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Radiation-Induced Differentiation in Human Lung Fibroblast

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1. Introduction

One of the most common tumors in many countries is lung cancer and patients with lung cancer may take radiotherapy. Although radiotherapy may have its own advantages, it can also induce serious problems such as acute radiation pneumonitis and pulmonary fibrosis (1, 2). Pulmonary fibrosis is characterized by excessive production of α-SMA and accumulation of extracellular matrix (ECM) such as collagen and fibronectin.

There has been a great amount of research about fibrosis but the exact mechanism causing the reaction is not elucidated especially in radiation-induced fibrosis. Until now it has been known that several factors such as transforming growth factor (TGF-β), tumor necrosis factor (TNF), interleukin (IL)-1, IL-6, platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) are related to fibrosis. Among them TGF-β with Smad signaling is known to be the main stream and other signaling molecules such as MAPK, ERK and JNK (3) also participates in the process. In addition to those above factors, it is thought that more diverse and complicate mechanisms may involve in the radiation-induced fibrosis. Therefore, to investigate the underlying mechanisms in radiation induced fibrosis, first of all, we confirmed whether radiation induces transdifferentiation in human normal lung fibroblasts.

Here, we suggest that not only TGF-β but also radiation can induce transdifferentiation in human lung fibroblast WI-38 and IMR-90.

2. Materials and Methods

2.1. Cell culture

IMR90 (ATCC CCL186) derived from human fetal lung fibroblast and WI38 (ATCC CCL75) derived from human lung fibroblast were obtained from the American Type Culture Collection (Rockville, MD, USA). IMR90 was grown in MEM medium and WI38 was cultured in RPMI medium supplemented with 10% fetal bovine serum and non-essential amino acid at 37°C in a humidified incubator with 5% CO_2.

2.2. Irradiation

Cells were irradiated with 0, 4, 8 and 16 Gy using a gamma-cell irradiator with 137Cs source (Atomic Energy of Canada, Ltd, Canada).

2.3. Collagen protein measurement

Cells were irradiated and incubated in growth medium. After 3days, cells were collected and examined for the amount of collagen using Sircol soluble collagen assay kit (Biocolor, Belfast, North Ireland)

2.4. Immunofluorescence staining

Irradiated cells were fixed with 3.7% formaldehyde and were stained with mouse anti-human α-smooth muscle actin (α-SMA) monoclonal antibody (Sigma) followed by Alexa Fluor 488 goat anti-mouse antibody (Molecular probes, USA) (1:200).

2.5. Western blot analysis.

Cell lysates were prepared by extracting proteins with RIPA supplemented with protease. Equal amounts of the proteins were separated on 10% SDS-PAGE and transferred to nitrocellulose membranes (Bio-Rad, CA). The membranes were blocked with 5% skim milk in Tris-buffered saline and then incubated with primary antibodies for 1 h at room temperature. Blots were developed by peroxidase-conjugated secondary antibody, and proteins were visualized by enhanced chemiluminescence (ECL) reagents according to the manufacturer's recommendation(Amersham Biosciences, England). The experiments were repeated at least three times.

2.6. Flow cytometric analysis

Cell viability was identified and quantified by flow cytometry with propidium iodide (PI) staining. Both adherent and floating cells were collected after radiation and fixed with 70% ice-cold ethanol overnight at -20°C. Fixed cells were washed twice with ice-cold PBS and incubated with 0.1 mg/ml RNase and 40ug/ml PI solution for 30 min at 37°C. Cells were analyzed by FACScan flow cytometer (Becton Dickinson, USA). Cells in sub-G1 phase were taken as apoptotic cells.

3. Results

3.1. Effects of radiation on cell viability

We first investigated the effects of irradiation on cell viability in human fibroblast cells. To find an optimal condition to induce transdifferentiation, IMR90 and WI38 cells were irradiated with 0, 2, 4, 8 and 16Gy, and then analyzed the cell viability by flow cytometry. The apoptotic cells were expressed in sub-G1 phase.

Based on the results of FACS analysis, the optimal dose that did not affect the cell viability was 4Gy and the cell viability was decreased when the radiation dose increased. Also, cell proliferation decreased in a radiation dose-dependent manner.

3.2. Effects of radiation on α-SMA expression

It has been reported that α-SMA is the key marker in fibrogenesis and during fibroblast transdifferentiation to myofibroblast their expression level increases. Therefore, we examined whether radiation increased α-SMA in human lung fibroblast IMR90 and WI38 cells by immunofluorescence staining and western blotting on 3 and 5 days after radiation. As a result, radiation increased α-SMA levels both in IMR90 and WI38 cells. However, the expression of α-SMA in IMR90 cells were remarkably increased compared to WI38 cells.

3.3. Effects of radiation on ECM synthesis

During transdifferentiation, it is well known that extracellular matrix such as collagen and fibronectin is synthesized and accumulated. Therefore, we evaluated the collagen protein synthesis and fibronectin expression by using sircol soluble collagen assay kit and western blotting. Notably, collagen and fibronectin were markedly increased compared to non-irradiated human fibroblasts suggesting that radiation did induce transdifferentiation from fibroblast to myofibroblast.

4. Conclusion

In this study, we showed that radiation induces transdifferentiaion in IMR90 and WI38 human lung fibroblasts. Although this may not be the first report for radiation-induced transdifferentiaton in fibroblasts, lung fibrogenesis has not been investigated thoroughly.

In the process of fibrosis, the increase of α-SMA expression and synthesis of extracellular matrix is well-characterized phenomena. Likewise, we observed markedly enhanced expression levels in α-SMA and extracellular matrix such as collagen and fibronectin in irradiated fibroblasts. However, α-SMA and ECM synthesis did not increase radiation dose dependently, and even though both IMR90 and WI38 cells were originated from human lung fibroblasts their reactivity and sensitivity to radiation were different. For the IMR90 cells, the optimal radiation dose was 8 Gy, whereas 4 Gy in WI38. In addition, irradiation at a dose of 16 Gy caused steep decrease on viability in WI38 cells but not in IMR90, insisting that IMR90 may be more resistant to radiation.

In conclusion, these results confirm the transdifferentiation in human lung fibroblast induced by radiation and the response seems to be cell dependant.

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