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Original article

Regulator of G protein signaling 20 contributes to radioresistance of non-small cell lung cancer cells by suppressing pyroptosis

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ABSTRACT

Objective: To investigate the potential role of the regulator of G protein signaling 20 (RGS20) in radioresistance of non-small cell lung cancer (NSCLC).

Methods: A total of 35 lung adenocarcinoma (LUAD) patients from The Cancer Genome Atlas (TCGA), who underwent radiotherapy, were enrolled and divided into radiosensitive ($n = 16$) and radioresistant ($n = 19$) groups based on clinical prognosis. The expression and prognosis of RGS20 were analyzed by Gene Expression Profiling Interactive Analysis (GEPIA) database. A radioresistant cell line (A549R) was constructed by irradiating A549 cells with 6 Gy X-rays for 10 fractions. Cell survival was measured by colony formation assay. The regulatory effect of RGS20 on pyroptosis were verified by LDH release and Western blot assay, and the underlying mechanism was investigated by transfecting RGS20 siRNA and applying a GSDMD inhibitor).

Results: A total of 2,181 differentially expressed genes (DEGs) were identified by analyzing the data of radiosensitive and radioresistant individuals from the TCGA-LUAD dataset. These DEGs were enriched in G alpha (z) signalling events analyzed by Reactome database. RGS20 exhibited significant upregulation among the DEGs, and its higher expression predicted poor prognosis in LUAD patients. In vitro, the expression of RGS20 protein was increased by irradiation in A549 cells, whereas it remained at much high levels in A549R cells regardless of irradiation. After irradiation, the expressions of pyroptosis-related proteins were significantly increased in A549 cells ($P < 0.05$), with no significant changes were observed in A549R cells. Treatment with LDC7559 significantly reduced LDH release ($P < 0.01$) and improved the survival rate of irradiated A549 cells ($P < 0.01$). Furthermore, knockdown of RGS20 gene in A549R cells significantly increased LDH release ($P < 0.001$) and enhanced radiosensitivity ($P < 0.01$), while LDC7559 administration reversed LDH release ($P < 0.01$) and radiation-induced cell death increased by siRGS20 ($P < 0.05$). Meantime, the increased expression level of GSDMD-NT was observed in A549 and A549R cells transfected with siRGS20 ($P < 0.05$).

Conclusion: RGS20 contributes to the radioresistance of NSCLC cells, which might be a potential target for NSCLC radiotherapy.

1. Introduction

Lung cancer remains a leading cause of cancer-related mortality globally, and non-small-cell lung cancer (NSCLC) accounts for approximately 85% of all cases.[1] The majority of patients are diagnosed in advanced stages, missing the optimal window for surgical intervention.[2] For advanced-stage NSCLC patients, radiotherapy is one of the mainstream modalities. With advancements in treatment technologies, such as stereotactic radiotherapy, intensity modulated radiotherapy, proton and heavy ion radiotherapy, as well as ultra-high dose rate (FLASH) radiotherapy, NSCLC can be effectively managed, leading to improved overall survival rate of patients. However, tumor radioresistance limits the efficacy of radiotherapy, often resulting in local recurrence.

RGS20, a member of the regulator of G protein signaling (RGS) family that acts as GTPase-activating protein (GAP), accelerates GTP hydrolysis on the α-subunits of heterotrimeric G proteins and results in termination of signaling pathways downstream of G protein-coupled receptors (GPCRs).[3] Through its regulatory role in GPCR signaling, RGS20 has been

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