Activation of insulin-like growth factor 1 receptor regulates the radiation-induced lung cancer cell apoptosis

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ABSTRACT

Background and aims: The prevalence of lung cancer is increasing in the recent decades. The underlying mechanism is unclear. The insulin-like growth factor (IGF) and p53 protein are important molecules involving the tumor immunity. This study aims to investigate the role of IGF intervene the radiation-induced lung cancer apoptosis.

Methods: Lung cancer cells were isolated from surgically removed lung cancer tissue. The lung cancer cell lines, A549 cells and H23 cells were irradiated. The expression of IGF1 receptor (IGF1R) by the lung cancer cells, and apoptosis, were assessed by flow cytometry.

Results: The results showed that human lung cancer cells expressed IGF1R. IGF1R played a critical role in the radiation-induced lung cancer cell apoptosis. The histone deacetylase-1 (HDAC1) phosphorylation was up regulated by irradiation. The phosphorylated HDAC1 bound the p53 promoter to inhibit the gene transcription, which was abolished by the presence of an inhibitor of HDAC1 or a STAT3 inhibitor.

Conclusion: The data suggest that activation of IGF1R plays a critical role in the radioresistance, which can be prevented in the presence of the inhibitors of HDAC1 or STAT3 inhibitors.

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1. Introduction

The prevalence of lung cancer was increasing in the last several decades (Milch et al., 2015). The underlying mechanism is unclear. To date, lung cancer has been one of the leading diseases causing human death. The therapeutic effect on lung cancer is unsatisfactory now (Castanon et al., 2014). Thus, it is urgent to find out more effective remedies to treat lung cancer. On the other hand, to elucidate the pathogenesis of lung cancer is also helpful to generate new therapeutic remedies for lung cancer. Radiotherapy is the treatment of cancer and other diseases with ionizing radiation. It is one of the therapeutic remedies for lung cancer. Ionizing radiation deposits energy that injures or destroys cells in the area being treated by damaging their genetic material, making it impossible for these cells to continue to grow. One of the drawbacks of radiotherapy is development of radioresistance. Radioresistance is the property of organisms that are capable of living in environments with very high levels of ionizing radiation (Wang et al., 2014). The precise mechanism of development of radioresistance is not fully understood. Activation of STAT3 in the radioresistant cells was found in laryngeal cancer (Choe et al., 2015). Phosphorylation of STAT3 on serine 727 regulates gene expression and is raised in many cancer cells (Yu et al., 2014). Targeting STAT3 enhances radiosensitivity in head and neck squamous cell carcinoma (Adachi et al., 2012). Yet, the relation between STAT3 and development of radioresistance remains to be further investigated.

The insulin-like growth factor 1 (IGF1) is a member of the growth factor family. IGF1 is a hormone similar in molecular structure to insulin. It plays an important role in childhood growth and continues to have anabolic effects in adults as well as immune cell activities (Yang et al., 2014a,b). IGF1 also interferes with apoptosis (Yang et al., 2014a,b). Since the basic mechanism by which radiation inducing cancer cell death is via the apoptosis pathway, we hypothesize that to enhance IGF1 activities develop radioresistance in cancer cells. To this end, we carried out the study; the results showed that high levels of IGF1 were detected in lung cancer tissue after radiotherapy. Treating lung cancer cells with IGF1 increased activities of STAT3 and histone deacetylase 1 (HDAC1), and inhibited p53 expression.

2. Materials and methods

2.1. Reagents

The antibodies of STAT3, pSTAT3, HDAC1, pHDAC1, IGF1, IGF1R and p53 were purchased from Santa Cruz Biotech (Shanghai, China). The antibody of pIGF1R was purchased from Cell Signal Technology (Shenzhen, China). The protein G, Annexin V kit, butyrate sodium,

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stattic and ChIP kit were purchased from Sigma Aldrich (Shanghai, China). The reagents for RT-qPCR and Western blotting were purchased from Invitrogen (Shanghai, China).

2.2. Cell culture

Human lung cancer cell lines, A549 cells and H23 cells were purchased from ATCC (Beijing, China). The cells were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 0.1 mg/ml streptomycin and 2 mM L-glutamine. The cell viability was greater than 98% before using for further experiments as assessed by Trypan blue exclusive assay.

2.3. Irradiation

Cells were exposed to radiation from a Gammacell 40 137Cs irradiator (dose rate, 0.82 Gy/min) at Central South University (Changsha, China).

2.4. Apoptosis assay

Cells were collected and stained with propidium iodide (PI) and Annexin V kit following the manufacturer's instructions. The cells were analyzed by flow cytometry. The data of "no staining" cells were used as a reference to set the gates. The Annexin V positive or PI positive and Annexin V positive cells were regarded as apoptotic cells.

2.5. Real time quantitative RT-PCR (RT-qPCR)

The total RNAs were extracted from the cells with TRIzol reagent. cDNA was synthesized using a reverse transcription kit. qPCR was carried out in a real time PCR device (MiniOptican, Bio-Rad) with SYBR Green Super Mix. The primers using in the present study include: IGF-1R, forward, tggagtgctgtatgcctctg, and reverse, cccttggcaactccttcata; p53, forward, tggccatctacaagcagtca; reverse, ggtacagtcagagccaacct; β-actin, catccgcaaagacctgtacg; reverse, cctgcttgctgatccacatc. The results were calculated with the deltadelta Ct method and normalized to folds of β-actin.

2.6. Western blotting

The total proteins were extracted from the cells, fractioned by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis), transferred onto a PVDF membrane. After blocking with 5% skim milk for 30 min, the membrane was incubated with the primary antibodies (300 ng/ml) overnight at 4 °C, and followed by the second antibodies (conjugated with peroxidase) for 1 h at room temperature. The membrane was washed with TBST (Tris-buffered saline Tween 20) after each incubation. The immune blots on the membrane were developed with ECL (Enhanced chemiluminescence). The results were photographed with an Image Station (4000 Pro, KODAK). The integrated density of the blots was determined by the software ImageJ.

2.7. Immunoprecipitation (IP)

Protein extracts were incubated overnight at 4 °C with anti-pSTAT3 (1 μg/ml) or anti-pHDAC1 (1 μg/ml), and normal rabbit IgG (1 μg/ml; as a control), respectively. Then, samples were incubated with protein G Sepharose 4B conjugate beads for 3 h at 4 °C. After washing and centrifugation, Immunoprecipitated proteins were eluted from the beads with Laemmli buffer and immunoblotted using the indicated primary antibodies.

2.8. Chromatin immunoprecipitation (ChIP)

The cells were fixed in 1% formaldehyde, and lysed. The fixed lysates were sonicated and precleared with Dynabeads Protein A (Invitrogen, Shanghai, China) and immunoprecipitated overnight at 4 °C with antibodies against pHDAC1 or pSTAT3 or non-specific IgG. The samples were treated with Proteinase K (45 °C for 2 h) in a buffered salt solution. DNA was purified with a ChIP purification kit (Invitrogen, Shanghai, China). Primers (gcctggaacactcttctcct and ggtggtaggattgggaggac) of p53 promoter sites were used to assess transcription factor pull-down of DNA via qPCR. An input control sample (sheared chromatin collected before antibody addition, equivalent to 1% of IP sample volume) was processed in parallel to standardize pull-down efficiencies.

2.9. Statistics

The data are presented as mean ± SD. Differences between two groups were determined by Student t test or ANOVA if more than two groups. A $p < 0.05$ was set as a significant criterion.

3. Results

3.1. Radiation increases IGF1 receptor (IGF1R) expression in lung cancer cells

Since IGF1 plays a role in development of adaptive radioprotection (Osuka et al., 2013), Inhibition of IGF1R can modulate activities of lung cancer cells (Nurwidya et al., 2014), we inferred that the IGF1/IGF1R interaction might develop radioresistance in lung
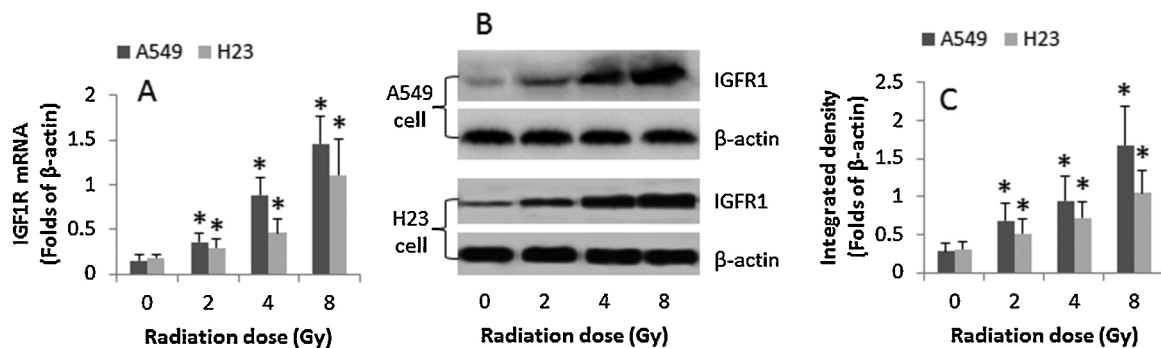

Fig. 1. **Radiation increases expression of IGF1R in lung cancer cells**. Lung cancer cells (A549 and H23) were exposed to X-ray irradiation at gradient doses for 4 h. The cells were collected and analyzed by RT-qPCR and Western blotting. A, the bars indicate the mRNA levels of IGF1R. B, the Western blots indicate the protein levels of IGF1R. C, the bars indicate the integrated density of the blots of panel B. The data of bars are presented as mean ± SD. *, $p < 0.01$, compared with the dose "0" group. The data are representatives of 3 independent experiments.