Urinary Proteome Changes in Patients with Advanced Lung Cancer Before and After Drug Therapy

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Abstract

Lung cancer, as a disease that poses a serious threat to human life and health, remains a challenge in terms of determining optimal treatment timing and identifying the most effective therapeutic agents despite the availability of multiple treatment modalities. The urine proteome can sensitively reflect physiological changes in the body and holds promise for revealing pathological or physiological alterations following drug therapy. To investigate changes in the urine proteome of advanced lung cancer patients receiving different therapeutic agents, urine samples were collected from these patients at various time points before and after treatment, and liquid chromatography-tandem mass spectrometry (LC-MS/MS) was employed to detect alterations in their urinary proteome. Comparative results demonstrated that differentially expressed proteins varied among different patients receiving different drug treatments, and the biological process changes reflected by these differential proteins were largely consistent with the patients' clinical manifestations. These findings indicate that (1) changes in urinary proteins can reflect patients' pathophysiological changes following different drug treatments; (2) when different patients receive the same drug treatment, changes in urinary proteins can reflect distinct pathophysiological changes within patients that are largely consistent with clinical assessment results. These discoveries may provide important information to assist clinical decision-making.

Full Text

Changes in Urine Proteome of Advanced Lung Cancer Patients Before and After Drug Treatment

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Abstract

Lung cancer poses a severe threat to human life and health. Despite numerous treatment options, determining the optimal therapeutic timing and selecting the most effective medication for each patient remains a significant clinical challenge. The urine proteome can sensitively reflect physiological changes in the body and holds promise for revealing pathophysiological alterations following drug therapy. To investigate changes in the urine proteome of advanced lung cancer patients undergoing different drug treatments, we collected urine samples from patients at various time points before and after treatment and analyzed them using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Our comparative analysis revealed that different patients exhibited distinct differential protein profiles after receiving different drug therapies. The biological process changes reflected by these differential proteins were largely consistent with clinical manifestations. These findings demonstrate that: (1) urine protein changes can reflect pathophysiological alterations in patients following different drug treatments; and (2) when different patients receive the same drug treatment, urine protein changes can reveal distinct pathophysiological changes that align with clinical disease assessments. These discoveries may provide valuable information to assist clinical decision-making in the future.

Keywords: lung cancer; urine proteome; drug therapy; pathophysiological changes

1. Introduction

Lung cancer incidence and mortality have been increasing rapidly worldwide, becoming a major threat to human life and health. According to reports, lung cancer is the most commonly diagnosed cancer (11.6%) and the leading cause of cancer-related death (18.4% of total cancer deaths)[1]. It is the second most common cancer among both men and women globally, and the most common cancer in the Chinese population[2],[3]. The two main histological subtypes are non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC), with NSCLC being the most common subtype, accounting for approximately 83% of all lung cancers .

Treatment options for lung cancer include surgery, radiotherapy, chemotherapy, targeted therapy, and immunotherapy, with selection depending on multiple factors including cancer type and stage . In current clinical practice for advanced lung cancer, platinum-based therapy remains the standard treatment. Platinum combination chemotherapy offers advantages in survival rates and symptom control, but its clinical effectiveness is limited by cumulative hematological and neurotoxicity, necessitating alternative treatments . Regarding side

effects, carboplatin most commonly causes thrombocytopenia, while cisplatin primarily induces nausea and vomiting. Therefore, the choice of platinum compounds should consider the expected toxicity profile, patient comorbidities, and preferences .

Angiogenesis inhibitors such as bevacizumab represent a promising personalized treatment approach for lung cancer. Bevacizumab, a commonly used lung cancer therapeutic, promotes tumor cell apoptosis but has been associated with toxic side effects including hypertension , hemorrhage , and proteinuria . In immunotherapy, pembrolizumab, a rapidly evolving immune checkpoint inhibitor, has been approved for first-line treatment of advanced NSCLC[1] . However, immune-related adverse events induced by immunotherapy can affect multiple organ systems, with reports of pembrolizumab-treated patients developing thyroid dysfunction, pneumonia, and severe skin reactions[11]. Additionally, some patients develop varying degrees of drug resistance after targeted therapy, further impacting clinical prognosis. Given these clinical challenges, determining the most effective treatment for each patient, selecting appropriate therapeutic methods and combination strategies, optimizing treatment timing, and maximizing efficacy while minimizing side effects represent critical and unavoidable challenges in modern clinical practice.

The urine proteome can sensitively reflect physiological changes in the body. Due to the lack of homeostatic regulation, urine can accumulate systemic changes at early stages[12]. Numerous studies have demonstrated that comparing urine proteome changes can provide clues for early diagnosis of many diseases, including myocarditis[13], Alzheimer' s disease[1] , liver fibrosis[1] , glioma[1] , pulmonary fibrosis[1] , and chronic pancreatitis[1] models. Furthermore, the urine proteome can sensitively distinguish subtle differences, with reports suggesting urinary proteins can reflect changes when identical tumor cells grow in different organs[1] and can detect changes induced by injecting a minimal number of cells subcutaneously in rats[2] . We hypothesize that the potential of urine proteomics extends beyond these applications. Theoretically, if urine samples from the same individual are compared at different time points, assuming no significant lifestyle changes during that period, the observed changes should reflect the individual' s physiological or pathological alterations during that timeframe. Careful analysis of urinary protein changes induced by various drugs with different effects and side effects may provide clues about disease-related pathways and biological processes, including helping patients avoid drugs that cause adverse side effects[21].

Therefore, in this study, we collected urine samples from eight advanced lung cancer patients before and after treatment and performed label-free quantitative analysis using data-independent acquisition mass spectrometry. Each patient served as their own control for comparing urine proteome differences. This study aimed to determine whether pathophysiological changes reflected by the urine proteome at different treatment stages align with clinical manifestations when advanced lung cancer patients receive different drug therapies, and to

explore the ability of urine proteomics to distinguish biological changes induced by different drugs.

2. Materials and Methods

2.1 Patient Samples

We collected 23 urine samples from eight advanced lung cancer patients (those with unresectable stage IIIB-IV disease) at Peking Union Medical College Hospital. All urine samples were morning urine specimens. Clinical information for all patients is summarized in . Lung cancer staging was performed according to the 8th edition of the International Lung Cancer TNM Staging System[22]. Patient response assessment followed RECIST 1.1 criteria[23]. All participants provided informed consent, and ethical approval was obtained from the participating hospital.

2.2 Urine Sample Collection and Preparation

Urine samples were collected from all patients at different time points before and after drug administration, with specific collection times and medication details provided in through . Urine samples were centrifuged to remove cellular debris and impurities, then stored temporarily at -80°C until analysis. Prior to LC-MS/MS analysis, 20 mL samples were thawed and centrifuged for 30 minutes. The supernatant was transferred to new centrifuge tubes, mixed thoroughly with three volumes of pre-cooled ethanol, and allowed to precipitate at -20°C for 12 hours to extract urinary proteins. After discarding the supernatant, the precipitate was dried and resuspended in lysis buffer (8 mol/L urea, 2 mol/L thiourea, 50 mmol/L Tris, and 25 mmol/L DTT). The resuspended sample was centrifuged, and the supernatant was retained. Protein concentration was measured using the Bradford assay. Urinary proteins were digested using the FASP method[2] . For each sample, 100 g of protein was added to a 10 kDa filter (Pall, Port Washington, NY, USA), washed sequentially with urea buffer (UA, 8 mol/L urea, 0.1 mol/L Tris-HCl, pH 8.5) and 25 mmol/L NH HCO solution. Proteins were reduced with 100 L of NH HCO solution containing dithiothreitol (DTT, Sigma) at a final concentration of 20 mmol/L at 37°C for 1 hour. Freshly prepared iodoacetamide (IAA, Sigma) was added to a final concentration of 50 mmol/L and incubated in the dark for 30 minutes. After centrifugation for 40 minutes, proteins were washed again with UA and NH HCO . Trypsin was added at an enzyme-to-protein ratio of 1:50, and digestion proceeded overnight at 37°C. The digested peptide mixture was collected as the filtrate. Peptide mixtures were desalted using Oasis HLB solid-phase extraction columns (Waters, Milford, MA), vacuum-dried, labeled, and stored at -80°C.

2.3 LC-MS/MS Analysis

All samples underwent data-dependent acquisition (DDA) mass spectrometry analysis. Dried peptide samples were reconstituted in 0.1% formic acid (FA) to a

concentration of 0.5 g/ L, with peptide concentration determined by BCA assay. For each peptide sample, 2 L was injected. Liquid chromatography parameters: samples were loaded onto the autosampler, equilibrated on a trap column for 4 minutes at 3 L/min, then eluted in reverse phase at 0.25 L/min (column flow). Mobile phase B gradient was 4% to 28% over 90 minutes. Eluted peptides were detected using an Orbitrap Fusion Lumos Tribrid mass spectrometer (Thermo Fisher Scientific, USA) with the following parameters: spray voltage 2.4 kV, ion transfer tube temperature 320°C. MS parameters: 350-1550 m/z, resolution 60,000; MS^2 scan: 200-2000 m/z, resolution 30,000, cycle time 3 s, top speed mode, 30% HCD collision energy.

2.4 Data Analysis

Mass spectrometry raw files were converted to mgf format using Proteome Discoverer software (version 2.1, Thermo Fisher Scientific). Mgf files were searched against the SwissProt database (taxonomy: Homo; containing 20,346 sequences) using Mascot software (version 2.4.1). Search parameters included: trypsin digestion, maximum 2 missed cleavages, carbamidomethylation of cysteine as fixed modification, methionine oxidation as variable modification, parent ion mass tolerance 10 ppm, fragment ion mass tolerance 0.02 Da. After searching, dat format files were exported and imported into Scaffold software (version 4.7.5) with parameters set at: protein FDR 1%, peptide FDR 1%, minimum 2 unique peptides per protein, total spectral count normalization, with subsequent analysis based on normalized spectral counts.

2.5 Statistical Analysis

Technical triplicate values for each sample were used for statistical analysis. For each patient, urinary proteins identified in pre-treatment urine samples served as self-controls for comparison with proteins identified in post-treatment samples. Differential proteins at each treatment time point were filtered using the following criteria: fold change 1.5 or 0.67, two-tailed paired t-test P-value <0.05. Statistical analysis was performed using GraphPad Prism (version 7.0, GraphPad Software).

2.6 Functional Annotation of Differential Proteins

The DAVID database was used to analyze differential proteins identified at different time points, with proteins categorized according to biological processes.

3. Results

3.1 Changes in Urine Proteome of Lung Cancer Patients

From October 2018 to June 2019, this study collected urine samples from eight patients at various time points before and after drug administration, totaling 23 samples for analysis. Detailed information on urine sample collection times,

medication regimens, and clinical manifestations is provided in through . For each sample, 1 g of peptide was analyzed using data-independent acquisition mass spectrometry. The number of urinary proteins identified in each of the eight patients is shown in . Screening criteria were: fold change 1.5 or 0.67, P-value <0.05.

3.2 Proteomic Functional Analysis of Patient P1

Four time-point urine samples were collected from Patient P1 before and after drug administration for mass spectrometry analysis, with detailed sample information and clinical symptoms provided in . Compared with pre-treatment proteome results, a total of 1,448 proteins were identified, with 472 differential proteins meeting screening criteria. Among these, 262, 223, and 241 differential proteins were identified at T1, T2, and T3 time points, respectively (). The Venn diagram shows the overlap of differential proteins across the three time points ([Figure 1: see original paper]A). At T1, 175 proteins were upregulated and 87 were downregulated. At T2, 142 proteins were upregulated and 81 were downregulated. At T3, 140 proteins were upregulated and 101 were downregulated. A total of 74 proteins showed continuous changes across all three time points, with 130, 66, and 96 unique differential proteins at T1, T2, and T3, respectively.

This experiment was single-blinded, meaning that during analysis of biological changes reflected by urinary proteins, all patient disease assessments and medication information were unknown. Differential proteins identified at T1, T2, and T3 were subjected to functional enrichment analysis using the DAVID database. Biological process analysis revealed changes in immune-related and tumor growth-related processes at all three time points (, [Figure 1: see original paper]B). At the first post-treatment time point T1, significant changes were observed in complement activation, cellular response to growth factor stimulus, classical complement activation pathway, cellular response to TNF, regulation of immune response, innate immune response, antigen processing and presentation, Fc- receptor signaling pathway, Fc- receptor signaling pathway involved in phagocytosis, and chronic inflammatory response. These prominent immune-related changes indicated immune system activation. At the second time point T2, complement activation, classical complement pathway, Fc- receptor signaling in phagocytosis, immune response, and alternative complement pathway showed significant changes, suggesting sustained immune recognition and tumor cell killing activity, consistent with the patient' s clinical assessment of tumor shrinkage. At the third time point T3, complement activation, classical and alternative pathways, immune response, regulation of complement activation, inflammatory response, and gluconeogenesis were altered. Gluconeogenesis has been reported to correlate with tumor growth[2] , and the patient was clinically assessed as stable at this time.

These results demonstrate that urinary protein changes reflected pathophysiological status consistent with clinical manifestations, indicating that the combi-

nation therapy of pemetrexed, cisplatin, and bevacizumab was effective for this patient.

3.3 Proteomic Analysis of Patient P2

Four time-point urine samples were collected from Patient P2 before and after drug administration, with detailed information provided in . Compared with pre-treatment results, 1,534 proteins were identified, with 491 differential proteins meeting criteria. Among these, 161, 302, and 232 differential proteins were identified at T1, T2, and T3, respectively (). At T1, 78 proteins were upregulated and 83 downregulated. At T2, 110 proteins were upregulated and 192 downregulated. At T3, 93 proteins were upregulated and 139 downregulated. The Venn diagram shows differential protein overlap across time points ([Figure 2: see original paper]A), with 27 proteins showing continuous changes across all three time points. Unique differential proteins numbered 79, 146, and 89 at T1, T2, and T3, respectively.

Functional enrichment analysis of differential proteins at each time point revealed biological process changes (, [Figure 2: see original paper]B). At T1 after initial drug administration, significant changes included proteolysis, cellular protein metabolic process, methionine biosynthetic process, fructose metabolic process, gluconeogenesis, neutrophil aggregation, serine family amino acid biosynthetic process, and positive regulation of inflammatory response. Notably, proteolysis showed continuous significant changes across all three time points. Compared with pre-treatment, fewer immune-related processes were identified at this stage, while tumor growth-related processes were more prominent, coinciding with a clinical assessment of stable disease. Several altered processes have reported tumor associations: (1) neutrophil aggregation is implicated in acute injury, chronic inflammation, cancer, and autoimmunity[2] ; (2) methionine biosynthesis is tumor-related, as many cancer cell types require exogenous methionine for survival[2] , and this methionine dependency has led to methionine restriction as a potential therapeutic strategy[2] ; (3) serine serves as an important one-carbon donor in the folate cycle with antioxidant functions, and many cancers are highly serine-dependent[2] .

At subsequent time points T2 and T3, shared altered biological processes included platelet degranulation, cell adhesion, carbohydrate metabolic process, proteolysis involved in cellular protein catabolism, Fc- receptor signaling in phagocytosis, Fc- receptor signaling pathway, TNF-mediated signaling pathway, Wnt signaling pathway and planar cell polarity pathway, regulation of immune system process, negative regulation of angiogenesis, cell recognition, cell-cell adhesion, complement activation, classical complement pathway, and L-methionine salvage. Additional processes at T2 included NIK/NF-kappaB signaling, regulation of immune response, positive regulation of Wnt signaling, glycolytic process, and regulation of inflammatory response. At T3, receptor-mediated endocytosis, acute inflammatory response, leukocyte migration, and immune response were also altered. Several processes are tumor-associated: (1)

tumor cells typically show reduced cell-cell and/or cell-matrix adhesion, with increasing evidence linking decreased cell adhesion to tumor invasion and metastasis[3] ; (2) NF- B has anti-inflammatory effects in vivo[31]; (3) aberrant Wnt signaling is recognized as a key factor in initiating and/or maintaining many cancers by affecting cancer stem cell behavior[32]; (4) angiogenesis is considered a hallmark of tumorigenesis[33].

These tumor growth-related changes likely reflect chemotherapy effects, while the increased immune-related processes at T2 and T3 may relate to sintilimab treatment, suggesting immune system activation. However, the overall significance of these changes was relatively low, and immune-related changes appeared later, suggesting that the drug combination did not effectively control tumor growth, consistent with the clinical assessment of progressive disease at these time points.

3.4 Proteomic Analysis of Patient P3

Two time-point urine samples were collected from Patient P3 before and after drug administration, with details provided in . Compared with pre-treatment, 1,049 proteins were identified, with 71 differential proteins meeting criteria (). Post-treatment changes included 39 upregulated and 32 downregulated proteins.

Functional enrichment analysis revealed biological process changes (, [Figure 3: see original paper]). Notably altered processes included platelet degranulation, platelet aggregation, blood coagulation and fibrin clot formation, coagulation, wound healing, and cell spreading, which may relate to the patient' s pre-treatment symptoms of cough and hemoptysis that improved after therapy. Immune-related changes included positive regulation of NF- B transcription factor activity, positive regulation of inflammatory response, neutrophil aggregation, and leukocyte migration involved in inflammatory response. Other changes potentially related to tumor biology included cell adhesion and positive regulation of nitric oxide biosynthetic process, as nitric oxide can have divergent effects on tumors by either stimulating growth or promoting cell death depending on its source[3] .

Notably, this patient' s biological processes did not include glycolysis or gluconeogenesis, which were observed in patients with progressive disease, suggesting that pembrolizumab treatment was effective. This aligns with the clinical assessment of stable disease, though continued monitoring is needed to confirm long-term efficacy.

3.5 Proteomic Analysis of Patient P4

Two time-point urine samples were collected from Patient P4 before and after drug administration, with details provided in . Compared with pre-treatment, 1,107 proteins were identified, with 315 differential proteins meeting criteria (). Post-treatment changes included 134 upregulated and 181 downregulated proteins.

Functional enrichment analysis revealed extensive biological process changes (, [Figure 4: see original paper]). Compared with Patient P3, who also had only one post-treatment time point, Patient P4 showed a larger number of differential proteins and altered biological processes, indicating a greater drug impact despite receiving the same pembrolizumab treatment. Among the most significantly altered processes were numerous immune responses, including classical complement pathway, acute-phase response, complement activation, regulation of complement activation, alternative complement pathway, innate immune response, and regulation of immune system process, indicating robust immune system activation after pembrolizumab treatment. Additional tumor-related processes included: (1) retinoid metabolic process, as retinoids and their derivatives can regulate cell growth, induce differentiation and apoptosis, and represent established anticancer therapies[3] ; (2) fibrinolysis, as fibrin promotes tumor cell migration by providing a matrix and interacting with adhesion molecules and integrins, with fibrinolytic components affecting tumor cell adhesion to ECM, proliferation, and survival[3] . In contrast, tumor growth-promoting processes showed relatively low significance, suggesting that differential protein changes were primarily driven by immune responses rather than tumor progression.

These results indicate that urinary protein changes reflected clinical status consistent with treatment efficacy, suggesting that pembrolizumab was effective for this patient.

3.6 Proteomic Analysis of Patient P5

Two time-point urine samples were collected from Patient P5 before and after drug administration, with details provided in . Compared with pre-treatment, 1,178 proteins were identified, with 233 differential proteins meeting criteria (). Post-treatment changes included 91 upregulated and 142 downregulated proteins.

Functional enrichment analysis revealed biological process changes (, [Figure 5: see original paper]). Similar to Patient P4, Patient P5 showed a substantial number of differential proteins. Significantly altered processes included numerous catabolic processes: chondroitin sulfate catabolic process, carbohydrate metabolic process, proteolysis, glycosphingolipid metabolic process, glycosaminoglycan catabolic process, retinoid metabolic process, oligosaccharide catabolic process, fibrinolysis, cellular protein metabolic process, and glycosaminoglycan metabolic process, which may be tumor-associated. Despite the large number of altered processes, immune-related processes were relatively few and included acute-phase response, alternative complement pathway, regulation of complement activation, and innate immune response, but with low significance, likely because this patient did not receive immunotherapy.

Based on these biological process changes, we infer that the etoposide plus cisplatin combination therapy had some efficacy, with the patient clinically assessed as having stable disease, though continued monitoring is needed to

confirm durability of response.

3.7 Proteomic Analysis of Patient P6

Four time-point urine samples were collected from Patient P6 before and after drug administration, with details provided in . Compared with pre-treatment, 850 differential proteins were identified, with 276, 330, and 632 differential proteins at T1, T2, and T3, respectively (). At T1, 163 proteins were upregulated and 113 downregulated. At T2, 246 were upregulated and 84 downregulated. At T3, 442 were upregulated and 186 downregulated. The Venn diagram shows differential protein overlap across time points ([Figure 6: see original paper]A), with 86 proteins showing continuous changes across all three time points. Unique differential proteins numbered 84, 109, and 359 at T1, T2, and T3, respectively.

Functional enrichment analysis revealed shared altered biological processes across time points (, [Figure 6: see original paper]B), including platelet degranulation, regulation of complement activation, alternative complement pathway, proteolysis, negative regulation of endopeptidase activity, complement activation, cell adhesion, glycosaminoglycan catabolic process, coagulation, classical complement pathway, negative regulation of cell adhesion, cellular amino acid metabolic process, acute-phase response, leukocyte migration, cell-cell adhesion, metabolic process, carbohydrate metabolic process, and oxidation-reduction process. These included both immune and tumor growth-related processes. Additional tumor-associated processes included: (1) tumor reprogramming of nutrient acquisition and metabolic pathways to meet bioenergetic, biosynthetic, and redox demands of malignant cells[3] ; (2) altered metabolic features appearing across many cancer types, making metabolic reprogramming a recognized hallmark of cancer[3] ,[3] .

At T1, immune-related processes including regulation of complement activation, alternative and classical complement pathways, and regulation of immune response were significantly altered, indicating immune system activation, with the patient clinically stable. At T2, with sustained immune activation, regulation of complement activation, leukocyte migration, alternative complement pathway, and TNF-mediated signaling pathway were significantly changed, while tumor growth-related processes including glycolysis and gluconeogenesis were also identified, with the patient remaining clinically stable. At T3, the number of differential proteins increased markedly, with metabolic decomposition processes, glycolysis, and gluconeogenesis showing significant changes, while immune-related processes persisted.

These temporal biological process changes indicate that immune-related alterations were prominent throughout treatment, consistent with the clinical assessment of tumor shrinkage, suggesting that the pemetrexed plus cisplatin combination therapy was effective for this patient.

3.8 Proteomic Analysis of Patient P7

Two time-point urine samples were collected from Patient P7 before and after drug administration, with details provided in . Compared with pre-treatment, 1,215 proteins were identified, with 212 differential proteins meeting criteria (). Post-treatment changes included 86 upregulated and 126 downregulated proteins.

Functional enrichment analysis revealed biological process changes (, [Figure 7: see original paper]). Unlike other patients, this patient developed severe fever and pneumonia after initial treatment, which proved fatal. The pneumonia was likely an adverse reaction to sintilimab treatment. Sintilimab is a human IgG4 monoclonal antibody that specifically binds to the PD-1 molecule on T cell surfaces, activating lymphocytes for tumor treatment but potentially triggering various autoimmune side effects termed immune-related adverse events (irAEs), including immune-related pneumonitis, myocarditis, nephritis, and hepatitis . Clinical trials of such drugs have reported fatal pneumonia in three cases [1]. Among the altered biological processes reflected by urinary proteins, the most significant changes included platelet degranulation, gluconeogenesis, leukocyte migration, retinoid metabolic process, cell adhesion, locomotion of cells or subcellular components, negative regulation of endopeptidase activity, extracellular matrix organization, cellular response to TNF, and glycosaminoglycan catabolic process.

Despite developing severe adverse reactions after sintilimab, pemetrexed, and carboplatin treatment, the urine sample collected after symptom onset did not show significant alterations in immune and cytokine-related biological processes. We speculate this may be because the immune adverse reaction developed suddenly and rapidly, while preceding biological processes remained relatively normal, leaving the urinary proteome unaffected.

3.9 Proteomic Analysis of Patient P8

Three time-point urine samples were collected from Patient P8 before and after drug administration, with details provided in . Compared with pre-treatment, a total of proteins were identified, with 408 differential proteins meeting criteria, including 194 and 329 differential proteins at T1 and T2, respectively (). At T1, 150 proteins were upregulated and 44 downregulated. At T2, 223 were upregulated and 106 downregulated. The Venn diagram shows differential protein overlap across time points ([Figure 8: see original paper]A), with 115 proteins showing continuous changes across both time points. Unique differential proteins numbered 79 and 214 at T1 and T2, respectively.

Functional enrichment analysis revealed shared altered biological processes across both time points (, [Figure 8: see original paper]B), including negative regulation of endopeptidase activity, platelet degranulation, glycosaminoglycan catabolic process, cellular protein metabolic process, fibrinolysis, carbohydrate metabolic process, positive regulation of nitric oxide biosynthetic process,

gluconeogenesis, glycolytic process, proteolysis, retinoid metabolic process, cell-cell adhesion, glycosaminoglycan metabolic process, glutathione metabolic process, acute-phase response, ATP biosynthetic process, classical and alternative complement pathways, complement activation, leukocyte cell-cell adhesion, and regulation of complement activation. These processes encompassed both immune and tumor growth-related responses. At T1 after initial drug administration, glycolysis, gluconeogenesis, and other metabolism-related processes were significantly altered, with immune-related processes also identified, and the patient was clinically stable. At T2, tumor growth-related processes showed significant changes, while immune-related processes were less prominent but numerous. The patient remained clinically stable at this time point.

These biological process changes indicate that tumor metabolic processes were prominently altered throughout treatment, consistent with the clinical assessment of stable disease, suggesting that the pemetrexed plus cisplatin combination therapy had some efficacy, though continued monitoring is needed.

4. Conclusion

In this study, by comparing urine proteome changes before and after treatment in lung cancer patients receiving different drug therapies, we found that: (1) urine protein changes can reflect pathophysiological alterations in patients following different drug treatments; and (2) when different patients receive the same drug treatment, urine protein changes can reveal distinct pathophysiological changes that align with clinical disease assessments. These findings may provide important information to assist clinical decision-making in the future.

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Note: Figure translations are in progress. See original paper for figures.