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Original Research Article

Small molecule inhibitor azd1480 reverses radiotherapy resistance in NSCLC by targeting JAK2/STAT3 pathway

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Abstract

Purpose: *To investigate the effect of small molecule inhibitor AZD1480 on radiotherapy resistance in non-small cell lung cancer (NSCLC), and the involvement of Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway in the process.*
Methods: *Radiation-resistant cell lines A549-20F, A549-30F, A549-40F and H460, H46040F, H460-40F, and H460-40F were established, and expressions of proteins related to JAK/STAT pathway, mitogen-activated protein (MAPK) pathway and transforming growth factor-β (TGF-β) were assayed. The JAK2V617FH460 overexpression cell line and JAK2H460 cell line were established, and expressions of ATGL and CPT1A were compared. The H460 cells and H460-40F cells were treated with JAK2 small molecule inhibitor AZD1480, and the expressions of ATGL, CPT1A and JAK2/STAT3 pathway-related proteins were compared. The survival and proliferation of cell lines were also compared.*
Results: *The JAK/STAT pathway was significantly enriched, and MAPK and TGF-β were up-regulated. In H460 cells, JAK2/STAT3 route was obvious, suggesting that radiotherapy activated JAK2/STAT3 pathway in NSCLC cells. Significant down-regulations of p-JAK2Y1007, JAK2, p-STAT3s727, STAT3, ATGL and CPT1A proteins expressions were seen in H460 + AZD1480 group, relative to H460 group, but protein levels of p-JAK2Y1007, JAK2, p-STAT3s727, STAT3, ATGL and CPT1A were significantly lower in H460-40F + AZD1480 group than in H460-40F group ($p < 0.05$). The survival and proliferation rates were significantly lower in A549 + AZD1480 group than in A549 and A549-40F groups ($p < 0.05$).*
Conclusion: *Radiotherapy up-regulates the expressions of ATGL and CPT1A in NSCLC cells by activating the JAK2/STAT3 pathway, while AZD1480, a small molecule inhibitor, reverses the radiation resistance of NSCLC by targeting JAK2/STAT3 pathway and key enzymes of lipid metabolism. Therefore, azd1480 may enhance clinical treatment efficacy in NSCLC patients by reducing radiation resistance.*

Keywords: *AZD1480, JAK2, STAT3, NSCLC, Radiotherapy resistance*

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INTRODUCTION

Lung carcinoma ranks among the most frequently occurring neoplasms worldwide, particularly NSCLC. The clinical prognosis of NSCLC patients is poor due to late diagnosis, which results in loss of the best opportunity for treatment [1,2]. Moreover, advancements in

medical techniques, in addition to chemoradiotherapy, targeted therapy, immunotherapy and other combined therapies provide newer directions for the clinical treatment of patients with advanced NSCLC, with good therapeutic effects [3]. Radiotherapy plays an important role in the whole course of treatment of NSCLC. However, local recurrence and disease progression caused by radiotherapy resistance are still major causes of treatment failure [4]. Therefore, it is particularly important to enhance the clinical treatment efficacy in NSCLC patients by reducing radiation resistance.

The Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway activation induce tumor formation and progression [5]. In addition, studies have confirmed that JAK/STAT3 pathway participates in the control of Warburg effect, lipogenesis and many other metabolism-related links, as well as immunotherapy tolerance by influencing immune monitoring [6]. It has been reported that the activation of fatty acid synthesis through the leptin-JAK/STAT3 pathway induces chemotherapy resistance in breast cancer stem cells [7]. However, not much is known about the role of the JAK/STAT3 pathway in the control of radiotherapy tolerance in NSCLC. The present study was aimed at investigating the influence of small molecule inhibitor AZD148 on radiotherapy resistance in NSCLC, and the involvement of the JAK2/STAT3 pathway in the process.

EXPERIMENTAL

Materials

Human lung adenocarcinoma cell line A549 was purchased from Shenzhen Haodi Huatuo Biotechnology Co. Ltd. Large cell lung cancer cell line H460 was bought from Shanghai Bohu Biotechnology Co. Ltd.

Reagents

Opti-MEM was bought from Shanghai Xinfan Biotechnol. Co. Ltd; siRNA fragment was bought from Baiao Maike Biotechnol. Co. Ltd, while AK2V617F was bought from Beijing Baiaolaibo Technology Co. Ltd. Wuhan Feien Biotechnology Co. Ltd was the source of STAT3 antibody; SYBR Green was product of Shanghai FanTai Biotechnol. Co. Ltd; BCA protein quantitative kit was obtained from Nanjing Novozan Biotechnol. Co. Ltd, while TRIzol was purchased from Shanghai Xige Biotechnol. Co. Ltd. Protein lysis kit was bought from Shanghai Xinyu Biotechnology Co. Ltd.

Procedures

A radiation-resistant cell line model was established. For this purpose, A549 and H460 cell lines were digested and passaged. The cells were exposed to x-rays at logarithmic growth stage. After experiencing radiation injury and growing again, the cells received X-ray irradiation again at repeated irradiation of 20, 30 and 40 cycles. The total radiation dose received by the cells was 40, 60 and 80 Gy, respectively. After passage to the third generation, the cells were cryopreserved. The successfully established radiation-resistant cell lines of A549 and H460 were tagged A549-40F and H460-40F, respectively. The KEGG pathway was analyzed via transcriptional sequencing of differential genes of radiation-resistant and parental cell lines, and the expressions of proteins of the JAK/STAT pathway, MAPK pathway, and TGF-β pathway in A459, A459-40F, H460, and H460-40F cell lines were determined.

Next, JAK2V617FH460 overexpression cell line (JAK2V617F group) and siRNA interference JAK2H460 cell lines (JAK2-#1 group and JAK2-#2 group, respectively) were established and the respective negative control group, vector group and scrambled group were set up. The expressions of triglyceride lipase (ATGL) and carnitine palmitoyl transferase 1A (CPT1A) were determined using Western blot assay. The H460 cells and H460-40F cells were treated with JAK2 small molecule inhibitor AZD1480 and the expression levels of ATGL, CPT1A and JAK2/STAT3 pathway-related proteins (p-JAK2Y1007, JAK2, p-STAT3S727 and STAT3) were determined using Western blot assay.

The A459 cells and A459-40F cells were treated with AZD1480, and the survival and proliferation of each cell line were determined with cloning test and EdU cell proliferation test.

Statistical analysis

Data were analyzed with SPSS 23.0. Measurement data in line with normal spread are shown as mean ± SD. Multiple groups were compared with 1-way ANOVA, while 2 groups were compared with SNK-q test. Statistical significance was assumed at $p < 0.05$.

RESULTS

Expression of JAK2/STAT3 in H460 cells and radiotherapy resistant cell lines

The JAK/STAT pathway as well as the MAPK and TG pathways were enriched. The JAK/STAT

pathway was the most obvious in H460 cells, suggesting that radiotherapy activated the JAK2/STAT3 pathway in NSCLC cells (Figure 1).

Expression levels of ATGL and CPT1A

In H460 cells, ATGL and CPT1A protein expression levels in JAK2V617F group were significantly higher than those in the vector group ($p < 0.05$). In contrast, in H460-40F cells, ATGL and CPT1A protein levels in JAK2-#1 and JAK2-#2 groups were significantly lower than the corresponding levels in scramble group ($p < 0.05$; Table 1).

Table 1: Levels of ATGL and CPT1A after overexpression/knockout of JAK2 in H460 and h460-40f cell lines

Group	ATGL	GPT1A
Vector	1.06±0.05	1.01±0.06
JAK2V617F	1.78±0.15	1.59±0.21
T	20.364	11.876
P-value	< 0.001	< 0.001
Scramble	1.02±0.12	1.01±0.12
JAK2-#1	0.23±0.05[a]	0.34±0.04[a]
JAK2-#2	0.25±0.06[a]	0.26±0.05[a]
F	593.85	550.16
P-value	< 0.001	< 0.001

***Note*:** [a]$P < 0.05$, vs. vector group; [b]$p < 0.05$, vs. scramble group

Effect of AZD1480 on the expressions of JAK2/STAT3 pathway and target ATGL and CPT1A in lipid metabolism

There were marked down-regulations of p-JAK2Y1007, JAK2 p-STAT3S727, STAT3, ATGL and CPT1A in H460 + AZD1480 group, relative to H460 group ($p < 0.05$). Moreover, there were significantly lower expressions of p-JAK2Y1007, JAK2, p-STAT3S727, STAT3, ATGL and CPT1A in H460-40F+AZD1480 group than in H460-40F group ($p < 0.05$; Table 2).

Survival rate after exposure to AZD1480

The survival rate of A549 + AZD1480 group was significantly lower than that of A549 group ($p < 0.05$). However, the survival rate of A549-40F + AZD1480 group was significantly lower than that of A54940F group (p < 0.05; Table 3).

Proliferation of radiation-resistant cells after exposure to AZD1480

Cell proliferation rate was significantly lower in A549 + AZD1480 group than in A549 group ($p < 0.05$). However, the proliferation rate was significantly lower in A549-40F + AZD1480 group than in A549-40F group ($p < 0.05$). These results are shown in Table 4.

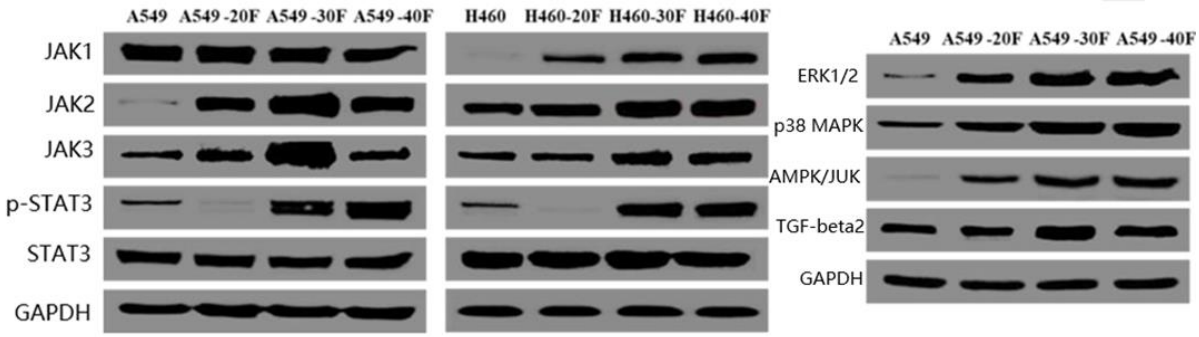

Figure 1: Expressions of JAK2/STAT3-associated proteins in radiotherapy-resistant cell H460

Table 2: Effect of AZD1480 on the expressions of JAK2/STAT3 pathway-associated proteins and ATGL and CPT1A

Group	p-JAK2Y1007	JAK2	p-STAT3S727	STAT3	ATGL	CPT1A
A459	1.16±0.15	1.05±0.24	1.02±0.05	1.04±0.16	1.05±0.08	1.01±0.03
A459+AZD1480	0.45±0.12	0.43±0.15	0.46±0.12	0.48±0.11	0.47±0.12	0.46±0.11
t	16.529	9.796	19.264	12.898	17.985	21.572
P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
A459-40F	1.15±0.21	1.21±0.21	1.08±0.16	1.14±0.15	1.16±0.21	0.18±0.16
A459-40F+AZD1480	0.51±0.18	0.52±0.14	0.58±0.12	0.56±0.12	0.54±0.11	0.57±0.08
t	10.348	12.226	11.180	13.503	11.696	15.250
P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Note: [a]$P < 0.05$, vs. H460 group; [b]$p < 0.05$, vs. H460-40F

Table 3: Survival rates of radiation-resistant cells after exposure to AZD1480

Group	Survival rate (%)
A459	0.45±0.16
A459+AZD1480	0.28±0.13
T	3.687
P-value	< 0.001
A459-40F	0.69±0.25
A459-40F+AZD1480	0.34±0.13
T	5.554
P-value	< 0.001

Note: [a]*P* < 0.05, vs. A459 group; [b]*p* < 0.05, vs. A459-40F group

Table 4: Proliferation of radiation-resistant cells after exposure to AZD1480

Group	Proliferation rate (%)
A459	0.35±0.15
A459+AZD1480	0.24±0.14
t	2.397
P-value	0.021
A459-40F	0.78±0.22
A459-40F+AZD1480	0.28±0.11
t	9.090
P-value	< 0.001

Note: [a]*P* < 0.05, vs. A459 group; [b]*p* < 0.05, vs. A459-40F group

DISCUSSION

Abnormal activation of intracellular signaling pathways induces malignant characteristics of tumor cells. The JAK/STAT pathway is present in all cells, and abnormal activation of JAK and its related transcription factor STAT3 are important characteristics of many types of human solid tumors [8]. The JAK/STAT pathway is closely related to the resistance of anti-tumor therapy. Studies have shown that JAK/STAT pathway regulates the characteristics of tumor stem cells, for example, chemoresistance of myxoid liposarcoma [9].

The JAK/STAT3 pathway induces tumor characteristics such as proliferation and angiogenesis. In addition, since it blocks anti-tumor immunity, blocking this pathway will be beneficial to cancer patients [10,11]. In particular, JAK-specific small molecule inhibitors have been effectively used in the treatment of hematopoietic malignancies with good outcomes [12]. The targeting of intracellular signaling pathways is an important measure in the search for and development of new drugs. Many drugs that act on signaling pathways have been applied in clinical practice, e.g., drugs targeting JAK2 mutation in myeloproliferative tumors [13,14].

Lu *et al* [15] reported that AZD1480, a JAK1/2 inhibitor, enhanced cell apoptosis, blocked cell proliferation and inhibited the growth of canine B-cell lymphoma [15]. Ding *et al*. demonstrated that the activation of extracellular signal-regulated kinase 1 and 2 (ERK1/2) and JAK2 signal in lung cancer cells enhanced the transformation of epithelial cells to mesenchymal cells, as well as cell invasion and migration and that these processes were reversed by many small molecule inhibitors such as PD98059 and AZD1480 [16].

Radiation resistance and parental cell line transcription sequencing of differential genes were used to study the KEGG pathway so as to unravel the regulatory mechanism of lipid metabolism in NSCLC cells. It was found that JAK/STAT pathway was significantly enriched, and the MAPK and TGF-β pathways were also enriched as well. Enrichment of the JAK2/STAT3 route was most prominent in H460 cells, suggesting that radiotherapy activated this signal route in NSCLC cells. The activity of triglyceride lipase is controlled by co-activator and blocker G0/G1 switch [17,18]. Moreover, CPT1A, the key rate-limiting enzyme in fatty acid oxidation is abnormally present in several tumors, and a correlation between CPTIA overexpression and radiation resistance of nasopharyngeal carcinoma has been demonstrated [19,20]. To further investigate whether JAK2/STAT3 pathway is involved in the regulation of ATGL and CPT1A (the key targets of lipid metabolism), JAK2 gene was overexpressed and knocked out in H460 cells. It was revealed that the expressions of ATGL and CPT1A in JAK2V617FH460 cells were significantly increased. In contrast, the expressions of ATGL and CPT1A were significantly down-regulated after JAK2 gene knockout, suggesting that JAK2/STAT3 pathway may be involved in regulating the expressions of ATGL and CPT1A.

A small molecule inhibitor of JAK2, i.e., AZD1480 was used to treat H460 and H460-40F so as to determine its effect on lipid metabolism targets. The results showed that the expressions of JAK2, p-JAK2, STAT3 and p-STAT3 in H460 and H460-40F cells were significantly blocked, and the expressions of ATGL and CPT1A were also decreased. Since AZD1480 blocked lipid metabolism in NSCLC cells, further studies were carried out to determine if it enhanced radio-sensitivity of the radiation-resistant cells. The results showed that AZD148 significantly inhibited the proliferation and survival of radiation-resistant cells, indicating that it played a radio-sensitizing role.

CONCLUSION

Radiotherapy upregulates the expression of ATGL and CPT1A by activating JAK2/STAT3 pathway in NSCLC cells. Small molecule inhibitor AZD1480 increases sensitivity to radiotherapy by targeting JAK2/STAT3 pathway and key enzymes of lipid metabolism, thereby reversing the radiotherapy resistance of NSCLC. Therefore, azd1480 may enhance treatment efficacy in NSCLC patients by reducing radiation resistance.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. Zhongmin Deng and Guijun Wei designed the study, supervised the data collection, and analyzed the data. Lei Qiu interpreted the data and prepared the manuscript for publication. Huifei Lu supervised the data collection, analyzed the data and reviewed the manuscript draft.

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