论著·基础医学

基于代谢组学与机器学习筛选高原失血性休克诊断标志物:D-inositol-4-phosphate、Phosalone与Methionine的鉴定与验证

周远群,向鑫明,欧阳杏楠,张 杰,张紫森,刘良明,李 涛 陆军军医大学(第三军医大学)大坪医院战伤休克与输血研究室,创伤与化学中毒全国重点实验室,重庆

[**摘要**] **目的** 整合代谢组学与机器学习算法研究模拟高原条件下失血性休克的代谢特征,筛选高原环境失血性休克的潜在诊断生物标志物。**方法** 10~12周龄、体质量180~220 g SPF级雄性SD大鼠80只按随机数字表法分为(n=40):假手术(Sham)组和休克(uncontrolled hemorrhagic shock,UHS)组。大鼠放置于低压缺氧舱(模拟海拔5 000 m高原环境)喂养48 h,出舱后立即经尾静脉注射油酸,30 min后,离断脾动脉使其自由出血,待平均动脉血压(mean arterial pressure,MAP)降至40 mmHg,建立模拟高原条件下的失血性休克模型。采集2组大鼠的血清,通过代谢组学分析鉴定差异代谢物。通过加权基因共表达网络分析(weighted gene co-expression network analysis,WGCNA)识别与休克相关的代谢物,利用LASSO(least absolute shrinkage and selection operator)回归、随机森林(random forest,RF)、支持向量机递归特征消除(support vector machine-recursive feature elimination,SVM-RFE)3种机器学习方法鉴定失血性休克的生物标志物。基于十折交叉验证进行受试者工作特征曲线(receiver operating characteristic curve,ROC)分析,计算平均曲线下面积(area under the curve,AUC),以评估生物标志物的诊断性能。**结果** 代谢组学数据主成分分析结果显示Sham组样本和UHS组样本明显分离,正交偏最小二乘法-判别分析(orthogonal projections to latent structures-discriminant analysis,OPLS-DA)进一步证实2组血清样本的代谢模式存在明显差异。共鉴定到5 398个代谢物,与Sham组相比,UHS组有391个代谢物显著下调(VIP>1,FC<1/1.5,P<0.05),1 181个代谢物显著上调(VIP>1,FC>1.5,P<0.05)。其中,变化最多的是脂质类代谢物,包括FFA(18:2)、FFA(18:1)、FFA(12:0)、FFA(14:0)、Ergosterol、Mesaconic acid、Suberic acid、gamma-Linolenic acid和PC[22:5(4Z,7Z,10Z,13Z,16Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z)]等,其次是有机酸及衍生物类代谢物,包括Methionine、Citrulline、Creatine、L-Lactic acid、Oxalacetic acid和2-Acetolactate等。代谢通路富集分析显示这些差异代谢物主要参与谷氨酰胺代谢,牛磺酸代谢,丙氨酸、天冬氨酸和谷氨酸代谢以及甘油磷脂代谢等。WGCNA分析结果显示MEturquoise模块与UHS呈最强正相关(R=0.95,P=8e-40),MEsalmon模块与UHS呈最强负相关(R=-0.67,P=2e-11)。进一步以|基因显著性(gene significance,GS)|>0.2和|模块隶属度(module membership,MM)|>0.8为标准进行筛选,共获得344个与高原环境失血性休克显著相关的特征代谢物。通过LASSO回归、RF和SVM-RFE 3种机器学习算法筛选出3种生物标志物,分别是Methionine、D-inositol-4-phosphate和Phosalone。与Sham组相比,UHS组中D-inositol-4-phosphate和Phosalone的含量显著升高,而Methionine的含量显著降低。ROC曲线分析结果显示D-inositol-4-phosphate、Phosalone和Methionine的平均AUC分别为0.988、0.950和0.988,表明这3种代谢物对模拟高原条件下失血性休克具有良好的预测能力。**结论** 模拟高原条件下失血性休克大鼠的代谢特征明显紊乱,多条代谢途径发生显著改变,代谢物D-inositol-4-phosphate、Phosalone和Methionine可能成为预测高原环境下失血性休克发生或判断休克程度的生物标志物。

[**关键词**] 高原环境;失血性休克;代谢产物;机器学习

[**中图法分类号**] R339.54;R363.21;R605.971 [**文献标志码**] A

[**基金项目**] 国家自然科学基金青年基金(82402549)

[**通信作者**] 李 涛,E-mail:lt200132@tmmu.edu.cn

2097-0927/Copyright © 2026,陆军军医大学(第三军医大学)。这篇开放获取文章遵循CC BY许可协议(https://creativecommons.org/licenses/by/4.0/)

引用格式:周远群,向鑫明,欧阳杏楠,等. 基于代谢组学与机器学习筛选高原失血性休克诊断标志物:D-inositol-4-phosphate、Phosalone与Methionine的鉴定与验证[J]. 陆军军医大学学报,2026,48(01):75-85. DOI:10.16016/j.2097-0927.202511028.

Citation:ZHOU Y Q,XIANG X M,OUYANG X N,*et al*. Biomarkers for hemorrhagic shock at high-altitude based on metabolomics and machine learning:Identification and validation of D-inositol-4-phosphate, phosalone and methionine[J]. J Army Med Univ,2026,48(01):75-85. DOI:10.16016/j.2097-0927.202511028.

Biomarkers for hemorrhagic shock at high-altitude based on metabolomics and machine learning: Identification and validation of D-inositol-4-phosphate, phosalone and methionine

ZHOU Yuanqun, XIANG Xinming, OUYANG Xingnan, ZHANG Jie, ZHANG Zisen, LIU Liangming, LI Tao
National Key Laboratory of Trauma and Chemical Poisoning, Department of War Trauma, Shock and Blood Transfusion, Daping Hospital, Army Medical University(Third Military Medical University), Chongqing, China

[**Abstract**] Objective To systematically investigate the metabolic characteristics of hemorrhagic shock (HS) under simulated high-altitude conditions by integrating metabolomics and machine learning algorithms, and screen the biomarkers for predicting HS under these conditions. Methods Eighty SPF-grade male SD rats (10 to 12 weeks old, weighing 180 to 220 g) were randomly divided into a sham operation group (Sham) and an uncontrolled HS group (UHS), with 40 animals in each group. All rats were placed in a hypobaric hypoxia chamber to simulate an altitude of 5 000 m for 48 h. After removal from the chamber, the rats were given an injection of oleic acid immediately *via* tail vein, and then in 30 min latter, those from the UHS group were induced to free bleeding by splenic artery transection, with the endpoint set at a mean arterial pressure (MAP) of 40 mmHg. Thus, a rat model of HS under stimulated high-altitude conditions was established. Metabolomic analysis was performed on the serum samples to identify differential metabolites between the Sham and UHS groups. Weighted gene co-expression network analysis (WGCNA) was carried out to identify metabolites associated with UHS. Three machine learning methods, including least absolute shrinkage and selection operator (Lasso) regression, random forest (RF), and support vector machine-recursive feature elimination (SVM-RFE) were employed to identify relevant biomarkers for HS at high-altitude. Based on 10-fold cross-validation, the diagnostic performance of the biomarkers was evaluated with receiver operating characteristic (ROC) curve analysis, and the area under the curve (AUC) was calculated. Results Principal component analysis (PCA) of the metabolomic data showed clear separation between the samples from the Sham and UHS groups. Orthogonal partial least squares-discriminant analysis (OPLS-DA) further confirmed significant differences in metabolic profiles between the 2 groups. There were 5 398 metabolites identified, with the UHS group having 391 metabolites significantly down-regulated (VIP>1, FC<1/1.5, P<0.05) and 1 181 metabolites obviously up-regulated (VIP>1, FC>1.5, P<0.05) when compared to the Sham group. Among the metabolites, the most altered metabolites were lipids, including FFA (18:2), FFA (18:1), FFA (12:0), FFA (14:0), ergosterol, mesaconic acid, suberic acid, gamma-linolenic acid, and PC [22:5 (4Z, 7Z, 10Z, 13Z, 16Z)/22:6 (4Z, 7Z, 10Z, 13Z, 16Z, 19Z)], and then followed by organic acids and derivatives, such as methionine, citrulline, creatine, L-lactic acid, oxalacetic acid, and 2-acetolactate. Pathway enrichment analysis showed that these differential metabolites were mainly involved in glutamine metabolism, taurine metabolism, alanine, aspartate and glutamate metabolism, as well as glycerophospholipid metabolism. WGCNA analysis found that MEturquoise module exhibited the strongest positive correlation with UHS (R=0.95, P=8e−40), while the MEsalmon module showed the strongest negative correlation with UHS (R=−0.67, P=2e−11). With thresholds of |Gene Significance (GS)|>0.2 and |Module Membership (MM)|>0.8, 344 characteristic metabolites were identified, and all of them were significantly associated with HS in a high-altitude environment. The 3 machine learning algorithms yielded 3 biomarkers, that is, methionine, D-inositol-4-phosphate and phosalone. Significantly increased D-inositol-4-phosphate and phosalone while decreased methionine were observed in the UHS group than the Sham group. ROC curve analysis revealed that the AUC values of D-inositol-4-phosphate, phosalone and methionine were 0.988, 0.950 and 0.988, respectively, indicating that the 3 biomarkers having good predictive efficiency for HS under simulated high-altitude condition. Conclusion HS rats under simulated high-altitude conditions present significantly disturbed

metabolic profiles, characterized by substantial changes across multiple pathways. The metabolites D-inositol-4-phosphate, phosalone and methionine may serve as potential biomarkers for predicting the occurrence or evaluating the severity of HS in high-altitude environments.

[**Key words**] high-altitude environment; hemorrhagic shock; metabolites; machine learning

Supported by the National Natural Science Foundation for Young Scholars of China(82402549). Corresponding author: LI Tao, E-mail: lt200132@tmmu.edu.cn

战创伤失血性休克的发生率极高,达50%以上,是导致战创伤早期死亡的重要原因之一[1-2]。在高原地区,因其缺氧寒冷的环境特点,进一步加重了失血性休克后的微循环障碍和器官功能损伤[3],导致死亡率较平原地区更高。在极端高原环境下,因战创伤引起的失血性休克对机体的生理平衡构成严峻挑战,其病理生理学机制复杂且进展迅速,常导致预后不良。高原低氧、低温、低气压等特殊环境因素,可能通过影响氧气输送、能量代谢、免疫调节及内分泌功能等多个层面,加剧战创伤后失血性休克的病理生理学改变[3-4],使得传统治疗策略面临巨大挑战。因此,深入理解高原环境失血性休克状态下机体代谢特征的演变,对于寻找可靠的早期预警指标和干预靶点具有至关重要的意义。

战创伤失血性休克的早期识别和精准分诊是提高救治成功率的关键环节。传统基于面容观察、血压监测和简单评分系统等的休克评估方法[5-6],虽可操作性强,能对失血性休克进行初步评估,但在复杂环境如战场或高原地区仍存在一定局限性。研究表明,休克导致的血压下降往往出现在失血性休克的失代偿阶段,因此,仅依据血压判断休克状态会导致评估滞后,无法对休克早期进行识别和监测,缺乏灵敏性和特异性。另一方面,在战场或高原等资源受限环境中,传统评估方法易受外界环境及主观因素干扰,且无法动态反映患者的病理进程与个体化差异,容易导致误判或治疗不及时。因此,急需开发能够实时、客观、灵敏地反映休克状态及预后的生物标志物,以提高战创伤或高原失血性休克的救治成功率。

生物标志物具有高敏感性与动态反映病理进程的能力,可在血压尚未显著下降时提示组织缺血、缺氧及炎症激活等早期改变,有助于区分患者的优先级,为实施针对性复苏及器官保护争取“黄金时间”。近年来,转录组学、代谢组学等技术为系统性挖掘此类标志物提供了有力工具。作为一种系统生物学技术,代谢组学通过对生物体内所有小分子代谢产物的定性与定量分析,能够快速、全面反映机体在特定生理或病理状态下的代谢活动及紊乱情况[7],已被广泛应用于疾病诊断、预后评估及药物作用机制研究等领域[8]。此外,机器学习算法在挖掘高维、复杂的组学数据方面展现出强大优势。通过将机器学习算法应用于代谢组学数据,可以从海量数据中精准识别核心代谢物组合,构建高精度的诊断模型[9-10]。如有研究利用机器学习算法从代谢组学数据中识别疾病特异性代谢指纹,为疾病诊断和早期识别提供了重要工具[11]。

本研究旨在整合代谢组学与机器学习算法,系统性地探究高原环境失血性休克大鼠的血清代谢特征,筛选出在高原失血性休克早期具有诊断和预后价值的特异性生物标志物。这些潜在生物标志物的发现将有助于揭示高原环境下失血性休克的独特病理生理机制,并为开发高原战创伤失血性休克的新型诊断工具和优化治疗策略提供重要的理论依据。

1 材料及方法

1.1 实验动物

10~12周龄、体质量180~220 g的SPF级雄性SD大鼠,由陆军特色医学中心实验动物中心提供[动物使用许可证号 SCXK(渝)2017-0002]。所有大鼠饲养期间自由饮水摄食,保持湿度40%~70%,温度22~24 ℃,12 h光照/12 h黑暗昼夜环境。本研究已获陆军军医大学实验动物福利伦理审查委员会批准(AMUWEC20194758)。

1.2 分组设计及模型制备

80只大鼠按随机数字表法分为(n=40):假手术(Sham)组和休克(uncontrolled hemorrhagic shock,UHS)组。大鼠置于低压氧舱(模拟海拔5 000 m)饲养48 h,其间可自由获取食物和水。出舱后,用3%戊巴比妥钠麻醉大鼠(30 mg/kg,腹腔注射),随后尾静脉注射油酸(50 μg/kg)[12],30 min后左股动脉插管并注入100 U肝素钠。UHS组大鼠从腹中线剖腹,暴露脾脏,从脾动脉第一、二分支之间切断脾实质,并剪断一条脾动脉分支,使血自由流至腹腔。同时,进行平均动脉血压(mean arterial pressure,MAP)监测,当MAP降至40 mmHg时即为高原失血性休克模型建立成功。实验结束后,在利用戊巴比妥钠进行深度麻醉下实施安乐死。

1.3 血清样本收集及代谢物检测

采集大鼠动脉血2 mL,室温静置1 h后,3 000 r/min离心10 min,取50 μL上清,加入300 μL 20%乙腈甲

醇内标提取液,12 000 r/min、4 ℃离心 10 min。取上清液静置 30 min(-20 ℃)。12 000 r/min、4 ℃再次离心 3 min,取上清液,上机分析。

1.4 代谢组学数据分析

采用超高效液相色谱仪,通过 Waters ACQUITY UPLC HSS T3 C18液相色谱柱分离目标化合物。在R语言中,利用XCMS(v3.12.0)包进行峰识别、峰提取、峰对齐处理,得到代谢物定量列表。通过基于质控样本(quality control,QC)的支持向量回归校正,消除系统误差,并基于QC样本的相对标准偏差(relative standard deviation,RSD)对特征进行过滤,剔除RSD>30%的特征,以确保数据的稳定性。对于在部分样本中缺失的峰值,采用均值进行填补。为减少异常值影响,增强数据正态分布属性,使用MetaboAnalyst 5.0软件的数据标准化流程,选择Normalization by sum和Auto scaling进行样本归一化和数据标准化,获得的数据矩阵用于后续分析。采用HMDB(human metabolome database)、massbank、Lipid-Maps、mzcloud等数据库进行物质鉴定,参数设置为ppm<30 ppm,得到代谢物定性结果。通过正交偏最小二乘法-判别分析(orthogonal projections to latent structures-discriminant analysis,OPLS-DA),以变量投影重要性(variable importance in projection,VIP)>1.0、P<0.05、差异倍数(fold change,FC)>1.5或<1/1.5为标准,筛选差异代谢物。使用MetaboAnalyst 5.0(http://metpa.metabolomics.ca)对差异代谢物进行通路富集分析。

1.5 加权共表达网络构建(weighted gene co-expression network analysis,WGCNA)

利用R语言中的WGCNA软件包构建加权基因共表达网络。首先,对样本进行层次聚类分析鉴定异常离群样本。利用pickSoftThreshold函数确定最佳软阈值为6,根据代谢物的表达模式构建共表达模块。采用Pearson相关性算法计算每个模块中代谢物与性状的相关系数(R)及P值,筛选出与UHS相关的特异性模块。最后,以|基因显著性(gene significance,GS)|>0.2,|模块隶属度(module membership,MM)|>0.8为标准筛选模块中的特征代谢物。

1.6 整合LASSO(least absolute shrinkage and selection operator)回归、随机森林(random forest,RF)、支持向量机递归特征消除(support vector machine-recursive feature elimination,SVM-RFE)筛选高原战创伤休克的核心生物标志物

将差异代谢物与WGCNA分析获得的特征代谢物取交集,获得99个差异特征代谢物。利用R语言中的glmnet函数对99个差异特征代谢物进行LASSO回归分析(α=1),通过lambda=lambda.1se选择最优λ,family设置为"binomial",nfolds设置为10,type.measure设置为"deviance",采用Logistic回归识别最终显著影响因素。为评估99个差异特征代谢物的相对重要性,利用randomForest函数构建随机森林模型(设定决策数量为100),importance设置为TRUE以计数变量重要性(Mean Decrease Accuracy),根据Mean Decrease Accuracy对各变量进行重要性排序,取Mean Decrease Accuracy>1的代谢物进行后续分析。采用"e1071""kernlab"及"caret"包完成SVM-RFE算法对差异特征代谢物的筛选,设置nfold=10,利用十折交叉验证对模型进行验证,通过PlotErrors和Plotaccuracy函数分别绘制错误率和准确率的变化曲线,以帮助直观评估模型性能。根据错误率最低点的特征数量,选择用于后续分析的特征。

1.7 统计学分析

借助SIMCA 14.1软件,建立主成分分析(principal component analysis,PCA)和OPLS-DA模型,对各组样本进行区分。对VIP>1的代谢物进行独立样本t检验,结合VIP>1,P<0.05、FC>1.5或<1/1.5筛选差异代谢物。用R软件中"caret"包和"pROC"包进行基于十折交叉验证的受试者工作特征曲线(receiver operating characteristic curve,ROC)分析,计算平均曲线下面积(area under the curve,AUC)及其95%CI。

2 结果

2.1 高原环境失血性休克血清代谢特征

对2组大鼠血清进行代谢组学分析,PCA结果显示2组样本明显分离,表明组间差异性良好(图1A)。进一步证实2组血清样本的代谢模式存在明显差异(图1B、C)。共鉴定到5 398个代谢物,VIP值描述了每个变量对模型的总体贡献(图1D),VIP>1表示该变量对模型的贡献大于平均值,对VIP>1的代谢物进行独立样本t检验,根据VIP>1,FC=1.5,P<0.05的标准筛选差异代谢物。与Sham组相比,UHS组有391个代谢物显著下调,1 181个代谢物显著上调(图2A、B)。其中,变化最多的是脂质类代谢物,包括FFA(18:2)、FFA(18:1)、FFA(12:0)、FFA(14:0)、Ergosterol、Mesaconic acid、Suberic acid、gamma-Linolenic acid和PC[22:5(4Z,7Z,10Z,13Z,16Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z)]等,其次是有机酸及衍生物类代谢物,包括Methionine、Citrulline、Creatine、L-Lactic acid、Oxalacetic acid和2-Acetolactate等(图2C)。代谢通路富集分析显示这些差异代谢物主要参与谷氨酰胺代谢,

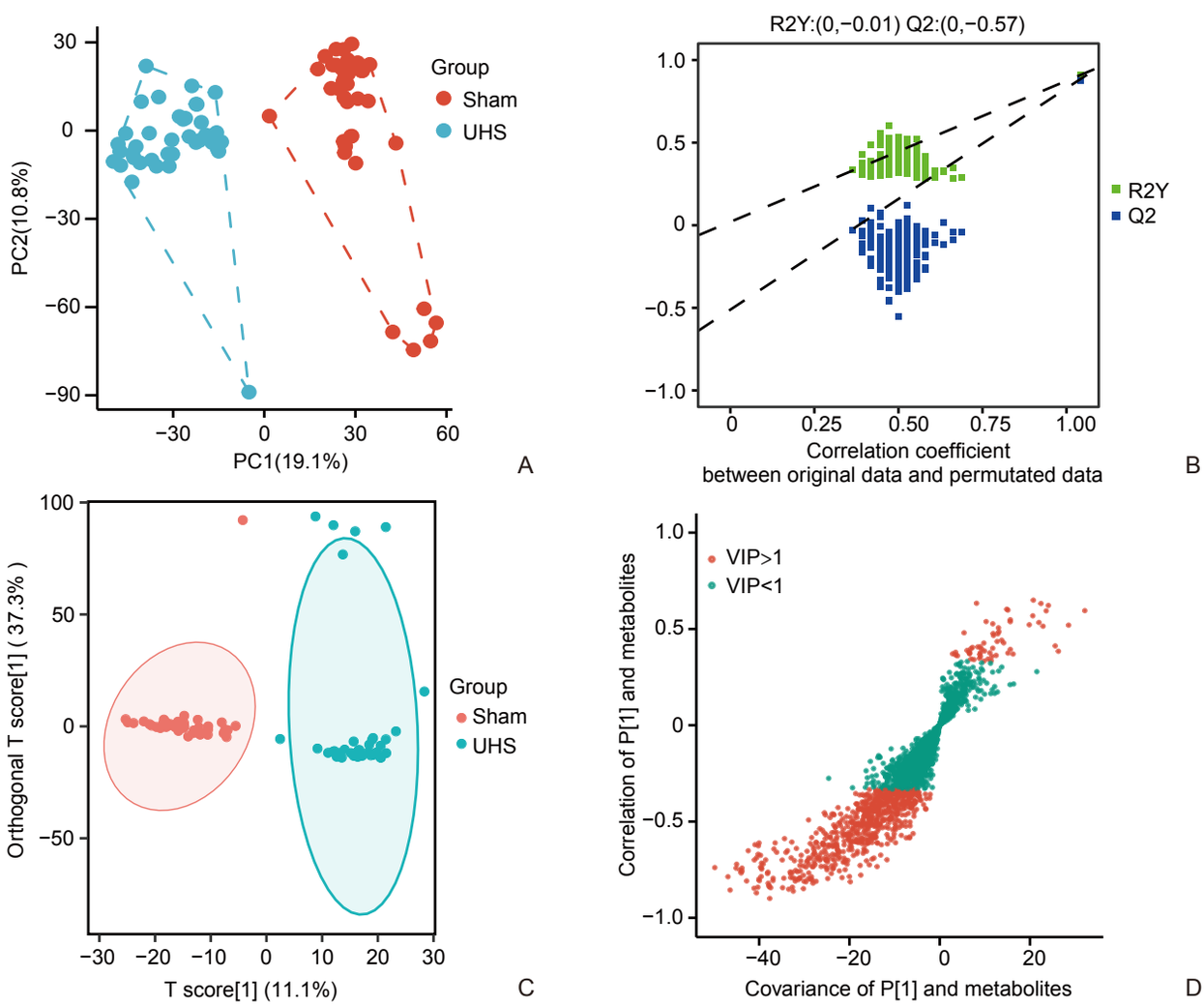
牛磺酸代谢,丙氨酸、天冬氨酸和谷氨酸代谢和甘油磷脂代谢等(图2D)。

2.2　识别高原环境失血性休克显著相关的特征代谢物

为进一步探索高原环境失血性休克相关的代谢变化,利用WGCNA方法建立了80个样本代谢组的无标度拓扑网络矩阵,根据表达模式将5 398个代谢物聚类到不同的代谢物共有模块中。根据拟合指数与power值散点图和平均连通性选出最佳power为6(图3A~C),建立了26个代谢物共有模块与2个性状的之间的相关性矩阵(图3D)。其中,MEturquoise模块与UHS呈最强正相关(R=0.95,P=8e−40),MEsalmon模块与UHS呈最强负相关(R=−0.67,P=2e−11)。进一步以|GS|>0.2和|MM|>0.8为标准筛选上述2个模块中的特征代谢物,共获得344个与高原环境失血性休克显著相关的特征代谢物(图3E、F)。

2.3　整合机器学习算法识别和鉴定高原环境失血性休克的生物标志物

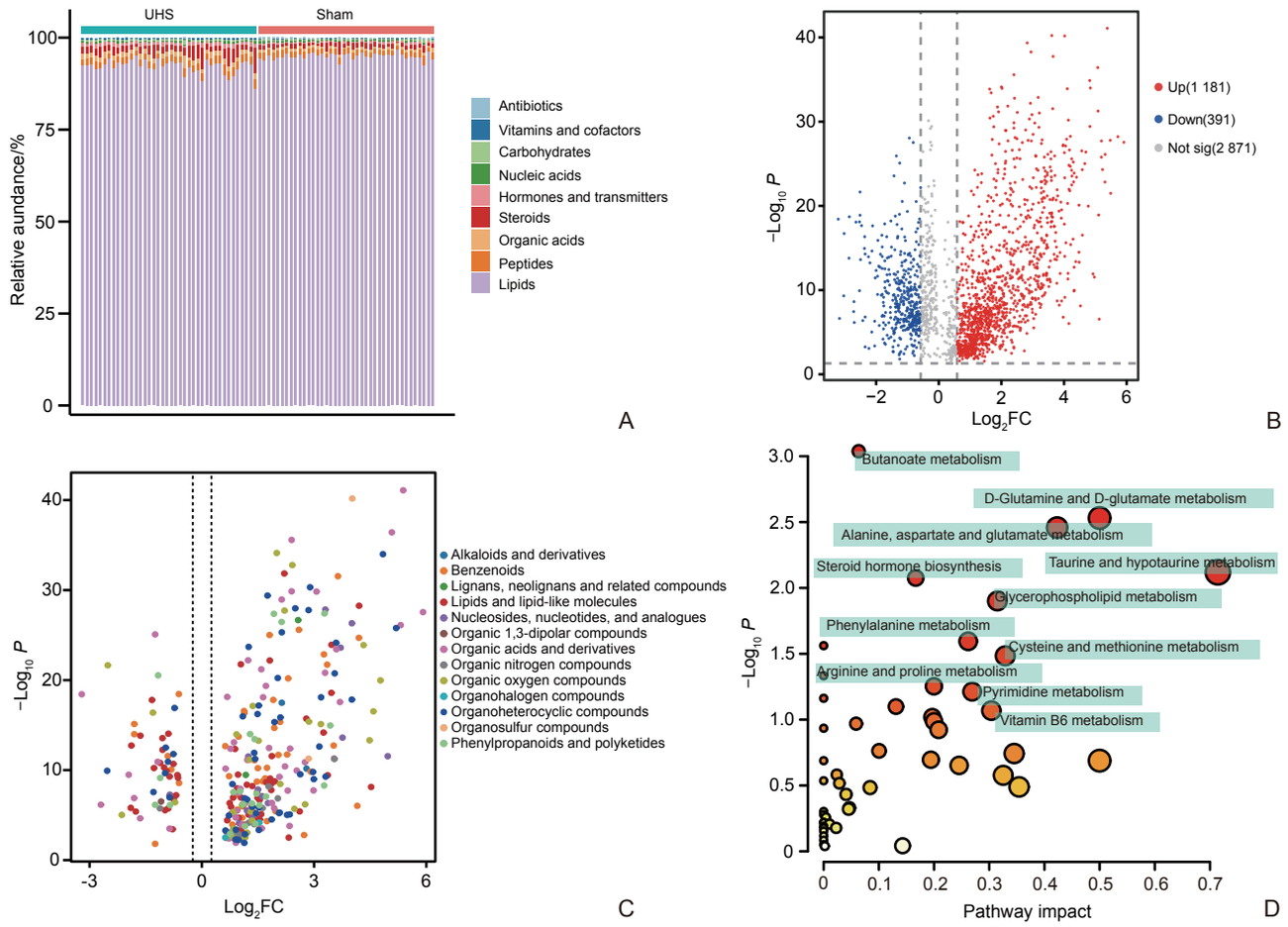
为了鉴定高原环境失血性休克的生物标志物,将差异代谢物与WGCNA分析获得的特征代谢物进行维恩分析,共获得99个差异的特征代谢物(图4A)。随后,整合LASSO回归、RF、SVM-RFE 3种机器学习算法,筛选高原环境失血性休克的生物标志物。其中,LASSO回归获得10个候选生物标志物,SVM-RFE获得13个候选生物标志物,RF获得44个候选生物标志物(图4B~G)。通过对3种算法选择的候选生物标志物进行维恩分析,获得3种高原环境失血性休克的重要生物标志



A: Principal component analysis; B: Validation plot of OPLS-DA model (when the Q2 fitted line intercepts the Y-axis below 0, it indicates the model results are reliable); C: Score plot of OPLS-DA model; D: S-plot of OPLS-DA model. UHS: Uncontrolled hemorrhagic shock; OPLS-DA: Orthogonal partial least squares-discriminant analysis

图1　高原环境下Sham和UHS大鼠血清代谢组学分析

Figure 1　Serum metabolomics analysis of Sham and UHS rats in high-altitude conditions



A: Relative abundance of metabolite in each group; B: Volcano plot of differential metabolites; C: HMDB super class classification volcano plot of differential metabolites; D: Functional enrichment analysis of differential metabolites

图2 高原环境失血性休克差异代谢物及功能富集

Figure 2 Differential metabolites and functional enrichment in hemorrhagic shock under high-altitude conditions

物,包括Methionine、D-inositol-4-phosphate和Phosalone。此外,基于任意2种机器学习算法获得的代谢物还包括 Mesaconic acid、Chrysosplenol D、Isopentenyl pyrophosphat、Diallyl disulfide、Uracil、4-Hydroxycinnamic acid、2-Acetolactate、D-Mannitol 1-phosphate、5′-Deoxy-5′-(Methylthio) Adenosine、O-Butanoylcarnitine 和(E)-2-methylgeranyl diphosphate(图4H)。这些代谢物有潜力作为高原环境失血性休克的诊断标志物,并可能在高原环境失血性休克的发生发展中发挥关键作用。

2.4 生物标志物的表达及诊断性能评估

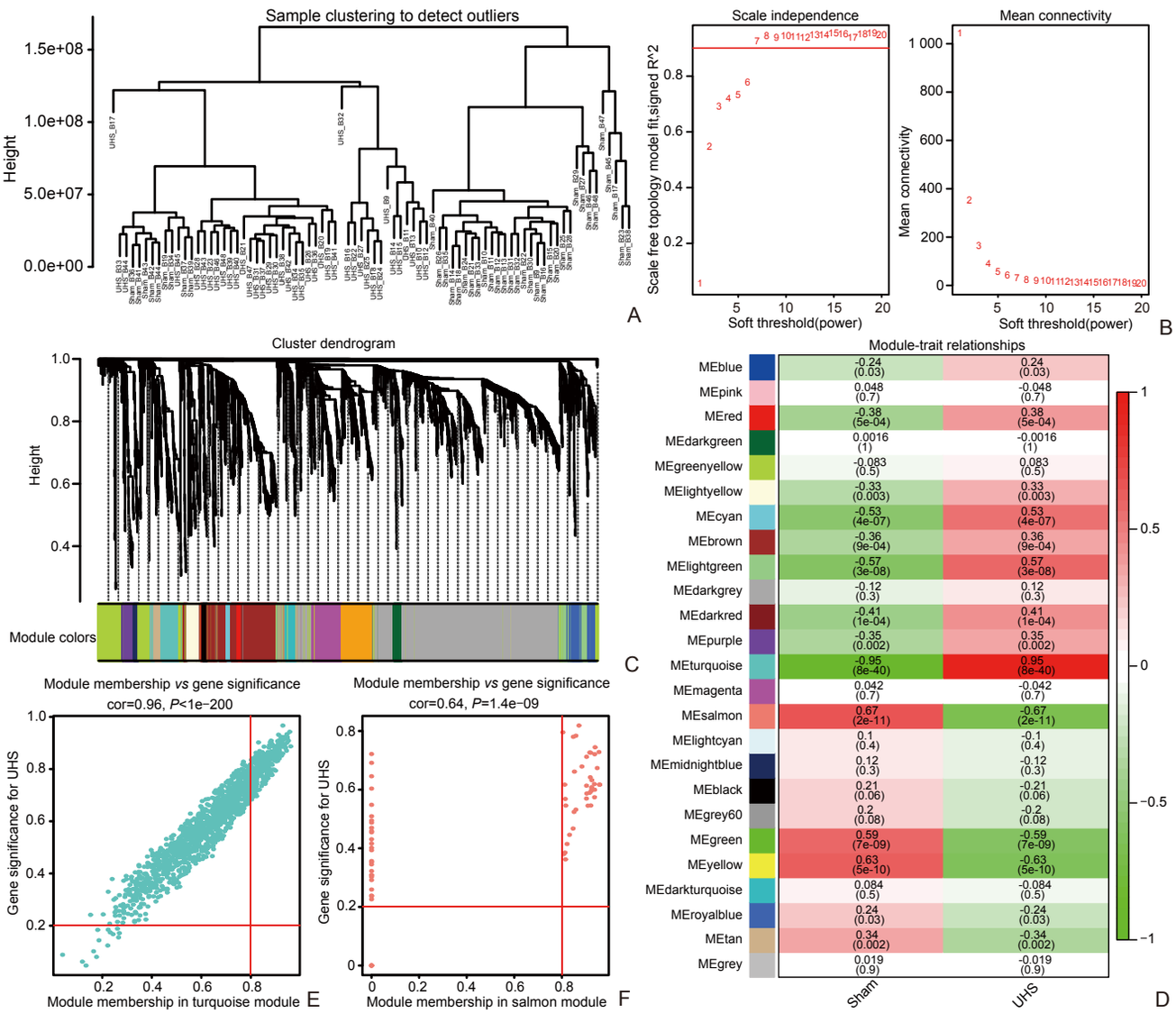
与Sham组相比,UHS组中D-inositol-4-phosphate和Phosalone的含量显著升高,而Methionine的含量显著降低(图5A)。进一步利用十折交叉验证构建3种代谢物的ROC曲线,并计算平均AUC,以评估其诊断性能。结果显示,D-inositol-4-phosphate、Phosalone和Methionine的平均AUC分别为0.988、0.950和0.988(图5B~D),提示通过3种机器学习算法获得的这3种代谢物对高原环境失血性休克具有良好的预测能力。

3 讨论

本研究通过整合代谢组学和机器学习算法,系统分析了模拟高原条件下失血休克大鼠模型的血清代谢谱,揭示了高原环境失血性休克后机体代谢网络发生了剧烈而广泛的紊乱,并成功筛选出D-inositol-4-phosphate、Phosalone和Methionine可能成为潜在的生物标志物,这不仅为早期、快速诊断高原战创伤休克提供了新的思路,也为其代谢调控机制的深入探讨提供了重要线索。

3.1 高原失血性休克引发的广泛代谢网络紊乱

在极端高原环境下,由致死性战创伤所致的失血性休克会对机体的生理稳态构成严重威胁,其病理机制复杂、进展迅速,常导致预后不良。本研究通过构

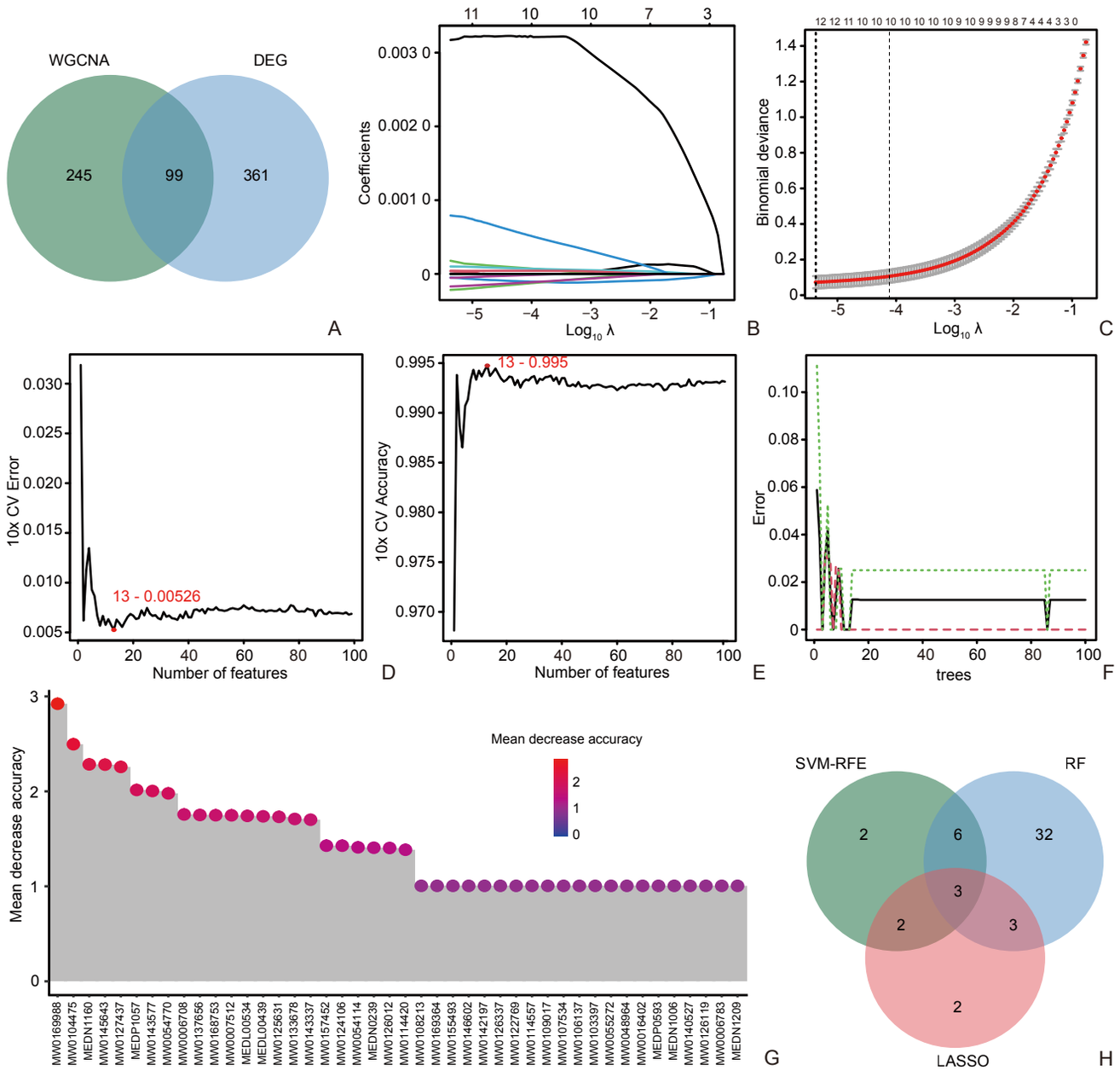


A: Sample clustering; B: Selection of power =6 as soft threshold through combined analysis of scale independence and mean connectivity; C: Metabolite co-expression modules; D: Heatmap of module-UHS correlations; E; Correlation plot in the turquoise module; F: Correlation plot between metabolite members and significance in the salmon module. UHS: Uncontrolled hemorrhagic shock

图3 高原环境失血性休克特征代谢物筛选

Figure 3 Screening of characteristic metabolites in hemorrhagic shock under high-altitude conditions

建模拟高原条件下的失血性休克大鼠模型并结合代谢组学分析,发现高原环境失血性休克导致机体出现了广泛而深刻的代谢紊乱。其中,脂质类代谢物、有机酸及其衍生物类代谢物的变化最为显著。研究表明,脂质代谢在机体应激与创伤反应中具有重要作用,其紊乱可能影响细胞膜的完整性、能量储存与供应以及信号转导等过程[13]。例如,甘油磷脂代谢的改变可能与细胞膜的结构破坏或修复机制有关[14]。有机酸及其衍生物是多种代谢途径的中间产物或调节因子,其含量的显著改变提示能量代谢、氨基酸代谢等基础生理过程可能出现了严重失衡。通过进一步代谢通路富集分析,我们发现这些差异代谢物主要参与谷氨酰胺代谢,牛磺酸代谢,丙氨酸、天冬氨酸和谷氨酸代谢以及甘油磷脂代谢等途径。谷氨酰胺、丙氨酸、天冬氨酸和谷氨酸是机体重要的氨基酸,在应激状态下可作为免疫细胞和肠道细胞的主要燃料,并参与维持氧化还原平衡[15]。高原失血性休克后,这些代谢途径的改变可能反映了机体在高能量需求下的代偿反应以及氧化应激状态的加剧,提示在缺氧与休克的双重打击下,机体线粒体功能受损、三羧酸循环受

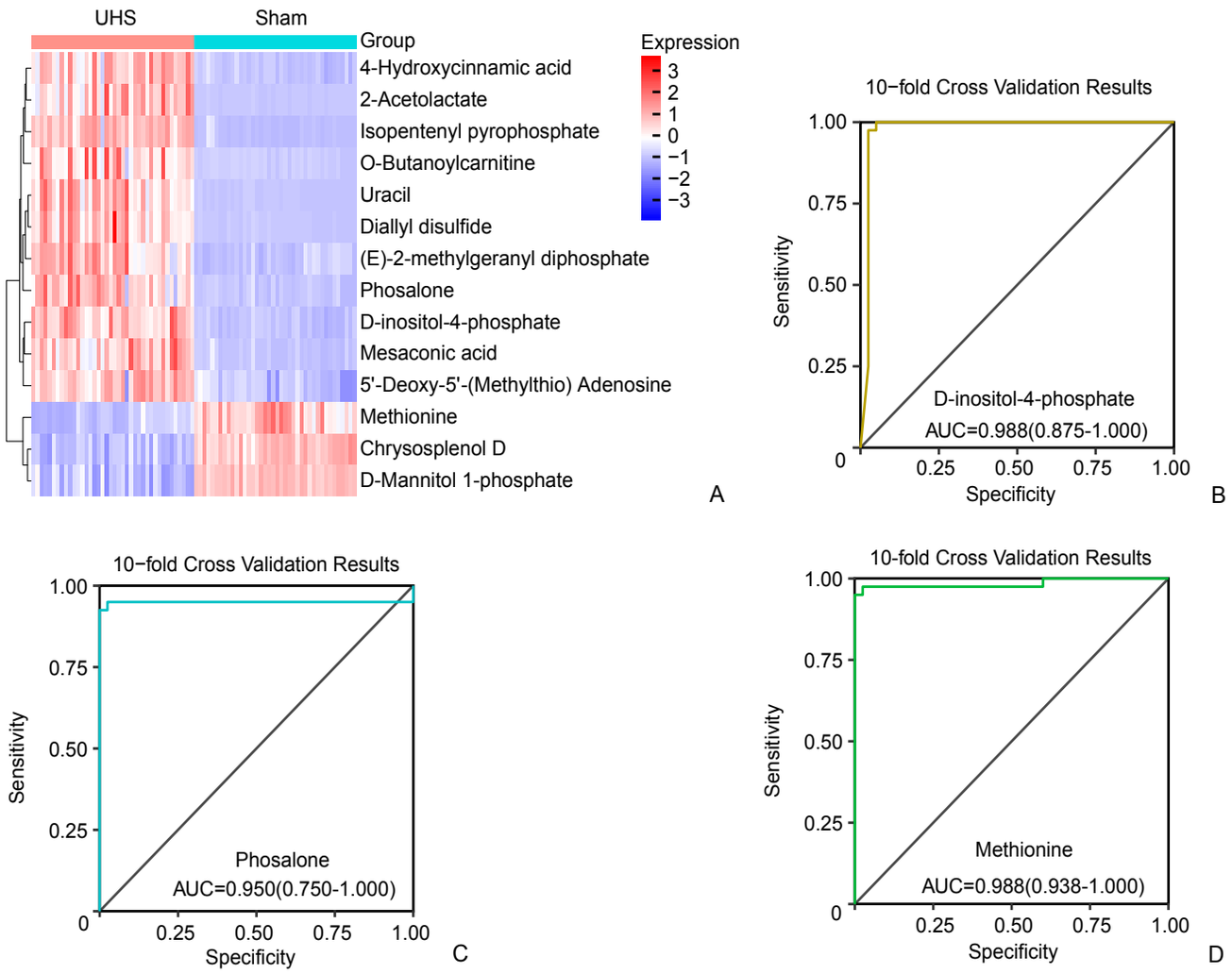


A: Venn diagram showing the intersection of differential metabolites and characteristic metabolites; B, C: LASSO regression algorithm identifies 10 candidate biomarkers; D, E: SVM-RFE algorithm identifies 13 candidate biomarkers; F, G: RF algorithm identifies 44 candidate biomarkers; H: Venn diagram showing the intersection of candidate biomarkers obtained by the 3 machine learning algorithms. LASSO: Least absolute shrinkage and selection operator; RF: Random forest; SVM-RFE: Support vector machine-recursive feature elimination

图4 3种机器学习算法筛选高原环境失血性休克生物标志物

Figure 4 Screening of biomarkers for hemorrhagic shock in high-altitude conditions using three machine learning algorithms

阻,迫使细胞转向以谷氨酰胺等氨基酸为主要碳源的"回补反应"以维持能量供应。同时,甘油磷脂代谢的紊乱直接反映了休克状态下细胞膜和线粒体膜的广泛损伤与重构,这与缺血缺氧导致的磷脂酶激活及脂质过氧化密切相关[16]。此外,本研究还发现牛磺酸代谢途径也发生了显著改变。牛磺酸作为重要的半必需氨基酸,具有抗氧化、调节渗透压、稳定细胞膜和参与神经调节等多重生理功能[17],其代谢改变可能与高原缺氧合并失血所引起的氧化应激增强及细胞损伤有关。总之,这些代谢通路的改变共同揭示了高原环境下失血性休克时机体所面临的能量危机与氧化损伤相互交织的复杂代谢失衡局面。



A: Heatmap displaying the expression of biomarkers identified by 2 or any 2 machine learning algorithms; B~D: ROC curves of potential biomarkers D-inositol-4-phosphate (B), phosalone (C) and methionine (D), with corresponding mean AUC and 95%*CI* values

图5 潜在生物标志物的表达及诊断性能评估

Figure 5 Expression and diagnostic performance evaluation of potential biomarkers

3.2 代谢物D-inositol-4-phosphate、Phosalone和Methionine可能成为高原环境下失血性休克的生物标志物

基于代谢组学分析与多种机器学习方法(WGCNA、LASSO回归、随机森林和支持向量机)的整合,我们发现代谢产物D-inositol-4-phosphate、Phosalone和Methionine可能作为高原环境下失血性休克的潜在生物标志物。通过基于十折交叉验证的ROC曲线分析,我们发现D-inositol-4-phosphate、Phosalone和Methionine的AUC均大于0.95,进一步支持这些代谢产物作为高原失血性休克早期诊断或严重程度评估的潜在生物标志物。与传统生理指标(如血压、心率)相比,这些代谢物分子能够更早、更直接地反映机体内部的代谢状态变化,有望为战场或高原地区的即时检验(POCT)技术开发提供新的靶点。

3.3 D-inositol-4-phosphate、Phosalone和Methionine在高原环境失血性休克中的作用及潜在调控机制

本研究的结果发现,D-inositol-4-phosphate和Phosalone的含量在高原失血性休克后显著升高,而Methionine的含量在高原失血性休克后显著降低。肌醇及其磷酸衍生物是重要的细胞内信号分子,参与调控细胞应激、凋亡、钙信号传导等多种生命活动[18]。在肾癌的生物标志物分析中,肌醇也被认为是区分癌症与正常样本的有效指标[19]。D-inositol-4-phosphate作为一种特定的肌醇磷酸异构体,其水平显著升高可能暗示着在休克状态下,磷脂酰肌醇信号通路被异常

激活。这种激活可能与缺氧、炎症因子风暴等应激信号有关,进而影响血管通透性、心肌收缩力及细胞存活,其在高原失血性休克中的具体作用及机制仍需进一步探究。甲硫氨酸是必需氨基酸,参与蛋白质合成、甲基化反应以及S-腺苷甲硫氨酸(S-adenosyl-L-methionine, SAM)的生成,后者是细胞内主要的甲基供体,对基因表达、神经递质合成和解毒过程至关重要[20]。有研究表明,在心脏病和神经退行性疾病中,甲硫氨酸的代谢异常与疾病进展密切相关[21-22]。在血管老化的研究中,甲硫氨酸也被确定为血管衰老的生物标志物和治疗靶点[23]。高原失血性休克后,甲硫氨酸水平的降低可能暗示着蛋白质合成受损、甲基化循环紊乱或氧化应激增加,标志着机体抗氧化储备的枯竭和代谢失衡的加剧。Phosalone通常被认为是一种有机磷杀虫剂,其在生物体内的内源性来源和生理病理意义目前尚不明确。我们推测,它可能是休克状态下某种内源性代谢物在酶作用或非酶促反应下的结构类似物或衍生物,抑或是机体在极端应激下产生的异常代谢副产物,但其确切的生物学本质和功能仍需进一步阐明。

本研究仍存在一定的局限性:①本研究仅基于大鼠模型在模拟的高原环境下研究失血性休克导致的代谢改变,与人体存在一定的生理和代谢差异,其结论仍需进一步验证。②本研究系统绘制了模拟高原环境下失血性休克的代谢图谱,并筛选了3种潜在生物标志物,但这些代谢改变及相关代谢物在高原休克病理生理过程中的具体作用及机制,仍需进一步功能验证。

综上所述,本研究系统阐明了高原环境失血性休克的独特代谢特征,并整合机器学习算法,鉴定出Methionine、D-inositol-4-phosphate和Phosalone可以作为高原失血性休克早期诊断或严重程度评估的潜在生物标志物。这些发现不仅为高原战创伤失血休克的分子机制提供了新的见解,也为开发其早期诊断工具和治疗靶点奠定了理论基础。

利益冲突 本文所有作者声明不存在利益冲突

参考文献:

[1] CANNON J W. Hemorrhagic shock[J]. N Engl J Med, 2018, 378(4): 370-379. DOI:10.1056/nejmra1705649.

[2] PALLAVICINI P, CARENZO L, ADAMS R, *et al*. An observational study of pre-hospital central venous access for patients with haemorrhagic shock due to major trauma[J]. Anaesthesia, 2025. DOI:10.1111/anae.16778. DOI:10.1111/anae.16778.

[3] XU J, YU W T, LI N, *et al*. The impact of high-altitude and cold environment on brain and heart damage in rats with hemorrhagic shock[J]. Brain Circ, 2024, 10(2): 174-183. DOI:10.4103/bc.bc_24_24.

[4] ZHAO J X, YOU G X, YIN Y J, *et al*. Acute high-altitude exposure shortens survival after uncontrolled hemorrhagic shock in rats[J]. J Surg Res, 2018, 226: 150-156. DOI: 10.1016/j.jss.2018.01.028.

[5] GUPTA S, ALAM A. Shock index is better than conventional vital signs for assessing higher level of care and mortality in severe sepsis or shock[J]. Am J Emerg Med, 2021, 46: 545-549. DOI:10.1016/j.ajem.2020.11.014.

[6] SILWAL S, DEVKOTA P, SINHA P, *et al*. Assessment of shock in emergency: narrative review[J]. Jgpeman, 2021, 8(11): 45-50. DOI:10.59284/jgpeman59.

[7] JOHNSON C H, IVANISEVIC J, SIUZDAK G. Metabolomics: beyond biomarkers and towards mechanisms[J]. Nat Rev Mol Cell Biol, 2016, 17(7): 451-459. DOI: 10.1038/nrm.2016.25.

[8] DI MINNO A, GELZO M, CATERINO M, *et al*. Challenges in metabolomics-based tests, biomarkers revealed by metabolomic analysis, and the promise of the application of metabolomics in precision medicine[J]. Int J Mol Sci, 2022, 23(9): 5213. DOI:10.3390/ijms23095213.

[9] XU Y D, CAO L L, CHEN Y F, *et al*. Integrating machine learning in metabolomics: a path to enhanced diagnostics and data interpretation[J]. Small Methods, 2024, 8(12): e2400305. DOI:10.1002/smtd.202400305.

[10] ZHAI J X, JIANG C, YAN H, *et al*. Integrative metabolomics and machine learning reveal diagnostic biomarkers for gelsenicine intoxication[J]. J Ethnopharmacol, 2025, 358:121022. DOI:10.1016/j.jep.2025.121022.

[11] CHEN Y Z, WANG B H, ZHAO Y Z, *et al*. Metabolomic machine learning predictor for diagnosis and prognosis of gastric cancer[J]. Nat Commun, 2024, 15: 1657. DOI: 10.1038/s41467-024-46043-y.

[12] 王易,朱娱,汤春琼, 等. 右美托咪定对高原环境暴露后失血性休克大鼠肺水肿的保护作用[J]. 陆军军医大学学报, 2023, 45(24): 2570-2578. DOI: 10.16016/j. 2097-0927.202307057.

WANG Y, ZHU Y, TANG C Q, *et al*. Protective effect of dexmedetomidine on pulmonary edema with hemorrhagic shock in rats exposed to plateau environment[J]. J Army Med Univ, 2023, 45(24): 2570-2578. DOI: 10.16016/j. 2097-0927.202307057.

[13] CLAYTON R P, HERNDON D N, ABATE N, *et al*. The effect of burn trauma on lipid and glucose metabolism:

implications for insulin sensitivity[J]. J Burn Care Res, 2018, 39(5): 713-723. DOI:10.1093/jbcr/irx047.

[14] TANG H R, KAN C X, ZHANG K X, *et al*. Glycerophospholipid and sphingosine- 1-phosphate metabolism in cardiovascular disease: mechanisms and therapeutic potential[J]. J Cardiovasc Transl Res, 2025, 18(4): 749-761. DOI:10.1007/s12265-025-10620-3.

[15] HEATH D F, GEORGE D R, ROSE J G. The effects of the stress caused by experimental procedures on alanine, aspartate, glutamate and glutamine in rat liver[J]. Biochem J, 1971, 125(3): 765-771. DOI:10.1042/bj1250765.

[16] SARKAR C, LIPINSKI M M. Glycerophospholipid dysregulation after traumatic brain injury[J]. Neurochem Int, 2024, 175: 105701. DOI:10.1016/j.neuint.2024.105701.

[17] SANTULLI G, KANSAKAR U, VARZIDEH F, *et al*. Functional role of taurine in aging and cardiovascular health: an updated overview[J]. Nutrients, 2023, 15(19): 4236. DOI:10.3390/nu15194236.

[18] CHENG Z L, MONTGOMERY M K. Physiological roles of phosphoinositides and inositol phosphates: Implications for metabolic dysfunction-associated steatotic liver disease[J]. Clin Sci, 2025, 139(19): 1095-1144. DOI: 10.1042/CS20257631.

[19] NIZIOŁ J, OSSOLIŃSKI K, TRIPET B P, *et al*. Nuclear magnetic resonance and surface-assisted laser desorption/ionization mass spectrometry-based serum metabolomics of kidney cancer[J]. Anal Bioanal Chem, 2020, 412(23): 5827-5841. DOI:10.1007/s00216-020-02807-1.

[20] LI T, TAN Y T, CHEN Y X, *et al*. Methionine deficiency facilitates antitumour immunity by altering m(6)a methylation of immune checkpoint transcripts[J]. Gut, 2023, 72(3): 501-511. DOI:10.1136/gutjnl-2022-326928.

[21] QIAN S X, BAO Y F, LI X Y, *et al*. Multi-omics analysis reveals key gut microbiota and metabolites closely associated with Huntington's disease[J]. Mol Neurobiol, 2025, 62(1): 351-365. DOI:10.1007/s12035-024-04271-9.

[22] HOFFMAN R M. Methioninase: a therapeutic for diseases related to altered methionine metabolism and transmethylation: cancer, heart disease, obesity, aging, and Parkinson's disease[J]. Hum Cell, 1997, 10(1): 69-80.

[23] GU Z H, WANG Y J, FANG Z Y, *et al*. Plasma metabolomics identifies S-adenosylmethionine as a biomarker and potential therapeutic target for vascular aging in older adult males[J]. J Pharm Biomed Anal, 2024, 243: 116097. DOI:10.1016/j.jpba.2024.116097.

(收稿:2025-11-05;修回:2025-12-01)

(编辑 栾 嘉)