Comparison of metabolic characteristics of lung cancer cells and normal somatic cells in low temperature environment

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Abstract: Objective: to compare the metabolic characteristics of lung cancer cells and normal somatic cells at low temperature. Methods: The low temperature (− 5.5℃ incubator) was used to simulate the in vivo environment of the tumor, and the energy metabolism of lung cancer cell line A549 and normal somatic cells at different low temperature was compared. Results: The establishment of three-dimensional culture model in vitro was confirmed through inverted microscope, electron microscope observation and photography. N-Cadherin and catenin (α, β) are highly expressed in normal human lung cells, and AJ junction distributed on the cell membrane and its adhesion spot on the inner surface of the membrane are connected with the end of stress fiber of Actin filament assembled in the cytoplasm, suggesting the existence of N-N-Cadherin/Catenins/Actin actin molecular complex connected with membrane AJ. After low temperature, lung cancer cell line A549 has higher survival rate and smaller increase of active oxygen compared with normal body cells. Conclusion: Under low temperature stress, normal cells mainly rely on aerobic oxidation to compensate for energy, while tumor cells mainly rely on glycolysis. ROS may promote the Warburg effect and tumor tolerance to low temperature by regulating tumor cell pathways and the activities of key glycolytic enzymes.

1. Introduction

Lung cancer is the most common primary malignant tumor of the lung. The vast majority of lung cancer originates from bronchial mucosal epithelium, which is a tumor composed of epidermal-like, glandular-like, or undifferentiated cells grown from bronchi, thus it is also called bronchial lung cancer. In recent decades, the incidence and mortality of lung cancer have increased significantly in all countries in the world, especially in the industrially developed countries.

A large number of studies have confirmed that the low temperature environment in some parts of the tumor will increase the aggressive behavior of the tumor and show a worse prognosis [1]. The strong tolerance of tumor cells to low temperature is considered to be an important factor for tumor development, invasion and metastasis [2]. In this study, the low-temperature environment (-5.5 C incubator) was used to simulate the low-temperature environment inside the tumor, to compare the metabolic characteristics of tumor cells and normal cells in the low-temperature environment, and to explore the mechanism of low-temperature tolerance of tumor cells, so as to provide new ideas for targeted therapy of tumors.

2. Conventional diagnostic methods for lung cancer

2.1. X-ray examination

X-ray examination is the most commonly used important means to diagnose lung cancer. We can know the location and size of lung cancer through line examination. Early case-line examination of lung cancer, although it has not yet revealed a mass, may reveal local emphysema due to bronchial obstruction, atelectasis, or invasive lesions or pulmonary inflammation in the vicinity of the lesion. In recent years, CT has been widely used in clinical practice. This examination method can display thin-layer cross-sectional three-dimensional structural images to avoid mutual overlap of lesions

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and images of normal tissues, and is very valuable for early detection of lung cancer lesions in general hidden areas X-ray examination (such as lung apex, septum, paravertebral column, areas close to chest wall pleura, the rear of the heart, mediastinum, and so on) and determination of whether the mediastinal lymph nodes are enlarged.

2.2. Cytologic examination

Exfoliated cancer cells can be found in sputum of most patients with primary lung cancer and the histological type of cancer cells can be determined [3–4]. Therefore, sputum cytology is a simple and effective method for the general investigation and diagnosis of lung cancer. After getting up, clean water was used to wash mouth. Fresh sputum coughed out from the deep part of lung or bronchial secretions sucked out through bronchoscopy washing can be used as examination specimens. Multiple sputum cytology examinations can improve the positive rate.

2.3. Bronchoscopy

Bronchoscopy is an important measure for the diagnosis of lung cancer [5]. Bronchoscopy can be used to directly observe the pathological changes of endobronchial membrane and lumen. If the tumor or carcinomatous infiltration is detected, tissues can be taken for pathological section examination, or bronchial secretions can be aspirated for cytological examination, in order to confirm the diagnosis and determine the histological type. However, bronchial adenoma is not suitable for bronchoscopy biopsy due to rich blood vessels, so as to avoid causing massive bleeding.

In addition, there are other conventional diagnostic methods for lung cancer [6–7]: mediastinoscopy, percutaneous lung biopsy, biopsy of metastatic lesions, mediastinal incision, and thoracotomy.

3. Materials and methods

3.1. Experimental materials

A549 cell line of lung cancer, Cultispher-s (Hyclone, the US), Spinner flask (bellcoglass, the UK), slow magnetic stirrer (Silla Instruments Co., Shanghai, China), desktop water bath thermostatic oscillator (Huangjing Communication Instruments Co., Ltd., Taicang, Jiangsu, China), FACS Calibur flow cytometer (Biorad, the US), and in-situ apoptosis detection kit (Beijing Zhongshan Biotechnology Co., Ltd.).

3.2. Cell culture and temperature

The lung cancer A549 cell line was cultured in DMEM containing 10% FBS (containing penicillin and streptomycin 100 U/mL each) at-5.5 C and 5% CO2 until the adherent area reached more than 80%. The sections were digested with 0.25% trypsin containing 0.02% EDTA, passaged in the ratio of 1: 2 or 1: 4, and adhered for 4–6 h for later use.

Normal somatic cells were reserved after 4 h of inoculation after isolation. The adherent cells were transferred into the conventional cell incubator for continuous culture for 24 h, as the control group at low temperature of 0 h. The medium was incubated in the conventional incubator for 18 h before being transferred to the-5.5 C 25.5% O2 low-temperature incubator for 6 h as the low-temperature 6 h group. The medium was incubated in the conventional incubator for 12 h before being transferred to the-5.5 C 25.5% O2 low-temperature incubator for 12 h as the low-temperature 12 h group. The samples were directly transferred to a low-temperature incubator at-5.5 C and 25.5% O2 for further culture for 24 h, as the low-temperature 24 h group.

3.3. Extraction and detection of cell culture solution

Firstly, 30m1 of prepared fresh DMEM culture solution was put into a 100m1 glass bottle with bottle stopper, which was washed and dried, and was also put into a 1cm long magnetic stirrer. Put the glass bottle on a magnetic stirrer and start stirring at a speed of 1200 rpm. The SPME was stoppered and extracted at-3 C and-5 C for 20, 40 and 60 minutes in head space air volatilized from the medium. At the end of the extraction, SPME was inserted into the inlet of the GC and analyzed

according to the GC working conditions previously set by us.

3.4. Detection of apoptosis

According to the instructions of the in situ apoptosis kit (cell counting was performed before grouping to ensure that the number of cells in each group was basically the same), and negative and positive controls were set according to the instructions. Apoptosis rate = number of apoptotic cells/total number of cells in the same field × 100%.

4. Result

4.1. In vitro culture of lung cancer A549 cells

After one to two days of solid culture, the cells grew in the spherical carrier under the inverted microscope, and the transmittance of the carrier was significantly decreased, with a small amount of cell adhesion on the surface. After 4–5 days of culture, a large number of cells grew in the carrier under the inverted microscope. The carrier was opaque and there were multiple layers of cell adhesion and clustering on the surface part of the carrier, forming a large spherical cell population. The scanning electron microscope showed that a large number of cells tightly adhered to the surface of a single spherical carrier to form a three-dimensional spherical structure, further confirming the success of the establishment of the three-dimensional model.

4.2. Western blot and immunoprecipitation analysis of protein

N-Cadherin immunoblotting showed that normal human lung cells had a strong positive band at 135 ku, showing high expression of N-Cadherin protein. PG cells of lung cancer had a weak positive band of N-Cadherin, and the expression of N-Cadherin protein was significantly inhibited as compared with normal RF cells.

The immunoblotting results of α-Catenin showed that normal human lung cells had a strong positive reaction band of RF at 102 ku, and the expression level of α-Catenin was high. The PG cells of lung cancer also had a positive reaction, but it was weaker than normal lung cells, and the expression was inhibited to a certain extent.

The results of β-Catenin immunoprecipitation showed that the cytoplasmic soluble fraction (TN extraction) of β-Catenin and the fraction that was tightly bound to the skeleton (RIPA extraction) of normal lung cell RF and lung cancer cell PG showed positive reactions at − 94 KU. The expression levels of β-Catenin in the two groups of cells were similar from the reaction intensity. The migration rate of the TN component in normal lung cells is slightly slower than that in PG cells. The two reaction bands of β-Catenin may represent the differences in protein posttranslational modifications (such as phosphorylation) [8].

4.3. Difference of ROS level and survival between lung cancer cells and normal somatic cells under low temperature

With the prolongation of hypothermia, the ROS levels of lung cancer cells and normal hepatocytes were gradually increased, but the normal cells increased faster and more. The ROS level of normal cells in the 24 h hypothermia group was four times that of normal cells in the 0 h hypothermia group, while the ROS level of lung cancer cell line A549 in the 24 h hypothermia group was only 1.8 times that of lung cancer cell line A549 in the 0 h hypothermia group. During this process, the ROS levels in each group of the lung cancer cell line A549 were always higher than those in the normal cell group under the same conditions (Figure 1a).

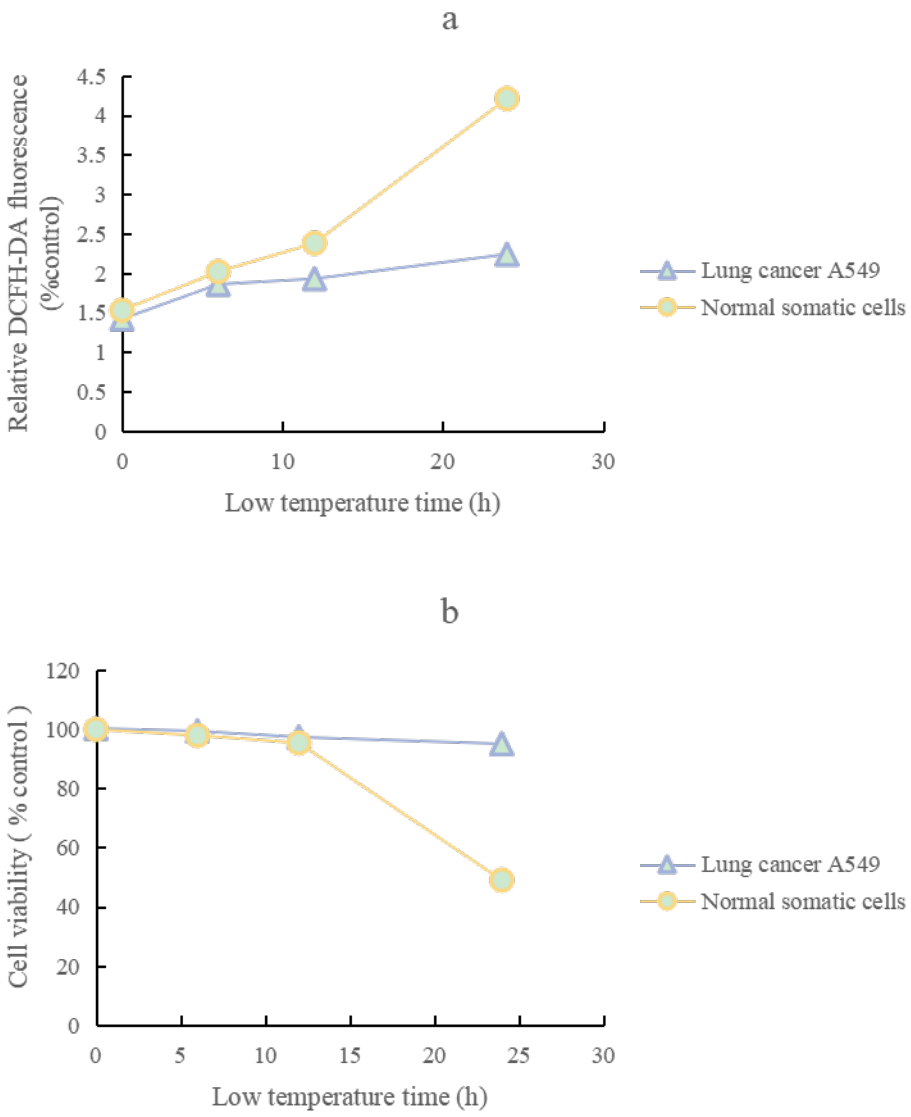

Figure 1 Survival curve and intracellular ROS level of hypothermic normal somatic cells and lung cancer cell line A549

Under low temperature, the viability of both cells decreased with the prolongation of low temperature, but that of normal somatic cells decreased more significantly. The viability of normal somatic cells in the low temperature 24 h group was reduced to less than 35%, while that of lung cancer cell line A549 in the low temperature 24 h group remained at about 80%, indicating that tumor cells had strong tolerance to low temperature (Fig. 1b).

5. Discussion

The living environment, surrounding spatial structure, mechanical characteristics of solid tumor cells in human body and the extensive signal communication between cells have an important impact on their biological behavior. However, previous in vitro experimental studies are mostly based on the planar monolayer culture system, focusing on the characteristics of individual tumor cells, ignoring the interaction between tumor cells and the overall characteristics of the special microenvironment in which they are located. At present, the research on in vitro three-dimensional culture of tumor cells in China is still rare. Although there are relevant reports abroad, most of them use expensive equipment such as rotary incubator and three-dimensional culture column, which is not conducive to its wide use in China. We used a rotating flask combined with a desktop water

bath thermostatic oscillator to conduct three-dimensional culture of lung cancer A549 cells in vitro on the basis of a spherical carrier. The observation under an inverted microscope and the detection under a scanning electron microscope showed that the three-dimensional culture model of lung cancer A549 cells in vitro was successfully established. It has important practical significance for studying the biological characteristics of solid tumors.

Based on the evidence of protein immunoblotting, immunoprecipitation analysis and immunofluorescence counterstaining, it was demonstrated that N-Cadherin and catenin (α, β) in normal human lung cells were highly expressed, distributed in the AJ junction of cell membrane and its intramembranous adhesion patch, and connected to the end of Actin microfilament stress fiber with good assembly in the cytoplasm, indicating the existence of the N-N-Cadherin/Catenins/Actin actin molecular complex with AJ junction. It was speculated that the AJ linker molecular complex played an important role in adhesion, communication, cytoskeletal and morphological maintenance of RF cells and in the phenotypic regulation of normal cell growth. The inhibition of AJ linker protein expression was associated with the destruction of the molecular complex and cytoskeletal disintegration, which were the important causes for the adhesion destruction and morphological changes in PG cells. In addition, it might interact with the communication dysfunction of PG cell gap junction and be closely related to the malignant growth and metastasis of PG cells.

After low temperature, normal cells will compensate for the lack of energy output caused by oxygen deficiency by improving the expression level of genes related to aerobic oxidation, indicating that normal cells are still mainly dependent on aerobic oxidation function in a low temperature environment. However, this obviously cannot be maintained for a long time. With the prolongation of the low temperature, the respiratory chain of oxygen as the final electron receptor was blocked, leading to the cell energy depletion and death. At the same time, it also prevents the large increase of ROS due to the blocked respiratory chain and electron overflow, and reduces cell low temperature damage. The results of this study also confirmed this viewpoint, that the ROS increase in tumor cells after hypothermia was smaller than that in normal cells (Figure 1a). These results suggest that normal cells still rely on aerobic oxidative compensation for increased supplemental energy at low temperature, while tumor cells rely on rapidly and highly activated anaerobic glycolysis. Previous studies [9] have shown that low temperature can induce ROS release and up-regulate the intracellular glycolysis pathway by low temperature induction factors, indicating that the Warburg effect of cancer may be related to the microenvironment of low temperature oxidative stress.

6. Conclusions

In summary, under low temperature stress, normal cells mainly rely on aerobic oxidation to compensate for energy, and tumor cells mainly rely on glycolysis. ROS may promote the Warburg effect and tumor tolerance to low temperature by regulating tumor cell pathways and the activities of key glycolytic enzymes.

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