Role and clinical significance of LncRNA16 in the malignant proliferation of lung cancer cells

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ABSTRACT

The highest mortality rate among cancer patients is lung cancer. A large proportion of cancer patients are lung cancer patients. The incidence rate of lung cancer is the highest in the world. In recent years, long-chain non-coding RNA, or LncRNA, has become more and more closely related to cancer and has gradually attracted the attention of many cancer researchers. LncRNA is the proto-oncogene of cancer. We can find out the cause of cancer and put forward effective methods to inhibit the occurrence of cancer by studying LncRNA. This study aimed to investigate the effect of noncoding long-chain RNA16 on the proliferation and molecular mechanism of lung cancer cells. During the operation, the distance between the excised lung cancer block and the adjacent tissue is 2cm from the corresponding lung cancer block. Then, the total RNA in lung cancer cells and tissues is extracted with a Trizole reagent. Then, the expression profile of LncRNA in three cases of lung cancer and the corresponding adjacent tissue is identified by RNA chip technology. The LncRNA related to proliferation and the expression difference is significant through bioinformatics. The conclusion was that the A549 and H1299 groups of shLncRNA16 could significantly reduce the incidence by 64% and 40% percentage points compared with the experimental group, which indicates that LncRNA16 can be used as a potential treatment target for patients.

Introduction

Lung cancer is characterized by the growth of lung cancer in lung cancer, which may be primary, originated from lung cells, or secondary, and transferred from another source. In the latest research, Engreitz has found that the peak age of lung cancer in Britain is between 75 to 80 years (1). His study found that 30% to 45% of all lung cancers were diagnosed in patients over 70 years of age, and many of the symptoms of the elderly were nonspecific, such as fever and weight loss, usually due to complications (2,3). Engreitz study found that the paraneoplastic phenomenon related to some types of tumors may be the first sign of lung cancer. When evaluating mediastinum, MRI of the chest is equivalent to CT, but it is usually superior in determining chest wall or vertebral body involvement (4,5). At present, the mainstream treatment of lung cancer will largely depend on the type of cell, the extent of diffusion and the physical condition of the patient, usually including surgery, chemotherapy and radiotherapy (6,7). This study has a certain reference value but has great limitations (8).

In recent years, there are more and more papers about the relationship between LncRNA and lung cancer. Xiao C found that LncRNA Hox transcription antisense RNA (hotair) can raise chromatin modifying factors to inhibit gene expression (9). Xiao C found that the expression of hotair increased in lung cancer and was related to metastasis and poor prognosis. In addition, hotair also promoted the proliferation, survival, invasion, metastasis and drug resistance of lung cancer cells (10,11). Xiao C reviewed the molecular mechanism of hotair mediated invasive phenotype of lung cancer, and finally discussed the potential of hotair in the diagnosis and treatment of lung cancer, as well as the challenge of using hotair for lung cancer intervention (12). The expression, biological function and clinical significance of pvt1 in lung cancer. Xiao C detected the expression of LncRNA PVT1 in 82 NSCLC tissues and 3 lung cancer cell lines by quantitative real-time PCR (QRT PCR). It was found that LncRNA in NSCLC tissues and lung cancer cells was higher than that in adjacent normal tissues and normal bronchial epithelial cells PVT1 expression was significantly up-regulated, indicating that the increase of PVT1 expression was closely related to histological grade and lymph node metastasis (13,14). This research progress provides a potential new treatment strategy for lung cancer treatment, but its feasibility still needs to be confirmed (15).

In this paper, we have previously examined the relationship between long-chain noncoding RNA16 and lung cancer cell proliferation. First of all, we use gene chip technology to detect the differential expression of LncRNA between lung cancer tissues and the corresponding adjacent tissues and then carry out follow-up cell function experiments on the long-chain non-coding RNA16 with
the largest expression difference to detect the long-chain non-coding RNA16. After that, we discussed the effect of genes on the proliferation and apoptosis of lung cancer cells and their molecular mechanism. The purpose of this study is to screen and verify the specificity and sensitivity of LncRNA related to the incidence of lung cancer. In order to identify and improve the molecular mechanism of lung cancer, the importance of long-chain non-coding RNA16 is a potential goal of lung cancer diagnosis and treatment and provides data support and a theoretical basis.

Lung cancer is very common, and the risk factors for lung cancer mainly include smoking, air pollution, genetics, etc., through the deletion of alleles (loss of heterozygosity), chromosome instability and imbalance, mutation of oncogenes and tumor suppressor genes and methylation of promoters, the resulting epigenetic gene silencing and abnormal gene expression of cell proliferation contribute to tumor development (16).

With the development of medical technology, many diagnostic methods for lung cancer have been invented, such as imaging technology, laparoscopic surgery, etc., but there are great limitations. The current gold standard for lung cancer diagnosis still depends on the histopathological diagnosis, but it is traumatic and patients must bear more pain, so there is an urgent need for less injury and early diagnosis of clinical methods. With the development of molecular biology, many tumor-related genes have been found. For example, TMEM74 is a membrane protein that induces autophagy, and it has growth effects on HeLa cells (cervical cells) and HepG cells (liver) and other tumor cell lines (17,18).

It is reported that about 50% of cancer patients receive some form of radiotherapy alone or in combination with chemotherapy and/or surgery. Although radiation can effectively kill cancer cells to achieve local tumor control, not all malignant tumors are effective. The poor clinical radiotherapy may be due to the insensitivity of the tumor to the dose of ionizing radiation or the ability of tumor cells to accept such insensitivity. Clinically, this phenomenon is defined as resistance to radiotherapy. Due to DNA damage and the repair of mutated genes, resistance to radiotherapy is often encountered, thus affecting the regulation of the cell cycle, activating various biological processes to protect cancer cells from radiation damage caused by tumor hypoxia, activating the signal pathway of survival before cell survival, promoting angiogenesis and Epithelial stromal transformation. Therefore, at present, radiation resistance is still a clinical problem that directly affects the development of cancer, recurrence and death (19).

LncRNA was once considered as a "junk gene" and did not play a biological role in biology, but with the birth and rapid development of gene chip technology and high-performance sequencing technology (20). The results show that there are similarities between LncRNA and mRNA. It is speculated that the main sources are: genetic structure destroyed and chromosome rearrangement. At present, antisense transcription of LncRNA, the most widely studied, plays an important role in many diseases. According to the literature, the functions of LncRNA mainly include transcription intervention, chromatin remodeling, promoter inactivation induced by transcription binding factors, auxiliary protein activation, transcription factor activation, oligomerization of activated proteins, transcription factor transport and epigenetics. The function of LncRNA will also be determined by the epigenetic inhibition of genes and the specific sub-location of LncRNA in cells. According to the location of LncRNA in the genome, it can be divided into four subtypes (21-22). Among them, LncRNA overlapped with protein mRNA molecules will affect the transcription level of neighboring genes, thus affecting many physiological and pathological activities. LncRNA controls gene transcription through promoters that competitively bind to p53 protein. Therefore, the molecular mechanism of LncRNA can be studied to find a new mechanism of action (23).

With the development of LncRNA research, the relationship between tumor and LncRNA is becoming clear. LncRNA can submerge PRC2 by recruiting cytosine, thus promoting the occurrence and development of lung cancer, over expressing in vivo, and inhibiting the proliferation of small cell lung cancer in vitro (24). It is found that LncRNA can not only be used as a photo-oncogene to stimulate the proliferation of tumor cells through appropriate ways, but also another part of LncRNA can be tumor suppressor gene, promote cell apoptosis or interact with other related genes, and inhibit the emergence and development of tumor. Therefore, lung cancer research can start with the main genes encoding proteins, and use high-performance sequencing technology to detect the corresponding LncRNA (25).

Materials and Methods

The study collected 80 lung cancer patients who underwent lung cancer surgery. The tumor was removed and immersed in dirt and kept in Marin. In addition to the mass, normal lung tissue was cut more than 2cm away from the mass as a negative control. All patients did not receive radiotherapy or chemotherapy before the operation. All patients signed informed consent and realized that tissue samples had been used for this scientific study. In this study, LncRNA chips were used to detect RNA in three lung cancer tissues and corresponding normal tissues.

Construction and transfection of lentivirus

The lentivirus used in this study was manufactured by Shanghai Jikai Biotechnology Co., Ltd. and constructed lentivirus (Lenti-LncRNA16) with full-length LncRNA16 sequence and lentivirus (LncRNA16-shRNA) with lncRNA16 gene. The screening method and stable transfectors of lentivirus were as follows: lentivirus-LncRNA16 and LncRNA16-shRNA lentivirus were added respectively, and cell culture was carried out. On the base, incubation continued for 24 hours to obtain transiently transfected cell lines. After transfection with Lenti-LncRNA16 and LncRNA16-shRNA lentiviruses, the stable transfectors were added to the culture medium together with puromycin and cultured for 2 weeks.

Tissue and cell RNA extraction

In this study, Trizol was used to extract 100 samples from lung cancer tissues in six pore plates per 100 ml of Trizol, or in 100 tissues of 50-80 mg. With Trizol, 5-15ugRNA could be extracted from one million cells, and 1-10ugRNA could be extracted from one mg of tissues. The method of extracting tissue RNA is as follows: Firstly, take out a tissue from formalin, cut tissue with scissors, then crush tissue with tissue homogenizer to make it homogeneous,
soak scissors and other tools in 0.1% DEPC water at 37 °C for 12 hours, and then sterilize at 120 °C for 30 minutes to remove the remaining DEPC. Operators also need to wear masks and hats to prevent RNA samples from being contaminated by tissue samples. After grinding the tissue, put about 200 mg of tissue powder into a centrifuge tube, add 3 ml of Trizol reagent and shake well to mix well. The supernatant contains RNA. After absorbing the supernatant, place it in a sterile centrifuge tube without enzyme. Then add 1 ml isopropanol to the supernatant, place it at room temperature for 15 minutes, and then centrifuge at low speed. After centrifugation at high speed for 15 minutes (12000 RPM), RNA was centrifuged and precipitated at the bottom of the centrifuge tube. The precipitate was white. Discard the supernatant, leaving the white precipitate as RNA, then add DEPC to the centrifuge tube, and dissolve the RNA in water to obtain different concentrations of RNA. The RNA sample is stored in an ultra-low-temperature refrigerator at - 80 °C for future reference. The process of RNA separation from tissues is described above. The method of cell RNA extraction is the same as that of tissue RNA extraction, but it is slightly different in the stage of cell processing. This is a brief description of the cell treatment method. 95-d and A549 cells in the logarithmic growth stage were selected and cultured in a 10 cm dish. After removing the medium, directly add 1-2 ml of triazole solution, and carefully pipette with a disposable pipette to make the cell completely contact with the triazole solution, and incubate at room temperature for 10 minutes, so that the cell can fully split. The subsequent steps of RNA extraction are the same as above.

**LncRNAChip detection**

The discovery of the LncRNA chip used by the Institute was completed by Shanghai Borui Biotechnology Co., Ltd. Trizol was used to isolate the total RNA from three cases of lung cancer and paracancerous tissues and then sent to the company for detection of the LNC RNA chip (Agilent company of the United States). The chip can detect 41053 LncRNAs and 29417 mRNA, and the test results are confirmed by real-time PCR.

**Statistical Analaysis**

Spss21.0 software was used for data analysis in this experiment, and the experiment process was repeated three times, so as to ensure the stability and accuracy of the experimental results. The Chi-square test was used in clinical correlation analysis, and the difference was statistically significant (P < 0.05).

**Results**

**Up-regulation of mRNA expression of LncRNA16 in lung cancer**

Real-time fluorescence quantitative PCR was used to detect the expression level of LncRNA16 mRNA in lung cancer and adjacent tissues. The results are shown in Table 1.

From Table 1, we can see that the expression level of 16 mRNA in LncRNA16 in tumor tissue is 0.94 (0.48, 1.70), and the expression level near cancer tissue is 0.65 (0.45, 0.86) (P = 0.019). It can be seen that compared with the lung cancer group and the adjacent cancer group, LncRNA16 is highly expressed in lung cancer tissue, P = 0.019.

**Cell cycle percentage, colony forming ability and cell proliferation activity of A549 cells in each group**

The results of flow cytometry showed that the percentage of cells in the G0 / G1 phase was 61.15% ± 1.07% in the Si-NC group and 24.28% ± 1.16% in the S phase. Compared with Si-NC group, the percentage of G1 phase cells (71.6% ± 0.96%) in Si-LncRNA16 group of G0 cells increased significantly (P < 0.05), while the percentage of cells (16.04% ± 0.92%) decreased significantly (P < 0.05). The results of cell colony formation are shown in Figure 1.

It can be seen from Figure 1 that the colony-forming ability (58.00 ± 7.37) of the Si-LncRNA16 group is significantly lower than that of the Si-NC group (317.00 ± 57.97) (P < 0.05). The results of the CCK8 experiment showed that compared with the Si-NC group (0.92 ± 0.07), the proliferation activity of the Si-LncRNA16 group (0.67 ± 0.08) decreased significantly (P < 0.01).

**mRNA and protein expression of LncRNA16, cyclinD1 and Cdkn2b in A549 cells of each group**

In this study, QRT-PCR was used to detect: Compared with the si-NC group, the expression level of LncRNA16 and CyclinD1 mRNA in A549 cells of the Si-LncRNA16 group was significantly decreased, and the expression level of Cdkn2b mRNA and Protein was significantly increased.

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group was significantly lower (P < 0.05 or P < 0.01), and the expression level of cdkn2b mRNA was significantly higher (P < 0.01). The experimental results are shown in Figure 2.

It can be seen from Figure 2 that according to the Western blotting detection method: compared with the Si-NC group, the expression level of cyclinD1 protein in A549 cells of the Si-LncRNA16 group was significantly lower (P < 0.05), while the expression level of cdkn2b protein was significantly lower (P < 0.05).

**Verification of down-regulation of stable lung cancer strains**

The cell lines A549 and H1299 of shLncRNA16 and SHNC plasmids transfected with the virus were observed under a fluorescence microscope, as shown in Figure 3.

By observing the efficiency of fluorescence expression in Figure 3 cells, we selected A549 and H1299 cell lines stably expressing shLncRNA16, extracted SHNC, extracted RNA and protein (for standby), and checked the expression level of LncRNA16 mRNA in transfected cells relative to SHNC cells by RT-PCR, as shown in Figure 4.

As can be seen from Figure 4, shLncRNA16 mRNA expression in A549 was reduced by 40% (P < 0.001) compared with normal cells, while LncRNA16 mRNA expression in H1299 was reduced by 70% (P < 0.001) compared with normal cells, so a stable cell line was successfully established.

**Discussion**

Lung cancer mortality ranks fourth in China. Most of the patients are diagnosed as advanced cancer with short survival time and poor prognosis. Traditional surgery or radiotherapy, chemotherapy have limitations, most patients will show drug resistance in the later stage, leading to cancer cell metastasis leading to treatment failure, so it is urgent to find the pathogenesis of lung cancer to ensure the subsequent clinical diagnosis and treatment, good reference basis.

In this study, we found the expression of iLncRNA16 in cancer tissues and paracancerous tissues of lung cancer patients, and found that incRNA was abnormally high in lung cancer, suggesting that it may be related to some biological functions of lung cancer. In subsequent experiments, shRNA expressing lentivirus was used to inhibit the proliferation of cancer cells, and puromycin was screened to obtain stable A549 and H1299 strains, which continued to inhibit LncRNA16. Through Transwell, cell clone test, CCK8 and other experiments, it was found that incRNA was inhibited in lung cancer.

In this study, we also found that LncRNA, as the antisense transcription of Dicer1 ribonuclease type III, is located on chromosome 14, so LncRNA16 may be transcribed from atcevw. It has been reported that in the absence of Dicer1 protein, up-regulation of sequence expression can be activated to promote the proliferation, invasion and metastasis of lung cancer cells.

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Not applicable.

**Interest conflict**

The authors declare that they have no conflict of interest.

**References**


