



Nodakenin Inhibits AURKA-CXCL5 Axis Induced Autophagy and Apoptosis in Non-Small Cell Lung Cancer and Promotes Radiosensitivity of Cancer Cells

Xiaoming Wang¹, Rui Tian², Kunpeng Duan³ and Xu Zhang^{4*}

¹Department of Western Medicine Pharmacy, Baoding No. 1 Central Hospital, 071000, Baoding, china

²Department of Oncology, Hospital of the People's Liberation Army: 82nd Group Army, Baoding, 071000, China

³General Surgery, Sanhe City Hospital in Hebei Province, Sanhe, 065200, China

⁴Department of Ultrasound, Affiliated Hospital of Hebei University, Baoding, 071000, China

Xiaoming Wang and Rui Tian contributed equally to this work as co-first author.

ABSTRACT

The objective of this study was to explore the role of nodakenin inhibiting AURKA-CXCL5 induced autophagy, cell death and promoting radiosensitivity in non-small cell lung cancer (NSCLC). The expression of AURKA-CXCL5 was determined in HBE, A549, H460 and H1299 cells. H460 cultured cells were divided into two groups: the nodakenin group (experimental group), and the control group. AURKA and CXCL5 mRNA expressions were detected by qRT-PCR in HBE and NSCLC cell lines. qRT-PCR was applied to detect the effect of *Angelica decursiva* on AURKA/CXCL5. LC3-II, P62 and the apoptosis-related factor expression were analyzed by Western blotting. The cell migration and invasion ability was detected by transwell assay. After the cells were irradiated with 4Gy and 8Gy for 24h, the sensitivity of the cells to radiotherapy was detected by MTT assay. Compared with HBE cells, AURKA and CXCL5 mRNA expressions were increased in A549, H1299 and H460 ($P < 0.05$). The AURKA and CXCL5 mRNA expressions in the experimental group were lower than in the control group ($P < 0.05$). Compared with the control group, the LC3-II in the experimental group increased ($P < 0.05$). The P62 protein expression in the experimental group was declined ($P < 0.05$). Compared with the control group, the Bax and Caspase-3 in the experimental group was added ($P < 0.05$). The Bcl-2 in the experimental group declined ($P < 0.05$). The number of cell migration and invasion in the experimental group decreased compared with the control group ($P < 0.05$). After the cells were irradiated with 4Gy and 8Gy for 24h, the cell viability of the experimental group was lower than the control group ($P < 0.05$). Nodakenin regulates autophagy and apoptosis of NSCLC by inhibiting the activation of the AURKA-CXCL5 axis, enhancing the radiosensitivity.

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Authors' Contribution

XW and RT conducted the experiments in this study. KD and XZ contributed to the design and interpretation of the current study and wrote the article. All authors read, revised, and approved the final manuscript.

Key words

Non-small cell lung cancer, Nodakenin, AURKA/CXCL5, Autophagy death, Radiosensitivity

INTRODUCTION

The incidence and mortality rate of lung cancer are both high (Lv *et al.*, 2021; Khan *et al.*, 2018). Non-small cell

lung cancer (NSCLC) has slow cell division and relatively late spread and metastasis, accounting for 85-90% of lung cancer. Autophagy is a process of cell self-degradation that plays a complex regulatory role in the development of tumors. In tumor cells, autophagy can promote cell survival, resist external stress and drug therapy (Feng *et al.*, 2019; Li *et al.*, 2019). However, when autophagy is excessively active, it may also lead to cell death, which to some extent has anti-tumor effects. Therefore, in-depth study on the regulation and mechanism of autophagy in lung cancer is crucial for discovering new therapeutic targets and strategies. Aurora kinase A (AURKA) is an important cell cycle regulatory protein (Ondrej *et al.*, 2020). It has important implications in cell mitosis and

* Corresponding author: xuzhang0312@163.com
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centrosome formation. The abnormal expression has been associated with the occurrence and development of various tumor types, including NSCLC. Meanwhile, C-X-C chemokine 5 ligand (CXCL5) is an inflammatory factor that has been over expressed in various cancers, which is associated with tumor growth, spread, and drug resistance (Chen *et al.*, 2019). However, the interrelationships between AURKA and CXCL5 in NSCLC and their role in regulating autophagy and tumor therapy are still unclear. Nodakenin, as a natural product, has rich pharmacological activities in various diseases, including anti-inflammatory, anti-tumor, and promoting radiosensitivity (Kong, 2020; Cui *et al.*, 2021). In recent years, according to some studies, nodakenin may affect the survival and proliferation of tumor cells through various pathways, including regulating autophagy and apoptosis pathways (Ma *et al.*, 2019). However, the specific action mechanism in NSCLC and the relationship with the AURKA-CXCL5 axis are still not fully understood. Therefore, the potential mechanism of nodakenin in the NSCLC treatment is deeply explored, with special attention to the regulatory effect on the AURKA-CXCL5 axis, and whether it can induce autophagic cell death and improve the sensitivity of NSCLC to radiation therapy. It is expected to provide new ideas and possibilities for the treatment of NSCLC, providing patients with more effective treatment options, thereby improving survival rate and quality of life.

MATERIALS AND METHODS

Experimental cells

HBE, A549, H460, and H1299 cells collected from ATCC were placed in RPMI-1640 medium with 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin at 37°C, 5% CO₂, and saturated humidity.

The H460 cultured cells were divided into nodakenin group (experimental group, EG) in which cells were treated with nodakenin solution (DMSO solvent), and control group (CG) in which H460 was mixed with DMSO solvent.

Quantitative PCR (qRT-PCR)

Based on the instructions (American Invitrogen Company, AIC), RNA was extracted with Trizol reagent (AIC), cDNA was prepared and qPCR analysis was performed. Relative RNA with the 2^{-ΔΔCt} was calculated, and normalized with GAPDH.

Western blotting

Cells were lysed using RIPA lysis buffer (Servicebio). The supernatant was collected and centrifuged at 4°C (12000 rpm, 30 min). The protein was separated on 10% or

15% PAGE and transferred to the Bio-Rad. TBS-Tween 20 treatment membrane containing 5% skim milk was used to cover and incubate overnight at 4°C. Western blotting (WB) was applied to detect protein expression. The following antibodies were used, including anti-LC3-II, anti-p62, anti-Bax, anti-Caspase-3, anti-Bcl-2. The membrane was washed with phosphate buffer solution (PBS). Servicebio was incubated with membrane at room temperature for one hour. Then it was washed with PBS. Lastly, enhanced chemiluminescence imaging was performed.

Migration and invasion analysis

Cells were inoculated into a six well plate. 400μL with 10% FBS was added to the six hole plate culture medium for migration experiments. 5×10⁴ cells were inoculated at 200μL serum-free RPMI-1640. For intrusion testing, Matrigel was added coating to the transwell cavity. 400μL with 10% fetal bovine serum was added to the bottom chamber. 2.0×10⁵ cells were added to the upper chamber with 200μL serum-free RPMI-1640. After cultivation for 24 h, the migrating and invading cells were fixed and stained with crystal violet, and then observe them.

Clonal cell survival assay

MTT assay was applied to detect the cell viability of H460 after exposure to radiation. The cells were cultured on 96 well plates at a concentration of 2×10³. The cells were incubated with 5% CO₂ at 37°C for 24 h. Then they were irradiated with 4 and 8Gy doses for 24, 48 and 72 h (5 wells). 4 h before the end of incubation, 20μl MTT was added, and then incubated for 4 h. After incubation, the density of each well plate was measured using a microplate reader at the detection wavelength of 570 nm.

Statistical analysis

All data are expressed in mean ± SD. The data were analyzed using SPSS 19.0 software. The unpaired two tailed Student's t-test was applied to evaluate the two groups. P<0.05 means a significant difference.

RESULTS

The HBE, AURKA and CXCL5 mRNA expressions in NSCLC cell were detected by qRT-PCR. Compared with HBE cells, the AURKA and CXCL5 mRNA expressions in A549, H1299 and H460 were increased (P<0.05). The AURKA/CXCL5 were up-regulated in NSCLC, as shown in Table I.

The effect of nodakenin on AURKA/CXCL5 axis expression was detected by qRT-PCR. The AURKA and CXCL5 mRNA expression in the EG was lower to the CG (P<0.05). Nodakenin inhibited the AURKA/CXCL5

expression, as shown in Table II.

The LC3-II and P62 was analyzed by WB. The LC3-II in EG exceeded the CG ($P<0.05$). The P62 protein expression in EG was lower than that in the CG ($P<0.05$), suggesting that nodakenin could activate autophagy in NSCLC.

Table I. The effect of nodakenin on AURKA/CXCL5 mRNA expression in NSCLC ($\bar{x}\pm s$).

Cells	AURKA	CXCL5
HBE	51.99 $\pm$ 4.75	84.34 $\pm$ 5.01
A549	39.96 $\pm$ 7.76	74.63 $\pm$ 4.48
H1299	50.12 $\pm$ 4.28	88.48 $\pm$ 3.01
H460	55.21 $\pm$ 2.96	88.54 $\pm$ 3.33
F	13.005	9.561
P	0.004	0.013

Table II. The effect of nodakenin on AURKA/CXCL5 axis (as detected by qRT-PCR), LC3-II and P62 protein (as detected by western blotting), cell migration and invasion (as detected by transwell assay) and radio-sensitivity (as detected by MTT assay) ($\bar{x}\pm s$).

Genes	Control group (CG)	Experimental group (EG)	t	P
AURKA/CXCL5 axis				
AURKA	1.97 $\pm$ 0.18	1.12 $\pm$ 0.03	11.665	0.014
CXCL5	2.01 $\pm$ 0.19	1.09 $\pm$ 0.02	9.103	0.028
LC3-II	1.12 $\pm$ 0.05	1.92 $\pm$ 0.15	15.822	0.017
P62	1.08 $\pm$ 0.08	1.97 $\pm$ 0.17	11.271	0.023
Bax	1.14 $\pm$ 0.08	1.85 $\pm$ 0.13	10.261	0.021
Caspase-3	1.07 $\pm$ 0.03	1.95 $\pm$ 0.18	13.251	0.018
Bcl-2	1.84 $\pm$ 0.13	1.08 $\pm$ 0.05	11.635	0.006
Cell migration	186.34 $\pm$ 23.59	115.57 $\pm$ 16.31	12.514	0.034
Cell invasion	139.44 $\pm$ 13.75	75.28 $\pm$ 10.66	9.201	0.027
Radio-sensitivity				
4Gy	17.65 $\pm$ 5.27	10.23 $\pm$ 3.44	10.354	0.003
8Gy	4.52 $\pm$ 1.35	0.57 $\pm$ 0.16	13.216	0.015

The expression of apoptosis related factors was analyzed by WB. The Bax and Caspase-3 protein expression in EG exceeded the CG ($P<0.05$). The Bcl-2 protein expression in EG was below the CG ($P<0.05$), suggesting that nodakenin could promote autophagy and apoptosis, as shown in Table II.

Transwell test was applied to detect the cell migration and invasion ability. The cell migration and invasion in EG

was below the CG ($P<0.05$), suggesting that nodakenin inhibited cell migration and invasion, as displayed in Table II.

MTT assay was applied to detect the radiosensitivity of cells. After 4Gy and 8Gy irradiation and culture for 24 h, the cell viability in the EG was below the CG ($P<0.05$), suggesting that nodakenin increased the radiosensitivity of cells, as shown in Table II.

DISCUSSION

Aurka and CXCL5 are key molecules in NSCLC. They regulate each other through the formation of AURKA-CXCL5 axis, playing an important implication in the NSCLC (Wang *et al.*, 2019). Aurka is a protein kinase, which is mainly involved in the regulation of cell division. It plays an important role in the G2/M phase of the cell cycle. By promoting the preparation before division and the formation of mitotic point, it can promote the transition of cells to division phase. The over expression of AURKA is related to the unrestrained growth and malignant transformation of tumor cells. CXCL5 is a chemokine belonging to the C-X-C chemokine family. It is significant for inflammation, immune response and tumor microenvironment. CXCL5 overexpression is associated with the tumor growth, metastasis and invasion. AURKA can directly or indirectly regulate the CXCL5 (Lee *et al.*, 2022). Specifically, AURKA can indirectly regulate the CXCL5 expression by affecting the activity of transcription factors. Meanwhile, AURKA can indirectly affect the release and biological activity of CXCL5 by affecting the cell cycle. Therefore, there is a complex interaction between AURKA and CXCL5 (Pandey *et al.*, 2020). The regulation of AURKA-CXCL5 axis in NSCLC is mainly to promote tumor growth, invasion and metastasis. The AURKA overexpression leads to the up regulation of CXCL5. It can promote the development of tumor by accelerating the chemotaxis, angiogenesis and anti-apoptosis of tumor cells. This regulatory mechanism is helpful to explain the malignant characteristics and treatment resistance of NSCLC. Nodakenin, also known as echinacoside, is a bioactive compound that exists naturally in some plants. It is usually classified as a flavonoid compound. It is most common in Echinacea and other plants. Echinacea is a plant extensively applied in traditional medicine and herbal medicine. It has a variety of medicinal properties (Xiong *et al.*, 2022). This study focuses on the potential mechanism of nodakenin in the treatment of NSCLC. Analyzing the regulation of AURKA-CXCL5 axis, the effects of autophagy and apoptosis pathways, the weakening of cell migration and invasion, and the improvement of radiosensitivity, the role

of nodakenin in the treatment of NSCLC can be better learned. The high expression of AURKA-CXCL5 axis in NSCLC suggests the importance in tumor development. AURKA is a protein related to cell cycle regulation. The abnormal expression is related to many cancers. CXCL5 is related to tumor growth and metastasis. These results highlight the possible key role of AURKA-CXCL5 axis in NSCLC. Nodakenin can significantly reduce the expression of AURKA and CXCL5, especially in NSCLC cells. This may be achieved by directly affecting the transcription or translation of AURKA and CXCL5. This finding provides a new idea for using nodakenin to interfere with AURKA-CXCL5.

According to the study, the LC3-II increased and the P62 decreased under the treatment of nodakenin. These changes suggested that nodakenin promoted autophagy. LC3-II is usually used as a marker of autophagy, and its rise indicates the activation of autophagy. P62 is a protein associated with autophagy degradation. The decrease of P62 indicates that the autophagy process may lead to the degradation of P62. For the effect of Bax, Caspase-3 and Bcl-2, the Bax and Caspase-3 increased, while the Bcl-2 decreased under the treatment of nodakenin. These changes suggested the activation of apoptosis. Bax is a pro apoptotic protein, and its rise may help to induce apoptosis. Caspase-3 is the executor of apoptosis, and the activation is a key step in the apoptosis. On the contrary, Bcl-2 is usually regarded as an inhibitor of apoptosis. The decline may make cells easier to enter apoptosis. Studies have found that the nodakenin can increase the autophagy. Autophagy is a protective mechanism within cells, which is usually used to remove damaged or abnormal proteins and organelles to maintain cell homeostasis (Yuwen *et al.*, 2019). Autophagy may be considered as a survival strategy in cancer treatment, because it can help cancer cells cope with treatment stress, such as chemotherapy or radiotherapy. Autophagy induced by nodakenin may help tumor cells escape death during treatment. On the other hand, nodakenin treatment was accompanied by changes in the expression of apoptosis related proteins, indicating the activation of apoptosis. Apoptosis is a programmed cell death. It is usually regarded as a favorable mechanism against cancer. When cells are under treatment or other stress, apoptosis can help clear damaged cells and prevent them from continuing to grow and spread. In addition, the migration and invasion of NSCLC cells were reduced after treatment with nodakenin. This related to the AURKA-CXCL5 axis inhibition, the autophagy activation and apoptosis pathways. Reducing the cell migration and invasion ability helps to prevent tumor metastasis and spread (Shen *et al.*, 2020). This is significant for the NSCLC treatment. MTT assay showed that NSCLC cells were more sensitive to radiotherapy after treatment with

nodakenin. With the synergistic effect of nodakenin, radiation therapy can more effectively kill tumor cells. This synergistic effect provides a new possibility for NSCLC patients, which can reduce the dose or time of radiation therapy, reduce the potential risk of side effects and complications, and improve the treatment effect. Nodakenin may increase the sensitivity through a variety of ways. The regulatory effects of nodakenin on autophagy and apoptosis are discussed. These effects may make tumor cells more vulnerable to radiation-induced DNA damage, thereby increasing the killing effect of radiation therapy. The anti-inflammatory and antioxidant properties of nodakenin may help to reduce the inflammation and oxidative stress caused by radiation (Zhao *et al.*, 2023), thereby reducing the resistance of tumor cells. In summary, nodakenin may affect the survival and treatment of NSCLC through a variety of mechanisms. These mechanisms include the regulation of AURKA-CXCL5 axis, the activation of autophagy and apoptosis pathway, the weakening of cell migration and invasion, and the improvement of radiosensitivity. If further studies confirm the potential efficacy of nodakenin, it may provide a new treatment option for NSCLC patients.

CONCLUSION

According to the research results, nodakenin regulates autophagy and apoptosis of NSCLC by inhibiting the activation of AURKA-CXCL5, and enhances the sensitivity of NSCLC to radiotherapy. These findings bring new ideas and possibilities for the treatment of NSCLC, providing more individualized and effective treatment for patients.

DECLARATIONS

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IRB approval

This study was approved by the Advanced Studies Research Board of Affiliated Hospital of Hebei University, Baoding, China.

Ethics approval

The study was carried out in compliance with guidelines issued by Ethical Review Board Committee of Affiliated Hospital of Hebei University, Baoding, China. The official letter would be available on fair request to

corresponding author.

Statement of conflict of interest

The authors have declared no conflict of interest.

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