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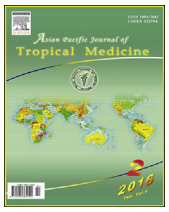

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Effect of lentivirus-mediated integrin αVβ3-shRNA on tumor growth of mice with lung cancer xenografts

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ABSTRACT

Objective: To study the effect of lentivirus-mediated integrin αVβ3-shRNA on tumor growth of mice with lung cancer xenograft.
Methods: Lung cancer tissue, paracancer tissue and normal tissue were collected and integrin αVβ3 expression was detected; BALB/c nude mice were selected, divided into integrin αVβ3 knockdown group (KD group) and negative control group (NC group), and inoculated with cells stably infected by integrin αVβ3-shRNA lentivirus and cells stably infected by negative control-shRNA lentivirus, respectively, the growth of tumor tissue was continuously observed, and the number of apoptosis cells as well as the expression of angiogenesis, apoptosis and invasion genes in tumor tissue were detected.
Results: mRNA content and protein content of integrin αVβ3 in lung cancer tissue were significantly higher than those in paracancer tissue and normal tissue; increasing trend of tumor tissue volume of KD group was weaker than that of NC group, and tumor volume at various points in time of KD group was lower than that of NC group; mRNA contents and protein contents of VEGF, FGF, EGF, Bcl-2, MMP-9, MMP-12 and MMP-13 in tumor tissue of KD group were lower than those of NC group, and apoptosis index as well as mRNA content and protein content of Bax were higher than those of NC group.
Conclusions: The expression of integrin αVβ3 increases in lung cancer tissue, and lentivirus-mediated integrin αVβ3-shRNA can inhibit tumor growth of mice with lung cancer xenografts.

1. Introduction

Lung cancer is one of the most common malignant tumors worldwide and death cases caused by it rank first in cancer-related deaths in China. Non-small cell lung cancer is the most common pathological type of lung cancer, and the cancer cells have strong ability of proliferation and invasion, which directly causes high recurrence rate and distant metastasis rate in non-small cell lung cancer patients after surgical resection or chemotherapy, thus leading to poor long-term prognosis of the disease [1,2]. At present, regulatory mechanisms of lung cancer cell proliferation and invasion haven't been fully elucidated. Integrins are a family of molecules discovered in recent years that are closely related to cell adhesion, proliferation, invasion and angiogenesis, are located in cell membrane and play a role in the form of membrane receptors. Integrin αVβ3 has been confirmed to participate in the clinical and pathological process of osteosarcoma, colon cancer and other malignant tumors [3,4], but whether the molecule is involved in the development of lung cancer has not been reported. In the following research, the effect of lentivirus-mediated integrin αVβ3-shRNA on tumor growth of mice with lung cancer xenograft was analyzed.

2. Materials and methods

2.1. Materials

2.1.1. Clinical and experimental subjects

Clinical specimens were lung cancer tissue, paracancer tissue and normal tissue, and tissue was from 100 cases of patients who received radical resection of lung cancer in our hospital from May 2012 to April 2015. Lung cancer cell lines A549 were

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purchased from the cell bank of Chinese Academy of Sciences, and BALB/c nude mice were purchased from Shanghai Slac Laboratory Animal Company.

2.1.2. Reagents and consumables

Serum and media for cell culture were from Gibco Company and consumables for culture were from Nest Company; RNA extraction and PCR amplification kits were from Beijing Tiangen Company, immunoblot kits and related antibodies were from US Sigma Company, and TUNEL kits were from US Roche Company.

2.2. Experimental methods

2.2.1. Cell culturing and transfecting methods

Lung cancer A549 cell lines were conventionally recovered and cultured, when cell density reached about 70–80%, 0.125% trypsin was used for digestion and sub-culture, and sub-cultured cells were inoculated in Petri dishes and infected with integrin αVβ3-shRNA lentivirus and negative control-shRNA lentivirus, respectively; four days after infection, 2 μg/mL puromycin was added for pressure screening, then media were replaced every 3 d to expand the culture, and cells stably infected by integrin αVβ3-shRNA lentivirus and negative control-shRNA lentivirus were obtained, respectively.

2.2.2. Establishment of mouse models with lung cancer xenografts

Sixty BALB/c nude mice were divided into integrin αVβ3 knockdown group (KD group) and negative control group (NC group), each group with 30, and 200 μL tumor cell suspension with density of 1×10^8/mL was subcutaneously injected in necks of two groups. KD group were inoculated with cells stably infected by integrin αVβ3-shRNA lentivirus, and NC group were inoculated with cells stably infected by negative control-shRNA lentivirus.

2.2.3. Detection of tumor growth

8 d, 12 d, 16 d, 20 d and 24 d after tumor cell inoculation, diameters of xenografts were measured in three directions and marked as a, b and c, respectively, and tumor tissue volume $V = \pi abc/6$.

2.2.4. Tumor tissue collecting and processing methods

Twenty-four days after tumor cell inoculation, mice were killed and anatomized, obtained tumor tissue was washed with normal saline and equally divided into two, one was frozen in liquid nitrogen and then preserved at −80 °C, and the other was fixed in formalin.

2.2.5. Detecting methods of gene expression in tumor tissue

Clinical lung tissue specimens and mouse tumor tissue preserved at −80 °C were taken, RNA extraction kits and protein extraction kits were used to extract RNA specimens and protein specimens, respectively, then PCR reaction and immunoblot test were performed, respectively, and mRNA contents and protein contents of related genes were detected.

2.2.6. Detecting methods of cell apoptosis index

Tumor tissue fixed in formalin was taken and made into paraffin slices, then TUNEL kits were used for staining, all procedures were conducted in accordance with the kit instructions, finally DAB was used for color development, 200 cells were observed under high power lens, cells with tan nuclei were taken as positive cells, the number of positive cells was counted, and the percentage of positive cells was calculated and used as apoptosis index.

2.3. Statistical methods

SPSS21.0 was used for data processing, comparison of measurement data between two groups was by t test, and differences were considered to be statistically significant at a level of $P < 0.05$.

3. Results

3.1. Integrin αVβ3 expression in lung cancer tissue and paracancer normal tissue

mRNA content and protein content of integrin αVβ3 in lung cancer tissue were significantly higher than those in paracancer tissue and normal tissue, and differences of pair wise comparison were statistically significant ($P < 0.05$) (Table 1).

3.2. Dynamic change of tumor tissue volume

After tumor cell inoculation, tumor tissue volume of both groups showed increasing trend, the increasing trend of tumor tissue volume of KD group was weaker than that of NC group, tumor tissue volume at various points in time of KD group was lower than that of NC group, and differences between two groups were statistically significant ($P < 0.05$) (Table 2).

3.3. Expression of angiogenesis molecules in tumor tissue

mRNA contents and protein contents of VEGF, FGF and EGF in tumor tissue of KD group were significantly lower than those of NC group, and differences between two groups were statistically significant ($P < 0.05$) (Table 3).

3.4. Cell apoptosis in tumor tissue

Apoptosis index in tumor tissue of KD group was higher than that of NC group, mRNA content and protein content of pro-apoptosis gene *Bax* were higher than those of NC group, and mRNA content and protein content of anti-apoptosis gene *Bcl-2* were lower than those of NC group ($P < 0.05$) (Table 4).

Table 1
Comparison of integrin αVβ3 expression in lung cancer tissue, paracancer tissue and normal tissue.

Indexes	Specimen no.	*Integrin αVβ3* mRNA content	Integrin αVβ3 protein content
Lung cancer tissue	100	278.75 ± 32.38	341.22 ± 39.58
Paracancer tissue	100	156.55 ± 18.48	176.64 ± 20.32
Normal tissue	100	100.00 ± 14.96	100.00 ± 11.74
F		17.686	23.692
P		<0.05	<0.05

Table 2
Dynamic change of tumor tissue volume of two groups.

Groups	8 d	12 d	16 d	20 d	24 d	28 d
KD group	0.44 ± 0.01	0.74 ± 0.04	1.02 ± 0.11	1.44 ± 0.12	1.74 ± 0.14	2.42 ± 0.21
NC group	0.87 ± 0.04	1.04 ± 0.08	2.35 ± 0.25	3.92 ± 0.38	7.52 ± 0.72	13.02 ± 1.03
T	9.292	6.039	13.822	16.484	35.863	23.944
P	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Table 3
Expression of angiogenesis molecules in tumor tissue of two groups.

Groups	mRNA content			Protein content		
	VEGF	*FGF*	*EGF*	VEGF	FGF	EGF
KD group	35.62 ± 4.29	23.52 ± 2.65	40.12 ± 4.65	18.58 ± 2.12	32.54 ± 4.42	37.49 ± 4.62
NC group	100.00 ± 13.54	100.00 ± 11.52	100.00 ± 12.47	100.00 ± 10.59	100.00 ± 14.12	100.00 ± 12.47
T	19.194	27.686	14.575	33.686	22.182	15.893
P	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Table 4
Comparison of cell apoptosis in tumor tissue of two groups.

Groups	Apoptosis index	mRNA content		Protein content	
		Bcl-2	*Bax*	Bcl-2	Bax
KD group	0.623 ± 0.074	22.46 ± 2.95	196.67 ± 20.19	35.42 ± 4.21	236.59 ± 30.22
NC group	0.309 ± 0.042	100.00 ± 12.85	100.00 ± 15.29	100.00 ± 10.93	100.00 ± 14.42
T	10.894	27.593	9.485	20.812	13.592
P	<0.05	<0.05	<0.05	<0.05	<0.05

Table 5
Expression of invasion-related molecules in tumor tissue of two groups.

Groups	mRNA content			Protein content		
	MMP-9	*MMP-12*	*MMP-13*	MMP-9	MMP-12	MMP-13
KD group	26.59 ± 3.12	34.28 ± 4.52	50.49 ± 5.92	31.52 ± 4.12	42.18 ± 4.86	23.25 ± 2.85
NC group	100 ± 11.96	100 ± 12.46	100 ± 14.17	100 ± 13.34	100 ± 12.91	100 ± 14.28
t	26.264	19.854	11.095	22.452	13.685	29.589
P	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

3.5. Expression of invasion-related molecules in tumor tissue

mRNA contents and protein contents of MMP-9, MMP-12 and MMP-13 in tumor tissue of KD group were significantly lower than those of NC group, and differences between two groups were statistically significant ($P < 0.05$) (Table 5).

4. Discussion

Integrin αVβ3 is one of the key members of the integrin family, it is a type of receptor on cell membrane and it mainly mediates intercellular adhesion, cell-matrix adhesion, cell invasion, angiogenesis and other processes [5–7]. In recent years, more and more studies have confirmed that integrin αVβ3 is overexpressed in osteosarcoma, colorectal cancer and other malignant tumors, and it is closely related to the clinical and pathological processes of tumors [3,4]. According to studies of foreign scholars, integrin αVβ3 can be combined with fibrinogen, type I collagen, LM, FN and so on, and increase the capacity of heterogeneous adhesion of tumor cells [8,9]. In addition, integrin αVβ3 can crosslink with VEGF receptor, enhance VEGF function and induce VEGF receptor activation, thus promoting angiogenesis [10].

Tumor tissue growth involves many complex links that specifically include large proliferation of cancer cells in tumor lesion, cell separation from primary lesion and invasion into extracellular matrix, formation of local new blood vessels and lymphatic vessels, and cancer cell invasion into vascular system as well as colonization and growth in distal tissue and organs. Lung cancer cells are with strong ability of proliferation and invasion, so the incidence of recurrence and metastasis is high after surgical resection. At present, there is no report about the relationship between integrin αVβ3 and the development of lung cancer. Based on the trend of integrin αVβ3 in osteosarcoma, colorectal cancer and other malignant tumors, it was speculated that there was abnormal expression of integrin αVβ3 in lung cancer tissue. Detection of integrin αVβ3 expression in lung cancer tissue and paracancer normal tissue showed that mRNA content and protein content of integrin αVβ3 in lung cancer tissue were significantly higher than those in paracancer tissue and normal tissue, which indicated that the overexpression of integrin αVβ3 was involved in the occurrence and development of lung cancer.

In order to verify the specific relationship between integrin αVβ3 and lung cancer occurrence and development, lung cancer cell lines were cultured at first, adenovirus-mediated integrin αVβ3-shRNA was transfected to inhibit the expression of integrin αVβ3, then cells transfected with integrin αVβ3-shRNA and cells transfected with negative control-shRNA were subcutaneously injected into the mice, respectively, and observation of tumor tissue growth showed that after tumor cell inoculation, tumor tissue volume of both groups showed increasing trend, the increasing trend of tumor tissue volume of KD group was weaker than that of NC group and tumor tissue volume at various points in time of KD group was lower than that of NC group, which indicated that transfection of integrin αVβ3-shRNA could transfer? the growth of lung cancer xenograft.

Large formation of local angiogenesis is the key link of the occurrence and development of tumors, and links such as tumor growth and metastasis are highly dependent on rich blood supply of tumor tissue. Physiological angiogenesis process specifically means that new blood vessels are formed in the vascular structures that already exist, the formation of new blood vessels in tumor tissue mainly begins with small lesions without vascular structures, and new blood vessels that are formed under the effect of pro-angiogenesis molecules such as VEGF, FGF and EGF can provide the necessary oxygen and nutrients for tumor tissue and thus be conducive to tumor growth [11,12]. After knocking down integrin αVβ3 expression in tumor cells, it was detected that mRNA contents and protein contents of VEGF, FGF and EGF in tumor tissue of KD group were significantly lower than those of NC group, which indicated that inhibiting integrin αVβ3 could reduce expression of pro-angiogenesis factors in lung cancer xenografts.

Formation of new blood vessels in local xenografts provides the necessary nutrients for the completion of a variety of biological behaviors of tumor cells. In the occurrence and development process of lung cancer, proliferation and invasion are the most important two biological behaviors that cause tumor recurrence and metastasis, and they involve the expression changes of a variety of related genes. Bcl-2 family is a family of apoptosis-regulating molecules located in mitochondrial outer membrane, Bcl-2 has the effect of inhibiting apoptosis and enhancing anti-apoptotic ability of cells, and Bax can antagonize Bcl-2 function and induce apoptosis [13,14]. MMPs are a group of protease molecules involved in the degradation of extracellular matrix, and among them, MMP-9, MMP-12 and MMP-13 are closely related to lung cancer invasion and metastasis [15,16]. Analysis of proliferation and invasion-related gene expression in tumor tissue of mice with xenografts showed that mRNA contents and protein contents of Bcl-2, MMP-9, MMP-12 and MMP-13 in tumor tissue of KD group were significantly lower than those of NC group, and mRNA content and protein content of Bax were significantly higher than those of NC group, which indicated that inhibiting integrin αVβ3 could regulate the expression of proliferation and invasion-related genes, induce apoptosis and inhibit invasion.

Based on above discussion, it can be concluded that the expression of integrin αVβ3 increases in lung cancer tissue, and lentivirus-mediated integrin αVβ3-shRNA can inhibit tumor growth of mice with lung cancer xenografts.

Conflict of interest statement

We declare that we have no conflict of interest.

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