



基于质谱分析的单细胞代谢组学研究进展

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摘要 细胞异质性是生物系统复杂性的重要组成部分, 在肿瘤微环境、免疫反应、干细胞分化, 以及疾病发生与发展等关键生物学过程中发挥了显著作用。基于质谱分析的单细胞组学研究近年来蓬勃发展。研究者在样本提取、质谱装置改进及数据分析方法上做出了大量努力, 为深入解析细胞异质性提供了丰富的科学数据。本文重点聚焦于质谱成像和毛细管电喷雾质谱分析两项技术, 综述其在单细胞代谢组学研究中的最新进展及其研究意义。同时, 本文也对该领域存在的挑战进行初步探讨。

关键词 细胞异质性, 单细胞分析, 代谢组学, 质谱成像, 毛细管电喷雾

细胞是所有生物体的基本组成成分, 细胞异质性是生物系统复杂性的重要因素之一。在生物体内, 即便是同一种类型的细胞, 其生物功能、代谢状态和响应刺激的能力也可能存在显著差异。这种异质性在肿瘤微环境、免疫反应、干细胞分化及疾病发生发展进程等关键生物学过程中尤为突出^[1-3]。细胞异质性有利于生物体的精细调控, 但在某些情况下(如癌症或自体免疫疾病)可能导致病理性免疫反应的发生。例如, 同类型或亚型的免疫细胞能够根据微环境信号作出适应性变化, 从而实现免疫系统的精准控制^[4]; 肿瘤组织中的不同细胞亚群对药物表现出不同的耐药性^[5]。随着多种单细胞组学技术的迅猛发展, 对单细胞异质性的探索已经成为理解这些复杂生物学过程的关键。

单细胞组学技术的兴起为解析单细胞异质性提供了强有力的工具。近年来, 单细胞基因组^[6]、单细胞转录组^[7]、单细胞蛋白组^[8]和单细胞代谢组^[9]等技术蓬勃发展^[10], 各类组学数据揭示了单细胞层面的多维信息。其中, 代谢组学旨在分析糖类、氨基酸、脂质、有

机酸、核苷酸及其衍生物等多种小分子物质。这些小分子代谢物作为基因调控网络和蛋白质作用网络的下游产物, 能够即时响应内外部环境的变化, 具有时效性强、动态响应快等特点。代谢物直接参与细胞的生命活动, 并对细胞的生理状态作出即时的反馈, 因此代谢组学是最接近生物表型的组学^[11]。单细胞代谢组学能够揭示不同细胞群体在代谢活性层面的异质性, 帮助深入理解细胞不同生物行为背后复杂的分子机制。

然而, 单细胞代谢组学研究面临着多重技术挑战。单个细胞体积小, 所含代谢物结构复杂, 种类繁多且含量极低, 使得传统的代谢组学分析方法无法满足单细胞检测需求^[12]。质谱仪器具有高灵敏度、高分辨率和宽动态范围等多种优势, 近年来尤其是在样本制备、微量采样、离子化技术、高灵敏度质量分析器等方面的突破, 使得单细胞水平的代谢物检测成为可能, 已成为单细胞代谢组学研究中最为核心的分析工具之一^[13-15]。为了应对不同的研究需求和应用场景, 基于质谱的单细胞代谢组学技术分为多种类型。根据其空间

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分辨能力、离子化模式、采样方式、实验通量的差异,常见的类型有基于质谱成像技术(mass spectrometry imaging, MSI)的单细胞代谢组学分析、基于毛细管进行原位取样并电喷雾离子化的单细胞代谢组学分析、基于微流控芯片的单细胞代谢组学分析和基于流式质谱的单细胞代谢组学分析. 不同类型的技术各有其优点与适用场景,构成了单细胞代谢组学研究的多样性. 先前已有研究者从基于环境质谱的单细胞分析^[16]、不同样品制备方法与细胞类型的单细胞分析^[17],以及单细胞数据分析工作流程^[18]等多个方面进行了综述. 本文将聚焦于应用最广泛的基于质谱成像以及基于毛细管电喷雾的单细胞代谢组学分析,综述其近五年来的最新进展,探讨它们在单细胞异质性研究中的应用与发展潜力. 通过对这些技术的系统梳理与分析,我们希望为未来的单细胞代谢组学研究提供有价值的参考.

1 基于质谱成像的单细胞代谢组学分析

以基质辅助激光解吸电离质谱(matrix-assisted laser desorption/ionization mass spectrometry, MALDI-MS)^[19]、二次离子质谱(secondary ion mass spectrometry, SIMS)^[20,21]、解吸电喷雾电离质谱(desorption electrospray ionization mass spectrometry, DESI-MS)^[22]为代表的质谱成像技术,能够在保持样本相对完整性的前提下,提供代谢物、脂质和蛋白质的空间分布图. 单细胞质谱成像是揭示细胞内生物分子空间分布的最先进工具. 越来越多的单细胞研究证据表明,描绘细胞内生物分子的空间分布是解决单细胞分子异质性和化学多样性难题的重要手段. 想要实现单细胞水平的质谱成像,需要克服很多技术难题,例如空间分辨率的提高、单细胞边界的划分、图像的还原整合、定制化的数据处理流程等.

基于MALDI-MS的MSI具有制备样品容易、扫描速度快、灵敏度高、光谱易于解释(主要产生单电荷离子)等优点,是目前应用最为广泛的MSI方法. 人类细胞的直径范围分布为5 μm (精子)~150 μm (卵子)^[23],平均约为10~20 μm . 商业化的MALDI-MS仪器的激光光斑大小在10~100 μm 之间,因此想要进行单细胞水平的代谢组学成像,提高空间分辨率是亟待解决的难题. 通常来讲,激光光斑的大小与电离效率成反比;缩小光斑直径虽然能提高空间分辨率,但是鉴定深度会大大下降. 为了解决这一问题, Dreisewerd等人^[24]发展了一种名为MALDI-2的策略(图1(a))^[25]. 与传统MALDI激光照

射后直接进行离子化方法不同, MALDI-2的创新点是在离子生成区域创造缓冲气体环境,形成一个临时的微反应器. 经过基质分子的共振双光子离子化后,能发生大量的离子-中性分子反应,启动二次电离过程. 该策略可将MALDI成像的灵敏度提高多达两个数量级,足以使研究者能够在植物和动物组织中识别出更多的生物分子,包括各种脂质种类、脂溶性维生素、低聚糖和糖苷,横向分辨率高达5 μm . 该团队后续将MALDI-2与轨道阱质谱仪联用,以600 nm的像素分辨率,成功对小鼠小脑,肾切片细胞中的磷脂和糖脂分布进行了可视化^[26].

为了实现更高的空间分辨率,不同光源及离子化的质谱成像技术也引起研究者的关注,例如SIMS(图1(b))^[27]和真空紫外激光解吸/电离(vacuum ultraviolet desorption/ionization, VUVDI)质谱成像(图1(c))^[28]. SIMS与MALDI的主要区别在于, MALDI的光源为激光光源, SIMS的光源为 $\text{Ga}^+/\text{Cs}^+/\text{C60}^+/\text{O}_2^+/\text{Ar}^+$ 等离子光源. SIMS通过聚焦的离子束直接轰击样品表面来释放二次离子进行检测,主离子束的类型在很大程度上决定了横向空间分辨率、溅射速率、深度分辨率,以及传递给解吸离子的能量. 这种方式产生的二次离子能够提供非常高的空间分辨率,通常可以达到亚微米级别,远高于传统MALDI质谱. SIMS分析时只需要样品的表层(通常在几纳米至几十纳米深度)即可进行分析. SIMS成像过程不需要基质,可防止基质的引入对信号产生的干扰,进一步提高分辨率. Bayır团队^[30]运用SIMS成像在单细胞/亚细胞水平上研究了心肌细胞冷冻水合样品铁死亡过程中的氧化磷脂酰乙醇胺分布,对极低丰度的过氧化脂质(20 pmol/ μmol 脂质)在病理条件下的异常积累进行了可视化. 但是SIMS也存在其局限性,比如昂贵的价格和较高的分子碎裂率,且仅能获得小质量的样品碎片成像信息($m/z < 500$). 南京大学陈洪渊院士及清华大学莫宇翔教授团队提出了VUVDI-MSI(图1(c)),可基于真空紫外光的高能量直接从样品表面解吸和电离分子,在亚微米的分辨率实现单个Hela细胞的MSI^[28]. VUV光源的波长为120~150 nm(单光子能量10.3~8.3 eV),可以提供更高的空间分辨率;具有足够的能量,可在无需基质辅助的情况下有效地电离目标分子,使得VUVDI-MSI非常适合直接分析生物样品中的小分子、脂类和代谢物. 通过调节激光通量,可获得对不同分子和碎片离子的选择性检测,实现深度分子鉴定. 与SIMS相比, VUVDI在亚微米水平上

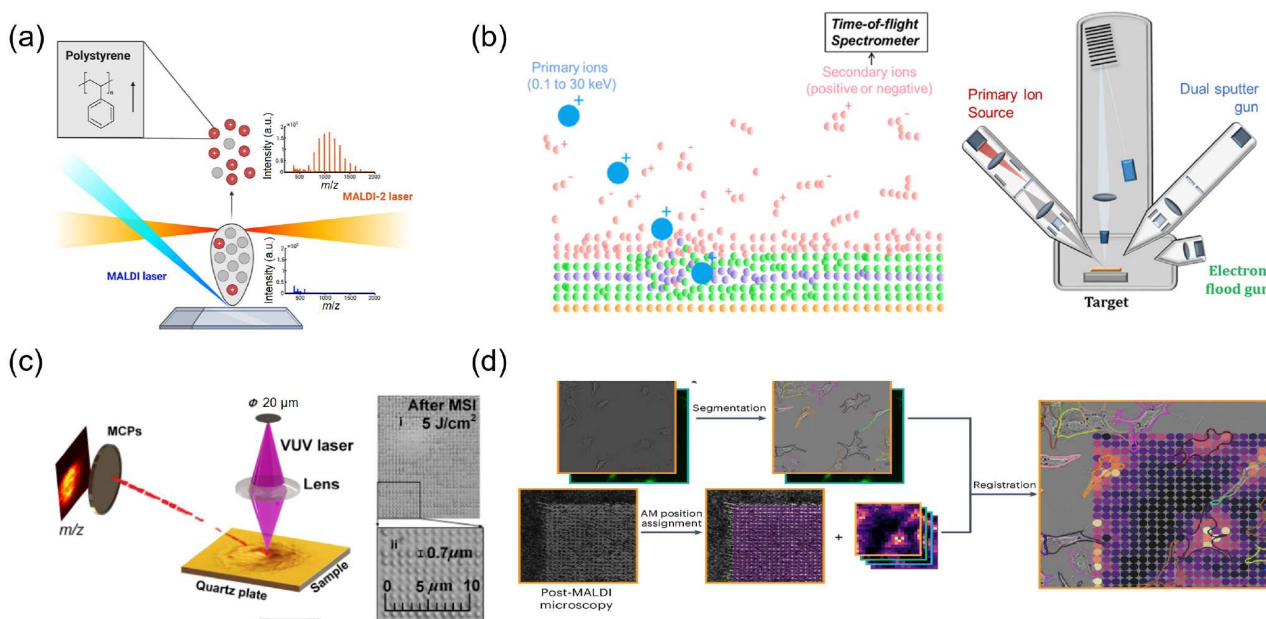


图 1 (网络版彩色) 基于质谱成像的单细胞代谢组学分析. (a) MALDI-2离子源的示意图. MALDI-2对多种聚合物的检测灵敏度显著高于MALDI^[25]. (b) SIMS的示意图, 左图显示产生二次离子的溅射过程, 右图仪器概述^[27]. (c) VUVDI-MSI系统的示意图^[28]. (d) 在¹³C-SpaceM中整合显微镜和IMS以获得单细胞谱^[29]

Figure 1 (Color online) Single-cell metabolomics analysis based on mass spectrometry imaging. (a) Schematic diagram of the MALDI-2 ion source. The detection sensitivity of MALDI-2 for various polymers is significantly higher than that of MALDI^[25]. (b) Schematic for analysis of a surface by time-of-flight secondary ion mass spectrometry (ToF-SIMS) showing (left) the sputtering process that generates secondary ions, (right) an overview of the instrumentation^[27]. (c) Schematic drawing of the VUVDI-MSI^[28]. (d) Integration of microscopy and imaging MS in ¹³C-SpaceM to obtain single-cell profiles^[29]

实现了更高的离子产率和更少的碎裂。

在提高空间分辨率的基础上, 如何对细胞边界和细胞类型进行精准地识别也是研究者关注的重点. Bal-luff等人^[19]联合数字图像分析技术, 使用机器学习算法对微观图像进行评估和注释. 他们运用形态计量学模型来统计每个细胞的大小和形状(如细胞核大小、细胞偏心率、细胞周长等), 从而对整个组织进行即时检测和分类, 然后将其与来自同一组织切片的MALDI-MSI数据准确关联. 这个工作流程使研究者能够在复杂的组织环境中确定每个细胞的详细分子特征, 以10 μm的成像分辨率对猪结肠和人胃癌标本进行分析, 最终产生10⁵个单细胞的形态和分子图谱. Alexandrov团队^[31]提出用于原位单细胞代谢组学分析的方案SpaceM, 无需定制质谱设备, 仅需市售的MALDI质谱以及荧光显微镜即可完成实验. 其实验步骤包括细胞样本的制备(细胞培养、固定、染色、干燥); 第一次获取显微图像(单个细胞的荧光强度和形态特征); MALDI成像; 第二次获取显微图像(根据烧蚀痕迹与第一轮图像进行共校准). 基于新开发的高精度图像校准方

法, 可实现亚微米精度的分辨率. 最终可绘制单细胞空间分子矩阵, 为每个细胞在原位提供多个维度的数据, 包括代谢物图谱、细胞荧光强度和空间形态特征. SpaceM展示了2840个人肝细胞的740个代谢物的谱图, 成功鉴定出人肝细胞脂肪变性的两个亚群. 该团队后续联合同位素示踪技术, 将该方法拓展为¹³C-SpaceM(图1(d)), 揭示了癌症中从脂肪酸合成的异质性^[29]. 这些细胞图形精准识别技术允许在单细胞水平上进行分子诊断, 展示了高通量, 自动化数字病理诊断的未来.

值得一提的是, 上述提到的质谱成像技术中, 大多数离子化过程都依赖于真空环境. 尽管这种方式在高精度和高灵敏度分析中具有独特的优势, 但其高昂的成本、复杂的操作流程以及样品适用性的局限性也不容忽视. 因此, 基于常压的电离方式(atmospheric pressure ionization, API)也是研究者关注的重点. 其中, DESI-MS是常压电离的典型代表. 它是一种软电离源, 样本制备方便, 可以在常压下得到较大分子的质谱信息; 但是其空间分辨率较差, 想要得到高分辨率的单细胞图谱难度较大. Burlingame团队在2000年^[32]提出了

常压-基质辅助激光解吸电离(atmospheric pressure ionization and matrix-assisted laser desorption ionization, AP-MALDI)这一技术,拓宽了MALDI MS的应用前景.在此基础上,Spengler团队^[33]进行了设备改进,实现了在接近生理条件下以1.4 μm 的横向分辨率进行单细胞分子成像的目标,在亚细胞水平对草履虫的脂类,代谢物以及肽类等物质的空间分布特征进行了可视化. AP-MALDI以其出色的兼容性以及较高的空间分辨率在单细胞质谱成像领域获得了一席之地.

此外,与高分辨率质谱仪(高分辨率轨道阱和傅里叶变换离子回旋共振质谱)的联用、基质的选择和优化^[34]、纳米材料辅助基质的离子化^[35]、机器学习人工智能方法的引入^[36]、离子淌度技术的引入以提高代谢物鉴定效率^[37]. 这些方面也是研究者为了高质量单细胞代谢组学成像这一目标而改进的研究方向.

2 基于毛细管电喷雾的单细胞代谢组学分析

虽然成像质谱可以提供空间信息,但是仍存在许多短板.例如其样本制备步骤较多,如洗涤、酶应用或衍生化过程会使分子离域;固定的细胞样品并不能在活细胞水平实现代谢物的检测;检测的代谢物类型主要聚集于脂质.与之形成互补的是基于毛细管原位取样并电喷雾离子化(electrospray ionization mass spectrometry, ESI-MS)的单细胞分析技术,可以直接对活细胞内的代谢物和酶活性^[38]进行分析,离子化效率高,取样操作过程灵活性高,在近年来产出了一些亮眼的成果.

毛细管作为取样的重要工具,其尖端直径的范围可以根据实验需要控制在微米到纳米范围.当直径范围在纳米水平时,产生nano-ESI效应,既可以提高离子化效率,减轻盐效应的影响^[39],还可以减少取样量,减少对细胞造成的损伤.我们课题组前期使用直径为150 nm的纳米毛细管,在维持细胞活性的情况下,实现了对单个活神经细胞突触的代谢物表征(图2(a))^[40].毛细管担任采样针和质谱喷针的双重角色,在显微操作装置的辅助下,操作过程具有灵活性,可与多种技术联用.本课题组^[41]通过交替使用纳米和微米直径针尖的毛细管,实现对同一单细胞进行单细胞代谢组和单细胞转录组同时检测的目标,为代谢物-基因相互作用网络的建立提供了全新的视角.黄光明和熊伟教授团队^[42]联合电生理膜片钳以及电喷雾离子源质谱技术,毛细管既作为膜片钳的探针,也作为细胞质溶液取样针和质谱喷针,成功建立一套单个神经元胞内组分取

样和质谱分析技术(图2(b)),对小鼠海马、前额叶、杏仁核、纹状体等脑区单个神经元内的数百种化学小分子进行了快速质谱检测,并且可以做到同步采集电生理信号,在单细胞水平上成功地完成了对神经元功能、代谢物组成及其代谢通路的研究.该团队继续应用该技术,结合神经科学的多种研究手段,揭示了一条脑内谷氨酸合成新通路及其参与日光照射改善学习记忆分子及神经环路机制^[43].由于谷氨酸在大脑内具有参与细胞内蛋白合成、能量代谢以及兴奋性神经信号传递等多种重要的生理功能,因此该通路的发现对于了解大脑工作机理以及探索相关疾病发生机制都将起到重要作用.更为难得的是,该团队利用vacuolin-1对溶酶体进行膨胀处理,建立了单个溶酶体水平的代谢物分析平台,首次实现基于单个溶酶体代谢表型的溶酶体分型,并深度探索了细胞衰老过程中溶酶体代谢表型的异质性改变.通过溶酶体与胞质溶液代谢表型存在显著差异,膨胀处理不同时间长度的溶酶体的代谢表型无显著差异以及膨胀后的溶酶体关键转运蛋白功能正常等多项结果,作者证明膨胀处理不会显著影响溶酶体本身的代谢活性.虽然已从多个角度证明膨胀过程对于溶酶体代谢的干扰较小,但是基于vacuolin-1的膨胀处理不具有普适性,仍需要开发可以兼容多种细胞器的、无干扰的分析方法^[44].

由于毛细管可以对活细胞模型指定目标区域进行微量取样,在胚胎以及肿瘤球等三维(3D)细胞模型的研究方面发挥关键作用.取样过程中控制取样量可维持细胞模型的活性,采样后继续进行培养,可实现基于3D细胞模型的时间分辨的代谢组学分析.本课题组^[45]通过基于纳米毛细管取样的单细胞代谢组学技术,在维持球体完整的前提下追踪单个肿瘤球在阿霉素给药模型下时间,空间分辨的细胞代谢异质性,阐明了球体底部细胞的特殊性(图3(a)). Lombard-Banek和Camille等人^[46]使用毛细管微采样(尖端直径约20 μm),在每个时间点对脊索动物胚胎进行两次采样,实现了活胚胎中时空分辨的单细胞的双重蛋白质-代谢组学研究(图3(b)).取样分析后,该胚胎仍可发育成视觉上行为正常的蝌蚪.刘震团队^[47]开发了一种基于毛细管修饰的固相萃取的显微活检平台,该平台能够定量,深入和最小破坏性地跟踪表征非洲爪蟾从细胞水平(单个卵母细胞)到组织水平(尾期胚胎的不同部位)发育过程中的内源性和外源性顺式二醇代谢物的变化(图3(c)).

虽然基于毛细管的单细胞代谢组学分析具有多重

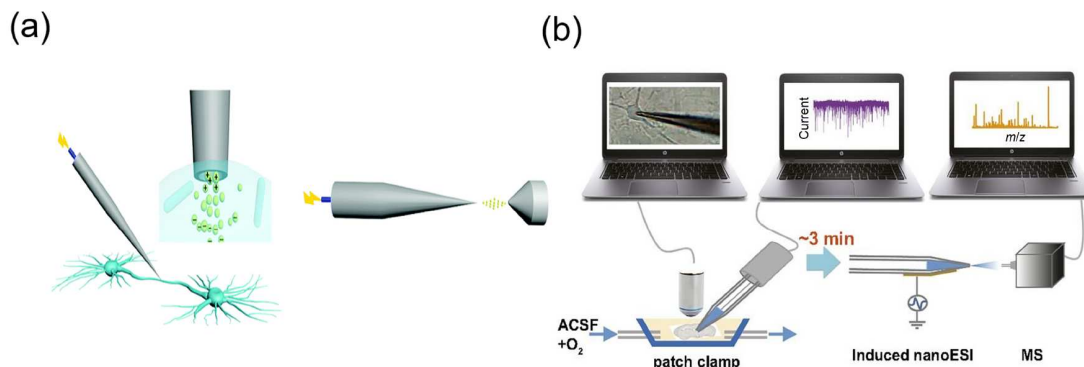


图2 (网络版彩色)基于毛细管电喷雾的单细胞代谢组学分析。(a)使用纳米毛细管进行神经细胞轴突样品提取及质谱分析的示意图^[40]。(b)使用毛细管进行单个神经元的细胞质溶液提取,电生理记录以及质谱分析的工作流程^[42]

Figure 2 (Color online) Single-cell metabolomics analysis based on capillary electrospray ionization. (a) Schematic diagrams of the electrosyringe for loading the sample into the nano-capillary, and the electrospray of the loaded sample for MS analysis^[40]. (b) Illustration of the workflow for single-neuron sample collection, electrophysiological recording and MS detection^[42]

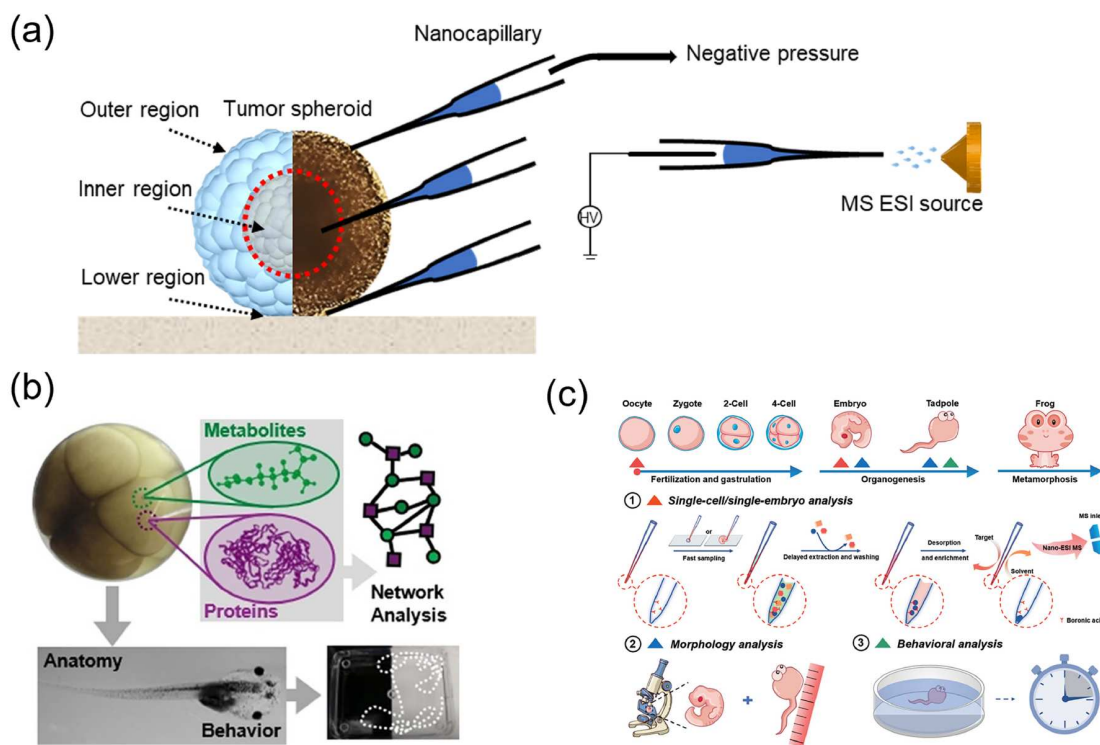


图3 (网络版彩色)对于3D细胞模型的单细胞代谢组学分析。(a)使用基于纳米毛细管的ESI-MS对单个活体肿瘤球体内部进行采样并进行分析的示意图^[45]。(b)对非洲爪蟾胚胎细胞中的蛋白质和代谢物进行双重表征的单细胞质谱技术^[46]。(c)单卵母细胞和尾芽期胚胎分析工作流程示意图^[47]

Figure 3 (Color online) Single-cell metabolomics analysis for 3D cell models. (a) Schematic setup for the sampling inside single living tumor spheroids using nanocapillary-based ESI-MS and the following ESI-MS analysis^[45]. (b) Single-cell mass spectrometry was advanced to enable dual characterization of proteins and metabolites in identified cells in *Xenopus laevis* embryos^[46]. (c) Schematic illustration of the workflow for single oocyte cells and tailbud-stage embryo analysis^[47]

优势,但是其取样过程需要专业人员的操作,造成通量低、采样重复性差等问题。为了解决该问题,自动化的毛细管取样-质谱进样装置的开发也是大家关注的重

点。张新荣和闻路红团队^[48]建立了一个视觉伺服机器人微操作平台,在该平台上通过自动的机械化操作,对单个细胞进行顺序提取、抽吸和质谱分析。该系统消

除了操作人员之间的差异,提高了工作效率,并且通过对膀胱癌细胞的实验,验证了该系统的性能.王文会团队^[49]通过定制成像、总线控制器、流体驱动等关键模块,实现了1 pL精度体积的细胞提取和电离系统自动控制方案,达到20个细胞每小时的工作通量.与人工操作相比,该系统在一致性(~21%)、灵敏度(~28%)和成功率(高达40%)方面都展示了更好的性能.上述两种自动化装置均是对裂解后的单细胞进行取样操作,想要实现对于活细胞的直接自动化取样,研究者仍需持续探索和努力.

3 总结与展望

本综述主要聚焦于质谱成像和毛细管电喷雾这两种技术在单细胞代谢组学领域的进展,对这两种技术的创新方向进行了系统的梳理与分析.除了这两种技术之外,基于微流控系统精准控制的自动化单细胞代谢组学技术^[50,51]、基于流式质谱的高通量单细胞代谢组学技术^[52,53]也是单细胞代谢组学分析的重要分支.虽然各种技术日新月异,如何在单细胞水平对代谢物进行深度注释,开发新的数据库和算法工具以提升数据的准确性,确保研究结果的可靠性,仍是各

种技术仍需改进的方向^[54,55].此外,单细胞代谢组学与其他单细胞组学技术的联用,如单细胞蛋白组、单细胞转录组,可以为研究人员提供更加全面的细胞功能图谱,揭示代谢网络与基因表达、蛋白质修饰等之间的相互作用,也是未来极具应用潜力的发展方向^[56~59].

高代谢物覆盖率、高通量的单细胞代谢组学分析可结合同位素示踪法,精准注释肿瘤微环境的细胞异质性,揭示肿瘤细胞代谢重编程的动态过程,为肿瘤细胞的生物学特性提供新见解,为开发新的抗肿瘤策略提供新靶点.同时,利用该技术可追踪胚胎发育过程中细胞代谢的动态变化,揭示决定细胞命运的分子基础,为再生医学、干细胞治疗等领域提供理论支持和研究方向.再次,还可从单细胞水平定义不同疾病分期病人的代谢特征,挖掘代谢标志物,为疾病早期诊断提供新的线索.总之,单细胞代谢组学已在临床诊断、疾病机制研究等方向显示其潜力.随着技术的进一步发展,该技术一定能够进一步推动其在生物学和医学领域的实用性,揭示关键的生物学过程,助力新型药物研发,加速新型诊疗方法的开发,为精准医学提供更具时效性和个体化的分子代谢信息支持.

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Summary for “基于质谱分析的单细胞代谢组学研究进展”

Advances in single-cell metabolomics based on mass spectrometry

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Cellular heterogeneity, a crucial factor contributing to the diversity and complexity of biological systems, plays a prominent role in processes such as the tumor microenvironment, immune responses, and stem cell differentiation. The emergence of single-cell omics technologies, encompassing single-cell genomics, proteomics, and metabolomics, has provided powerful tools for exploring cellular heterogeneity. Metabolites, being the downstream products of gene and protein action networks, offer a direct reflection of cellular functional changes in real time. Consequently, metabolomics is regarded as the omics platform most closely aligned with biological phenotypes. Single-cell metabolomics currently enables the analysis of various small molecules, including amino acids, lipids, nucleotides, and their derivatives, to uncover metabolic heterogeneity at the single-cell level. However, the field of single-cell metabolomics faces several technical challenges, including the small size of cells, the complexity of metabolite structure identification, and the low intracellular content of metabolites. Mass spectrometry (MS) has emerged as the primary tool in single-cell metabolomics due to its high sensitivity and resolution, enabling the detection of a wide range of metabolites within individual cells and providing detailed insights into cellular metabolism and function. To address diverse research needs, single-cell metabolomics technologies based on mass spectrometry are categorized into various types. This categorization is based on factors such as spatial resolution, ionization mode, sampling method, and experimental throughput. Among all the developed methods, MS imaging and direct electrospray ionization-based MS analysis are widely employed for single-cell analysis, which will be overviewed in this article.

MS imaging provides spatial distribution information of metabolites within tissues or cells. Matrix-assisted laser desorption/ionization MS imaging (MALDI-MSI) is renowned for its simple sample preparation and rapid scanning capabilities, although its spatial resolution remains an area for improvement. New technologies, such as MALDI-2, secondary ion MS, and vacuum ultraviolet desorption ionization MS, have attained higher spatial resolution by employing advanced ionization techniques and innovative light sources. Furthermore, the integration of machine learning and advanced image analysis has enabled accurate identification of cell boundaries and molecular characterization at the single-cell level, significantly advancing the capabilities of MS imaging in single-cell metabolomics.

In situ sampling combined with direct electrospray ionization using capillary allows for the direct analysis of metabolites from living cells, offering high ionization efficiency and flexible sampling. This approach has demonstrated considerable success in the study of neuronal function, organelle metabolic profiling, and the analysis of metabolic heterogeneity in complex three-dimensional cell models. However, the sampling process requires skilled operation, and its low throughput presents a challenge. The development of automated sampling systems remains a key area of focus for further improving this technology and increasing its applicability in single-cell metabolomics research.

Despite the rapid advancements in these technologies, several challenges persist, including the need for improved annotation of metabolites at the single-cell level, the development of new databases and algorithms to enhance data accuracy, and ensuring the reliability of results. Moreover, integrating single-cell metabolomics with other single-cell omics technologies, such as proteomics and transcriptomics, holds great promise. This multi-omics approach could provide a more comprehensive map of cellular function, revealing intricate interactions between metabolic networks, gene expression, and protein modifications. This integration represents a promising direction for future research in the field of single-cell metabolomics. With ongoing advancements in single-cell metabolomics, the technology is poised to have a profound impact on biology and medicine. It will aid in uncovering key biological processes, accelerating drug development, advancing diagnostic and therapeutic methods, and providing personalized metabolic information for precision medicine, ultimately revolutionizing our understanding of cellular heterogeneity and its implications in health and disease.

cellular heterogeneity, single-cell analysis, metabolomics, mass spectrometry imaging, capillary electrospray

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