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评 述 质谱分析专刊

基于质谱技术的代谢组学研究及其在中国的发展

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摘要 代谢组学是关于生物系统代谢物组成及变化规律的科学, 是系统生物学的重要组成部分. 质谱技术是目前代谢组学研究中最主要的分析手段之一, 广泛应用于代谢组学各个领域. 本文阐述了基于质谱技术的代谢组学方法及其应用, 重点介绍和评论了近年来我国在该领域取得的进步和成果, 并对基于质谱技术的代谢组学研究目前存在的问题及未来的发展进行了分析与展望.

关键词
代谢组学
质谱技术
系统生物学

1 引言

后基因组时代如何解析基因组功能是生命科学研究的热点问题, 基于基因功能的复杂性和生物系统的完整性, 有必要从整体层面上来理解构成生物体系的各个模块功能[1]. 由基因组衍生出来的转录组学、蛋白质组学以及代谢组学等整体性研究方法成了解析功能基因组的新的重要手段[2~4]. 代谢组学作为系统生物学的重要组成部分, 可定义为对生命体系因环境刺激、病理生理扰动或基因改变等所引起的体现为所有代谢物动态应答的质和量及其变化规律. 由于代谢(物)处于生物系统生化活动调控的末端, 涵盖反映生理表型的直接而全面的生物标记物信息, 因此, 代谢组学作为系统生物学中最下游的“组学”, 可反映生物化学变化的信号放大效应, 且日益成为整体性研究生命体系功能变化的重要学科分支.

由于生物体系的复杂性, 代谢产物数目多、差异大、浓度分布范围广、组成复杂, 代谢组学也是一门技术驱动的科学. 现代质谱技术具有高选择和灵敏性、普适性和分析速度快等特点, 可同时检测鉴定多种代谢物, 提供丰富的数据信息. 因此, 基于质谱技术的代谢组学研究文献不断增加, 已超过了基于核磁共振技术的文献量, 成为最有效的研究手段之一. 目前, 基于质谱技术的代谢组学研究在药物开发、临床疾病、植物学、营养学和环境毒理学等领域发挥着重要的作用[5].

2 质谱技术在代谢组学中的研究进展

气相色谱-质谱和液相色谱-质谱联用可以检测数千种化合物, 包括糖类、有机酸、氨基酸、脂肪酸、环境外源污染物和植物的次级代谢产物, 这是其他任何一项技术所无法比拟的[5~7].

超高效液相色谱与质谱联用扩展了分析物(代谢物)的覆盖率, 因此成为当今代谢组学的主要方法之一. 多维气-质和液-质联用的发展, 在对复杂体系中的靶标分析(如标记物分析)代谢组学研究中有着突出的优势. 纳升级[8, 9]和芯片纳流液相色谱-质谱系统也成为高效快速、高灵敏度的分离分析复杂代谢组样品的新技术[10]. 2013 年, *Science* 报道的高密度芯片-质谱接口技术, 如质谱微阵列芯片, 可解决单细胞代谢组学质谱检测中高通量制备单细胞样本的瓶颈问题[11].

复杂体系中代谢产物的结构鉴定是富有挑战性的问题, 具有高分辨率的质谱系统(如四极杆串联飞行时间质谱仪[12]、傅里叶变换离子回旋共振质谱仪[13]和 Orbitrap 等)在此方面有着突出的优势. 准确质量的质谱和串联质谱检测与数据库检索、标准品验证及其他实验结果相结合, 提供了更高水平的代谢物结构识别和确认能力, 对生物标记物的确定非常重要.

到目前为止, 大部分整体代谢谱(轮廓)分析采用电喷雾电离源. 最近, Shrestha 等[14]以电喷雾电离为基础开发了一种新的激光剥离电喷雾电离方法, 在常压下可用质谱原位分析单细胞的代谢谱图.

质谱分析中, 精确质量测定、化合物元素组成、串联质谱特征离子碎片信息、气相或液相色谱分离保留时间等大量的多元性数据, 不仅可以帮助鉴定代谢物的结构, 而且与化学计量学结合, 构建合理的数学模型, 选用有效的数理统计方法对数学模型进行计算和归类分析, 挖掘有用信息, 对代谢组学分析结果的解释, 在相关疾病生物标记物的发现、阐明代谢物的生物功能、疾病的发病机理等研究中有重要的意义[15].

目前, 基于质谱技术的代谢组学研究已广泛应用于疾病诊断、药物毒性和效果评价、微生物和植物的细胞代谢以及基因功能的阐明等. 作为新的研究思路和手段, 基于质谱技术的代谢组学方法近年来又在中药成分的安全性评价、环境毒理分析、营养基因组学、整合药物代谢和系统毒理学等方面取得了新的突破和进展. 例如, 最近在癌症代谢组学方面的新突破包括发现细胞的快速增殖需要大量的非必需甘氨酸, 对癌症治疗有着清晰直接的影响[16]. 在这项工作中, Jain 等[17]首次采用液相色谱-串联质谱方法从 60 种不同的人体癌细胞株中检测了 219 种代谢产物.

3 我国的研究成果

近年来, 基于质谱技术的代谢组学研究在中国快速发展, 建立了多个代谢组学技术平台和研究组, 开展了与重大疾病、中药和环境等相关的一系列各具特色的代谢组学研究, 在技术突破和应用方面取得了一定的成果.

许国旺研究组针对代谢组的特点, 采用“分而治之”的策略, 建立完善了一系列基于色-质联用技术的代谢组学分析平台, 其中包括非靶向的代谢组、脂类的组学分析平台以及针对痕量代谢物的靶向分析平台等, 并在提高代谢组学分析覆盖的化合物种类, 实现高通量、高灵敏度代谢组分析以及发展复杂代谢组数据解析方法, 解决代谢物定量及未知代谢物结构定性等关键问题上开展了一些创新性研究. 通过发展新型同位素标记衍生化液-质联用方法, 实现了 24 种类固醇激素的快速高灵敏检测[18]; 发展了多维色-质联用技术, 提高了色谱的分离能力[19~21]. 结合代谢组学非靶向分析及靶向分析的特点, 该研究组提出了一种“拟靶向”的分析策略, 以多反应监测方式实现一次进样定量超过 500 种代谢物, 显著提高了代谢组分析的数据质量[22], 发展了包括色谱峰匹配策略[23]、代谢表型识别以及筛选潜在标志物的新算法等[24~26]. 该研究组将色-质联用和高分辨质谱代谢组学方法应用在疾病诊疗的领域, 如糖尿病及其并发症的相关代谢标志物[27~40], 以及开展了肝癌[41~43]、卵巢癌[44]、肺癌[45]、大肠癌及其他肿瘤[46~49]的代谢组学研究. 同时研究了中医证候[50, 51]、中药配伍以及疗效评价等[20, 28, 29, 52]中医药相关的代谢组学, 以及不同水稻亚种的代谢特征以及转基因水稻代谢组特征[53, 54]等. 该研究组近年来也注重关键差异代谢物的功能研究, 揭示了代谢组对复杂生物现象的影响[55], 开发了基于脂质代谢动力学的分析策略[56~58], 为功能代谢组研究提供了新的研究思路.

再帕尔课题组研究了恶性肿瘤代谢组学的分析方法和生物标志物的发现. 以快速高分辨液-质联用技术为主要分析手段, 通过构建高效、整合式的高通量分析技术平台, 开展了食管癌[59]、肺癌[60]、乳腺癌[61]和宫颈癌[62]等恶性肿瘤的代谢组学分析方法及发现小分子生物标志物的研究. 分别建立了“组合式”离子化方式的代谢组学新型分析方法[60]、模式识别与偏相关网络分析相结合的“整合式”数据处理方法[61]、 整体轮廓与靶向代谢组学相结合的分析方法和定量代谢轮廓分析方法[59], 以及尿液代谢组学的前处理及数据分析与校正新方法[63]. 运用这些方法在更可靠、更全面地发现与恶性肿瘤诊断或疗效评价密切相关的潜在小分子标志物方面取得了重要进展. 通过基于动物肿瘤模型的差异代谢物动态行为分析与药物代谢组学相结合的方法研究[64], 发现了与肿瘤发展密切相关的潜在生物标志物以及与药物药效或毒性作用相关的药物敏感性标志物. 研究了血浆中内源性代谢物稳定性以及相关代谢物的变化规律,

发现了显著性变化的不稳定代谢物, 从而辅助潜在生物标志物的正确识别[65].

近6年来, 本研究组通过开发和建立基于质谱技术代谢组学方法, 开展了与环境毒理和重大疾病相关的代谢组学研究[66~78]. 采用超高效液相色谱-四极杆串联飞行时间质谱分析了 2,3,7,8-二噁英在芳香烃受体敏感型的 C57BL/6J 小鼠与不敏感型的 DBA/2J 小鼠中的不同毒性反应[72, 74]. 同时, 还对大脑中的海马组织与小脑组织的代谢物解析中发现, 这两个组织有诸多类似的地方, 也呈现出二噁英可促进衰老的现象[67, 73]. 用高分辨液-质联用法研究了体内代谢中马兜铃酸的毒性和致毒机制[70, 76, 78]. 结合动物模型与生物标记物的发现, 研究了灌胃葡萄糖的健康大鼠与糖尿病大鼠的代谢表型的相似度[71].本研究组还对流感病毒感染的细胞模型[69]、阿尔茨海默病转基因小鼠[68]及人类肝癌手术后的血清进行了代谢组学分析[77]. 此外, 本研究组与德国赫姆霍兹中心合作, 建立了超高分辨的傅里叶变换离子回旋共振质谱代谢组学分析方法, 高准确度的质谱数据有利于快速鉴定代谢物作为潜在的生物标记物.

唐惠儒研究组[79~81]建立了液-质与核磁共振联用代谢组学研究技术平台, 开展了多方面研究: 与重要疾病发生发展及治疗愈后相关的代谢变化规律与机制、宿主代谢组和寄生生物间相互作用的机制及其与健康的关系, 以及应激对生长发育过程中代谢组的影响规律及其与健康的关系等代谢基础研究. 刘虎威研究组首次将基于停流技术的反相色谱和正相色谱的二维分析系统与质谱联用应用于代谢物分析[82], 开展了生物样本的脂质轮廓图谱分析等脂质组学研究[82~85]. 陈焕文研究组[86, 87]致力于质谱仪器和方法的开发研究, 将质谱技术应用于生物样品的代谢指纹图谱和癌症诊断中等研究工作. 林金明研究组[88]开发了微流控芯片与质谱联用技术, 对细胞分泌物进行检测分析以期得到细胞生命活动中扮演重要角色的物质的结构和含量信息. 张金兰研究组[89]研究和建立了一种中药体内成分和代谢产物发现和鉴定的新策略(质谱树状图过滤技术), 研究了淫羊藿的代谢物和代谢途径. 鞘脂类的代谢研究为麻风病和肺结核等过敏性疾病的发病机制和病理机制提供了证据[90]. 王喜军研究组[91~94]采用多维色谱-质谱及高分辨质谱联用的方法开展了中医方证代谢组学的研究, 考察了中药体内代谢物及其动态变化规律, 利用代谢组学对整体代谢轮廓的描述来评价复杂性多元效应. 我国从事代谢组学和质谱学研究的学者和团队众多, 由于篇幅有限, 在此无法一一介绍.

4 结论和展望

代谢组学是研究基因组功能的重要手段之一, 是系统生物学不可或缺的部分. 基于质谱技术的代谢组学方法, 如超高效液相色谱-高分辨质谱、毛细管电泳-质谱联用、多维色谱-质谱联用、质谱-核磁共振联用和超高分辨质谱等技术, 在灵敏度、分辨率、动态范围和高通量等方面均有显著的改善, 并且与其他学科方法的配合和交叉, 推动了代谢组学的发展. 近 10 年来, 中国研究者在基于质谱技术的代谢组学新方法的创新和完善方面取得了一定成果, 并将其应用于更广泛的代谢组学相关研究领域.

整体代谢组学研究范围的不断拓宽导致新生物标记物的发现和新生化现象的阐释. 因此, 质谱技术将继续发挥主导作用. 但是, 功能完善的代谢产物数据库的构建、多维数据验证方法、代谢组学研究的标准化和进一步的技术突破仍是代谢组学尚需解决的问题. 另一方面, 只有将代谢组学技术与基因组学、蛋白质组学数据整合在一起, 才能提供完整的系统生物学认知. 这样的平台必将产生庞大的数据, 如何合理有效地对基于质谱技术的代谢组学方法进行数据挖掘, 与不同平台和不同组学的数据进行整合, 尽量全面准确地对代谢物定性定量分析也是未来代谢组学的研究重点.

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Mass spectrometry-based metabolomics and their developments in China

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Abstract: As an important component of systems biology, metabonomics (or metabolomics) is a science of studying metabolites and the corresponding changes in living systems. Mass spectrometry is one of the primary analytical tools of metabolomic studies and has been widely applied in many research areas. This paper overviews the recent developments in technologies and applications of mass spectrometry-based metabolomics, highlights major progress and achievement that have been recently gained in China in this field, and discusses the existing problems of mass spectrometry-based metabolomics and the future perspectives.

Keywords: metabonomics, mass spectrometry, systems biology