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评 述

阿尔茨海默病患者尿液标志物的多肽组学研究

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深港脑科学创新研究院(批准号: 2023SHIBS0003)和深圳大学分析测试中心资助

摘要 采用尿液多肽组学技术, 系统分析了阿尔茨海默病(Alzheimer's disease, AD)和轻度认知障碍(mild cognitive impairment, MCI)患者的尿液样本, 揭示了尿液多肽组在AD筛查和诊断中具有潜在应用价值. 本研究纳入32例AD患者、34例MCI患者及20例健康对照者, 采用高效液相色谱-串联质谱(high-performance liquid chromatography-tandem mass spectrometry, HPLC-MS/MS)技术进行多肽组分析. 发现尿液多肽组呈现"20%高丰度降解蛋白主导"的分布规律, 即前20%高丰度蛋白贡献了约80%的肽段数目和90%的信号强度. 进一步筛选出基于磷酸组氨酸磷酸酶1(phosphohistidine phosphatase 1, PHPT1)、神经氨酸酶1(sialidase-1, NEU1)和纤维蛋白原β链(fibrinogen beta chain, FGB)的三种降解蛋白质的AD特异性标志物组合, 它们与调控线粒体功能、抑制神经炎症和阻断淀粉样纤维诱导的补体激活相关; 而表皮生长因子(epidermal growth factor, EGF)和Ⅲ型胶原蛋白α1链(collagen alpha-1(III) chain, COL3A1)的组合被筛选为MCI的多肽组标志物, 可通过激活EGFR-MAPK通路促神经元存活、介导基底膜重塑抑制整合素信号异常渗入反映早期代偿. 此外, 在AD/MCI患者尿液中肽段的甲硫氨酸和脯氨酸残基氧化修饰水平显著升高, α1-抗胰蛋白酶(alpha-1-antitrypsin, SERPINA1)降解多肽的甲硫氨酸氧化和胶原蛋白α-1(I)链(collagen alpha-1(I) chain, COL1A1)/胶原蛋白α-2(I)链(collagen alpha-2(I) chain, COL1A2)降解多肽的脯氨酸氧化可区分疾病组与对照组, 机制涉及氧化应激和破坏胶原-细胞骨架交互等. 以上结果表明, 尿液多肽组从降解蛋白质和多肽翻译后修饰两方面反映AD的病理机制.

关键词 阿尔茨海默病, 轻度认知障碍, 尿液多肽组学, 氧化修饰, 生物标志物

阿尔茨海默病(Alzheimer's disease, AD)是神经退行性疾病的典型代表, 发病机制复杂, 目前尚不能治愈, 早期发现和干预是延缓AD的重要手段[1~5]. 脑脊液(cerebrospinal fluid, CSF)和血液是AD生物标志物的主要来源, 但CSF侵入性强, 不易获取[1~3]. Grande等人[6]研究显示, p-tau181(磷酸化tau蛋白181)、p-tau217(磷酸化tau蛋白217)、NfL(神经丝轻链)和GFAP(胶质纤维酸性蛋白)等血液生物标志物能预测全因痴呆和AD 痴呆, 但单独使用时阳性预测值偏低, 寻找其他生物标志物仍有迫切的临床需求. 尿液由于无需承担生

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理功能和维持稳态, 是一种理想的替代生物样本[7].

多肽组学是蛋白质组学方法[8]的拓展和延伸, 体液中的多肽反映了蛋白质的降解情况, 是生物标志物的新来源. 早期的多肽组生物标志物研究通常采用基质辅助激光解吸电离飞行时间质谱(matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, MALDI-TOF)技术, 例如, M'Koma等人[9]通过MALDI-TOF技术在前列腺癌中筛选出4种特异性标志物(*m/z* 1373.1~2484.6), Wang等人[10]利用MALDI-TOF/TOF MS检测出4种肽段(*m/z* 1196.47, 3222.17, 4640.35, 5072.14)作为重度抑郁症的标志物, 分别归属于为血清白蛋白、α-1-微球蛋白(α-1-microglobulin/bikunin precursor, AMBP)、基底膜特异性硫酸乙酰肝素蛋白聚糖核心蛋白(basement membrane-specific heparan sulfate proteoglycan core protein, HSPG)和载脂蛋白A-I(apolipoprotein a-I, APOA1). Abe等人[11]开发了一种基于BLOTCHIP®的血清多肽组分析技术, 通过直接转移电泳结合MALDI-TOF/MS技术, 在AD患者中鉴定出归属于纤维蛋白原β链(fibrinogen beta chain, FGB)和α2-HS糖蛋白(alpha-2-heremans-schmid glycoprotein, AHSG)的多肽组合标志物, 对AD的诊断敏感性达87%、特异性达65%. 高分辨串联质谱技术提供了更高的灵敏度和动态范围[12], Kononikhin等人[13]采用高效液相色谱-串联质谱(high-performance liquid chromatography-tandem mass spectrometry, HPLC-MS/MS)在先兆子痫患者尿液中鉴定到35种差异肽段, Good等人[14]利用毛细管电泳质谱(capillary electrophoresis-mass spectrometry, CE-MS)筛选出273种慢性肾病标志物. 本课题组[15]曾经用定量蛋白质组学技术发现在AD患者尿液中凝溶胶蛋白和胰岛素样生长因子结合蛋白7的表达上调. 但是, 目前尚未见有关AD尿液多肽组生物标志物的报道. 本研究利用基于高分辨串联质谱的多肽组学方法寻找在降解蛋白质水平以及甲硫氨酸和脯氨酸的氧化修饰水平变化的AD和MCI的潜在生物标志物.

1 实验部分

1.1 仪器与试剂

EksigentnanoLC-Ultra™ 2D系统、TripleTOF 5600质谱仪(AB SCIEX, 美国)、ProteinPilot4.5软件(AB SCIEX)、真空冷冻干燥机(Thermo Savant)、低温离心机(Eppendorf)、乙腈(质谱纯, Sigma-Aldrich, 美国)、三氟乙酸(质谱纯, Sigma-Aldrich)、氧化石墨烯-磷酸镧纳米磁性复合材料(graphene oxide-lanthanum phosphate magnetic nanocomposite, LaGM, 本实验室合成), 多肽分离技术采用文献[10]方法.

1.2 尿液多肽的分离和富集

纳入深圳大学总医院32例AD、34例轻度认知障碍(mild cognitive impairment, MCI)及20例年龄相近的对照者尿液样本, 实验所有样本来自深圳大学总医院志愿者, 均知情同意, 并经深圳大学医学部伦理委员会批准. 表1汇总了本研究受试者基本信息, 数据以均值±标准差表示. 尿液样本经0.22 μm滤膜过滤后−80℃保存. 取1.5 mL尿液, 4℃, 13475×*g*离心10 min, 上清加入30 μL氧化石墨烯-磷酸镧纳米磁性复合材料(LaGM)(30 mg/mL), 涡旋10 min后离心去上清, 依次用500 μL去离子水洗涤两次、80%乙腈-0.1%三氟乙酸洗脱, 冻干后备用.

1.3 液相色谱-质谱联用分析

纳升液相色谱流动相A(nano-reverse phase liquid chromatography buffer A, Nano-RPLC Buffer A)为含0.1%甲酸的水-乙腈溶液(98:2, V/V), 纳升液相色谱流动相B(nano-reverse phase liquid chromatography buffer B, Nano-RPLC Buffer B)为含0.1%甲酸的乙腈-水溶液(98:2, V/V). 色谱条件: AcclaimPepMapC18预柱(75 μm×2 cm), 分析柱为ChromXPC18(75 μm×15 cm); 梯度洗脱(0~42 min, 5%~25% B; 42~56 min, 25%~40% B; 56~64 min, 80% B; 64~70 min, 5% B). 质谱参数: 喷雾电压2.4 kV, TOF-MS扫描时间250 ms, 二级图谱累积时间80 ms, 动态排除11 s.

1.4 数据分析

将质谱数据保存为wiff图谱文件, 传送到Protein Pilot Software 4.5软件的analysis文件夹中, 打开ProteinPilot Software 4.5, 选择新建项目, 然后选择离子化方式, 选择需要搜库的wiff文件, 选择人蛋白库, 搜库条件为非酶切、可变修饰和氨基酸取代. 搜库完成后, 将假阳性率控制为1%, 输出Excel数据文件.

对Protein Pilot Software 4.5软件输出的Excel数据

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表 1 受试者基本信息与认知评分[a)]

Table 1 Demographic characteristics and cognitive scores of the enrolled subjects[a)]

指标	AD	MCI	Control
样本数(n)	32	34	20
女性比例(%)	40.6	44.1	45.0
MMSE	16.3±3.3$^{**,\#\#}$	24.2±3.7**	28.05±1.3
年龄	73.6±8.9	70.1±7.4	70.3±7.1

a) 数据以mean±SD表示, 进行t检验, 与对照组相比, $^{**}P<0.01$, 与MCI相比, $\#\#P<0.01$

文件进行数据处理. 以置信度(conference)为筛选条件, 去除分数低于95以下的肽段, 随后以肽段序列为筛选条件去除重复肽段, 统计所有数据中肽段和归属蛋白的数目和种类, 采用COUNTIF函数进行归类分析, 得到每个蛋白质和肽段在各个样品中的频率.

通过基于肽段数目的筛选策略降低个体差异影响, 以疾病状态为分层变量从而进行随机分层抽样, 按7:3比例构建训练集(AD 23例、MCI 24例、对照14例)与验证集(AD 9例、MCI 10例、对照6例). 运用t检验分析差异, 标志物筛选实施多算法特征选择: (ⅰ) LASSO回归(glmnet包)通过10折交叉验证优化正则化参数入, 压缩无关特征; (ⅱ) 随机森林(random forest包)基于基尼不纯度评估特征重要性; (ⅲ) Boruta算法通过“阴影特征”比较识别真实相关变量; (ⅳ) XG Boost(xgboost包)采用早停法防止过拟合. 最终在独立测试集评估性能. 多肽-蛋白对应关系基于ABSCIEX公司的Protein Pilot软件(置信度≥95%), 只选择目标蛋白质的特异性肽段.

为提升AD与MCI诊断模型的区分效能, 本研究对磷酸组氨酸磷酸酶1(phosphohistidine phosphatase 1, PHPT1), 神经氨酸酶1(sialidase-1, NEU1)与纤维蛋白原β链(fibrinogen beta chain, FGB)的多种权重组合, 以及表皮生长因子(epidermal growth factor, EGF)与Ⅲ型胶原蛋白α1链(collagen alpha-1(III) chain, COL3A1)的权重组合进行了系统评估. 具体而言, 先基于各单标志物的AUC值分配初始权重, 再通过网格搜索法(grid search)在步长为0.1的权重空间中进行组合筛选(网络版附表1), 计算各组合的AUC值及其95%置信区间(confidence interval, CI).

验证集评估采用四象限分类法统计真阳性(true positive, TP)、真阴性(true negative, TN)、假阳性(false positive, FP)和假阴性(false negative, FN), 准确率计算公式为:

$$\text{准确率} = \frac{TP+TN}{TP+TN+FP+FN}.$$

在搜库条件中, 设置筛选发生氧化修饰的肽段, 通过一级质谱的精确分子量、串联质谱的打分和手工比对确定. 我们将发生氧化修饰肽段的强度进行标准化平均, 将降解蛋白中的所有氧化修饰肽段的强度除以氧化修饰肽段的数目的商作为衡量氧化程度的标准. 氧化修饰分析聚焦甲硫氨酸(oxidation(M))和脯氨酸(oxidation(P))修饰, 筛选频数均值前10的候选降解蛋白(α1-抗胰蛋白酶(methionine in alpha-1 antitrypsin, SERPINA1)、胶原蛋白α-1(I)链(collagen alpha-1(I) chain, COL1A1)和胶原蛋白α-2(I)链(collagen alpha-2 (I) chain, COL1A2), 显著性差异通过正态性检验确认数据呈非正态分布, 继而采用适用于非参数数据的Kruskal-Wallis检验进行组间差异分析($^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$), 误差棒表示均数标准误(standard error of mean, SEM).

2 结果与讨论

2.1 尿液多肽组的总体特征和个体差异

如表2所示, 在AD, MCI和对照组尿液中, 均为前20%的高丰度降解蛋白贡献了73%~86%的肽段数目和89%~95%的肽段强度, 与血液多肽组的分布相似[16,17]. 此外, 从整体来看, 肽段数目与归属蛋白数目呈正相关趋势(图1), 但在个体水平上肽段数目与其归属的降解蛋白质数目并非严格正相关(如AD训练集中样本12鉴定到86条肽段对应35种蛋白质, 样本14鉴定到137条肽段仅对应29种蛋白质). 如网络版附表2所示, 本研究检测的尿液多肽组荷质比(m/z)范围为400~1247, 分子量范围为535~7454 D, 其中分子量在2000~3000 D的肽段数目最多. 尿液中降解蛋白数目呈AD>MCI>Control的趋势(表2), 这可能是由于AD病理中神经元凋亡[18]、神经炎症[19]和细胞骨架破坏[20]共同驱动的广泛蛋白水解, 从而使它们更多地进入尿液.

2.2 基于尿液多肽组的生物标志物筛选与验证

2.2.1 差异降解蛋白的筛选与初步分析

运用R语言(网络版附录1)对三组样本进行两两比

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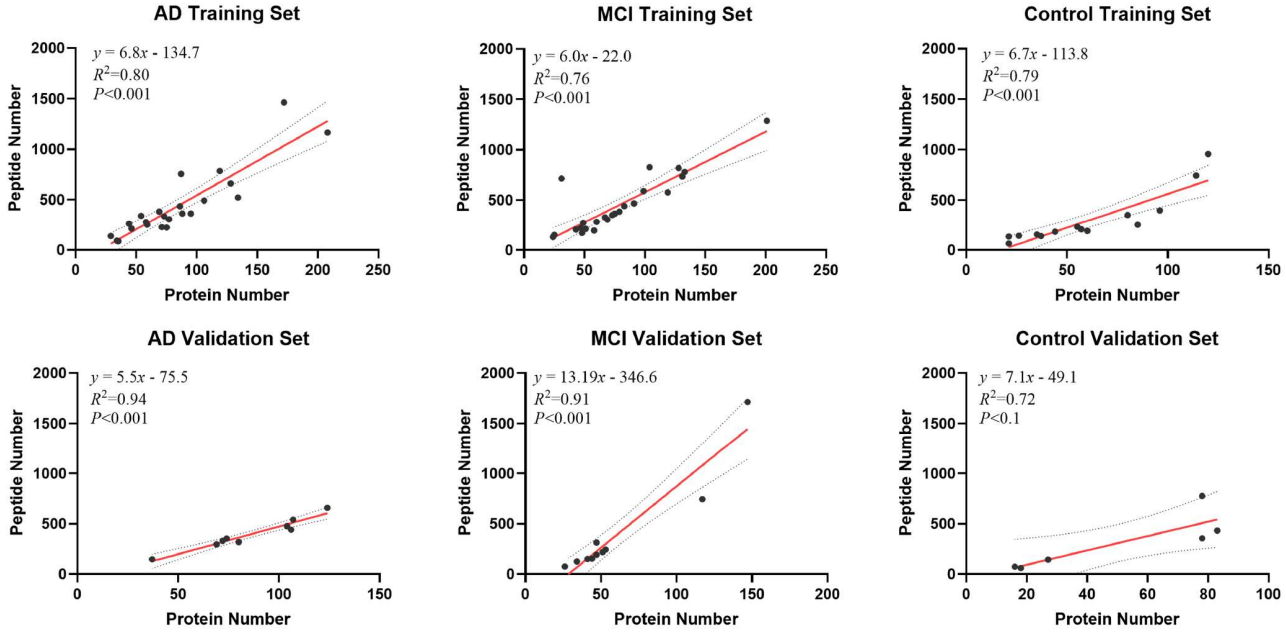

图 1 尿液多肽组中肽段数目与其归属的降解蛋白数目的相关性分析
Figure 1 Correlation between peptide counts and the number of source-degraded proteins in the urinary peptidome

表 2 不同组别尿液多肽组降解蛋白及肽段总体特征
Table 2 Global characteristics of urinary peptidome-derived degraded proteins and peptides across groups

组别	数据集	样本量	降解蛋白数目均值(范围)	肽段数目均值(范围)	前20%蛋白贡献肽段数目占比(%)	前20%蛋白贡献肽段强度占比(%)
AD	Training Set	23	85(34~208)	441(86~1463)	82	93
	Validation set	9	86(37~124)	396(147~659)	76	95
MCI	Training Set	24	79(24~201)	451(131~1287)	86	89
	Validation set	10	61(26~147)	393(75~1714)	81	89
Control	Training Set	14	61(21~114)	296(65~744)	73	93
	Validation set	6	50(16~83)	307(60~776)	79	91

较, 采用双尾*t*检验, 筛选出每组间差异降解蛋白(网络版附表3). 通过综合评估灵敏度、特异度、AUC和$\log_2$FC等指标, 最终确定了具有显著区分能力的降解蛋白标志物(表3). 在AD与对照组的比较中, 确定PHPT1, NEU1和FGB三种降解蛋白质为AD的筛查标志物; 在MCI与对照组的比较中, EGF和COL3A1为标志物.

2.2.2 组合模型设计

(1) AD *vs*. Control诊断模型. 将磷酸组氨酸磷酸酶1、神经氨酸酶1和纤维蛋白原β链组合后, 诊断模型的区分能力(AUC=0.7981, *P*=0.0026)较PHPT1单独检测(AUC=0.7174, *P*=0.0284)显著增加(图2), 验证组的准确率由53.3%提高至66.7%.

PHPT1是一种磷酸组氨酸磷酸酶, 通过调控线粒体代谢关键酶(如ATP-柠檬酸裂解酶的去磷酸化[21])影响能量稳态, 其过表达可能激活凋亡通路, 并间接促进tau蛋白异常磷酸化[22~25], 可能与AD神经元丢失及线粒体功能障碍关联[1,2]. NEU1通过调控Trem2受体的唾液酸化状态[26], 影响小胶质细胞转化[27], 促进促炎因子(TNF-α, IL-1β)释放[26,28], 进而间接加β-淀粉样蛋白(amyloid β, Aβ)沉积及tau病理进程[25,29]. FGB可与Aβ相互作用形成异常凝块[30,31], 并通过破坏血脑屏障(blood-brain barrier, BBB)的完整性, 加剧神经炎症及

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表 3　核心生物标志物统计指标[a)]

Table 3　Summary statistics of the core biomarkers[a)]

	标志物	灵敏度	特异度	log_2FC	AUC	*P*值
AD *vs*. Control	PHPT1	0.48	0.93	3.69↑	0.72	0.008
	NEU1	0.39	1.00	6.23↑	0.70	0.035
	FGB	0.91	0.14	0.81↑	0.71	0.039
MCI *vs*. Control	EGF	0.67	0.71	1.72↑	0.70	0.052
	COL3A1	0.63	0.50	1.76↑	0.66	0.030

a) 灵敏度为正确识别患者的概率; 特异度为正确排除健康人的概率; AUC为综合诊断能力指标; log_2FC表示患者与健康人相比降解蛋白质的肽段数目的变化倍数, 如果降解蛋白的肽段数目为0, 给数据加上极小常数(0.01)避免对数运算无意义; *P*值为*t*检验的值

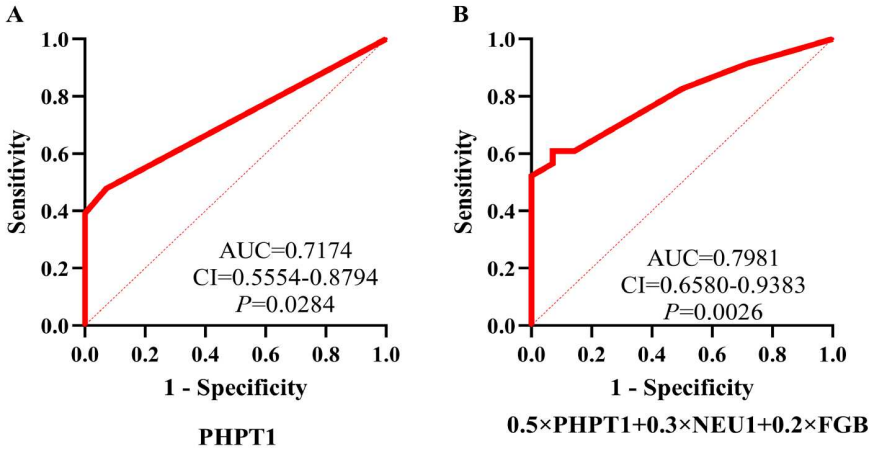

图 2　PHPT1单指标(A)和AD-对照标志物组合(B)的ROC曲线诊断效能分析

Figure 2　ROC curve diagnostic efficacy analysis of PHPT1 single indicator (A) and AD-control marker combination (B)

血管病理[32,33]. PHPT1和NEU1的高特异性可弥补FGB的低特异度. 此外, 在肽段水平, Abe等人[11]提出血清中FGB的多肽GHRPLDKKREEAPSLRPAPPPISGGGY是AD标志物, 我们在各组尿液中也都检测到该序列及其相关序列肽段, 提示血清和尿液中肽段具有相关性.

(2) MCI *vs*. Control诊断模型. 将EGF和Ⅲ型胶原蛋白组合后, 诊断模型的区分能力(AUC=0.8115, *P*=0.0013)较EGF单独检测(AUC=0.7039, *P*=0.0382)显著增加(图3), 诊断模型的区分能力较表皮生长因子单独检测诊断效能更优, 验证组准确率从43.8%提升至50%, 更具优势.

EGF可通过激活EGFR-MAPK通路增强神经元抗凋亡能力[34,35], 并维持BBB稳态以延缓神经营养失衡[36,37], 其表达异常可能反映MCI早期神经营养代偿机制的失调[38]. COL3A1在血管基底膜异常累积, 可通过整合素信号介导BBB基底膜增厚[39,40], 促进炎症因子渗入及胶质细胞活化[32], 提示该标志物参与MCI脑血管微环境紊乱. EGF与COL3A1的组合分别从神经营养支持与血管基质重塑角度对应MCI多维度病理特征.

与以往基于完整蛋白质的尿液蛋白组学研究相比, 本研究在标志物类型与技术路径上具有一定的互补性. 例如, Wang等人[41]采用LASSO联合SVM构建的完整蛋白标志物组合(如DDC, PLD3), 在训练集中AUC达0.998; Hällqvist等人[42]则通过靶向蛋白组学筛选出MIEN1和ABCA7等标志物, 其研究更强调脂质代谢与炎症通路的关联. 本课题组前期工作表明, AD患者尿液蛋白质组中凝溶胶蛋白和胰岛素样生长因子结合蛋白7的表达高于正常对照组, 凝溶胶蛋白表达上调是为保护神经元突触而启动的一种内在防御机制, 而后者上调则促进神经炎症, 加重AD病理[15]. 因此, AD的尿液蛋白质组标志物与多肽组标志物可能从不同侧面反映AD的病理机制和进程.

机制上, 多肽组学研究的是蛋白质的降解后状态,

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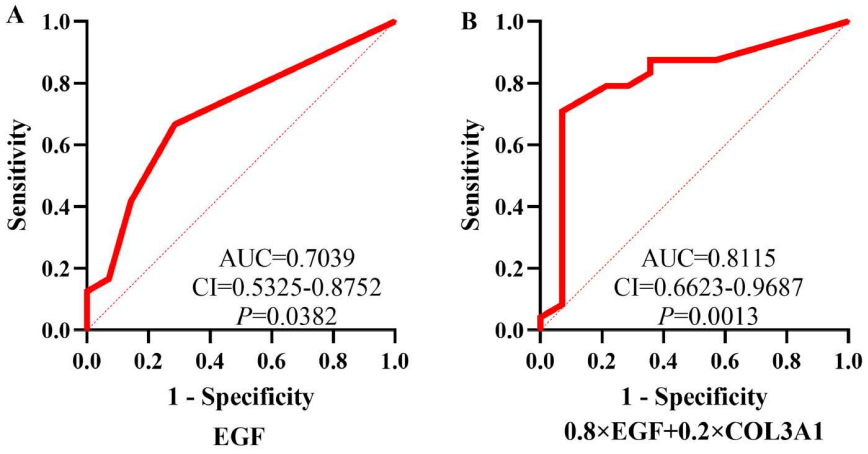

图 3 EGF单指标(A)与MCI-对照标志物组合(B)的ROC曲线诊断效能分析
Figure 3 ROC curve diagnostic efficacy analysis of EGF single indicator (A) and MCI-Control marker combination (B)

而蛋白质组学研究的是体液或组织中的完整蛋白质, 二者共同指向线粒体功能障碍、神经炎症及血管病理等核心病理机制. 本课题组[43,44]前期研究也表明, 唾液的蛋白质组和多肽组成分差别很大, 因此, 针对同一样本, 这两种技术路线具有互补性. 而多肽组学在方法学上, 如翻译后修饰的富集、稳定同位素标记定量和DIA采集分析等还需进一步探索. 未来可通过多组学策略, 进一步提升标志物的诊断特异性.

2.3 氧化修饰肽段oxidation(M)和oxidation(P)作为AD尿液诊断标志物

2.3.1 选择氧化修饰靶标的理论依据

氧化应激是AD的早期病理事件, 早于Aβ沉积及tau蛋白磷酸化[45,46]. 它会引发蛋白质、脂质和DNA的损伤, 进一步加剧神经退行性病变[47]. 此外, 在AD患者的脑脊液和血液中, 氧化应激标志物的水平显著升高, 如蛋白质羰基含量在AD患者大脑皮质和血浆中显著增加[46]. 然而, 这些氧化应激标志物的动态还原性(如受MSR系统调控[48])以及传统检测方法的局限性, 阻碍了其在临床诊断中的应用. 本研究表明, 尿液不仅具有无创性和临床可操作性优势[49], 而且尿液多肽的氧化修饰也可能反映AD全身性氧化应激状态.

2.3.2 选择氧化修饰靶标的实验依据

如图4所示, 训练集与验证集分析表明, AD组和MCI组的甲硫氨酸氧化修饰总体水平显著高于对照组(***P<0.001), 但AD与MCI组间无显著差异. 进一步针对SERPINA1的甲硫氨酸氧化修饰研究发现, 训练集中AD组水平显著高于MCI及对照组(***P<0.001), 而验证集中MCI组水平反超AD组(****P<0.0001), 提示该修饰的跨队列稳定性较弱. 因此, SERPINA1氧化修饰仅适用于疾病组(AD+MCI)与对照组的区分. 病理机制上, 甲硫氨酸代谢途径的异常与谷胱甘肽耗竭密切相关[50], 后者是中枢神经系统抗氧化防御的关键分子, 其功能受损可能加速神经退行性病变[51]. 甲硫氨酸氧化与甲硫氨酸亚砜还原酶(methionine sulfoxide reductases, MSR)系统的功能缺陷密切相关. MSR活性随年龄增长下降, 导致氧化修饰产物累积, 突破细胞稳态阈值[52], 靶向MSR被认为是治疗AD的新靶点[53].

如图5所示, 在训练集中, AD组与MCI组的脯氨酸氧化修饰水平均显著高于对照组(****P<0.0001); 验证集中, AD组修饰水平显著高于对照组(**P<0.01)及MCI组(*P<0.05), 其中COL1A1与COL1A2与总体趋势一致, 跨数据集一致性表明, 该特征对疾病组与对照组的区分效能稳定, 而AD与MCI组间差异未呈现跨队列重现性(或受样本量影响). 脯氨酸氧化与蛋白质羰基化相关[54], 后者是氧化损伤的标志, 可导致蛋白质交联、聚集及功能丧失[55,56]. 研究显示, 脯氨酸氧化可能更直接反映晚期结构性损伤(如神经原纤维缠结的形成)[57~59], 与MSR系统修复能力衰退导致的不可逆损伤相关[60].

值得注意的是, AD组与MCI组间修饰差异的跨队列一致性较弱, 可能是样本量较小, 也可能是由于病理

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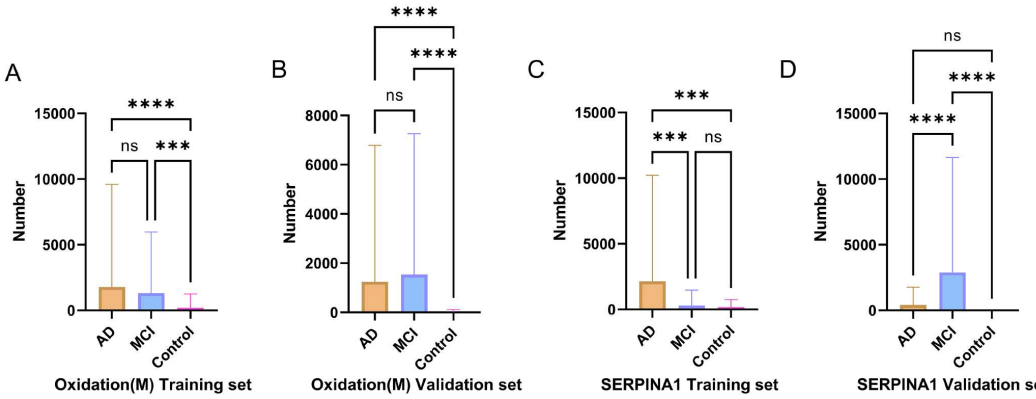

图 4　AD, MCI和对照中甲硫氨酸氧化修饰水平的训练集与验证集分析. A: 总降解蛋白肽段的甲硫氨酸氧化修饰水平训练集分析; B: 总降解蛋白肽段的甲硫氨酸氧化修饰水平验证集分析; C: SERPINA1肽段的甲硫氨酸氧化修饰水平训练集分析; D: SERPINA1肽段的甲硫氨酸氧化修饰水平验证集分析. ***, P<0.001; ****, P<0.0001; ns, 无显著性

Figure 4　Analysis of methionine oxidation modification levels in AD, MCI and Control. A: Training set analysis of methionine oxidation modification levels on peptides of total degraded proteins; B: validation set analysis of methionine oxidation modification levels on peptides of total degraded proteins; C: training set analysis of methionine oxidation modification levels on peptides of SERPINA1; D: validation set analysis of methionine oxidation modification levels on peptides of SERPINA1. ***, P<0.001; ****, P<0.0001; ns, not significant

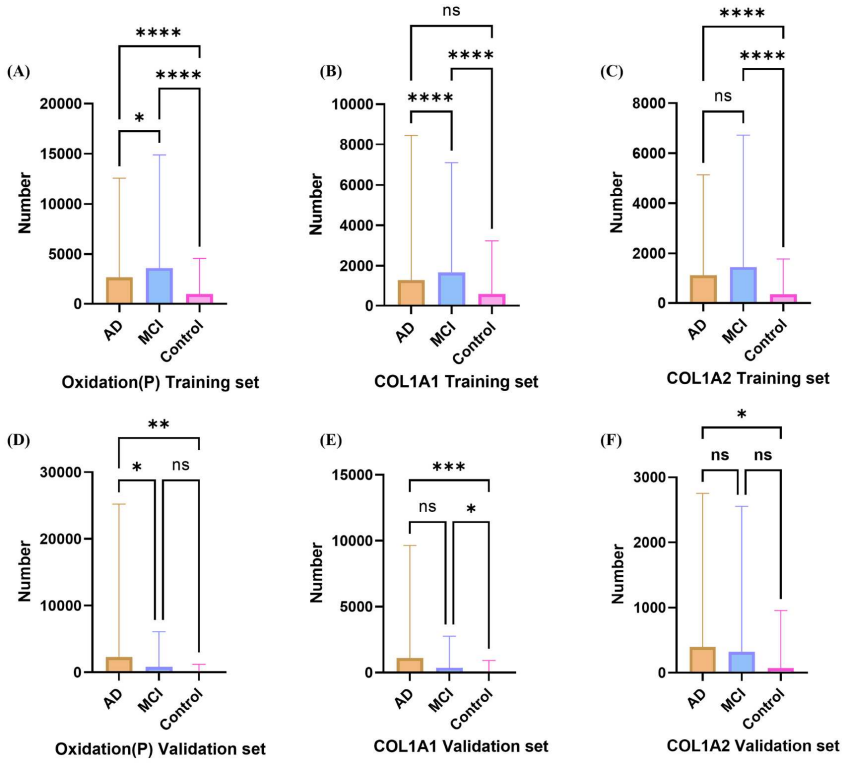

图 5　AD, MCI和对照中脯氨酸氧化修饰水平的训练集与验证集分析. A: 总降解蛋白肽段的脯氨酸氧化修饰水平训练集分析; B: COL1A1肽段的脯氨酸氧化修饰水平训练集分析; C: COL1A2肽段的脯氨酸氧化修饰水平训练集分析; D: 总降解蛋白肽段的脯氨酸氧化修饰水平训练集分析; E: COL1A1肽段的脯氨酸氧化修饰水平训练集分析; F: COL1A2肽段的脯氨酸氧化修饰水平验证集分析. *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001; ns, 无显著性

Figure 5　Analysis of proline oxidation modification levels in AD, MCI and Controls. A: Training set analysis of proline oxidation modification levels on peptides of total degraded proteins; B: training set analysis of proline oxidation modification levels on peptides of COL1A1; C: training set analysis of proline oxidation modification levels on peptides of COL1A2; D: training set analysis of proline oxidation modification levels on peptides of total degraded proteins; E: training set analysis of proline oxidation modification levels on peptides of COL1A1; F: validation set analysis of proline oxidation modification levels on peptides of COL1A2. *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001; ns, not significant

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特征与生化指标并非完全同步. 但疾病组与对照组的区分效能在跨数据集中保持稳定. 此外, 尿液多肽组中其他翻译后修饰也值得注意, 例如, 在MCI组泛素化修饰(GlyGly)水平显著高于对照组(网络版附图1), 提示泛素化可能通过调控蛋白质降解途径参与AD早期病理. 蛋白质的泛素化修饰与氧化修饰具有正相关性, 在氧化应激条件下, 泛素化修饰的水平会发生显著变化, 发生半胱氨酸和甲硫氨酸氧化的蛋白是蛋白酶降解的良好底物, 氧化应激调节蛋白质的泛素化修饰[15]. 本研究检测到α1-抗胰蛋白酶同时发生了氧化修饰和泛素化修饰. α1-抗胰蛋白酶能够减少由Aβ诱导的小胶质细胞中的促炎介质[61], 能够时间依赖性地减轻Aβ1~42诱导的NLRP3炎症体的上调, 减少caspase 1及其裂解产物IL-1β的表达[62]. 但是, 在α1-抗胰蛋白酶发挥抗炎作用的过程中, 本身会被氧化, 氧化后的AAT会形成甲硫氨酸亚砜, 导致其构象变化和功能丧失, 并导致神经炎症加剧.

这些发现强调了多肽组翻译后修饰网络的复杂性及其在AD生物标志物研究中的重要性. 尽管本研究筛选出具有潜力的尿液多肽标志物, 但需指出以下局限, 训练集(AD 23例、MCI 24例、对照14例)与验证集(AD 9例、MCI 10例、对照6例)样本量较小, 尤其验证集样本量有限可能导致模型性能评估(如AD准确率66.7%, MCI准确率50%)存在波动风险. 未来需通过多中心、大样本队列进一步验证标志物的稳健性.

3 结论

本研究提出了可用于与脑脊液和血液标志物相互验证的尿液标志物, 确定了可用于区分AD与健康对照的降解蛋白质组合PHPT1, NEU1和FGB, 以及能够区分MCI与健康对照的EGF和COL3A1组合. 在AD和MCI组中, 上述蛋白的降解多肽在尿液中均显著增加. 此外, 在尿液多肽组中甲硫氨酸和脯氨酸的氧化修饰水平呈现上升趋势, 特别是SERPINA1的甲硫氨酸氧化修饰及COL1A1/COL1A2的脯氨酸氧化修饰可稳定区分AD/MCI患者与健康对照, 但尚不能可靠区分AD与MCI. 以上尿液多肽组生物标志物涉及线粒体功能障碍、神经炎症、氧化应激和血脑屏障受损等AD病理特征, 提示通过尿液多肽组可以反映AD和MCI的病理特征.

参考文献

1 Zetterberg H, Bendlin B B. Biomarkers for Alzheimer's disease—preparing for a new era of disease-modifying therapies. Mol Psychiatry, 2021, 26: 296–308

2 García-Morales V, González-Acedo A, Melguizo-Rodríguez L, et al. Current understanding of the physiopathology, diagnosis and therapeutic approach to Alzheimer's disease. Biomedicines, 2021, 9: 1910

3 Kukull W A, Bowen J D. Dementia epidemiology. Med Clin N Am, 2002, 86: 573–590

4 Zheng Y P, He J S, Hong T. Research progress on biological markers in body fluids of Alzheimer's disease (in Chinese). Sci China C Life Sci, 2009, 39: 821–829 [郑妍鹏, 何金生, 洪涛. 阿尔茨海默病体液生物学标记物研究进展. 中国科学C辑: 生命科学, 2009, 39: 821–829]

5 Chen F, Wang N, Tian X, et al. The potential diagnostic accuracy of urine formaldehyde levels in Alzheimer's disease: a systematic review and meta-analysis. Front Aging Neurosci, 2022, 14: 1057059

6 Grande G, Valletta M, Rizzuto D, et al. Blood-based biomarkers of Alzheimer's disease and incident dementia in the community. Nat Med, 2025, 31: 2027–2035

7 Li M L, Zhao M D, Gao Y H. Changes of proteins induced by anticoagulants can be more sensitively detected in urine than in plasma. Sci China Life Sci, 2014, 57: 649–656

8 Wang J X, Chang C, Ma J, et al. Statistical strategies for selection of differentially expressed proteins based on mass spectrometry technology (in Chinese). Sci Sin Vitae, 2015, 45: 347–358 [王锦霞, 常乘, 马洁, 等. 基于质谱技术筛选差异表达蛋白的统计学策略研究进展. 中国科学: 生命科学, 2015, 45: 347–358]

9 M'Koma A E, Blum D L, Norris J L, et al. Detection of pre-neoplastic and neoplastic prostate disease by MADI profiling of urine. Biochem Biophys Res Commun, 2007, 353: 829–834

10 Wang Y, Chen J, Chen L, et al. Urinary peptidomics identifies potential biomarkers for major depressive disorder. Psychiatry Res, 2014, 217: 25–

https://www.sciengine.com/doi/10.1360/SSV-2025-0162

33
11 Abe K, Shang J, Shi X, et al. A new serum biomarker set to detect mild cognitive impairment and Alzheimer's disease by peptidome technology. J Alzheimer's Dis, 2020, 73: 217–227
12 Di Meo A, Batruch I, Brown M D, et al. Identification of prognostic biomarkers in the urinary peptidome of the small renal mass. Am J Pathol, 2019, 189: 2366–2376
13 Kononikhin A S, Starodubtseva N L, Bugrova A E, et al. An untargeted approach for the analysis of the urine peptidome of women with preeclampsia. J Proteomics, 2016, 149: 38–43
14 Good D M, Zürbig P, Argilés À, et al. Naturally occurring human urinary peptides for use in diagnosis of chronic kidney disease. Mol Cell Proteomics, 2010, 9: 2424–2437
15 Yao F, Hong X, Li S, et al. Urine-based biomarkers for Alzheimer's disease identified through coupling computational and experimental methods. J Alzheimer's Dis, 2018, 65: 421–431
16 Zheng W L, Wang L L, Li S M , et al. Recent advances in peptidomics of body fluid biomarkers (in Chinese). J Chin Mass Spectr Soc, 2021, 42: 926–941 [郑琬琳, 万磊磊, 李水明, 等. 多肽组学技术在体液生物标志物中的研究进展. 质谱学报, 2021, 42: 926–941]
17 Fang X F, Bai L Y, Li S M, et al. Study of characteristics and distribution patterns of human serum peptidome based on high resolution tandem mass spectrometry (in Chinese). Chin J Anal Chem, 2024, 52: 1337–1363 [方秀峰, 白璐雅, 李水明, 等. 基于高分辨串联质谱的人血清多肽组总体特征和分布规律研究. 分析化学, 2024, 52: 1337–1363]
18 Xu M R, Dai R F, Wei Q Q, et al. Urinary AD7c-NTP evaluates cognition impairment and differentially diagnoses AD and MCI. Am J Alzheimers Dis Other Demen, 2022, 37: 15333175221115247
19 Dumont M, Roy M, Jodoin P M, et al. Free water in white matter differentiates MCI and AD from control subjects. Front Aging Neurosci, 2019, 11: 270
20 Finer G, Schnaper H W, Kanwar Y S, et al. Divergent roles of Smad3 and PI3-kinase in murine adriamycin nephropathy indicate distinct mechanisms of proteinuria and fibrogenesis. Kidney Int, 2012, 82: 525–536
21 Ek P, Ek B, Zetterqvist Ö. Phosphohistidine phosphatase 1 (PHPT1) also dephosphorylates phospholysine of chemically phosphorylated histone H1 and polylysine. Upsala J Med Sci, 2015, 120: 20–27
22 McCullough B S, Wang H, Barrios A M. Inhibitor screen identifies covalent inhibitors of the protein histidine phosphatase PHPT1. ACS Med Chem Lett, 2022, 13: 1198–1201
23 Wang H, Gaston Jr. R, Ahmed K T, et al. Derivatives of the fungal natural product illudalic acid inhibit the activity of protein histidine phosphatase PHPT1. Chemmedchem, 2023, 18: e202300187
24 Zhang H, Wei W, Zhao M, et al. Interaction between Aβ and Tau in the pathogenesis of Alzheimer's disease. Int J Biol Sci, 2021, 17: 2181–2192
25 Busche M A, Hyman B T. Synergy between amyloid-β and tau in Alzheimer's disease. Nat Neurosci, 2020, 23: 1183–1193
26 Fremuth L E, Hu H, van de Vlekkert D, et al. Neuraminidase 1 regulates neuropathogenesis by governing the cellular state of microglia via modulation of Trem2 sialylation. Cell Rep, 2025, 44: 115204
27 Liddelow S A, Guttenplan K A, Clarke L E, et al. Neurotoxic reactive astrocytes are induced by activated microglia. Nature, 2017, 541: 481–487
28 Ge W X, Zhang L, Qi L W. Progress of neuraminidase 1 in cardiovascular diseases (in Chinese). Prog Pharm Sci, 2023, 47: 884–896 [葛文欣, 张蕾, 齐炼文. 神经氨酸酶1在心血管疾病中的研究进展. 药学进展, 2023, 47: 884–896]
29 Annunziata I, Patterson A, Helton D, et al. Lysosomal NEU1 deficiency affects amyloid precursor protein levels and amyloid-β secretion via deregulated lysosomal exocytosis. Nat Commun, 2013, 4: 2734
30 Sulimai N H, Brown J, Lominadze D. Fibrinogen, fibrinogen-like 1 and fibrinogen-like 2 proteins, and their effects. Biomedicines, 2022, 10: 1712
31 Zamolodchikov D, Strickland S. Aβ delays fibrin clot lysis by altering fibrin structure and attenuating plasminogen binding to fibrin. Blood, 2012, 119: 3342–3351
32 Sweeney M D, Sagare A P, Zlokovic B V. Blood-brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. Nat Rev Neurol, 2018, 14: 133–150
33 Zhao Z, Nelson A R, Betsholtz C, et al. Establishment and dysfunction of the blood-brain barrier. Cell, 2015, 163: 1064–1078
34 Yang S, Jin H, Zhao Z. Epidermal growth factor treatment has protective effects on the integrity of the blood-brain barrier against cerebral ischemia injury in bEnd3 cells. Exp Ther Med, 2019, 17: 2397-2402–2402

https://www.sciengine.com/doi/10.1360/SSV-2025-0162

35 Zhang Y, Tang X W, Xiao W H, et al. Correlations of serum epidermal growth factor and C-reactive protein with cognitive function in patients with schizophrenia (in Chinese). J Clin Med Pract, 2022, 26: 76–80+86 [张玥, 唐小伟, 肖文焕, 等. 血清表皮生长因子和C反应蛋白与精神分裂症患者认知功能的关系. 实用临床医药杂志, 2022, 26: 76–80+86]
36 Xian C J. EGF family of growth factors: essential roles and functional redundancy in the nerve system. Front Biosci, 2004, 9: 85
37 Mahzarnia A, Lutz M W, Badea A. A continuous extension of gene set enrichment analysis using the likelihood ratio test statistics identifies vascular endothelial growth factor as a candidate pathway for Alzheimer's disease via ITGA5. J Alzheimer Dis, 2024, 97: 635–648
38 Marksteiner J, Kemmler G, Weiss E M, et al. Five out of 16 plasma signaling proteins are enhanced in plasma of patients with mild cognitive impairment and Alzheimer's disease. Neurobiol Aging, 2011, 32: 539–540
39 Montagne A, Barnes S R, Sweeney M D, et al. Blood-brain barrier breakdown in the aging human hippocampus. Neuron, 2015, 85: 296–302
40 Pei X, Luo Y, Zeng H, et al. Identification and validation of key genes in gastric cancer: insights from *in silico* analysis, clinical samples, and functional assays. Aging, 2024, 16: 10615–10635
41 Wang Y, Sun Y, Wang Y, et al. Identification of novel diagnostic panel for mild cognitive impairment and Alzheimer's disease: Findings based on urine proteomics and machine learning. Alzheimers Res Ther, 2023, 15: 191
42 Hällqvist J, Pinto R C, Heywood W E, et al. A multiplexed urinary biomarker panel has potential for Alzheimer's disease diagnosis using targeted proteomics and machine learning. Int J Mol Sci, 2023, 24: 13758
43 Kong X Y, Du J S, Xu J L, et al. Comparison of two different separation methods for analysis of salivary peptidome (in Chinese). Chin J Anal Chem, 2019, 47: 1816–1822 [孔祥怡, 杜建时, 徐金玲, 等. 两种不同分离方法的唾液多肽组分析结果比较. 分析化学, 2019, 47: 1816–1822]
44 Shi K, Kong X Y, Du J S, et al. Proteomic identification and high abundant proteins analysis of human saliva by nano-liquid chromatography-high resolution tandem mass spectrometry (in Chinese). J Chin Mass Spectr Soc, 2016, 37: 401–407 [石锴, 孔祥怡, 杜建时, 等. 人唾液蛋白质组的纳升液相色谱-高分辨串联质谱鉴定及高丰度蛋白质分析. 质谱学报, 2016, 37: 401–407]
45 Gilgun-Sherki Y, Melamed E, Offen D. Antioxidant treatment in Alzheimer's disease: current state. J Mol Neurosci, 2003, 21: 1–12
46 Chen Z, Zhong C. Oxidative stress in Alzheimer's disease. Neurosci Bull, 2014, 30: 271–281
47 Butterfield D A, Halliwell B. Oxidative stress, dysfunctional glucose metabolism and Alzheimer disease. Nat Rev Neurosci, 2019, 20: 148–160
48 Lim J M, Kim G, Levine R L. Methionine in proteins: it's not just for protein initiation anymore. Neurochem Res, 2019, 44: 247–257
49 Butterfield D A, Swomley A M, Sultana R. Amyloid β-peptide (1–42)-induced oxidative stress in Alzheimer disease: importance in disease pathogenesis and progression. Antioxid Redox Signal, 2013, 19: 823–835
50 Kaddurah-Daouk R, Zhu H, Sharma S, et al. Alterations in metabolic pathways and networks in Alzheimer's disease. Transl Psychiatry, 2013, 3: e244
51 Ballatori N, Krance S M, Notenboom S, et al. Glutathione dysregulation and the etiology and progression of human diseases. Biol Chem, 2009, 390: 191–214
52 Vogt W. Oxidation of methionyl residues in proteins: tools, targets, and reversal. Free Radic Biol Med, 1995, 18: 93–105
53 Chandran S, Binninger D. Role of oxidative stress, methionine oxidation and methionine sulfoxide reductases (MSR) in Alzheimer's disease. Antioxidants, 2023, 13: 21
54 Yang G F, Ji J G, Peng L W, et al. Oxidatived proteins in human brains separated and identified by proteomics technology (in Chinese). Chin J Tissue Eng Res, 2011, 15: 1990–1993 [杨国锋, 纪建国, 彭立威, 等. 蛋白质组学技术分离及鉴定人脑氧化蛋白质. 中国组织工程研究与临床康复, 2011, 15: 1990–1993]
55 Estévez M, Díaz-Velasco S, Martínez R. Protein carbonylation in food and nutrition: a concise update. Amino Acids, 2022, 54: 559–573
56 Dalle-Donne I, Aldini G, Carini M, et al. Protein carbonylation, cellular dysfunction, and disease progression. J Cell Mol Med, 2006, 10: 389–406
57 Hooper C, Killick R, Lovestone S. The GSK3 hypothesis of Alzheimer's disease. J Neurochem, 2008, 104: 1433–1439
58 Arikanoglu A, Akil E, Varol S, et al. Relationship of cognitive performance with prolidase and oxidative stress in Alzheimer disease. Neurol Sci, 2013, 34: 2117–2121
59 Arber C, Belder C R S, Tomczuk F, et al. The presenilin 1 mutation P436S causes familial Alzheimer's disease with elevated Aβ43 and atypical clinical manifestations. Alzheimers Dement, 2024, 20: 4717–4726
60 Stadtman E R, Levine R L. Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. Amino Acids, 2003, 25: 207–218
61 Alam S, Mahadev R. The role of alpha-1 antitrypsin in emphysema. In: Mahadeva R, ed. Emphysema. London: IntechOpen, 2012

https://www.sciengine.com/doi/10.1360/SSV-2025-0162

62 Ebrahimi T, Rust M, Kaiser S N, et al. α1-antitrypsin mitigates NLRP3-inflammasome activation in amyloid β1–42-stimulated murine astrocytes. J Neuroinflamm, 2018, 15: 282

Peptidomics of urinary biomarkers for Alzheimer's disease

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Urinary peptidomics has been applied to comprehensively analyze urine samples from patients with Alzheimer's disease (AD) and mild cognitive impairment (MCI), highlighting its potential for AD screening and diagnosis. This study included 32 AD patients, 34 MCI patients, and 20 healthy controls. Peptidomic analysis was performed on each urine sample using high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). The urinary peptidome displayed a distribution in which the 20% of highly abundant proteins accounted for ~80% of peptide counts and 90% of signal intensity. An AD-specific biomarker panel was identified based on three degraded proteins—14 kD phosphohistidine phosphatase 1 (PHPT1), sialidase-1 (NEU1), and fibrinogen beta chain (FGB)—which regulate mitochondrial function, suppress neuroinflammation, and block complement activation induced by amyloid fibers. Epidermal growth factor (EGF) and collagen alpha-1(III) chain (COL3A1) were selected as biomarkers for MCI. These proteins promote neuron survival through the epidermal growth factor receptor-mitogen-activated protein kinase (EGFR-MAPK) pathway and inhibit abnormal integrin signaling via mediating basement membrane remodeling, reflecting early compensatory mechanisms. Additionally, in AD/MCI patients, the oxidation levels of methionine and proline residues within urinary peptides were markedly higher. The oxidation of methionine in alpha-1 antitrypsin (SERPINA1) degradation peptides and proline in collagen alpha-1(I) chain (COL1A1)/ collagen alpha-2(I) chain (COL1A2) degradation peptides distinguishes diseased groups from controls. The mechanisms involved were oxidative stress and disrupted collagen-cytoskeleton interactions. Overall, the study indicates that the urinary peptidome reflects AD pathological mechanisms through both protein degradation and post-translational peptide modifications.

Alzheimer's disease, mild cognitive impairment, urine peptidomics, oxidative modification, biomarkers

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