

## **Ionizing Radiation Promotes the Migratory and Invasive Potential of Lung Cancer Cells by Different Mechanisms**

Jin Nyoung Ho, Ga Young Kang, and Hong-Duck Um\*

Laboratory of Radiation Cancer Research, Korea Institute of Radiological and Medical Sciences, Seoul, Korea

\*Corresponding author: hdum@kcch.re.kr

### **1. Introduction**

Although radiation therapy is a major therapeutic modality for cancer treatment, previous reports have suggested that ionizing radiation (IR) can promote the invasive and metastatic potential of cancer cells [1]. It was consistently reported that IR can induce certain types of matrix metalloproteinases, which are critical to the degradation of extracellular matrix [2-4]. Given that the motility of cancer cells is an additional requirement for their metastasis, this study investigated whether IR can also influence the migratory potential of cancer cells.

### **2. Methods**

#### *2.1 Cell culture*

A549 human lung adenocarcinoma cells were obtained from American Type Culture Collection and grown in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum and gentamicin (50 µg/ml) at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>.

#### *2.2 Irradiation*

Cells were plated in 60-mm dishes and incubated at 37°C under humidified 5% CO<sub>2</sub> and 95% air in culture medium until 70~80% confluent. Cells were then exposed to γ-rays from a <sup>137</sup>Cs γ-ray source [Atomic Energy of Canada] at a dose rate of 3.06 Gy/min.

#### *2.3 Invasion and migration assays*

Invasion of A549 cell was measured by the invasion of cells through Matrigel-coated transwell inserts (Corning Inc., Corning, NY). Briefly, transwell inserts with 8-µm pore size were coated with Matrigel (1 mg/ml). Cells were trypsinized, and 200 µl of cell suspension (2×10<sup>5</sup> cells/ml) were added to the Matrigel-coated chamber; the lower compartment was filled with RPMI 1640 conditioned media (1 ml). After incubation for 16~20 h at 37°C, cells on the surface of Matrigel-coated polycarbonate membrane (non-invading cells) were removed with a cotton swab and the migrating cells remaining on the bottom part of the filters were fixed and stained with Diff-Quick kit (Fisher Scientific, Pittsburgh, PA) and then counted under a microscope. Migration assays were done using the same procedure but with uncoated polycarbonate filters.

### **3. Results**

#### *3.1 IR differentially promotes the migratory and invasive potential of lung cancer cells.*

It was reported that IR can promote the invasiveness of cancer cells [2-4]. To explore whether IR also influences their migratory potential, human A549 lung adenocarcinoma cells were irradiated with various doses of γ-rays. At the end of 24 hr of incubation, their migratory and invasive potentials were compared using uncoated and Matrigel-coated filters, respectively. IR promoted both the migration and invasion of the cancer cells (Fig. 1). However, the optimal doses of γ-rays required for these effects were different: While the promotion of cell migration was observed using γ-rays as low as 2.5 Gy, 10 Gy or higher doses were required to increase the invasive potential. Therefore, the migratory machinery of cancer cells appears to more sensitively respond to IR than the invasive machinery.

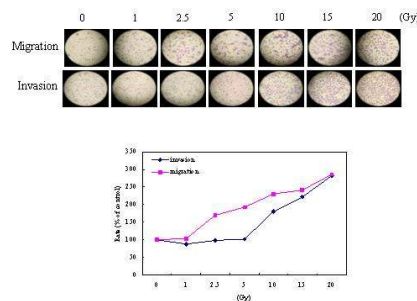


Fig. 1. IR stimulates both the migration and invasiveness of A549 lung cancer cells.

#### *3.2 PI3K mediates the ability of IR to promote the invasion, but not migration, of the cancer cells*

To determine signaling molecules involved in the IR-induced responses, the cells were irradiated with 10 Gy of γ-rays in the presence or absence of SB202190, PD98059, SP600125, and LY294002 which inhibit p38 MAPK, ERK, JNK, and PI3K, respectively. All these inhibitors did not, or minimally, suppress the ability of IR to promote cell migration (Fig. 2A). However, IR-induced cell invasion was effectively attenuated by the addition of LY294002 (Fig. 2B). This effect was not observed using other inhibitors tested. These results

suggest that PI3K is selectively involved in IR-induced invasion, but not migration of the cancer cells.

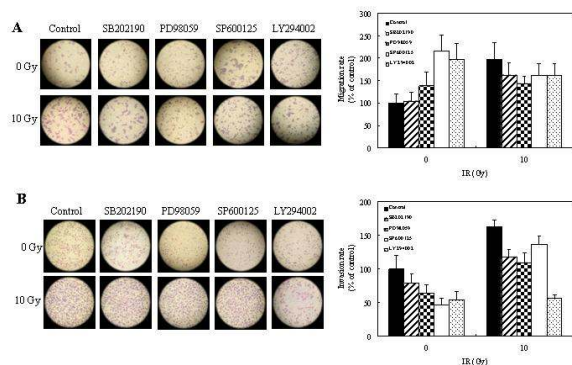


Fig. 2. Effects of specific inhibitors on the migration and invasion of cancer cells. A549 Cells were pretreated with p38 inhibitor (SB202190, 5  $\mu$ M), Erk inhibitor (PD98059, 5  $\mu$ M), JNK inhibitor (SP600125, 5  $\mu$ M), and PI3K inhibitor (LY294002, 5  $\mu$ M) for 30 min, and then irradiated with 10 Gy of  $\gamma$ -rays. At the end of 24 hr of incubation, Migration (A) and Invasion assays (B) were performed.

#### 4. Conclusions

In this study, we showed that IR can increase the migratory potential of cancer cells. This effect could be observed even using the relatively low doses of IR which fail to promote the invasion of the same cell types. Moreover, PI3K was found to selectively mediate IR-induced invasion, but not migration. Taken together, IR appears to promote the migration and invasion of cancer cells by different mechanisms.

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