Journal of Translational Medicine

RESEARCH

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Metabolomics approach reveals key plasma biomarkers in multiple myeloma for diagnosis, staging, and prognosis

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Abstract

Background Multiple myeloma (MM) is the most aggressive and prevalent primary malignant tumor within the blood system, and can be classified into grades RISS-I, II, and III. High-grade tumors are associated with decreased survival rates and increased recurrence rates. To better understand metabolic disorders and expand the potential targets for MM, we conducted large-scale untargeted metabolomics on plasma samples from MM patients and healthy controls (HC).

Methods Our study included 33 HC, 38 newly diagnosed MM patients (NDMM) categorized into three RISS grades (grade I: $n=5$; grade II: $n=19$; grade III: $n=8$), and 92 MM patients post-targeted therapy with bortezomib-based regimens. Simultaneously, MM cell lines were employed for validation studies. Metabolites were analyzed and identified using ultra high liquid chromatography coupled with Q Orbitrap mass spectrometry (UPLC-HRMS), followed by verification through a self-built database.

Results Compared with HC participants, a total of 70 metabolites were identified as undergoing significant changes in NDMM. These metabolites were significantly enriched in citrate cycle, choline metabolism, glycerophospholipid metabolism, and sphingolipid metabolism, etc. Notably, a panel of circulating plasma metabolite biomarkers, including lactic acid and leucine, has emerged not only as diagnostic indicators but also as valuable tools for tumor surveillance, aiding in the assessment of disease stage and prognostic evaluation. Moreover, 14 differential metabolites were identified in both MM cell lines and MM patients. Among these, intracellular levels of lactate and leucine significantly decreased in vitro, aligning with the plasma results.

Conclusion Our findings on key metabolites and metabolic pathways provide novel insights into the exploration of diagnostic and therapeutic targets for MM. A prospective study is essential to validate these discoveries for future MM patient care.

Keywords Multiple myeloma, Biomarkers, Metabolomics, Peripheral plasma, Metabolic pathways

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Introduction

Multiple myeloma (MM) ranks as the second most prevalent hematological malignancy, characterized by the accumulation of plasma cells in the bone marrow, leading to bone destruction and marrow failure [1]. Globally, approximately 155,688 new cases of MM are diagnosed each year [2], with male patients accounting for 54.3% (estimated range 70,924–94,910). The median age at diagnosis is 69 years, with 37%, 26%, and 37% of MM patients falling under the age categories of under 65, between 65 and 74 years, and over 75 years, respectively [3, 4]. Despite a 5-year survival rate of 56% [5], MM is deemed incurable due to its recurrent relapsing course, necessitating diverse treatment options [6].

Over the past few decades, numerous efforts have focused on identifying markers related to the pathogenesis, diagnosis, and risk stratification of MM patients, including serum β_2-microglobulin (β_2M), lactate dehydrogenase (LDH), creatinine (Cr), and genomic features t(4;14), t(14;16), del17p, et al. [7–9]. These markers have significantly improved the diagnosis and treatment of MM. Despite advancements in early molecular diagnostics, most patients are still diagnosed in intermediate or late disease stages [10, 11]. Existing prognostic stratification methods, such as the revised International Staging System (RISS), face limitations in predicting actual clinical outcomes due to the high heterogeneity of MM [12]. Concurrently, patients with newly diagnosed MM (NDMM) are typically treated with multi-drug chemotherapy based on bortezomib or lenalidomide, but only a subset of them derive clinical benefits from this treatment [13]. Therefore, there is a pressing need to develop biomarkers or algorithms that can facilitate early diagnosis and risk prediction in NDMM patients, as well as identify chemo-sensitive patients benefiting from targeted therapy.

Metabolic reprogramming, a hallmark in non-solid tumors like lymphoma [14, 15], MM [16], and leukemia [17], has spurred the use of metabolomics, a systemic tool focusing on endogenous metabolites. Metabolomics has significantly contributed insights into cancer biology, aiding in the understanding of molecular disease bases and uncovering new pathways for diagnosis, classification, and treatment [18–21]. Previous studies primarily concentrated on MM diagnosis, revealing potential utility in differentially profiling key metabolites such as choline, creatinine, leucine, tryptophan, and valine to discriminate MM patients from healthy controls [22–24]. Only few studies have identified biomarkers for both diagnosis and progress monitoring [25], or for treatment response and prognosis in MM patients [26]. However, none of these studies have revealed abnormal metabolites that can be used to simultaneously assess diagnosis, grading, and therapeutic response of MM patients simultaneously.

To address these gaps, we aimed to identify potentially minimally-invasive metabolic biomarkers for MM diagnosis, severity, and treatment response, advancing our understanding of MM-associated metabolites. Ultra-performance liquid chromatography coupled with high-resolution Orbitrap mass spectrometry, Q Exactive TM (UPLC-HRMS) was used to profile the samples of NDMM patients, on-chemotherapy MM patients, healthy controls and MM cells. Principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) characterized the differences in the metabolomics data. Finally, we examined MM-induced metabolite alterations, shedding light on the entire MM process, including diagnosis, severity, and treatment response, offering insights into pathogenesis and new prognostic factors for MM.

Materials and methods

Patients and samples

A total of 176 plasma samples from 166 subjects, including 133 MM participants and 33 healthy volunteers, were used in this study. They were obtained from Beijing Chao-yang Hospital between 2019 and 2021, and each patient provided written informed consent before their participation. The diagnosis and response criteria were based on the International Myeloma Working Group (IMWG) diagnostic criteria [1]. Symptomatic MM patients were newly diagnosed and received chemotherapy with a bortezomib-based regimen for one to six cycles, with an average of four to six cycles. For patients who achieved a complete response (CR) or partial response (PR), the regimen was repeated for two to four cycles, or autologous stem cell transplantation was completed as consolidation therapy.

Peripheral blood samples of 2–4 mL were collected at preset time points, including baseline and during chemotherapy (on-CT). Longitudinal time points were taken at every cycle starting from the first on-CT time point. Blood was collected in EDTA-coated tubes and plasma separation from the blood was achieved by centrifuging samples at 1500×*g* for 5 min within 2 h. The plasma was then stored at -80 ℃ until metabolite extraction and analysis.

Cell culture

The EB virus transformed human B lymphocyte line KM932, human myeloma cell lines (HMCLs) RPMI-8226, AMO-1, MM.1R, MM.1 S and LAMA-84 were purchased from the Cell Resource Center, IBMS, CAMS/PUMC. All cells were cultured in RPMI-1640 medium containing 10% fetal bovine serum, under meticulous conditions of 5% CO_2 at a constant temperature of 37 °C. These cells are mycoplasma-free.

Sample preparation

For metabolite extraction of plasma samples, 50 μL plasma of each patient was mixed with mixture of methanol and acetonitrile (v/v, 1:1) with the addition of 200 ng/mL propranolol and tolbutamide as internal standards (IS). The mixture was vortexed for 1 min and centrifuged at 15,000 ×g for 10 min at 4 °C. A 200 μL aliquot of the resulting supernatant was subsequently transferred to a new tube and kept at 4 °C until LC-MS analysis.

For metabolite extraction of cell samples, each cell sample was subjected to a mixture of methanol and water (v/v, 1:1) at a 1:10 weight-to-volume (w/v) ratio. The cell sample was vigorously vortexed for 1 min, followed by 30 min of ultrasonic treatment. Subsequently, the mixture was centrifuged at 15,000 ×g for 10 min at 4 °C to separate the supernatant from the cellular debris. A 50 μL aliquot of supernatant was subsequently transferred to a new tube and added 450 μL mixture of methanol and acetonitrile (v/v, 1:1) with the addition of 200 ng/mL propranolol and tolbutamide as internal standards (IS). The mixture was vortexed for 1 min and centrifuged at 15,000 ×g for 10 min at 4 °C. A 200 μL aliquot of the final supernatant was transferred to a fresh tube and stored at 4 °C until further analysis by LC-MS.

Mass spectrometry analysis

The LC-HRMS analyses were conducted via an Ultimate 3000 LC system coupled with a Q Orbitrap mass analyzer (Q Exactive, Thermo Fisher Scientific, USA). Chromatographic separation was performed via an ACQUITY BEH C18 column (Waters, 2.1 × 50 mm, 1.7 μm) at a flow rate of 0.25 mL/min, maintained at 30 ℃. Mobile phase A consisted of water with 0.1% formic acid and 2.5 mmol/L ammonium formate, while mobile phase B was acetonitrile. The gradient conditions were as follows: 0–1.0 min, 95% A; 1.0–5.0 min, 95%-40% A; 5.0–8.0 min, 40%-0% A; 8.0–11.0 min, 0% A; 11.0–14.0 min, 0-40% A; 14.0–15.0 min, 40-95% A; 15.0–18.0 min, 95% A. The spectrometric settings for positive/negative ion modes were as follows: scan mode, full MS over the m/z scan range of 70-1050; resolution, 70,000; spray voltage (+/-), 3.0 kV; capillary temperature, 350 ℃; S-lens RF, 50; full MS/dd-MS2 over the resolution of 17,500; AGC target, $1e^{5;}$ maximum TT, 50 ms; NCE, 20, 40, 60.

Metabolite identification and statistical analysis

Metabolite identification was performed via Compound Discoverer 3.3 (ThermoFisher, CA, USA). The identification criteria involved exact mass, retention time, fragmentation spectra and isotopic pattern. An in-house library [27, 28] and the online library mzCloud were utilized for this purpose. The final output data included the compound name and peak area.

Pattern recognition analysis, including principal component analysis (PCA), orthogonal partial least squares discriminant analysis (OPLS-DA), and topology analysis, were carried out to identify key metabolic features via MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/). The differentially abundant metabolites were screened (variable importance for the project, VIP > 1.0 and $p < 0.05$). Metabolic pathway analysis was conducted via the "MS Peaks to Pathways" of MetaboAnalyst 5.0. Significant pathways were computed on the basis of the spectral features with an impact greater than 0.1. SPSS 16.0 (Armonk, New York, USA) was used for t-test analysis. All the data were expressed as mean ± standard deviation (mean ± SD). One way ANOVA was used to analyze the differences between multiple groups, and Tukey's test was employed for pairwise comparisons. * represents $P < 0.05$, ** denotes $P < 0.01$, ***denotes $P < 0.001$.

Results

Metabolic fingerprint of plasma from newly diagnosed MM patients

To analyze the metabolic changes between newly diagnosed MM patients and normal control (HC) group, untargeted metabolomics analysis was carried out using UPLC-Orbitrap-MS in both ESI positive (ESI+) and negative (ESI-) ion modes. PCA, an unsupervised model, was performed to reveal differences in the metabolic profiles of samples across groups. The workflow of our work is depicted in Fig. 1. The PCA score plots clearly demonstrated a distinct separation between the NDMM and HC groups (Fig. 2A). For a more detailed analysis of metabolic profiling discrepancies between the NDMM and HC groups, we employed the supervised pattern recognition method, OPLS-DA. The OPLS-DA score plot illustrated a marked separation between the NDMM and HC groups (Fig. 2D). To screen potential biomarkers, VIP values were calculated for each metabolite using the OPLS-DA models. Meanwhile, fold change (FC) and P values were obtained by assessing the magnitude and statistical significance of the variations between different groups, respectively. Intriguingly, our analysis yielded a total of 70 differentially abundant metabolites (VIP > 1.0 and $p < 0.05$) when comparing NDMM to the HC. To offer a more intuitive visualization, we crafted a volcano plot, illustrating log2 FC against -log10 (p-value). Highlighted within this plot are 23 pivotal metabolites, which not only exhibited remarkable statistical significance ($p < 0.0001$) but also have been frequently implicated in MM (Fig. 2B). To provide a visual representation of the differentially expressed metabolites identified through LC-MS analysis, a heatmap was generated (Fig. 2C). These data confirmed that significant differences in metabolites exist between NDMM patients and the HC group.

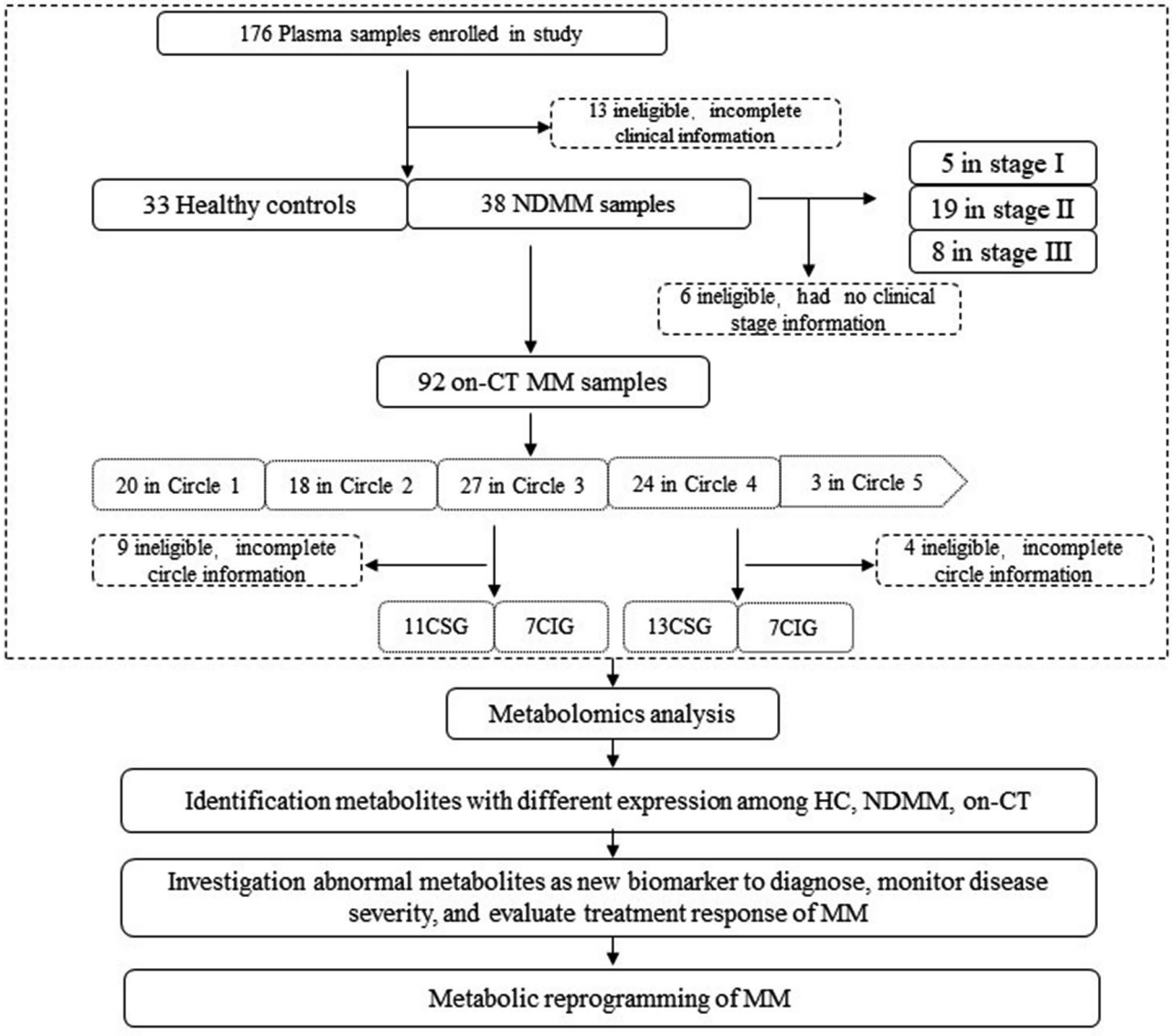

Fig. 1 Outline of the workflow for untargeted metabolomics and bioinformatics analysis. MM, Multiple myeloma; NDMM, newly diagnosed multiple myeloma; CT, Chemotherapy; CSG, Chemotheray sensitive group; CIG, Chemotheray insensitive group

Performance of plasma metabolites for the early diagnosis of MM patients

Given that the majority of MM patients are diagnosed in advanced stages, early detection significantly improves the likelihood of successful MM treatment. In contrast to the traditional bone marrow aspiration method used for screening, a blood-based test is minimally-invasive and relatively cost-effective. Thus, we examined the clinical application value of metabolite detection in MM diagnosis, with a particular emphasis on early MM. To evaluate the discriminatory capacity of the 70 aforementioned differentially abundant metabolites significantly altered in the MM cohort, ROC analyses were employed to calculate the area under the curve (AUC). Among these metabolites, 6 exhibited high diagnostic value in distinguishing plasma samples of the MM group from those of the HC group. As illustrated in Fig. 3, the AUC values for pyroglutamic acid, arginine, lactic acid, choline, acetylcholine, and leucine were 0.999, 0.999, 0.994, 0.984, 0.935, and 0.852, respectively. These values clearly indicated the ability to differentiate early MM patients from healthy controls.

Metabolic biomarkers associated with disease risk in MM patients

To gain precise insights into biomarkers associated with the severity of the disease, patients with NDMM were categorized into R-ISS-I ($n=5$), R-ISS-II ($n=19$), and R-ISS-III groups ($n=8$), according to the IMWG diagnostic criteria [9]. First, we identified statistically differential

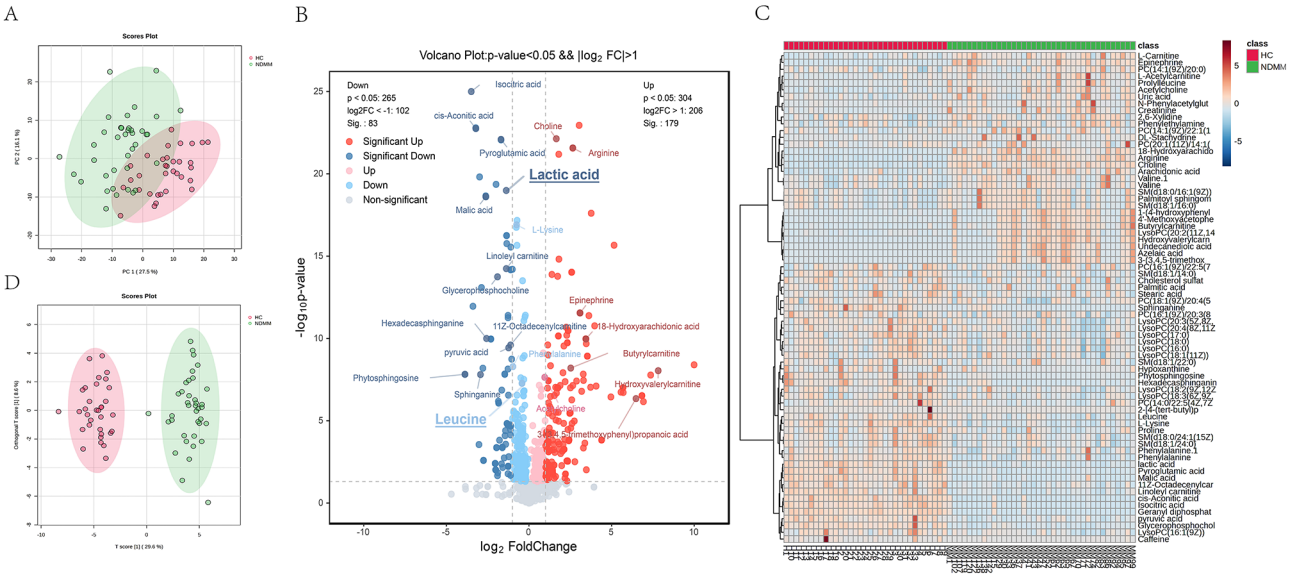

Fig. 2 Multivariate statistical analysis of untargeted metabolomics. The red dot represents the healthy control group (HC). The green dots represent the newly diagnosis MM group before treatment, namely NDMM. (**A**) It represents the difference between NDMM and HC in PCA score plot. (**B**) Volcano plots of the significantly differential metabolites in HC group versus NDMM were shown, with the top 20 compounds in AUC annotated. (**C**) Heatmap of metabolite alterations from NDMM and HC. Two-sided Kruskal-Wallis tests with $P < 0.05$. (**D**) It represents the difference between NDMM and HC in OPLS-DA score plot

metabolites ($p < 0.05$) between R-ISS-III group samples and healthy group samples, with FC exceeding 2.0 or falling below 0.5. Second, the abundance of metabolites needed to be positively or negatively correlated with risk.

As depicted in Fig. 4A, the levels of acetylcholine significantly increased, whereas the levels of lactic acid and leucine markedly decreased in the R-ISS groups (R-ISS-I, R-ISS-II, R-ISS-III) compared with those in the healthy group. Notably, the substantial increase in acetylcholine and decrease in lactic acid and leucine in the R-ISS I/II/III group compared with those in the healthy group align with previous findings indicating that the levels of acetylcholine, lactic acid, and leucine allow discrimination between NDMM patients and healthy controls. Furthermore, the panel of acetylcholine/lactic acid/leucine could serve as both diagnostic and risk biomarkers for MM. These findings underscore the potential of metabolomics for biomarker discovery, enabling more precise and accessible early detection of MM.

Metabolic biomarkers associated with chemotherapy sensitivity in MM patients

We identified two subtypes among MM patients undergoing 3 cycles of chemotherapy: the chemo-sensitive group (CSG) and the chemo-insensitive group (CIG). Correlation analysis between the aforementioned 70 candidate metabolites and 7 common clinical indexes was further performed (Fig. 4B). The clinical variables included hemoglobin (Hb), M protein, clonal plasma cells, immature plasma cells, plasma cells, serum creatinine (Scr) and lactic dehydrogenase (LDH). The level of lactic acid was significantly positively correlated with Hb and negatively correlated with Scr, immature plasma cells and plasma cells. On the other hand, the abundance of leucine was positively correlated with only Hb, whereas cholesterol sulfate was negatively correlated with the level of the M protein. Furthermore, in MM patients undergoing chemotherapy for both 3 and 4 cycles, the levels of lactic acid, leucine, and cholesterol sulfate were significantly greater in the CSG group than in the CIG group (Fig. 4C and D). Surprisingly, lactic acid and leucine demonstrated utility for diagnosis, severity assessment, and prediction of chemotherapy sensitivity (Fig. 4E).

Metabolic pathway analysis

The metabolic networks, based on the statistically and functionally integrated metabolomics data, were visualized via Cytoscape software. The observed state of identified metabolites in NDMM are shown in the metabolic networks (Fig. 5). To explore the metabolic pathways involved in MM development, the differentially abundant metabolites were enriched for the related metabolic pathway analysis via the MetaboAnalyst online tool. As depicted in Fig. 6 and Supplementary Fig. 1, differentially abundant metabolites were significantly enriched in multiple metabolic pathways, including central carbon metabolism in cancer, choline metabolism in cancer, citrate (TCA) cycle, glycerophospholipid metabolism, valine, leucine and isoleucine biosynthesis, sphingolipid

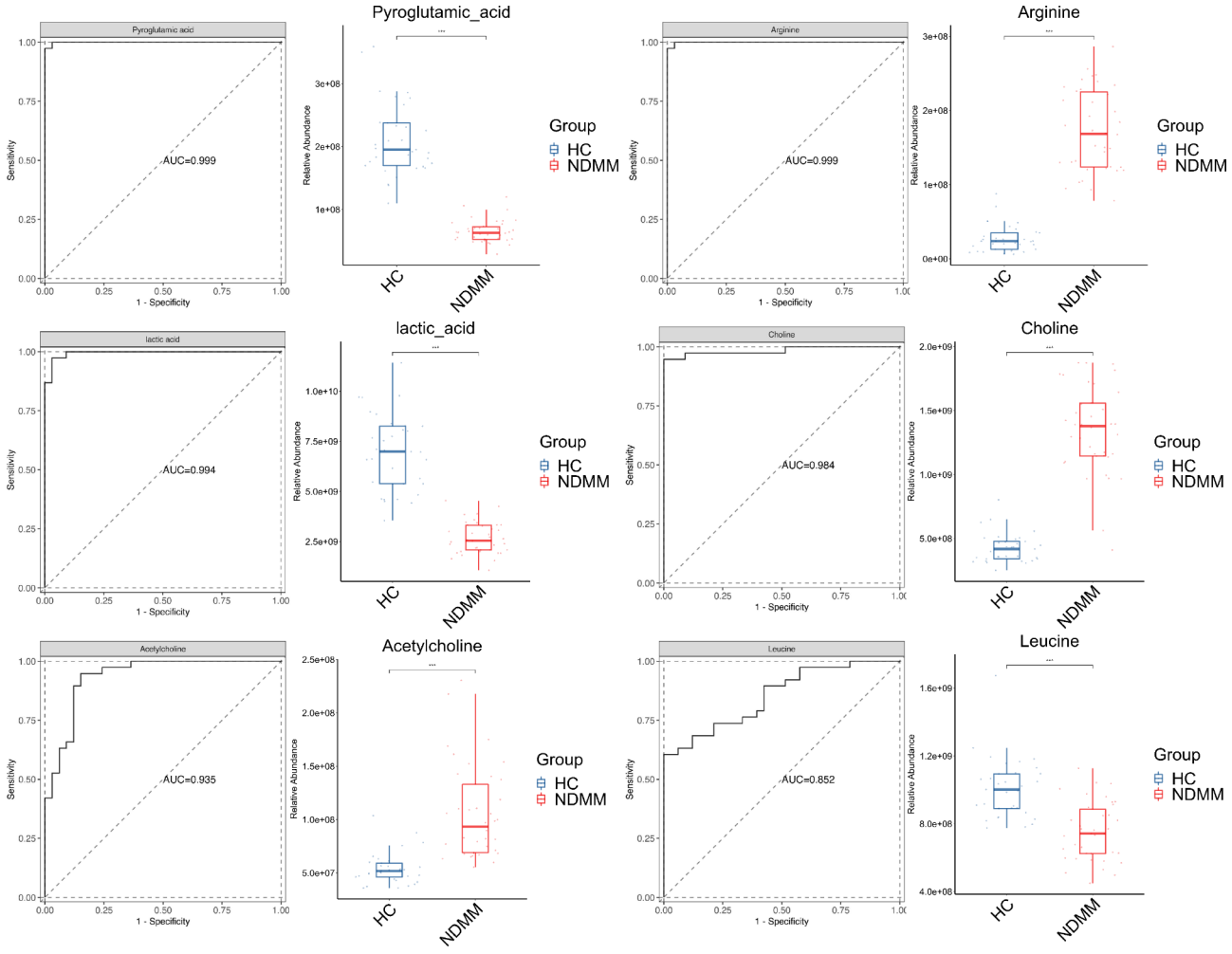

Fig. 3 The alteration of circulating metabolites in plasma between NDMM and HC. The receiver operating characteristic (ROC) curve and abundance of pyroglutamic acid, arginine, lactic acid, choline, acetylcholine and leucine (HC, $n = 33$; NDMM, $n = 38$). AUC, Area Under Curve

metabolism, phenylalanine metabolism, purine metabolism, arginine and proline metabolism, thermogenesis, and biosynthesis of amino acids. Glycolysis and the TCA cycle, as two major energy metabolism pathways, attracted our attention. Additionally, we observed a substantial reduction in lactic acid levels, a marker associated with glucose metabolism, in patients with MM.

Metabolic reprogramming of MM cells

Untargeted metabolomics studies were conducted on human MM cell lines, which included RPMI-8226, AMO-1, MM.1R, MM.1 S, and LAMA-84, in comparison with the control group, KM932, a human B cell line. The PCA score plots clearly demonstrated a distinct separation between MM cells and control group (Fig. 7A). The OPLS-DA score plot illustrated a marked separation between the MM cells and control group (Fig. 7B). 80 differentially abundant metabolites (VIP > 1.0, |log2 FC| > 1, and $p < 0.05$) were identified in MM cells versus the control group (Fig. 7C). In keeping with the metabolic analyses of MM patients, human MM cells exhibited 3 identical metabolic pathways, including citrate (TCA) cycle, sphingolipid metabolism, and g lycerophospholipid metabolism (Fig. 7D).

Lactic acid and leucine are downregulated in MM cells

A total of 14 differential metabolites were identified as overlapping between MM cells and MM patients, as depicted in Fig. 8A. To provide a visual representation of the 14 differential metabolites, a heatmap was generated (Fig. 8B). This heatmap substantiated the significant disparities in metabolite profiles between MM cells and the control group. Intriguingly, the marked decrease in lactic acid and leucine in MM cells is consistent with prior finding that plasma levels of those allows discrimination between MM patients and healthy control. Moreover, Fig. 8C highlights a remarkable decline in the levels of the 2 crucial metabolites, lactic acid and leucine, in the majority of MM cells compared to the control group.

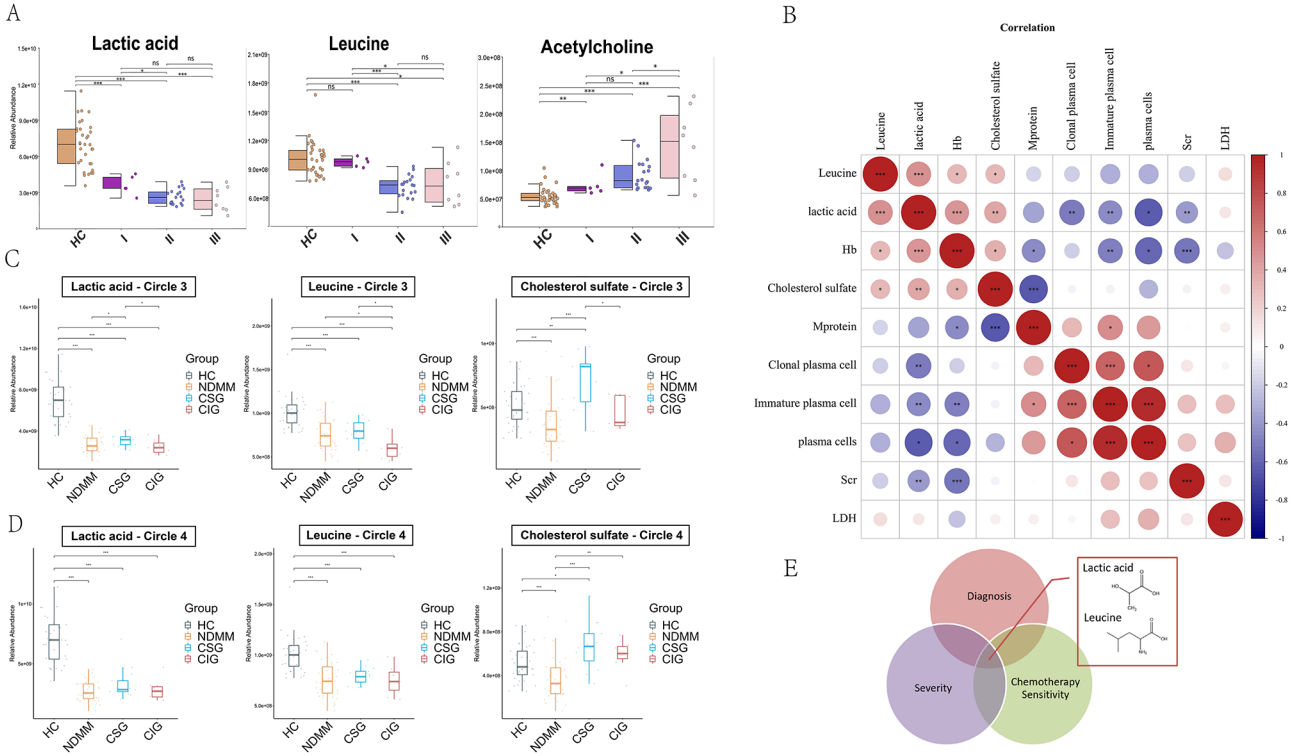

Fig. 4 (**A**) The violin plots of the screened biomarkers (lactic acid, leucine, and acylcholine) expressions in MM patients of ISS stages and healthy controls. (**B**) Pearson's correlation coefficient between candidate metabolites and potential risk indexes in clinic. The heatmap showed the correlation coefficients between candidate metabolites (leucine, lactic acid, and cholesterol sulfate) and risk factors (Hb, LDH, Scr, M protein, and PCs) in plasma. (**C**) The violin plots of the screened biomarkers (lactic acid, leucine, and cholesterol sulfate) expressions of CSG & CIG in the circle 3. (**D**) The violin plots of the screened biomarkers (lactic acid, leucine, and cholesterol sulfate) expressions of CSG & CIG in the circle 4. (**E**) Venn diagram showed that 2 metabolites (leucine, and lactic acid) were overlapped when comparing MM with HC

These findings underscore the potential of lactic acid and leucine as diagnostic biomarkers for MM.

Discussion

Early detection and intervention play crucial roles in enhancing the clinical outcomes of MM patients [5, 29], emphasizing the pressing need to identify potential minimally -invasive biomarkers. Considering the intratumoral heterogeneity and systemic changes induced by hematological malignancy, the analysis of blood samples offers insights into the overall phenotype of MM. Moreover, in comparison to invasive detection methods such as bone marrow puncture or biopsy, an ideal biomarker for MM clinical diagnosis should exhibit optimal sensitivity and specificity when obtained from patients through minimally invasive means, such as blood.

Prior studies have explored metabolic biomarkers of MM in blood [24, 30, 31], indicating that molecular predictive classifiers could offer valuable insights for future targeted MM therapy. However, these studies focused primarily on identifying and describing the metabolic landscape of MM on the basis of single or specific clinical factors. In this retrospective metabolomics analysis of MM, we sought to address three key questions: (i) which patients could be screened early, (ii) which patients could be precisely staged, and (iii) which patients could benefit from chemotherapies. Notably, we observed a consistent decline in lactic acid and leucine levels in the plasma of MM patients at diagnosis, staging, and prognosis, suggesting that these metabolites are potential plasma biomarkers associated with active MM disease. Amazingly, the same phenomenon was also observed in vitro within MM cells.

To the best of our knowledge, this is the most comprehensive analysis demonstrating the extent of metabolic reprogramming in MM. Importantly, the samples included in this study are real-world samples without strict enrollment criteria, demonstrating the robustness of our analysis. Following univariate analyses, we identified a biomarker panel that includes lactic acid and leucine.

Lactic acid is often considered a marker of the "Warburg effect" in tumor cells [32, 33]. Warburg's observation revealed that, unlike most normal cells, tumor cells tend to ferment glucose to lactate even in the presence of sufficient oxygen to support mitochondrial oxidative phosphorylation. The acidic microenvironment formed by lactic acid is conducive to the rapid growth and distant

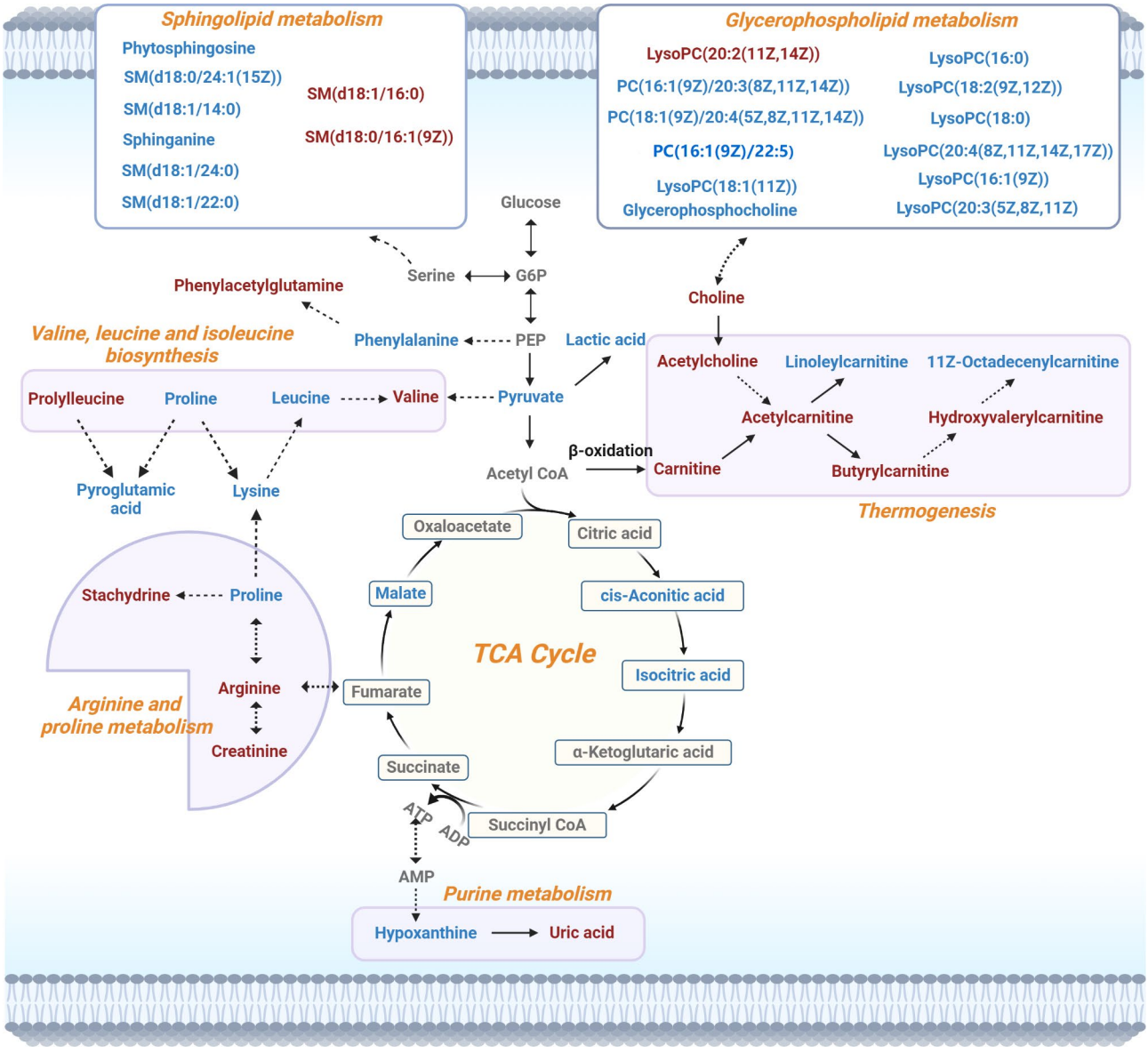

Fig. 5 Metabolic activity network of plasma metabolites in early MM. Red/blue names represent higher/lower intensity in NDMM. Metabolites that had no direct relationship were linked by dotted lines

metastasis of tumor cells. Wiled et al. [33] reported that lactate is also supplied to cancer cells from the surrounding environment, referring to this phenomenon as the reverse Warburg effect. Our observation of a decline in lactate levels in MM patients and cells suggests that the reverse Warburg effect might be applicable to the microenvironment in MM, as previously reported [34]. Furthermore, in patients with MM who achieve complete remission, the increase in lactate concentration is particularly pronounced [22]. Notably, the elevated level of lactate dehydrogenase (LDH), the enzyme catalyzing the conversion of pyruvate to lactate, serves as a marker of poor prognosis at the time of MM diagnosis. An increase in LDH is associated with worse overall survival (OS), progression-free survival (PFS), aggressive disease, and a higher tumor burden [35, 36]. Correspondingly, our research demonstrates that a decreased lactate level is closely related to MM initiation and progression, and it is expected to become an important biomarker for clinical diagnosis and treatment of MM in the future.

Another biomarker, leucine, one of the branched chain amino acids (BCAAs), is an important amino acid that plays crucial roles in the body. Compared with the HC group, MM patients presented lower levels of leucine, and a similar downward trend was also observed in MM cells in vitro. In another related study, the amino acid profiles of MM presented relatively low concentrations of leucine [31]. Furthermore, the concentration

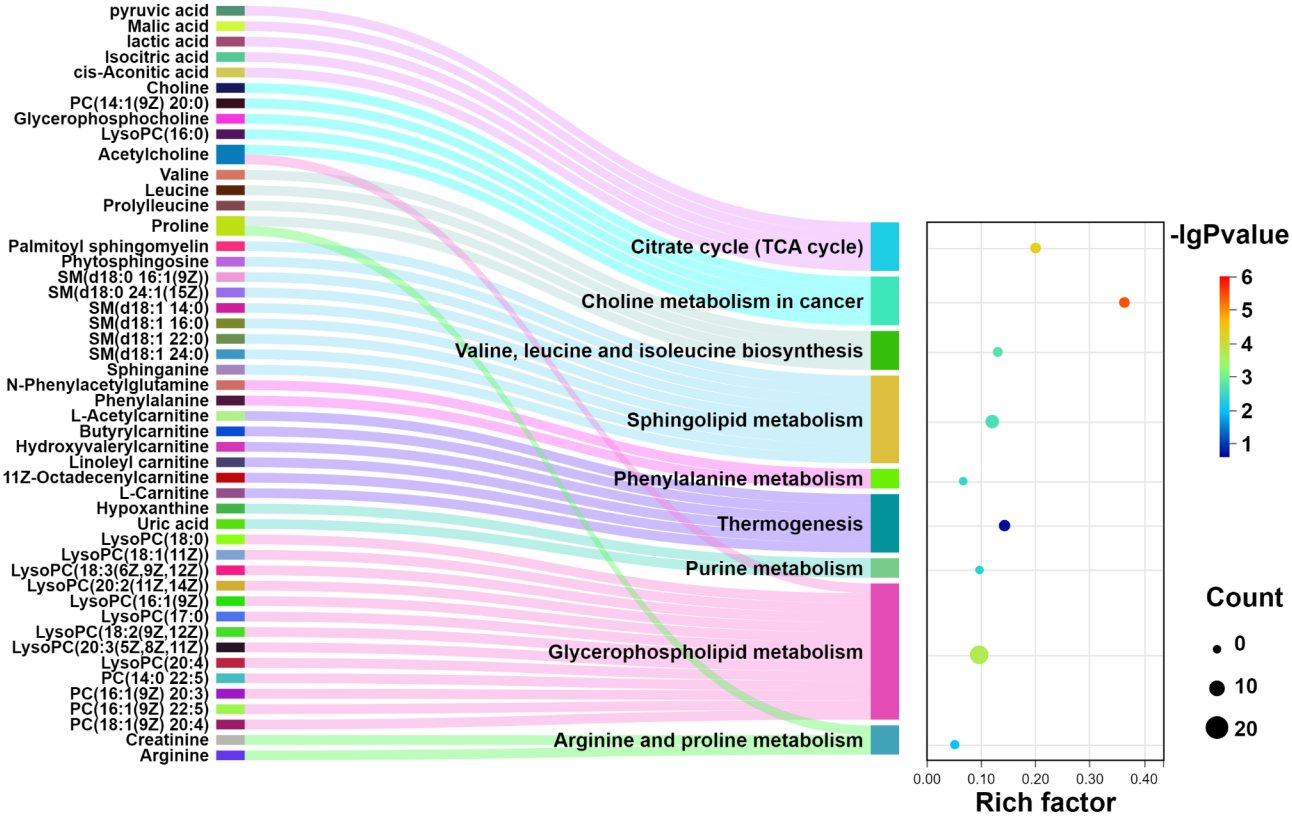

Fig. 6 Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolic pathways enriched by significantly differential metabolites in HC versus NDMM

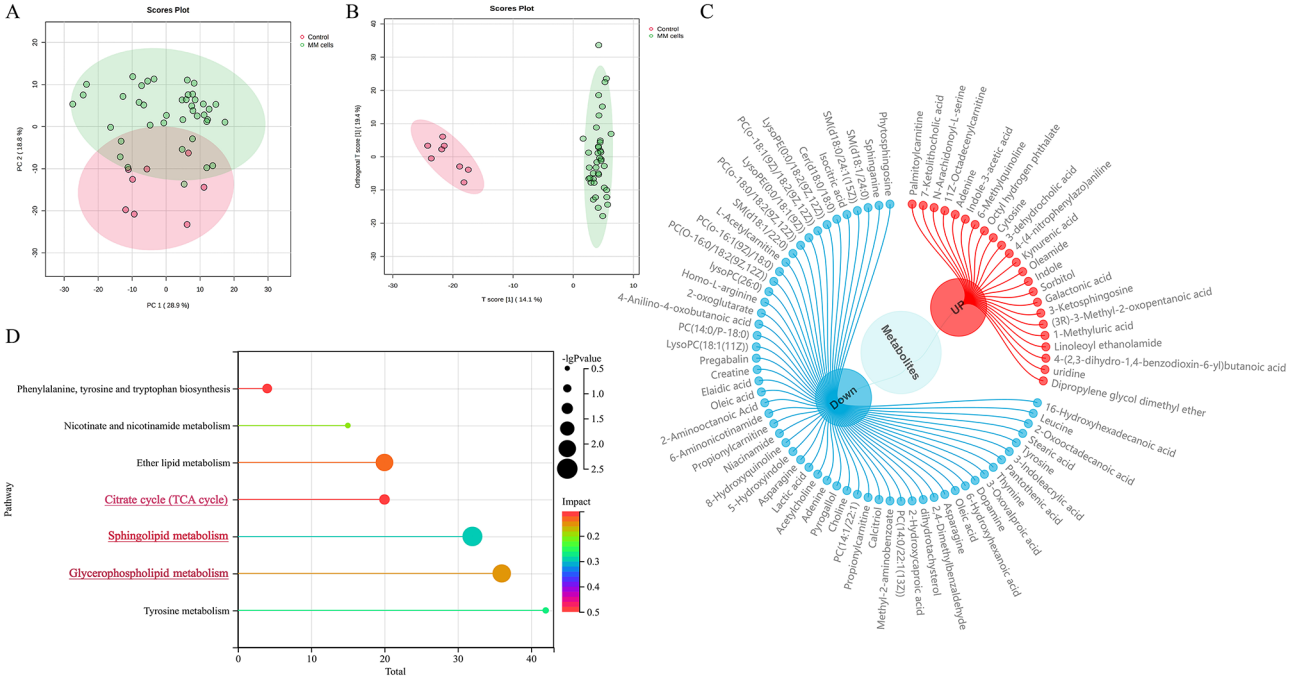

Fig. 7 Multivariate statistical analysis of untargeted metabolomics in human myeloma cell lines (HMCLs). (**A**) It represents the difference between HMCLs and control group in PCA score plot. The red dot represents control group, the green dots represent HMCLs. (**B**) It represents the difference between HMCLs and control group in OPLS-DA score plot. The red dot represents control group; the green dots represent HMCLs. (**C**) The alteration of intracellular metabolites between HMCLs and control group. Red/blue names represent higher/lower intensity in HMCLs. (**D**) Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolic pathways enriched by significantly differential metabolites in HMCLs versus control group

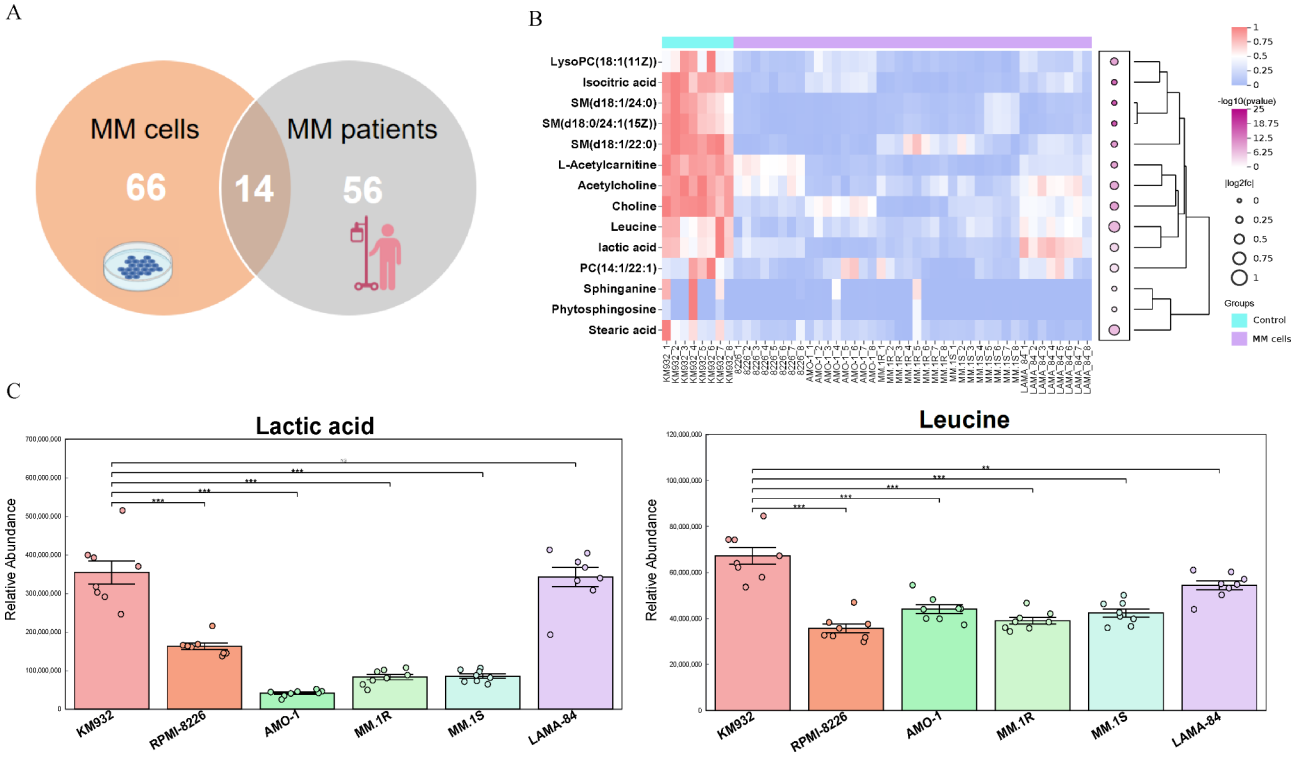

Fig. 8 (**A**) Venn diagram showed that 14 differential metabolites were overlapped between MM cells and MM patients. (**B**) Heatmap of the 14 differential metabolites in control group versus HMCLs were shown. (**C**) Changes in intracellular lactic acid and leucine of each HMCL and control group

of essential amino acids, especially leucine, was significantly decreased in MM patients [37], which was consistent with our findings. Therefore, leucine appears to be a potential biomarker that should be evaluated in future studies addressing the diagnosis, staging, follow-up, prognosis, and treatment of MM.

Interestingly, a recent study identified genes related to lactic acid and BCAAs metabolism as potential prognostic biomarkers independently associated with the overall survival of MM patients [38], which strongly supports our conclusion. Overall, we hypothesize that the diagnostic value may be improved by combining the examination of clinical indicators, lactate, and leucine levels.

Metabolic pathways constitute a highly organized network of sequential chemical reactions in an organism, playing a vital role in maintaining the energy and material balance necessary for life processes. Deeper insights into how these abnormalities disrupt normal metabolic pathways will aid in better prevention, diagnosis, and treatment of associated diseases. Gonsalves et al. [31] reported that BCAAs metabolism, tryptophan metabolism, phospholipid metabolism, and nucleotide turnover were potentially affected by MM, whereas Chanukuppa et al. [24] reported alterations in pyrimidine metabolism, purine metabolism, amino acid metabolism, nitrogen metabolism, sulfur metabolism, and the citrate cycle. Wei et al. reported significant serum metabolic disorders in 46 pairs of pre- and post-therapy MM patients, specifically in arginine, proline and glycerophospholipid pathways [26]. These findings confirmed the vital role of certain metabolites and metabolic pathways in the pathogenesis of MM.

The citric acid cycle (TCA cycle), also known as the Krebs cycle, is a crucial biochemical pathway that occurs in the mitochondria of eukaryotic cells. It generates energy in the form of ATP by coupling the breakdown of substrates to the phosphorylation of ADP. The TCA cycle is a highly regulated process that responds to changes in nutrient availability and energy demand [39]. It can be upregulated during periods of high energy demand or downregulated during periods of nutrient abundance or energy excess. We found that isocitric acid, malic acid, pyruvic acid, and cis-aconitic acid in the TCA cycle were significant downregulated in MM patients, possibly suggesting that the TCA cycle was inhibited. This finding is consistent with Warburg's suggestion that even when oxygen is sufficient, tumor cells rely on massive glucose uptake, converting it to lactate for energy [34].

Lipids serve not only as essential components of cell membranes and energy storage systems but also as crucial signaling molecules that regulate biological processes under both normal and diseased conditions. In our study, we observed significant disturbances in choline metabolism, glycerophospholipid metabolism, and

sphingolipid metabolism in MM patients. Choline, an integral part of acetylcholine synthesis and a precursor to phospholipid synthesis [40], undergoes transformation into phosphocholine, which is coupled with diacylglycerol to form phosphatidylcholine (PCs), a major component of cell membranes. Therefore, choline is considered to reflect the intensity of cell membrane synthesis. Our findings revealed a significant increase in choline and acetylcholine in the MM group compared with those in the HC group, whereas the abundances of PC(16:1(9Z)/20:3(8Z,11Z,14Z), PC(18:1(9Z)/20:4(5Z, 8Z, 11Z, 14Z), and PC(16:1(9Z)/22:5) were notably decreased in the MM group compared with those in the HC group. High choline uptake and downregulated PCs are believed to lead to the hydrolysis necessary for forming lipid messengers, responsible for the replication of clonal plasma cells and tumor dissemination [40]. Members of the lysophosphatidylcholines (LPCs), including LysoPC(16:0), LysoPC(18:2(9Z, 12Z)), LysoPC(18:0), LysoPC(20:4(8Z,11Z,14Z,12Z)), LysoPC(18:1(11Z)), LysoPC(16:1(9Z)), LysoPC(20:3(5Z,8Z,11Z)), and LysoPC(20:2(11Z, 14Z)), exhibited decreased levels in MM patients, consistent with previous research [41]. LPCs are crucial LDL/bioactive lipids that contribute to the inflammatory impact of oxidized LDL on endothelial cells. They are involved in inflammatory stimuli, and promote the release of IL-6 and other inflammatory factors, ultimately contributing to the development and progression of MM [25, 39]. Sphingolipids (SPs), another family of bioactive lipids with a structural role in the plasma membrane, have products of their metabolism (sphingosine, sphingosine-1-phosphate, ceramides, ceramide-1-phosphate) that play crucial roles in MM migration and adhesion, survival and proliferation, as well as angiogenesis and invasion [42]. In our study, most SPs exhibited significantly decreased levels in plasma, suggesting that SPs' hydrolysis can be part of the systemic metabolic regulation/reprogramming of MM.

Amino acids play an essential role in the synthesis of various biomolecules necessary for cell proliferation [43]. Moreover, targeting amino acid metabolism has been proposed as a potential cancer therapy, highlighting the importance of amino acid metabolism in cancer [44, 45]. Alterations in plasma amino acid profiles are relatively common in MM processes [23–26, 40]. Consistent with previous findings, we also discovered the main perturbed amino acid metabolism pathways in MM plasma, including valine, leucine, and isoleucine biosynthesis, phenylalanine metabolism, and arginine and proline metabolism [26].

Leucine, valine and isoleucine, classified as branched-chain amino acids (BCAAs), are crucial for human life and are particularly involved in stress, energy and muscle metabolism [45]. BCAAs follow different metabolic routes, with valine exclusively contributing to carbohydrates (glycogenic), leucine solely to fats (ketogenic) and isoleucine being both a glucogenic and a ketogenic amino acid. The catabolism of valine begins with the removal of the amino group by transamination, producing alpha-ketoisovalerate, an alpha-keto acid, which is converted to isobutyryl-CoA through oxidative decarboxylation by the branched-chain alpha-ketoacid dehydrogenase complex. This is further oxidized and rearranged to succinyl-CoA, which can enter the TCA cycle. The elevated level of valine in MM patients may be due to the inhibited TCA cycle, as previously demonstrated.

Arginine and proline metabolism was significantly enriched in MM. Furthermore, our findings revealed notable elevations in both arginine and creatinine levels, accompanied by a conspicuous decline in proline levels. This pattern of alterations underscores the intricate metabolic shifts occurring within the MM group, particularly focusing on arginine metabolism, which has garnered significant attention in prior research [26]. Arginine, a fundamental amino acid, plays a pivotal role in the urea cycle, serving as a precursor for protein synthesis, polyamine production, creatine synthesis, and nitric oxide (NO) biosynthesis. Arginine deprivation has been demonstrated to have a direct pro-survival effect on myeloma cells, with potential therapeutic implications. Renal failure is a frequent clinical feature in MM patients. Creatinine, a key end product of arginine and proline metabolism, is transported to the kidneys via blood plasma, and serum creatinine is commonly used as an indicator of renal function. Previous studies have reported an obvious up-regulation of serum creatinine and arginine levels in MM patients compared with healthy controls [22, 25, 46], which is consistent with our findings. The elevated levels of creatinine in the plasma of MM patients may be attributed to impaired renal function during the progression of MM, thereby impeding the elimination of toxins [47].

Hydrovalerylcarnitine, butyrylcarnitine, L-acetylcarnitine, and L-carnitine, which play key roles in thermogenesis and fatty acid oxidation (FAO), were significantly elevated in MM patients compared with healthy controls. Acylcarnitines play a primary role in the FAO process within mitochondria, converting fatty acids into energy. When the body requires energy, fatty acids undergo β-oxidation reactions, breaking down into shorter groups and eventually transforming into acetyl-CoA to enter the tricarboxylic acid cycle, generating substantial energy for the cell. Carnitine and acetylcarnitine have been recognized as novel biomarkers for active diagnosis, relapse, and mediators of disease-associated pathologies in MM [48]. Additionally, carnitine may enhance plasma cell immunoglobulin (Ig) secretion, promoting B lymphocytes to differentiate into plasma cells and participate in

antibody-mediated immune responses [49, 50]. Therefore, the increased levels of plasma carnitine and, to a greater extent, acetylcarnitine and hydrovalerylcarnitine in MM patients could entail increased lipid oxidation in highly metabolically active myeloma cells.

In conclusion, a deeper understanding of the metabolic profiles of MM could aid in identifying cases resistant to specific agents, preventing repetitive errors and cumulative toxicity, and exploring new experimental strategies for these cohorts.

Despite these insights, the study has several limitations. While we summarized differentially abundant metabolites and explored their value in MM, more functional validations in vivo and animal models are necessary. Additionally, the patient sample size is relatively small, validation and further investigation in a larger, independent cohort are warranted to better comprehend the mechanisms of MM. Further research is essential to support these results and verify the underlying biological functions of key amino acid metabolites through large-scale and mechanistic studies.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12967-024-05848-7.

Supplementary Material 1: Supplementary Fig. 1. Compared with healthy controls, fold change (FC) and VIP value of different metabolites found in plasma from newly diagnosed multiple myeloma patients. The red columns represent the FC of the metabolite in pregnant women were up-regulated (log2 FC > 0); blue columns (log2 FC < 0) represent down-regulated metabolites in early multiple myeloma; The larger the circle, the higher the VIP value. The differential pathways were as follows: citrate cycle, choline metabolism in cancer, glycerophospholipid metabolism, sphingolipid metabolism, valine, leucine and isoleucine biosynthesis, phenylalanine metabolism, purine metabolism, arginine and proline metabolism, and thermogenesis

Acknowledgements

We would like to publicly thank all participating patients and the staff of department of Hematology of Beijing Chao-yang Hospital, for their selfless collaboration in this study. We also would like to express our gratitude to EditSprings (https://www.editsprings.cn) and https://www.BioRender.com for the expert linguistic services provided and drawing implement.

Author contributions

Xiaoxue Wang and Guolin Shen designed the study, constructed the graphics/tables, performed the statistical analyses and wrote the first draft. Xiaoxue Wang and Longhao Cheng participated in the metabolomics experiments. Aijun Liu and Longhao Cheng collected the clinical samples. Lihong Liu proofread this manuscript. Lili Gong and Guolin Shen conceived and supervised the entire project.

Funding

This work was supported by grants from the National High Level Hospital Clinical Research Funding (2023-NHLHCRF-PY-06, 2023-NHLHCRF-YXHZ-ZRMS-04), CAMS Innovation Fund for Medical Sciences (CIFMS) (2021-I2M-1-049), and China International Medical Foundation-Chinese Clinical Pharmacy Funding (Z-2021-46-2101-2023).

Data availability

The data presented in this study are available on request from the corresponding author. The data are not publicly available due to ethical and privacy restrictions.

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the guidelines of the Declaration of Helsinki and was approved by the Ethics Committee of Beijing Chao-yang Hospital, Capital Medical University, and informed written consent was obtained from all patients and healthy individuals. (No. 2019-4-15-4).

Informed consent

Informed consent was obtained from all study participants.

Conflict of interest

The authors declare no conflict of interest.

Received: 26 July 2024 / Accepted: 30 October 2024
Published online: 06 February 2025

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