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Case Report

Case Report: Phosphorylation Analysis of a Chemotherapy-resistant Lung Squamous Cell Carcinoma Patient Specimen

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Abstract

Lung squamous cell carcinoma (LUSC) is one of the most common subtypes of non-small cell lung cancer, accounting for approximately 25-30%. Chemotherapy remains one of the main treatment options for lung squamous cell carcinoma, but long-term use often leads to the development of drug resistance in LUSC, resulting in reduced chemotherapy efficacy. This report described a patient with lung squamous cell carcinoma who underwent albumin paclitaxel+carboplatin chemotherapy after being diagnosed with right primary bronchogenic carcinoma (LUSC, cT3N1M0 stage IIIA). Two months later, examination revealed that the tumor volume seemed to have increased, indicating the development of chemotherapy resistance. Phosphorylation omics analysis was performed on adjacent and tumor tissues of patients, and it was found that the phosphorylation abundance of some loci in genes such as TRAF2 (T7) and PAK2 (S2) changed, which may be a key factor in the development of chemotherapy resistance.

Keywords: Lung Squamous Cell Carcinoma; Chemotherapy; Drug Resistance; Phosphorylation Omics; Kinase.

INTRODUCTION

In the past few years, the incidence and mortality of lung cancer have been on the rise [1]. Lung cancer can be divided into non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) according to histopathological features. NSCLC accounts for approximately 85% of lung cancer cases [2]. Lung squamous cell carcinoma (LUSC) is one of the most common subtypes, accounting for approximately 25-30% of lung cancer cases [3]. Chemotherapy is still one of the main treatment options for LUSC, but a large number of patients are resistant to chemotherapy [4]. Phosphorylation analysis of a chemotherapy-resistant patient specimen was carried out, and the results provide some insights into the mechanism of drug resistance.

CASE DESCRIPTION AND DIAGNOSTIC ASSESSMENT

Medical Records

The patient, male, was born in 1958. He had intermittent cough for 2 months and went to the hospital for examination on July 14th, 2023. Chest CT (July 14th, 2023) revealed a tumor shadow in the right hilum area with a soft tissue mass. The boundary was unclear, and the shadow was surrounded by blood vessels. The size of the shadow was approximately 3.1*2.5 cm. An irregular soft tissue nodule shadow was observed along the right lung middle and lower lobe bronchus, and the image of the lesion was slightly clearer after enhanced scanning (Figure 1). On July 17, 2023, the fiberoptic bronchoscope was perfected. The fiberoptic bronchoscopy results revealed malignant tumor cells in the <middle lobe of the right lung>, and the cell morphology was consistent with that of squamous cell carcinoma. Pathological biopsy revealed squamous cell carcinoma in the <middle lobe of the right lung>. On July 19, 2023 PET/CT was performed. The results confirmed that the patient had right hilar central lung cancer invading the bronchial opening of the middle and lower lobes with right hilar lymph node metastasis; was in good health and did not have a history of infectious diseases, such as hepatitis, tuberculosis, typhoid fever and malaria; and had a medical history of hypertension, coronary heart disease and diabetes. The patient was diagnosed with primary bronchogenic carcinoma on the right (stage cT3N1M0 IIIA squamous cell carcinoma). After 2 courses of chemotherapy with albumin, paclitaxel and carboplatin, a chest CT scan and enhancement were performed on September 08, 2023. The scan revealed a large right hilum shadow with a soft tissue mass and unclear boundary, The shadow was surrounded by blood vessels and was approximately 3.0 cm×2.3 cm in size, and the CT value was approximately 39 HU. Part of the bronchus in the lower lobe of the right lung was occluded, and a soft tissue density shadow in the nodule was observed (compared with the previous film on July 14, 2023: the total volume of the mass seemed to be increased) (Figure 2). On September 15, 2023, under general anesthesia, video-assisted thoracoscopy and right middle and lower lobe resection+mediastinal lymph node dissection was performed. The postoperative pathology report showed squamous cell infiltration and moderate differentiation in the right middle and lower lung, with no lung membrane infiltration, nerve bundle invasion (+), vascular invasion (+) or air cavity spread (-); no cancer was found in the bronchial stump; and metastatic carcinoma was found in the lymph nodes of < Group 10 > (1/3) but not in the lymph nodes of < Group 2, Group 4,

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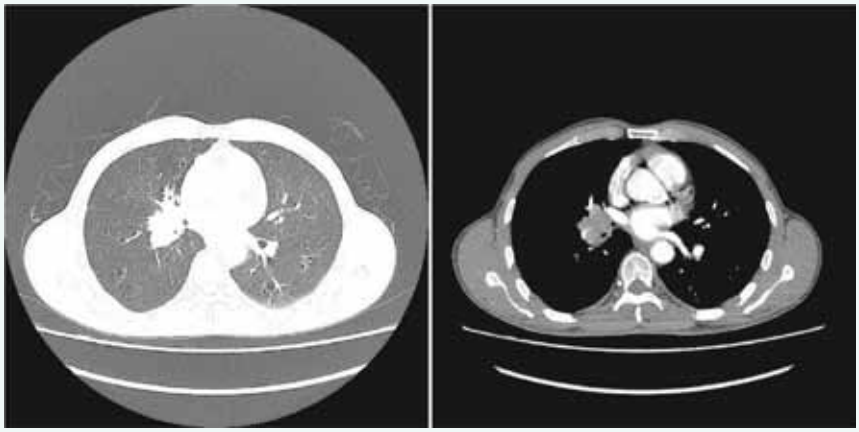

Figure 1: Chest CT plain scan + enhancement on July 14, 2023. Large right hilar shadow with a soft tissue mass, unclear boundaries and surrounding adjacent blood vessels, with a size of approximately 3.1*2. 5 cm and a CT value of approximately 39 HU.

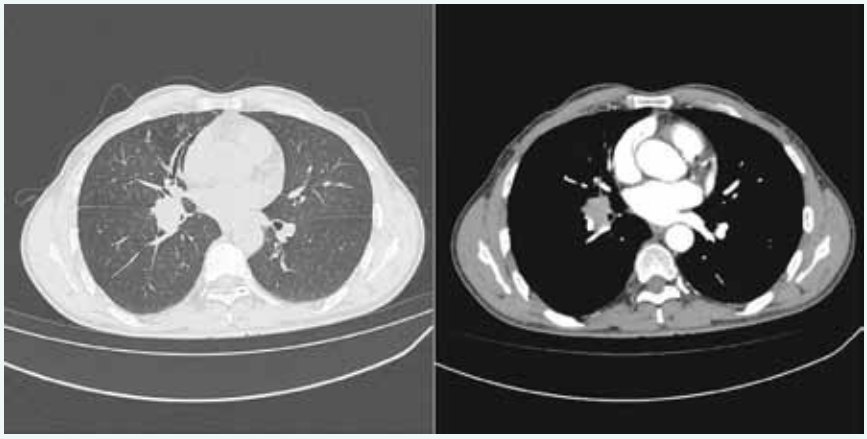

Figure 2: Chest CT plain scan + enhancement on September 8, 2023. Large right hilar shadow with a soft tissue mass, unclear boundaries and surrounding adjacent blood vessels, with a size of approximately 3.0 cm × 2.3 cm and a CT value of approximately 39 HU. (Compared with the previous films on July 14, 2023, the but the overall volume of the mass seemed to be increased.)

Group 7, Group 11 or Group 12 > (0/4, 0/5, 0/7, 0/1 and 0/1). The cancer cell lines were ALK-F (-), CD56 (-), CK5/6 (+), CK8/18 (+), CgA (-), Ki-67 (+, 30%), NSE (-), NapsinA (-), P63 (+), Syn (-), and TTF-1 (+) (Figure 3).

RESULTS OF THE PHOSPHORYLATION ANALYSIS

We sequenced the tumor tissues (CA) and adjacent tissues (P), selected sites with confidence scores ≥ 0.7, and screened differentially phosphorylated proteins according to a change ≥ 1.5 (upregulated 1.5 times) or ≤ 1/1. 5 (downregulated 1.5 times), and these proteins were subsequently annotated by Gene Ontology (GO) biological process analysis. Figure 4A shows that the differentially phosphorylated proteins were involved in 15 biological processes, among which 928 proteins were closely related to the occurrence and development of tumor cells. To explore the specific signaling pathways associated with these proteins, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was carried out. KEGG analysis revealed that 15 signaling pathways were enriched in 928 proteins, including cancer-related pathways, the MAPK signaling pathway, and pathways involving ErbB, chemokines, T/B-cell receptors, Ras, and Rap1 (Figure 4B).

We selected five signaling pathways closely related to tumor cell growth, the top 20 genes with the greatest CA/P expression and the top 20 genes with the lowest CA/P expression in each signaling pathway were analyzed and visualized in a heatmap. From Figure 4C, it can be seen that TARF2, PAK, PML, FLNA, MAP3K5, MAP3K20, STAT2 and other proteins had significantly increased abundance of phosphate sites in tumor tissue, with most genes associated with apoptosis; on the contrary, proteins such as ERBB2, RAF1, MAPK3, MAP2K2, MAPK1, and EGFR had significantly lower expression of phosphate sites in tumor tissues, mainly related to cell proliferation. These altered genes were likely key factors in the occurrence, development, and chemotherapy resistance of LUSC.

DISCUSSION

Chemotherapy can effectively kill tumor cells and prolong the life of patients, so it has been widely used in the clinical treatment of LUSC [5]. Although chemotherapy can improve the prognosis of patients with LUSC, the long-term use of chemotherapy often leads to drug resistance in LUSC patients, which leads to a decreased curative effect of chemotherapy. Therefore, it is necessary to actively explore the related mechanism of drug resistance in LUSC patients in the clinic to improve the curative effect of chemotherapy [6].

This patient completed a chest CT examination on July 14, 2023, and improved fiberoptic bronchoscopy and PET/CT examinations were

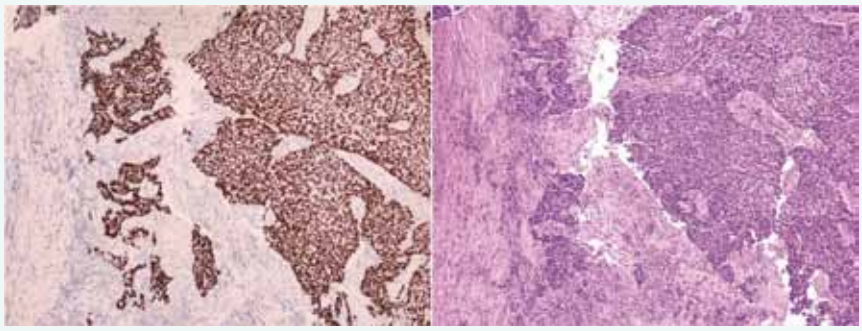

Figure 3: Pathology report on September 19, 2023. Invasive moderately differentiated squamous cell carcinoma in the < right middle lower lung >, with no lung membrane infiltration, nerve bundle invasion (+), vascular invasion (+), or air cavity dissemination (-); no cancer was found in the bronchial stump. Metastatic carcinoma (1/3) was found in the lymph nodes of < Group 10 >, but no metastatic carcinoma (0/4, 0/5, 0/7, 0/1, 0/1) was found in the lymph nodes of < Group 2, Group 4, Group 7, Group 11 or Group 12. The cancer cell lines were ALK-F (-), CD56 (-), CK5/6 (+), CK8/18 (+), CgA (-), Ki-67 (+, 30%), NSE (-), NapsinA (-), P63 (+), Syn (-), and TTF-1 (-).

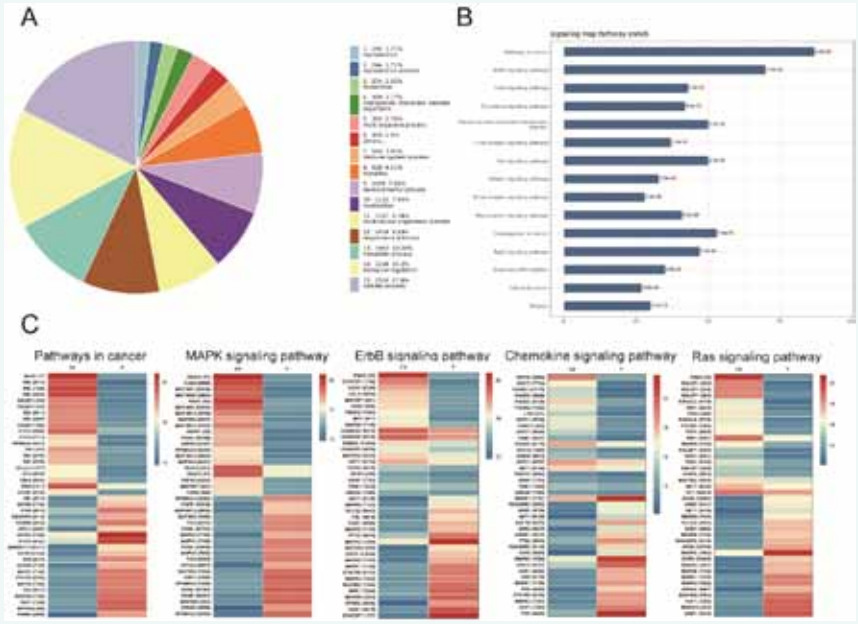

Figure 4: Phosphorylation analysis results of the lung squamous cell carcinoma patient specimen. A. GO biological process annotation of the differentially phosphorylated proteins. B. KEGG pathway enrichment analysis of 928 genes from the signaling process in Figure A. C. Thermogram of the top 20 genes with the maximum and minimum CA/P expression in 5 selected signaling pathways identified by the KEGG analysis.

performed on July 17 and 19, respectively. The patient was diagnosed with right primary bronchogenic carcinoma (stage cT3N1M0 IIIA squamous cell carcinoma), which invaded the bronchial opening of the middle and lower lobes with right hilar lymph node metastasis. Two courses of albumin paclitaxel + carboplatin chemotherapy were administered, and a plain chest CT scan + enhancement was performed on September 8, 2023. Compared with July 14, 2023, it was suggested that the volume of the soft tissue mass shadow increased, indicating that this patient had LUSC drug resistance. Therefore, surgery was performed on September 15th, 2023.

To clarify the mechanism of drug resistance in this patient with LUSC, phosphorylation analysis was performed. Protein phosphorylation is one of the most widely studied posttranslational modifications and can play a role in various biological processes of cells [7]. Phosphorylation analysis is helpful for identifying phosphorylated proteins and their related sites, exploring the mechanism of physiological and pathological signaling pathways, and laying a foundation for clarifying the occurrence, progression and drug resistance of diseases. The signaling process is closely related to the occurrence and development of tumors, and the results of KEGG enrichment confirmed that the MAPK, ErbB, chemokine and Ras signaling pathways are interrelated, and abnormal activation can induce apoptosis, reduce or decrease differentiation ability, lead to the malignant proliferation and transformation of cells, and cause drug resistance in tumor cells.

As key genes for tumor cell growth, EGFR, MAPK3, MAP2K2, MAPK1 and others exhibited reduced phosphorylation levels at certain sites in LUSC tumor tissue. Although the specific functions of most of the detected sites were uncertain due to a lack of research, MAPK3 (T202/Y204) had been clearly positively correlated with cell proliferation. Therefore, we speculated that the drug still played a role in LUSC cell growth after receiving chemotherapy, but it was not enough to kill the cells and lead to drug resistance. Interestingly, the majority of genes with the most significant increase in phosphorylation abundance in

LUSC tumor tissue were associated with apoptosis, such as TARF2, PAK2, MAP3K5, MAP3K20, and some had detected more than one locus. Due to the lack of research on the detected sites, it cannot be determined whether these protein activities were activated or inhibited. Therefore, there were currently two speculations: 1) After receiving chemotherapy, LUSC patients would successfully take effect with the drug. The growth of tumor cells was inhibited, and the apoptosis program was activated, but a bypass pathway occurred, causing tumor cells to develop resistance; 2) After receiving chemotherapy, LUSC patients experienced drug inhibition of tumor cell proliferation, but the apoptotic pathway was also inhibited, resulting in slow cell proliferation and drug resistance. The specific mechanism of drug resistance need to further explore in the future.

Among the 5 signaling pathways, TRAF (T7) and PAK2 (S2) were involved in more than one signaling pathway and exhibited the most significant changes. TRAF2 is a TNF receptor-related factor that usually cooperates with other members of the TRAF protein family to participate in activating the classical NFκB pathway, stimulating the MAPK cascade, and participating in endoplasmic reticulum stress signal transduction, autophagy regulation and cell death program control [8]. It has been found that TRAF2 can promote tumor growth by promoting the Wnt/β-catenin signaling pathway in colon cancer [9]. PAK2 belongs to the P-21 activated kinase family and plays a role in various signaling pathways, including cytoskeleton regulation, cell motility, cell cycle progression, cell apoptosis or proliferation [10-12]. Previous studies have shown that PAK2 is significantly associated with the proliferation, adhesion and migration of tumor cells in glioblastoma, ovarian cancer and pancreatic cancer [13-15]. TRAF (T7) and PAK2 (S2) were likely key factors in LUSC chemotherapy resistance and were worthy of further research.

The mechanism involved in the drug resistance of LUSC is very complicated. The phosphorylation analysis results in this report showed that the increased activity of tumor signaling channels and related proteins may be involved in the mechanism leading to the drug resistance of LUSC, and genes such as TRAF2 (T7) and PAK2 (S2), may be the key targets of drug resistance in LUSC.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by West China School of Public Health/West China Fourth Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

XZ and FZ conceived and designed the case report. RH collected the case and conducted case diagnosis. LC was responsible for the detection and data analysis of phosphorylation omics. JX had written the manuscript. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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