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Mechanism of SNHG12 in Regulating Human Angiostatin Binding Protein Through MicroRNA-497 in the Migration and Invasion of Human Lung Cancer Cells

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Abstract: *Objective:* To investigate the effect of small nucleolar host gene 12 (SNHG12) on the migration and invasion of human lung cancer cells by regulating human angiostatin binding protein through microribonucleic acid (microRNA)-497. *Methods:* A549, H1299, and PC9 cells were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium containing 10% fetal bovine serum, and human bronchial epithelial (HBE) cells were cultured in Dulbecco's modified eagle medium (DMEM) containing 10% fetal bovine serum. The incubator conditions were as follows: saturated humidity, 37°C, and 5% carbon dioxide (CO_2). *Results:* The gene expressions of small nucleolar host gene 12 (SNHG12) in HBE, A549, H1299, and PC9 were 1.00 ± 0.02, 5.61 ± 0.42, 3.78 ± 0.29, and 3.51 ± 0.23, respectively. The gene expressions of microRNA-497HBE, A549, H1299, and PC9 were 1.00 ± 0.13, 0.21 ± 0.04, 0.35 ± 0.05, and 0.37 ± 0.06, respectively, with $P < 0.05$. The microRNA-497 gene expression and cell apoptosis rate in the microRNA-497 group and the microRNA-497 + pcDNA3.1 group were significantly higher than those in the miR-NC group, whereas the A value and cell invasion number were significantly lower than those in the miR-negative control (NC) group, with $P < 0.05$. Compared with the microRNA-497 + pcDNA3.1 group, the microRNA-497 gene expression and cell apoptosis rate in the microRNA-497 + SNHG12 group were significantly lower, whereas the A value and cell invasion number were significantly higher, with $P < 0.05$. *Conclusion:* SNHG12 can inhibit the migration and invasion of human lung cancer cells by regulating human angiostatin binding protein through microRNA-497.

Keywords: SNHG12; MicroRNA-497; Lung cancer; Cell migration and invasion

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1. Introduction

At present, lung cancer has become a major threat to the health of the nation. Existing treatment modalities such as surgery, radiotherapy, and chemotherapy, which are guided by histomorphological changes in the development of lung cancer to a certain degree, have not made any breakthroughs despite the improvement and advancement in the past century. There are limitations in the treatment effect, the survival rate is low, the treatment situation is critical, and the treatment intensity and modalities need to be thoroughly addressed and improved [1–5]. Small nucleolar ribonucleic acids (RNAs) and microRNAs play important roles in tumor growth regulation. In lung cancer tissues, small nucleolar host gene 12 (SNHG12) is significantly

upregulated, whereas miR-497 is significantly downregulated. In lung cancer, the expression of angiomotin (AMOT) is significantly upregulated, and it is known to be negatively correlated with miR-497 expression. In addition, SNHG12, which is significantly upregulated in lung cancer tissues, seems to upregulate the expression of AMOT in lung cancer cells, and subsequently promote the proliferation and migration of tumor cells. It inhibits the expression of miR-497, thus leading to the significantly upregulated expression of AMOT in lung cancer. It also initiates angiogenesis and cell migration to promote the migration and survival of tumor cells. We need to investigate the signal transduction pathway of microRNA-497, which promotes the proliferation and migration of tumor cells through the upregulation of AMOT expression in lung cancer cells, and thus elucidate the mechanism by which SNHG12 affects the migration and invasion of human lung cancer cells through microRNA-497 regulation of human angiostatin binding protein. This would be a novel approach for investigating the invasion and migration of lung cancer cells and also provide a new therapeutic target for clinical treatment.

2. Data and methods

2.1. Materials

Normal human bronchial epithelial (HBE) cells and lung cancer A549, H1299, and PC9 cells (ATCC) were used in this study.

2.2. Methods

A549, H1299, and PC9 cells were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium containing 10% fetal bovine serum, and HBE cells were cultured in Dulbecco's modified eagle medium (DMEM) containing 10% fetal bovine serum. The incubator conditions were as follows: saturated humidity, 37℃, and 5% carbon dioxide (CO_2). Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was used to detect the expression of SNHG12 and microRNA-497 genes, 2,5-diphenyl-2H-tetrazolium bromide (MTT) assay was used to detect cell proliferation, transwell chamber was used to detect cell invasion, Annexin V-FITC/PI double staining method was used to detect cell apoptosis, and dual-luciferase reporter gene assay was used to detect cyclin D1, matrix metalloproteinase (MMP)-2, and sur-vivin expressions.

2.3. Statistical analysis

Each experiment was repeated three times. All experimental data were analyzed by SPSS 21.0, and the measurement data were expressed as mean ± standard deviation ($\bar{x} \pm s$). One-way analysis of variance was used to compare differences between multiple groups, and Student–Newman–Keuls (SNK)-q test was used for pairwise comparison. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Expression of SNHG12 and microRNA-497 in lung cancer cell lines

The gene expressions of SNHG12 in HBE, A549, H1299, and PC9 were 1.00 ± 0.02, 5.61 ± 0.42, 3.78 ± 0.29, and 3.51 ± 0.23, respectively. The gene expressions of microRNA-497HBE, A549, H1299, and PC9 were 1.00 ± 0.13, 0.21 ± 0.04, 0.35 ± 0.05, and 0.37 ± 0.06, respectively, with $P < 0.05$ (**Table 1**).

Table 1. Expression of SNHG12 and microRNA-497 in lung cancer cell lines

Cell	SNHG12 gene expression	microRNA-497 gene expression
HBE	1.00 ± 0.02	1.00 ± 0.13
A549	5.61 ± 0.42	0.21 ± 0.04
H1299	3.78 ± 0.29	0.35 ± 0.05
PC9	3.51 ± 0.23	0.37 ± 0.06
P	< 0.001	< 0.001

3.2. Expression of SNHG12 attenuates the effects of microRNA-497 on the proliferation, apoptosis, and invasion of A549 cells

The microRNA-497 gene expression and cell apoptosis rate in the microRNA-497 group and the microRNA-497 + pcDNA3.1 groups were significantly higher than those in the miR- negative control (NC) group, and the A value and cell invasion number were significantly lower than those in miR-NC group, with $P < 0.05$. Compared with the microRNA-497 + pcDNA3.1 group, the microRNA-497 gene expression and cell apoptosis rate in the microRNA-497 + SNHG12 group were significantly lower, and the A value and cell invasion number were significantly higher, with $P < 0.05$ (**Table 2**).

Table 2. SNHG12 regulates the effects of microRNA-497 on the proliferation, apoptosis, and invasion of A549 cells

Group	microRNA-497 gene expression	A value	Cell invasion number	Apoptosis rate (%)
miR-NC group	1.00 ± 0.03	0.76 ± 0.04	196.12 ± 6.21	2.53 ± 0.12
microRNA-497 group	7.14 ± 0.51	0.50 ± 0.04	141.58 ± 3.87	24.01 ± 2.14
microRNA-497 + pcDNA3.1 group	7.16 ± 0.52	0.51 ± 0.06	142.16 ± 3.49	23.89 ± 2.13
microRNA-497 + SNHG12 group	3.01 ± 0.22	0.71 ± 0.06	178.21 ± 5.02	11.25 ± 1.69
P	< 0.001	< 0.001	< 0.001	< 0.001

4. Discussion

Lung cancer has become a serious threat to the nation's health. Existing treatment modalities such as surgery, radiotherapy, and chemotherapy, which are guided by the histomorphological changes to a certain degree, have not made any breakthroughs despite the improvement and advancements over the past century. The treatment effect is clearly limited, and the survival rate is low. The critical treatment situation, the treatment modalities, and the treatment intensity need to be addressed and improved. In recent years, an increasing number of studies have found that SNHG12 and microRNA (miRNA) play important roles in regulating tumor growth. SNHG12 expression has been found to be upregulated in lung cancer tissues and cell lines, along with the expression of human angiostatin binding protein (AMOT), and the two expressions have also been found to be positively correlated. Researchers are interested in finding out whether SNHG12 could promote the proliferation and migration of tumor cells by upregulating the expression of AMOT in lung cancer cells. In addition, the regulatory signaling pathway has also become a focus for researchers.

SNHG12 has been found to be significantly upregulated in lung cancer tissues. This may be closely related to the migration and invasion capacities of lung cancer cells. There are many genes in the biological genome that encodes RNA instead of protein. These genes are called non-coding genes, and the

corresponding RNA is known as a non-coding RNA, which cannot be translated into protein but is widely involved in almost all physiological and pathological activities of the human body, including regulating the occurrence and development of tumors. Small nucleolar RNA (snoRNA) is a non-coding RNA with 60–300 nucleotides in the nucleolus of eukaryotic cells. It is mainly involved in the maturation process of ribosomal ribonucleic acid (rRNA) and other small regulatory RNAs after transcription. The mature rRNA processed by snoRNA first binds to ribosomal proteins in the nucleolus, and then leaves the nucleus through further complex maturation and transport processes; it finally forms functional mature ribosomes in the cytoplasm. Ribosome is the site of protein synthesis. It controls the synthesis of almost all proteins in cells. Therefore, snoRNA is extremely important for cell growth and even life activities [6,7]. Since snoRNA is closely related to the occurrence and development of tumors, it is involved in tumorigenesis in many ways. Some snoRNAs, such as U50, SNORD12, SNORD12b, SNORD12c, SNORD44, H5SN2, etc., have tumor-suppressive properties, while other snoRNAs, such as SNORD33, SNORD66, SNORD76, SNORD112, SNORD113, SNORD114, SNORA42, U70C, and ACA59B, have cancer-promoting properties [8–12]. In addition, abnormalities in gene encoding snoRNA have also been found to be associated with tumorigenesis. SNHG12 is an snoRNA, which regulates the migration of endothelial cells and plays an important role in cell proliferation and migration. MicroRNA (miRNA) is a non-coding regulatory RNA with 17–25 nucleotides. It may act as an oncogene, tumor suppressor gene, or even both to regulate various key biological processes of tumor cells, including cell differentiation, development, apoptosis, proliferation, and so on. Many literatures have reported that mirR-497 promotes cell apoptosis and inhibits cell proliferation through key molecules in the cell cycle and some cell signal transduction pathways, such as PI3K-AKT-mTOR and MAPK/ERK signaling pathways. AMOT has the function of regulating cell movement. It inhibits the migration and formation of endothelial cells by binding with angiogenesis inhibin [13,14]. The protein AMOT is composed of 675 amino acid residues and is mainly located within the endothelial cell membrane. It has two topoisomers, which play different roles in different stages of angiogenesis, namely AMOT1 (also known as JEAP protein) and AMOT2 (also known as MASCOT protein). Together, the two proteins constitute the AMOT protein family. AMOT2 promotes the formation of neoplastic blood vessels by promoting the migration of endothelial cells and stabilizing and regulating the polar arrangement of vascular cells. AMOT is an angiostatin binding protein, which plays an important role in regulating endothelial cell migration and tubular structure formation. It binds to angiostatin and mediates its inhibitory effect on endothelial cell migration. AMOT is highly expressed in malignant tumors, promotes the growth of malignant tumors and the vascular invasion of cancer cells, as well as leads to the formation of tumor thrombus. This protein enhances cell migration and stabilizes tubular structures, and it is associated with actin and affects cell shaping. The expression of AMOT in mouse embryonic retina during angiogenesis mainly occurs during the cell migration stage. AMOT plays a crucial role in embryogenesis and tumor invasion. Moreover, the regulatory switch between the phenotypes of migrating and non-migrating cells may be determined by the expression level of AMOT protein, which initiates angiogenesis and cell migration, thereby promoting neovascularization.

The rapid proliferation and metastasis of malignant cells are mainly caused by the abnormal activation of oncogenes and the inactivation or mutation of tumor suppressor genes, which may further reduce the adhesion of cancer tissues and result in the abnormal migration and apoptosis of cancer cells. Moreover, due to the degradation of intercellular adhesion molecules and the dissolution of extracellular matrix, tumor cells invade lymph nodes, blood vessels, and other tissues, and then metastasize, especially after penetrating the basement membrane and entering the connective tissue. It has been recognized that proto-oncogenes and tumor cell surface adhesion molecules are important factors affecting the occurrence, invasion, and metastasis of tumor cells, in which both of them have their own roles, respectively. Since the formation of new blood vessels may promote tumor growth, researchers anticipate that the growth of malignant tumors

may be inhibited by inhibiting tumor angiogenesis, thus achieving the goal of cancer treatment. AMOT may be associated with the degree of malignancy and invasive properties of cancer. The expression and transcription levels of AMOT increase with the decrease of cell differentiation, and the expression of AMOT has been found to be significantly higher in invasive tumors, which is in sharp contrast to its relatively low expression in non-invasive tumors. AMOT, a common marker of angiogenesis, is positively correlated with its expression level. Although lymphatic metastasis is acknowledged as the primary mechanism for tumor metastasis, hematologic spread, particularly in patients with advanced malignancies, is not uncommon; it is in fact an important mechanism by which disease deteriorates and aggravates in cancer patients. If selectively targeted antibodies can inhibit tumor growth and metastasis by inhibiting physiological and pathological neovascularization, or even degrade and downstage malignant tumors, it may become a promising treatment used to inhibit neoplastic vascular formation. When AMOT antibody binds to the surface of endothelial cells, it acts like an angiogenic statin, thereby inducing endothelial migration. With systemic or local treatment, AMOT antibody can inhibit pathological angiogenesis, thus inhibiting tumor growth. Under the action of AMOT antibody, the number of endothelial cell pseudopods manifested as neovascularization *in vivo* significantly reduces, and the migration of endothelial cells is also inhibited.

In conclusion, SNHG12 can inhibit the migration and invasion of human lung cancer cells by regulating human angiostatin binding protein through microRNA-497.

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Disclosure statement

The authors declare no conflict of interest.

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