcomes in the radiosensitivity or not. The result might contribute to the understanding of a potential regulatory mechanism of cell death induced by radiation.

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