JBUON 2021; 26(3): 802-811
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
Email: editorial_office@jbuon.com

ORIGINAL ARTICLE

AKR1C3 and β-catenin expression in non-small cell lung cancer and relationship with radiation resistance

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Summary

Purpose: *To search the AKR1C3 and β-catenin expression in non-small cell lung cancer (NSCLC) and to explore the correlation between AKR1C3 and β-catenin and radiation resistance.*

Methods: *Paraffin specimens from 61 patients with NSCLC were evaluated. These patients could not receive operation but received radical radiotherapy. The patients were divided into effective group and ineffective group with reference to RECIST evaluation criteria. The sites and intensity of AKR1C3 and β-catenin protein expression were detected by immunohistochemistry. The relationship between AKR1C3 and β-catenin and radiation resistance was analyzed by Mann-Whitney U test. The correlation between AKR1C3 and β-catenin was analyzed by Spearman's correlation test. Mann-Whitney U test was used to analyze the AKR1C3 overall expression in the effective group and the ineffective group after radiotherapy.*

Results: *The nuclear expression in the two groups was statistically significant (p=0.033). The β-catenin protein was mainly expressed in the cytoplasm and the nucleus of tissues with NSCLC. The β-catenin nuclear expression was different between the two groups, with statistical significance (p=0.008). AKR1C3 nuclear expression was positively correlated with β-catenin nuclear expression (rs=0.382, p=0.002).*

Conclusions: *High AKR1C3 nuclear expression in NSCLC is related to radiation resistance. The higher the AKR1C3 nucleus expression, the worse short-term curative effects after radiotherapy. High β-catenin nuclear expression is related to radiation resistance, and the higher the β-catenin nuclear expression, the worse the short-term curative effects after radiotherapy. The nuclear aggregation of AKR1C3 during radiation resistance of non-small cell lung cancer (NSCLC) may have some synergistic relationship with nuclear aggregation of β-catenin.*

Key words: *AKR1C3, β-catenin, non-small cell lung cancer (NSCLC), radiation resistance*

Introduction

Lung cancer is one of the most common fatal cancers worldwide [1], which can be divided into two categories: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). The incidence of NSCLC is high, accounting for about 85% of lung cancer cases. It is still rising in recent years, and has become the leading cause of death from cancer in China [2]. Finding ways to improve the survival rate of patients with NSCLC has become the focus of cancer prevention and treatment. At present, the treatment on NSCLC mainly includes surgery, chemotherapy and radiotherapy [3]. Among them, radiotherapy is still the first choice in the treatment of advanced NSCLC [4]. Radiotherapy for patients with advanced NSCLC can effectively inhibit the spread of cancer cells *in vivo*. However, patients

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Received: 27/11/2020; Accepted: 29/12/2020

with NSCLC are usually accompanied by varying degrees of radiation resistance, which limits the efficacy of tumor radiotherapy. At present, radiation resistance has become one of the important reasons for the failure of patients with NSCLC. Therefore, it is important to clarify the NSCLC mechanism of radiation resistance to improve the survival rate of patients with NSCLC.

In the early stage, the research group found that the AKR1C3 gene can regulate radiation resistance of tumors by scavenging reactive oxygen species (ROS) in esophageal cancer cells [5]. ROS is the main mechanism by which radiation can kill tumor cells *in vivo*. It is inferred that AKR1C3 genes associated with esophageal cancer radiation resistance may also play a role in other types of tumors. At the same time, it was also found in a study that there was high β-catenin gene expression [6] in radiation-resistant Seg-1R cell lines that were confirmed to be lung cancer cell lines [7]. On the basis of the above research background, 61 patients with NSCLC who received radical radiotherapy in our hospital from 2010 to 2016 were collected and evaluated. The expression and site of AKR1C3 and β-catenin in tissues were detected, and the relationship between them and short-term curative effects after radiotherapy for NSCLC was analyzed.

Methods

Experimental subjects

Sixty-one elderly patients with advanced NSCLC treated in our hospital from January 2010 to December 2016 were selected and evaluated. Inclusion criteria: All patients were confirmed to have advanced NSCLC on admission and all of them were treated for the first time without any radiotherapy or chemotherapy; They were unable to undergo surgical resection after medical examination and were unable or unwilling to receive chemotherapy, with lesion diameter ≥1 cm and the expected survival time was greater than 6 months. Exclusion criteria: Patients with contraindications for chest radiotherapy.

Experimental reagents

1. AKR1C3 rabbit anti-human polyclonal antibody was purchased from American ABCAM Co.
2. β-catenin mouse anti-human monoclonal antibody was purchased from Beijing Zhongshu Jinqiao Biotechnology Co., Ltd. (Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd.).

Experimental materials

1. 61 patients were diagnosed as NSCLC by 2 experienced pathologists, and tumor tissues were biopsied under tracheal microscope.
2. Fixation: The pathological tissue masses obtained after bite examination by tracheoscopy were put into the pre-matched fixation solution (10% formalin, Bouin's fixation solution). This was to denature and solidify the proteins of tissues and cells to prevent autolysis or bacterial decomposition after cells died, so as to maintain the original morphological structure of cells.
3. Dehydration: The gradual removal of water from tissue masses by using alcohol with low to high concentrations as a dehydrating agent.
4. Transparency: The tissue block was placed in the transparent agent xylene, which was dissolved in alcohol and paraffin, and the middle alcohol of the tissue block was replaced by xylene.
5. Paraffin immersion and embedding: The transparent tissue masses were placed in the melted paraffin and placed in the dissolving box for insulation. When the paraffin was completely immersed in the tissue masses, it was embedded: the stainless steel embedded frame was poured into the dissolved paraffin, and the soaked paraffin tissue masses were quickly clamped and cooled and solidified into masses.

AKR1C3 and β-catenin expression of NSCLC was detected by two-step immunohistochemistry

1. Section: Paraffin masses of the pathological tissues to be examined were cut into 4 μM sections and placed on clean polylysine-treated slides.
2. Drying: The tissue sections were dried at 80°C for 2 h, until the paraffin was melted.
3. Deparaffinating:
   a. The dried sections were placed in xylene solution I and II for 10 min each.
   b. Then, they were placed in 100% alcohol solution I and II, each solution for 10 min.
   c. The sections were sequentially passed through 90%, 80% and 70% alcohol solution, each solution for 5 min.
   d. The sections were taken out and placed in distilled water for 5 min.
4. Two-step process
   a. 3% hydrogen peroxide was added to the treated specimens until all the specimens were covered to block endogenous peroxidase. Then the mixture was placed in wet box at 37°C for 10 min.
   b. The sections were rinsed with distilled water and soaked in phosphate buffered saline (PBS) for 3 min.
   c. Then, the sections were placed in citrate buffer and repaired under high pressure. After that, they were naturally cooled to room temperature, and soaked in citrate buffer.
   d. 10μL of primary antibody fluid was added on different sections of the same specimen, which were incubated in wet box at 4°C for 18-24 h, rinsed with PBS, and soaked for 3 min. This procedure was repeated 3 times.
   e. According to the operating procedures of the kit, the secondary antibody fluid was dropped on the sections, which were incubated in wet box at 37°C

for 45 min, rinsed with PBS and soaked for 3 min. This procedure was repeated 3 times.

f. DAB chromogenic solution after preparation was placed on the sections, which were observed at any time under optical microscope, with the membrane (cytoplasm) and the nucleus shown as brown.
g. The sections were rinsed with running water until no claybank.
h. All the sections were re-dyed with hematoxylin for 3 min and rinsed with water.

5. The sections were dehydrated and transparent
   a. The sections were sequentially passed through 70%, 80% and 90% alcohol solutions for 5 min each.
   b. Dehydration: Then, the sections were passed through 100% alcohol solution I and II, each solution for 10 min.
   c. Transparency: The dehydrated sections were placed in xylene I and II, each solution for 10 min.
   d. The sections were dropped with neutral gum to seal, covered with the slides and observed under optical microscope.
6. Criteria for determining the results of immunohistochemistry
   The tested results were scored according to the percentage of positive cells expressing the target protein and the staining intensity: 3 high power fields (×400) were selected in each case, with at least 200 cells per field. Scoring criteria: 1: Percentage of positive cells<25%, 2: Percentage of positive cells between 25-50%, 3: Percentage of positive cells between 50-75%, 4: Percentage of positive cells between>75%. The staining intensity was determined as follows: proteins expressed in membrane (cytoplasm) and nucleus, which were roughly claybank particles, with dark and light color. The criteria: 0: no coloring, 1: The cells were light claybank particles, 2: there were claybank particles in cells, 3: there were brown particles in cells. The results were determined as the product of the above two [8].

Target delineation and radiotherapy

Image guided three-dimensional conformal radiotherapy was performed in the patients. The specific operation was as follows: The patient was asked to lie at supine position, with hands crossing on the top of the head. The body was fixed at the position with body model. Sixty-four rows of spiral CT were used to scan the patient's static state, from the 6th cervical vertebra to the 2nd lumbar vertebra for target delineation. And then, the therapeutic regimen was formulated according to the scope outlined. Three-dimensional conformal radiation or imRT was performed to the patients, the conventional segmentation was 2.0 Gy/time, 60-66 Gy/30-33 times.

Evaluation criteria for short-term curative effects after radiotherapy

At the end of the 3rd month after radiotherapy, two or more experienced radiotherapy chief physicians were employed to evaluate short-term curative effects of the patients. They discussed the decision together with those who disagreed. Complete response (CR) was defined as the complete disappearance of all detectable tumors. Partial response (PR) was defined as a ≥ 50% reduction in the maximal diameter of the tumor. And stable disease was defined as no decrease or increase in the tumor diameter. Progressive disease (PD) was defined as enlargement of the primary tumor or the appearance of new lesions [9]. According to short-term curative effects after radiotherapy, the patients were divided into two groups: effective group: complete response (CR) plus partial response (PR), and ineffective group: progressive disease (PD).

Statistics

SPSS17.0 statistics package was used to process the data. Mann-Whitney U test was used to analyze the relationship between AKR1C3 and β-catenin and radiation resistance. P<0.05 showed that the difference was statistically significant. The correlation between AKR1C3 and β-catenin was analyzed by Spearman's correlation test. Bilateral α=0.05 was for significant test level.

Table 1. Clinical information of 61 patients with NSCLC

General Indicator	*Group*	*Number of patients*	*Constituent ratio (%)*
Gender	Male	35	57.37
	Female	26	42.62
Age, years	≤60	3	4.92
	>60	58	95.08
Type of cancer	Squamous carcinoma	15	24.59
	Adenocarcinoma	36	59.02
	Large cell carcinoma	10	16.39
Clinical stage	I	0	0
	II	0	0
	III	38	62.30
	IV	23	37.70

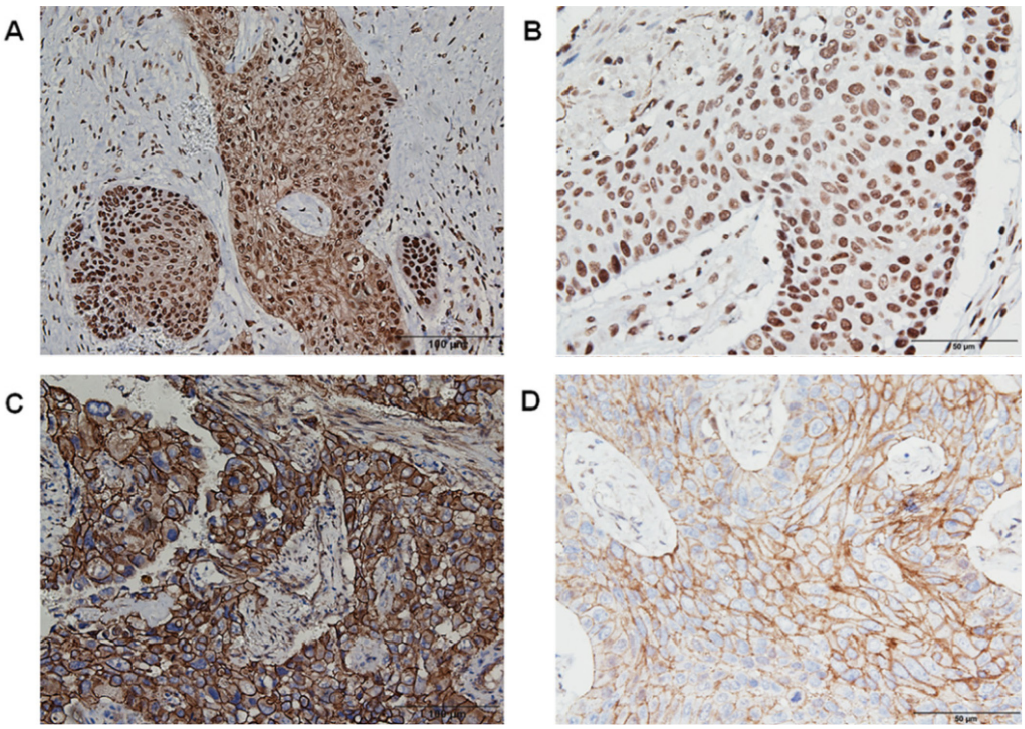

Figure 1. AKR1C3 and β-catenin protein expression in tissues with NSCLC. **A:** AKR1C3 was mainly expressed in cytoplasm and nucleus. **B:** AKR1C3 was mainly expressed in the nucleus. **C:** β-catenin was mainly expressed in membrane, cytoplasm and nucleus. **D:** β-catenin was mainly expressed in membrane.

Results

General conditions and short-term curative effects in patients

General conditions in patients with NSCLC are shown in Table 1.

AKR1C3 and β-catenin expression in NSCLC

1. The positive AKR1C3 proteins were expressed as claybank particles, which were mainly located in the cytoplasm and nucleus of tissues and NSCLC cells. A typical positive expression of the specimen is shown in Figure 1A,B.
2. The positive β-catenin proteins were expressed as claybank particles, which were mainly located in the membrane, cytoplasm and nucleus of tissues and NSCLC cells. The typical positive expression of the specimen is shown in Figure 1C,D.

Relationship between AKR1C3 and β-catenin and short-term curative effects after radiotherapy on NSCLC

1. Correlation analysis of AKR1C3 expression in NSCLC and short-term curative effects:
   a. Relationship between AKR1C3 expression and short-term curative effects after radiotherapy in patients.
   Mann-Whitney U test was used to determine the AKR1C3 overall expression in the effective group and the ineffective group (which refers to the expression in the cytoplasm and nucleus, and the immunohistochemical staining intensity score multiplied by the percentage score of stained positive cells). The median of AKR1C3 overall expression was 8.000 in the ineffective group and 7.000 in the effective group after radiotherapy. The results of Mann-Whitney U test showed that there was no significant difference in the AKR1C3 overall expression in the effective and the ineffective group (Z=-0.088, U=459.000, p=0.930), as shown in Table 2 and Figure 2.

   b. Relationship between AKR1C3 nucleus expression and short-term curative effects after radiotherapy in patients.
   Mann-Whitney U test was used to determine the AKR1C3 nuclear expression in the effective and the ineffective group. The median of AKR1C3 nuclear expression was 6.000 in the ineffective group and 4.000 in the effective group after radiotherapy. The results of Mann-Whitney U test showed that there was significant difference in the AKR1C3 nuclear expression between the effective group and the ineffective group after radiotherapy (Z=-2.138, U=319.500, p=0.033), as shown in Table 3 and Figure 2.

Table 2. Relationship between short-term curative effect after radiotherapy and AKR1C3 overall expression

	Groups	*Number of patients*	*AKR1C3 overall expression*			*Z*	*p*
			P25	*P50*	*P75*		
Short-term curative effect	Ineffective group	31	3.000	8.000	9.000	-0.088	0.930
	Effective group	30	3.000	7.000	9.750		

Table 3. Relationship between short-term curative effects after radiotherapy and AKR1C3 nuclear expression

	Groups	*Number of patients*	*AKR1C3 nuclear expression*			*Z*	*p*
			P25	*P50*	*P75*		
Short-term curative effect	Ineffective group	31	2.000	6.000	8.000	-2.138	0.033
	Effective group	30	2.000	4.000	4.000		

Table 4. Relationship between short-term curative effect after radiotherapy and β-catenin overall expression

	Groups	*Number of patients*	*β-catenin overall expression*			*Z*	*p*
			P25	*P50*	*P75*		
Short-term curative effect	Ineffective group	31	6.000	9.000	12.000	-0.857	0.392
	Effective group	30	3.000	9.000	12.000		

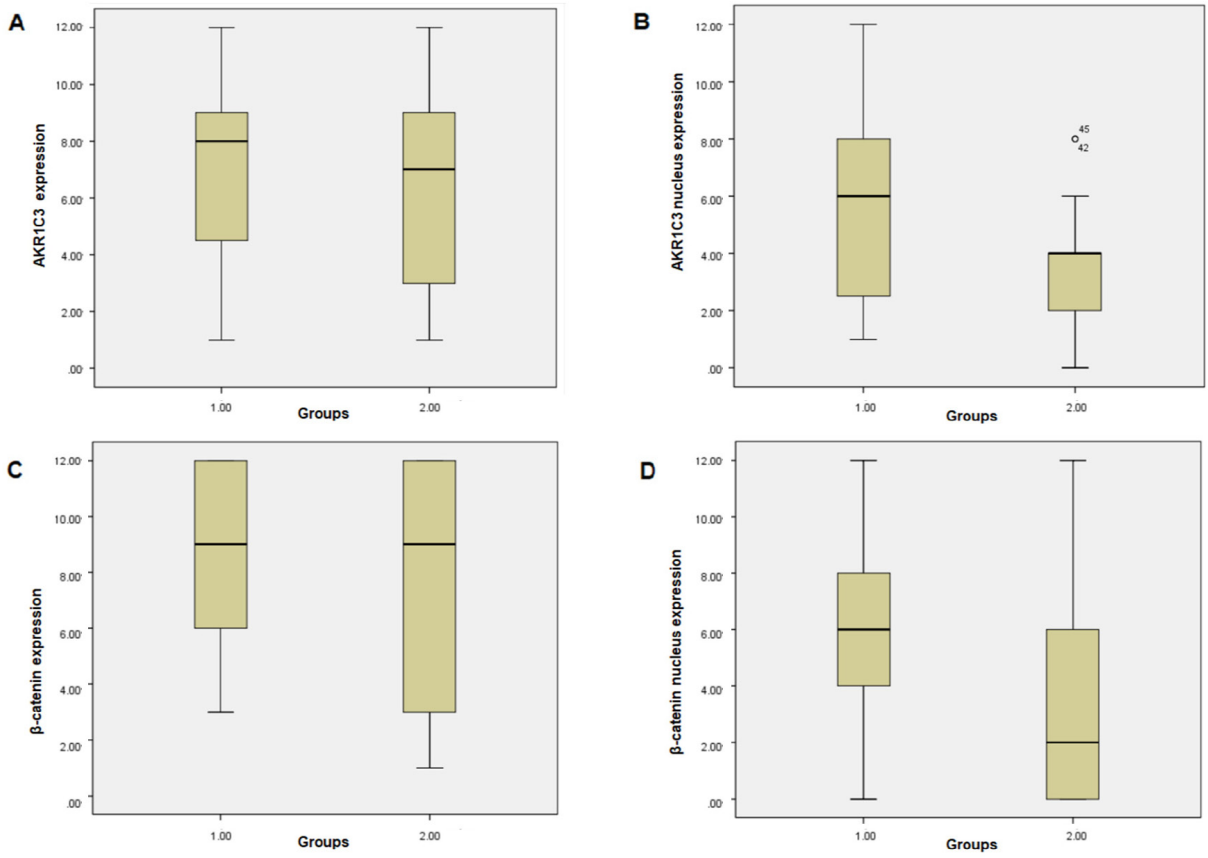

Figure 2. AKR1C3 and β-catenin expression in effective group and ineffective group after radiotherapy. **A:** AKR1C3 protein expression in the effective group and the ineffective group after radiotherapy (p=0.93). **B:** Expression of AKR1C3 nuclear protein in the effective group and the ineffective group after radiotherapy (p=0.033). **C:** Expression of β-catenin proteins in the effective group and the ineffective group after radiotherapy (p=0.392). **D:** Expression of β-catenin nuclear proteins in the effective group and the ineffective group after radiotherapy (p=0.008). In each part of the Figure, Group 1 was the ineffective group and Group 2 was the effective group.

2. Correlation analysis of β-catenin expression in NSCLC
   a. Relationship between β-catenin expression and short-term curative effects after radiotherapy:
   Mann-Whitney U test was used to determine the β-catenin overall expression in the effective and the ineffective group. The median of β-catenin overall expression was 9.000 in the ineffective group and 9.000 in the effective group. The results of Mann-Whitney U test showed that there was no significant difference in the β-catenin expression between the effective and the ineffective group (Z=-0.857, U=407.500, p=0.392), as shown in Table 4 and Figure 2.
   b. Relationship between β-catenin nucleus expression and short-term curative effects after radiotherapy:
   Mann-Whitney U test was used to determine the β-catenin nuclear expression in the effective and the ineffective group. The median of β-catenin nuclear expression was 3.000 in the ineffective group and 1.000 in the ef-

Table 5. Relationship between short-term curative effects after radiotherapy and β-catenin nuclear expression in patients

	Groups	*Number of patients*	*β-catenin nuclear expression*			*Z*	*p*
			P25	*P50*	*P75*		
Short-term curative effect	Ineffective group	31	2.000	3.000	4.000	-2.672	0.008
	Effective group	30	0	1.000	3.000		

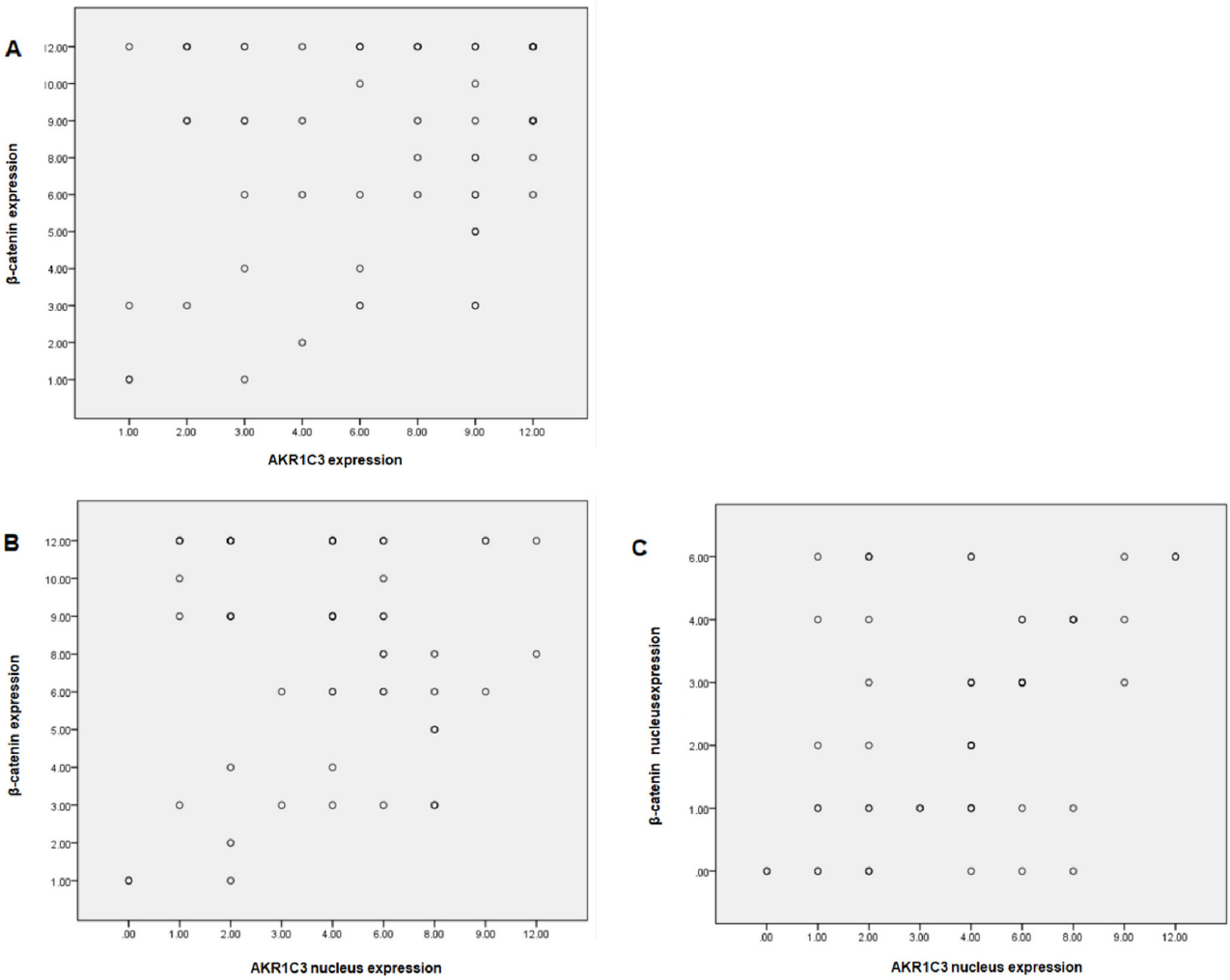

Figure 3. Scatter diagram of correlation between AKR1C3 and β-catenin expression in NSCLC. **A:** Correlation between AKR1C3 and β-catenin protein expression (p=0.161). **B:** Correlation between AKR1C3 nuclear expression and β-catenin expression (p=0.659). **C:** Correlation between AKR1C3 nuclear expression and β-catenin nuclear expression (p=0.002).

fective group after radiotherapy. The results of Mann-Whitney U test showed that the β-catenin nuclear expression was statistically significantly different between the effective and the ineffective group (Z=-2.672, U=282.500, p=0.008), as shown in Table 5 and Figure 2.

Relationship between AKR1C3 and β-catenin expression in NSCLC

The positive AKR1C3 proteins were expressed as claybank particles, which were mainly located in the cytoplasm and nucleus of tissues and cells with NSCLC. The positive β-catenin proteins were expressed as claybank particles, which were mainly located in the membrane, cytoplasm and nucleus of tissues and cells with NSCLC. Spearman's correlation analysis was used in this research to determine the correlation between AKR1C3 and β-catenin expression in NSCLC, as shown in Figure 3.

1. The correlation between AKR1C3 overall expression and β-catenin overall expression in tissues with NSCLC: The monotony relationship between the overall expression of AKR1C3 and β-catenin was directly judged by drawing scatter plots. The results showed that there was no correlation between the two expressions (rs=0.182, p=0.161).
2. The correlation between AKR1C3 nuclear expression and β-catenin overall expression in tissues with NSCLC: There was a monotony relationship between AKR1C3 nuclear expression and β-catenin overall expression by drawing scatter plots. The results showed that there was no correlation between the two expressions (rs=-0.058, p=0.659).
2. The correlation between AKR1C3 nucleus expression and β-catenin nucleus expression in tissues with NSCLC: There was a monotony relationship between AKR1C3 nuclear expression and β-catenin nuclear expression by drawing scatter plots. The results showed that the two expressions were correlated (rs=0.382, p=0.002).

Discussion

NSCLC is one of the most common malignant tumors, accounting for 85% of the overall number of lung cancers. This incidence is still increasing in recent years, and has become the main cause of cancer deaths in China [10,11]. According to the statistics, the annual increase in the incidence of NSCLC in China has reached 4.7%, and most patients are in the middle and advanced stage at the time of diagnosis. Radiotherapy is one of the important treatment methods [12,13]. With the popularity of radiotherapy in the treatment of patients with NSCLC, more and more cases have proved that radiotherapy is insensitive, due to radiation resistance. It is one of the most important reasons restricting the realization of NSCLC curative effect and the recurrence and metastasis of tumor [14,15]. It is found that the radiation resistance of this tumor may be related to the presence of some resistance factors in the patients themselves. But there is still a lack of effective targets for radiotherapy of sensitized lung cancer, which is an urgent problem in clinical practice [16]. Radiation resistance is the result of a multi-genes, multi-factors and multi-mechanisms involved in the process of radiation treatment. The sensitivity is mainly affected by the following four biological factors: repair of subfatal and potentially fatal injuries, cell proliferation, cell cycle redistribution and reoxygenation [17]. It was found that the AKR1C3 gene could scavenge reactive oxygen species (ROS) in NSCLC cells, suggesting that AKR1C3 gene may regulate radiation resistance of tumors by affecting the reoxygenation of cells after radiation. The high expression of β-catenin gene in radiation-resistant lung cancer cells also suggests that the gene may be associated with radiation resistance.

AKR1C3 is a C3 member of aldo-keto reductase (AKR) family 1, which is a redox enzyme. Recent studies have confirmed that AKR1C3 is closely related to radiotherapy tolerance in some cancer patients. A previous research found that AKR1C3 gene overexpression was also found in NSCLC cells tolerant to radiotherapy [5]. Sun et al have shown in their studies that AKR1C3 overexpression reduces the sensitivity of prostate cancer patients to radiotherapy by activating the cell MAPK signaling pathway [18]. At the same time, AKR1C3 has also been shown to be associated with resistance to some antitumor drugs. Shiiba research group suggested that AKR1C3 is associated with resistance to cisplatin and 5-fluorouracil [19]. The results of Matsunaga experiment showed that the down-regulation of AKR1C3 expression also significantly increased the drug sensitivity of JCT-15 to cisplatin in colorectal cancer cells [20]. Furthermore, overexpression of AKR1C3 gene can cause resistance to anthracycline in tumor cells [21].

In order to test the AKR1C3 expression in NSCLC and whether it is related to radiation resistance or not, 61 patients diagnosed as NSCLC squamous by 2 pathology experts were selected in this study. They received radical radiotherapy without operation. Bronchoscopic biopsy was used for the detection of tumor tissue. Paraffin block was embedded after two-step immunohistochemical detection of AKR1C3 expression. According to short-term curative effects of

radical radiotherapy, the patients were divided into effective group and ineffective group after radiotherapy. AKR1C3 protein was expressed in claybank particles in the cytoplasm and nucleus of NSCLC. The results showed that there was no difference in the AKR1C3 expression in the two groups, but the high AKR1C3 nuclear expression might enhance the radiation resistance of patients with NSCLC. Experimental results were consistent with previous reports on the expression of AKR1C3 in NSCLC and radiation resistance of tumors. This experiment found and confirmed that the aggregation of nuclear AKR1C3 may enhance the resistance of radiotherapy on NSCLC. The results provide a searchable basis for the next research of the mechanism of AKR1C3 role in radiation resistance of NSCLC.

β-catenin is an evolutionarily conserved multifunctional protein. It binds to E-cadherin to form a complex to participate in intercellular connections to maintain the normal morphology of epithelial cells. It is also involved in Wnt signaling pathways. As a key link, regulating gene transcription plays an important role in physiological and pathological processes such as embryonic development, tumorigenesis and invasion and metastasis. Specifically, a research found that in Wnt/β-catenin signaling pathways, when phosphorylated at Tyr654, the 12th Arm motif near the C terminal of β-catenin is negatively charged. Its affinity for E-cadherin decreased significantly, and it transferred into the nucleus, promoting gene transcription, cell proliferation, and tumorigenesis [22,23]. When phosphorylation of β-catenin at Ser191 and Ser246 occurred, there would be an increase in β-catenin nucleation. It promotes downstream gene involvement in cyclin D1 and C-MyC expression, thereby playing a role in cell cycle regulation [24]. In this research, it was found that β-catenin protein was mainly expressed in the cytoplasm and nucleus by detecting the β-catenin protein expression in tissues and NSCLC cells. And high nuclear expression of β-catenin protein is associated with radiation resistance in patients with NSCLC. The results of this research were consistent with previous reports on high expression of β-catenin gene in radiation-resistant NSCLC cell lines [7].

At present, the mechanism of AKR1C3 enhancing radiation resistance of cells with NSCLC has not been clear. We speculated that it may be related to its nuclear intervention in the transcription of some genes, thus activating or participating in the activation of some radiation resistance related signaling pathways. β-catenin can be dissociated from the complex on the membrane and aggregated in the cytoplasm and translocated into the nucleus after the phosphorylation of certain sites. It has been reported that phosphorylation of different sites may be related to cell proliferation, tumorigenesis and cell cycle regulation, but the specific functions need to be further explored [25,26]. β-catenin is a key gene in Wnt/β-catenin signaling pathways. It has been reported that after activation of this pathway, β-catenin would transfer from the cytoplasm to the nucleus and bind to a member of TCF/LEF1, which can recruit β-catenin as a co-activator into the enhanced hadronic element of the gene they target [27,28]. So far, nearly 50% of known human tumors have been shown to be associated with abnormal regulation of Wnt/β-catenin signaling pathways by affecting hyperplasia [29], apoptosis [30], DNA damage repair [31,32] of tumor cells, which are involved in the formation of radiation resistance of tumors. Wnt pathways have been shown to induce radiation resistance to colorectal cancer by activating COX2 upregulation [33,34]. The AKR1C3 and β-catenin protein expression was detected in NSCLC tissues and cells and the AKR1C3 nuclear expression was positively correlated with β-catenin nuclear expression. So, presumably, AKR1C3 nuclear aggregation may be involved in triggering Wnt/β-catenin activated signaling pathways. It is involved in the formation of radiation resistance of tumor cells by regulating the downstream originals of pathways such as Cyclin D1, c-MYC, PLA2G2, and so on. Another presumption is that the activated Wnt/β-catenin signaling further promotes AKR1C3 nuclear aggregation, triggering the ROS clearance mechanism in tumor cells and exerting radiation resistance by influencing cell reoxygenation. However, its specific mechanism needs further exploration.

To sum up, the formation of radiation resistance in NSCLC cells may be accomplished by a series of genes through multiple signal transduction pathways. The AKR1C3 nuclear aggregation may be involved in triggering the activation Wnt/β-catenin signaling pathways, or activated Wnt/β-catenin signaling pathways to further induce AKR1C3 nuclear aggregation, which specifically implement the formation of radiation resistance. Next, our research group intends to conduct RNA interference on the above genes, detect the expression changes of the upstream and downstream genes, further clarify the specific mechanism of action, and provide a molecular biological basis for future clinical judgment of prognosis and gene intervention therapy. The detection of AKR1C3 and β-catenin1 and the development of related drugs may be important for the evaluation of curative effects after radiotherapy and targeted volume therapy in patients with NSCLC.

Conflict of interests

The authors declare no conflict of interests.

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