

质谱成像技术在肿瘤空间蛋白质组学研究中的进展与应用

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摘要: 肿瘤组织呈现高度空间异质性和微环境复杂性, 依靠传统转录组与蛋白质组等整体分析方法难以揭示其内部细胞互作与空间分布特征。近年来, 具备空间解析能力的空间蛋白质组学方法逐渐兴起, 其中非靶向技术可实现全景式检测, 但灵敏度和特异性相对不足; 传统靶向技术具备多重性与特异性, 但受限于通量、定量精度和分辨率。因此, 质谱成像技术应运而生, 其代表平台包括成像质谱流式、多重离子束成像、基质辅助激光解吸电离质谱成像和二次离子质谱成像等, 具备亚细胞级分辨率和多靶标同步定位能力。质谱成像平台的数据获取与分析流程主要包括肿瘤样本标准化制备、标签标记与多重染色、激光或离子束逐点成像、信号预处理、基于机器学习或深度学习的细胞分割与表型注释, 以及一阶、二阶与高阶空间结构分析等。大量临床研究表明, 质谱成像技术可揭示免疫细胞、肿瘤细胞与基质细胞之间的空间互作模式, 识别与预后或治疗反应相关的空间微域, 并为肿瘤空间标志物的发现和疗效评估提供依据, 从而助力精准治疗策略优化。未来, 需要优化高通量采集流程、整合多平台空间组学数据、联用动态时空成像技术, 并构建人工智能驱动的统一分析框架, 实现肿瘤微环境的多模态检测, 进一步推动临床应用转化。

关键词: 肿瘤异质性; 肿瘤微环境; 空间蛋白质组学; 质谱成像 (MSI); 细胞互作; 临床转化

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Advances and Applications of Mass Spectrometry Imaging in Tumor Spatial Proteomics Research

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Abstract: Tumor tissues exhibit pronounced spatial heterogeneity and a complex microenvironment, making it difficult for traditional bulk-level analytical approaches such as transcriptomics and

proteomics to capture the intricate cellular interactions and spatial distribution patterns within tumors. In recent years, spatial proteomics methods with spatially resolved capabilities have emerged. Nontargeted techniques allow for panoramic molecular detection but are often limited by insufficient sensitivity and specificity, while conventional targeted approaches provide multiplexing capacity and specificity but remain constrained by throughput, quantitative accuracy, and spatial resolution. To overcome these limitations, mass spectrometry imaging (MSI) has been developed, with representative platforms including imaging mass cytometry (IMC), multiplex ion beam imaging (MIBI), matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI), and secondary ion mass spectrometry (SIMS) imaging. These platforms offer unique advantages by achieving subcellular-level resolution, enabling simultaneous multi-target quantification, and delivering robust quantitative performance, thereby addressing key challenges in spatial proteomics research. The data acquisition and analysis workflow of MSI typically involves standardized preparation of tumor samples, target-specific labeling and multiplex staining, point-by-point imaging through laser or ion beams, signal preprocessing, machine learning or deep learning-based cell segmentation and phenotype annotation, and higher-order spatial structure analyses. Through these processes, antibody-based MSI enables the detailed mapping of cellular architecture and interactions within tumor tissues. Clinical studies have demonstrated its ability to uncover spatial interaction networks among immune cells, tumor cells, and stromal cells, as well as to identify spatial microdomains associated with prognosis and treatment response. These findings not only contribute to the discovery of tumor spatial biomarkers but also provide valuable evidence for treatment evaluation, ultimately facilitating the optimization of precision oncology strategies. Looking ahead, further advancements will require the optimization of high-throughput data acquisition workflows, integration of multi-platform spatial omics data, incorporation of dynamic spatiotemporal imaging techniques, and the development of unified artificial intelligence-driven analytical frameworks. Together, these innovations will enable multimodal characterization of the tumor microenvironment and accelerate the translation of MSI into clinical applications, thereby advancing personalized cancer diagnosis and therapeutics.

Key words: tumor heterogeneity; tumor microenvironment; spatial proteomics; mass spectrometry imaging (MSI); cell-cell interactions; clinical translation

近年来,尽管肿瘤分子机制解析和治疗策略不断进步,但肿瘤微环境的复杂性与肿瘤组织结构的不均质性仍是制约精准治疗的关键难题^[1-2]。肿瘤组织由多样化的细胞群体与非细胞成分构成,其空间分布不均质性不仅影响肿瘤生物学演进,更显著干扰药物反应与治疗效果^[3-5]。传统组学技术(如转录组学和蛋白质组学)多采用整体组织匀浆分析,忽略了肿瘤内部关键空间信息,难以真实反映细胞之间的互作状态与组织微环境背景^[6]。因此,发展具备空间分辨能力的组学技术成为突破肿瘤研究瓶颈的关键路径。空间蛋白质组学因能够直观呈现蛋白质在组织中的表达状态与空间定位,尤其适用于揭示肿瘤内

部复杂的空间特征,正逐步成为该领域的研究热点^[7]。在单细胞甚至亚细胞尺度上获取空间多重蛋白质图谱,不仅有助于深入理解肿瘤生态系统的组织构型,也为精准治疗中的标志物发现与疗效预测提供了重要支撑^[8-11]。

空间蛋白质组学研究方法可分为非靶向与靶向2大类^[11]。非靶向方法基于质谱直接检测组织样本的内源性分子,其核心优势在于免标记检测能力及未知蛋白质筛选潜力,代表性技术包括基质辅助激光解吸/电离质谱成像(matrix-assisted laser desorption/ionization mass spectrometry imaging, MALDI-MSI)和激光显微切割-液相色谱质谱(laser capture microdissection-

liquid chromatography-mass spectrometry, LCM-LC-MS)等^[12-13]。MALDI-MSI通过基质吸收激光能量使样品分子解吸电离,可直接在组织切片上原位获取生物分子的空间分布信息^[14]。相较于其他质谱成像技术, MALDI-MSI借助基质辅助不仅可促进大分子高效解吸与离子化,结合飞行时间(time of flight, TOF)分析器更可拓展质量检测范围,因此尤其适用于蛋白质及多肽分析^[15]。然而,该技术的空间分辨率存在一定局限(通常为10~50 μm),主要用于解析多细胞尺度的分子表达^[16-17];同时,由于非靶向检测信息量庞大、背景信号复杂且耗时较长,导致单细胞原位成像实际应用难度较高^[18-19]。与MALDI-MSI原位分析不同, LCM-LC-MS需通过选择性切割特定组织区域对切割区域进行酶解处理后结合液相色谱-串联质谱,实现目标区域蛋白质分子的鉴定与定量^[20-21]。该方法的优势在于激光显微切割可精确定位目标区域,而液相色谱-串联质谱能够提供高灵敏度和高检测深度的蛋白质覆盖范围^[20,22]。然而,该技术的操作流程较为复杂耗时^[23];同时,切割区域的蛋白质组数据仅能以整体平均值呈现,难以解析区域内部的空间异质性^[24]。

尽管存在上述局限,非靶向空间蛋白质组学方法凭借免标记特性与广谱检测能力,在空间分子图谱构建——尤其是未知蛋白的发现性研究与空间分布解析中仍具有不可替代性。然而,临床研究样本队列大、组织异质性强、蛋白复杂度高特征放大了其固有瓶颈:首先,非靶向方法操作流程长、数据规模大,制约了大规模队列数据处理效率^[25];其次,离子抑制等基质效应与背景噪声在异质环境中被加剧,导致检测灵敏度与定量精度下降^[26];此外,翻译后修饰等蛋白质异构体在高复杂度体系中的存在进一步增加了数据库匹配不确定性,具有升高假阳性/假阴性风险^[27]。这些特征使该方法更适用于深度机制探索等基础研究场景,而在临床转化所需的高通量验证、多重靶点分析及规模化应用中存在制约。

与之互补,靶向空间蛋白质组学方法通过抗原-抗体特异性结合的鉴别体系,结合荧光检测、核酸编码测序以及质谱检测系统,展现出目标蛋白质分子精准靶向识别、高灵敏度检测、多参数同时检测、细胞及亚细胞级分辨率原位成像等优势^[28]。这些特性能够提高疾病诊断与疗效预

测的准确性,整合多维度分子信息以支持个体化治疗决策,揭示组织微环境中靶点的空间病理特征,在临床转化中具有广泛适用性。靶向空间蛋白质组学研究方法根据信号读出机制(readout)可分为3类:第一类是基于荧光或化学显色的免疫染色技术,如多重免疫荧光和多重免疫组化,其操作相对成熟,但标记通量受限^[29-30];第二类是基于抗体-DNA条码并结合信号放大的扩增检测平台,代表性方法包括索引共检测(co-detection by indexing, CODEX)^[31]、迭代漂白扩展多路复用(iterative bleaching extends multiplexing, IBEX)^[32]以及数字空间解析(digital spatial profiling, ISP)平台^[33]等,可实现较高的多重性与特异性,但在信号定量精确度及亚细胞空间解析能力方面存在局限。相比之下,第三类基于质谱的成像技术不仅突破了光学检测中光谱重叠、通道受限的瓶颈,还兼具亚细胞级空间分辨率、原位多靶标同步检测能力、良好的定量性能及与多组学数据整合的高度兼容性,在空间蛋白质组学研究中展现出显著优势^[11,34-35]。这类技术已成为推动肿瘤空间蛋白质组学深入研究的关键工具,也是本文后续重点讨论的方向。

基于此,本文聚焦质谱成像核心技术,系统梳理其在肿瘤空间蛋白质组学研究中的进展。首先,解析其原理与代表性平台,概述数据采集与分析流程;然后,阐述其在肿瘤微环境空间相互作用研究中的应用,包括免疫细胞间、免疫-肿瘤细胞及免疫-基质互作网络解析;最后,探讨该技术在临床生物标志物发现、治疗响应监测及精准治疗决策中的应用前景与挑战,以期质谱成像技术的临床转化提供参考。

1 质谱成像核心技术及主要技术路线

1.1 质谱成像核心技术

为满足对组织中蛋白质空间分布的高维度、高分辨率检测需求,多种质谱成像技术相继发展,主要包括成像质谱流式、多重离子束成像、基质辅助激光解吸/电离质谱成像及二次离子质谱成像等,这些技术已成为空间蛋白质组学研究的重要技术支撑,示于图1。

成像质谱流式(imaging mass cytometry, IMC)是在质谱流式细胞术(mass cytometry)基础上发展而来的空间蛋白质成像平台,融合了金属标记

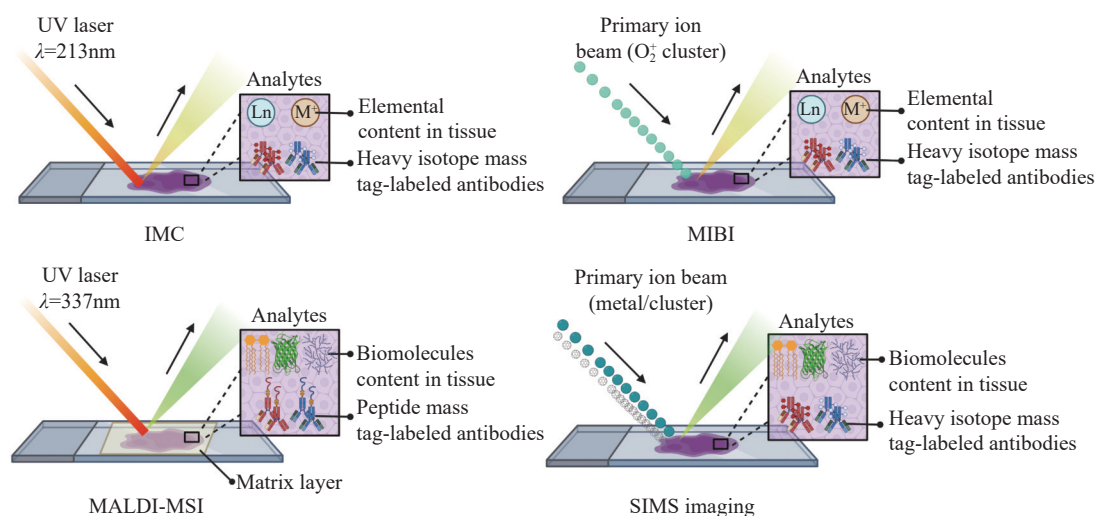


图1 质谱成像核心技术

Fig. 1 Representative core technologies of mass spectrometry imaging

免疫染色与电感耦合等离子体质谱技术,具备高通量、多参数和单细胞空间分辨率等特点^[10]。其基本原理是利用稀土金属同位素标记的抗体对组织切片中的靶蛋白进行标记,再经激光逐点烧蚀释放金属标签,随后通过电感耦合等离子体电离并进入飞行时间质谱检测器,实现多通道同步检测^[10,36-37]。现有的IMC空间分辨率约1 μm ,可在单细胞水平精细描绘组织结构^[10]。与传统荧光染色相比,金属同位素标签具有信号稳定、光谱重叠少、背景噪声低等优点,显著提升了检测通量与灵敏度^[38-41]。目前,IMC在单次实验中能同步检测50种以上蛋白质分子,理论检测通道数可进一步扩展至100种以上^[10,37,41-42]。然而,该方法受限于抗体使用导致的多重挑战:首先,抗体来源显著影响检测可靠性,不同供应商及克隆号抗体的抗原识别特异性可能存在差异^[43-44];同时,国产抗体对细胞膜表面或高表达抗原的检测表现稳定,但对胞浆或细胞核内功能性蛋白质的检测性能仍需提升,导致部分靶点依赖高价进口试剂,间接推升检测成本;进一步地,由于金属偶联效率在不同抗体间存在差异,且偶联修饰可能改变抗体构象并产生空间位阻效应,进而影响抗原结合位点的可及性与亲和力,对免疫染色结果的灵敏度和重现性造成潜在影响^[36];另外,实验前期需要进行详尽的抗体准确性验证与浓度滴定,以避免假阳性或假阴性信号的产生^[43]。为克服这些问题,在实验前期需投入大量时间和资源进行抗体筛选与优化,这在一定程度上增加了

实验设计的复杂性和操作成本。值得注意的是,IMC的应用并不仅限于抗体介导的蛋白质组学检测,还同样适用于外源性金属药物、内源性微量元素、元素标记的小分子探针等物质的空间成像,在药物作用机制与元素代谢研究中具有独特价值^[45-49]。

在基于激光烧蚀的IMC平台广泛应用的同时,另一类以离子束为激发源的质谱成像技术——多重离子束成像(multiplexed ion beam imaging, MIBI)亦成为空间蛋白质组学的重要补充手段。MIBI同样采用稀土金属同位素标记抗体对靶蛋白进行识别,通过氧簇离子束逐点溅射组织切片,再利用飞行时间质谱检测溅射出的金属离子信号^[34,50-51]。与IMC相比,MIBI具备更高的表面解吸能力,其空间分辨率可达约200~300 nm,在亚细胞尺度的组织结构成像中更具优势^[18,52]。此外,MIBI不仅可检测高质量数金属标记抗体,还具备检测低质量数内源性元素(如Ca、P、Fe、Zn等)的能力,这一特性使MIBI在组织内离子代谢、金属元素富集及细胞内稳态研究中具有独特的技术潜力^[8]。然而,由于其图像采集速度较慢、通量较低,限制了大样本组织的快速成像,目前主要适用于对分辨率需求优先的应用场景^[9,51,53]。当前,MIBI已广泛应用于肿瘤微环境中细胞表型与空间构筑的可视化研究,尤其适用于边缘浸润区、免疫排斥区域等肿瘤组织内精细异质结构的解析场景^[8,53-54]。作为与IMC互补的空间蛋白质组学工具,MIBI通过更

高的空间分辨率与内源性元素检测能力,在多尺度空间生物学研究中发挥独特作用。

除 IMC 与 MIBI 外, MALDI-MSI 同样在空间蛋白质组学研究中展现出独特的技术价值^[55]。MALDI-MSI 通过在组织表面覆盖基质,使基质吸收激光能量并将其转移至分析物,同时基质作为电荷供体促进电离,最终实现生物分子的温和解吸与检测^[12,18,56-57]。该方法无需探针即可实现对代谢物、脂质、肽段及部分蛋白的非靶向空间分析^[58-59]。然而,由于对生物大分子的电离效率较低, MALDI-MSI 主要适用于低分子量蛋白质(≤ 25 ku)或原位酶解肽段的检测,对大分子及低丰度蛋白的检测灵敏度有限^[60-61]。为解决这一难题,基于抗体的标签化检测手段逐渐兴起。光裂解质谱标签(photo-cleavable mass tags)由光裂解连接子和质量报告单元组成,当携带该标签的抗体与目标蛋白质结合后,可经紫外照射断裂释放特定 m/z 的肽段,从而实现高特异性和高通量的大分子蛋白质空间成像,现已应用于乳腺癌等肿瘤组织中蛋白质与代谢产物共定位分析^[12,62]。常规 MALDI-MSI 的空间分辨率约为 $10\sim 50\ \mu\text{m}$ ^[16-17],随着光学系统与离子化技术的进步(如高数值孔径透镜、MALDI-2 激光辅助电离等),其分辨率已提升至亚微米水平,最高可达约 $600\ \text{nm}$ 。这种提升使 MALDI-MSI 在脑组织等精细结构的多组学成像中展现出显著优势,已成为肿瘤空间多组学研究,特别是代谢物与蛋白质共定位分析的重要工具^[18,63-64]。

相较于 MALDI-MSI 适用于大分子分析的软电离特性,二次离子质谱(secondary ion mass spectrometry, SIMS)成像通过高真空离子束轰击样本高效生成小分子或离子,更适用于代

谢物、脂质片段及元素的空间分析^[65-66]。生物样品分析中常用的 SIMS 离子源包括金属/原子离子束(metal/atomic ion beams)和离子簇束(ion cluster beams)等^[67-68]。金属/原子离子束(如 Ga^+ 、 In^+ 等)直径小、稳定性高,具有极高的空间分辨率($< 100\ \text{nm}$),但冲击能量集中,常导致严重的分子碎裂,多适用于元素与小分子分析^[69-70];离子簇束(如 Ar_n^+ 、 C_{60}^+ 等)能量密度较低,轰击过程中引入的损伤较小,可在保持一定空间分辨率($1\sim 20\ \mu\text{m}$)的同时提高对脂质、肽段等分子的检测能力^[67,70-71]。近年来,新型离子源的出现进一步改善了 SIMS 的离子化过程,其中 $(\text{H}_2\text{O})_n^+$ 可以显著增强生物分子的离子化效率,结合冷冻水合样品制备,可在约 $1\ \mu\text{m}$ 分辨率下增强脂质、代谢物及肽段信号,从而推动 SIMS 成像在空间多组学中的应用^[72-73]。然而,高能轰击引发生物大分子碎裂的固有局限仍阻碍 SIMS 成像对完整蛋白质的空间解析。为此,研究者创新性地采用镧系金属-抗体标记策略,将蛋白质检测转化为高稳定性金属离子信号进行检测。在现有肿瘤研究中,已在使用 C_{60}^+ -SIMS 特异性检测镧系金属标签的同时,使用 $(\text{H}_2\text{O})_n^+$ -GCIB-SIMS 采集代谢物信号,实现特异性蛋白质-代谢分子空间共定位,拓展了 SIMS 成像在空间多组学联合分析中的应用^[74-75]。

综上,IMC、MIBI、MALDI-MSI、SIMS 成像等方法构成了当前质谱成像核心技术,其在电离方式、空间分辨率和应用范围等方面的差异列于表 1。

1.2 质谱成像平台数据获取流程与代表性成像系统

作为空间蛋白质组学研究的重要手段,质谱

表 1 质谱成像核心技术对照表

Table 1 Comparison of core technologies in mass spectrometry imaging

技术 Technology	电离方式 Ionization method	常规空间分辨率* Typical spatial resolution	检测目标 Detection target	适用领域 Application field
IMC	电感耦合等离子体	$\sim 1\ \mu\text{m}$	标记蛋白分子 ^[37,41] 、 免标记金属离子 ^[49]	单细胞空间蛋白质组学 ^[76-78]
MIBI	一次离子束溅射	$200\sim 300\ \text{nm}$	标记蛋白分子 ^[9,79] 、 免标记金属离子 ^[8]	单细胞空间蛋白质组学 ^[8,79]
MALDI-MSI	基质辅助激光解吸	$10\sim 50\ \mu\text{m}$	免标记与标记蛋白分子 ^[12,62] 、 免标记小分子 ^[58]	蛋白与代谢组学分析 ^[63-64]
SIMS imaging	一次离子束溅射	$< 100\ \text{nm}/1\sim 20\ \mu\text{m}$	标记蛋白分子 ^[74-75] 、 免标记小分子与金属离子 ^[70]	空间多组学联合分析 ^[74-75]

注:在优化实验条件及特定仪器配置中可进一步提升空间分辨率

成像平台的数据获取通常包括以下流程, 示于图 2a。首先, 构建具有代表性的患者队列, 结合临床特征和疾病分型选取肿瘤样本, 为后续分析提供生物学基础^[80]; 随后, 将肿瘤组织制备成组织切片或组织芯片, 便于多样本的高通量处理与标准化分析; 接着, 利用金属同位素标记抗体对目标蛋白质进行多重染色, 在单张切片上实现数十种蛋白质的同时标记与识别^[76,81]; 随后, 通过激光或离子束逐点消融汽化组织, 结合质谱检测实现高灵敏度、无光谱串扰的多蛋白定量^[82]; 最终, 借助计算方法将信号数据重建为空间图谱, 呈现细胞类型、功能状态及其在组织中的空间分布, 为解析肿瘤微环境结构、细胞互作关系及其与临床结局的关联提供关键信息^[83-84]。

以 IMC 为例, 目前广泛应用的单细胞空间蛋白质组学成像平台是 Standard BioTools 公司的 Hyperion™ 系列, 示于图 2b。该系统利用金属标记抗体结合激光烧蚀质谱技术, 通过高维空间图像重建, 可在单张组织切片上同时检测数十种蛋白质的共定位分布, 实现细胞微环境的高通量、多参数解析^[85]。最新一代 Hyperion XTi™ 在扫描成像速度、检测灵敏度和自动化程度等方面较前代显著提升。与传统的 Hyperion™ (200 Hz) 和 Hyperion+™ (400 Hz) 相比, Hyperion XTi™ 以 800 Hz 的高速成像能力显著缩短了扫描时间。此外, 该系统引入的预览模式和组织扫描模式能够在短时间内完成组织切片样本扫描, 快速识别和选取目标区域, 进一步提升成像效率和精确度^[86-87]。IMC 系统为单细胞空间蛋白质组学研究提供了

可靠平台, 尤其适用于在大型临床队列中开展肿瘤微环境高维参数分析及细胞空间互作模式的精细解析。

1.3 质谱成像平台数据分析流程

质谱成像数据分析的主要流程示于图 3。其首要环节为信号预处理, 主要包括溢出校正 (spillover correction)、去噪 (denoising) 及热点去除 (hot-spot removal)。溢出校正通过数学建模修正因金属同位素纯度不足、氧化产物形成 (如¹⁶O 引起的 +16 u 偏移) 及质谱分辨率限制 (+1 u 信号串扰) 所致的通道间污染, 提升定量准确性^[40,88-89]。去噪通过滤波与背景扣除增强信噪比^[90]; 热点去除则剔除由抗体聚集等异常引起的非组织特异性高强度信号, 以减少分析误差^[91-92]。随后, 通过评估抗体的特异性和敏感性确保数据质量^[41]。在多重染色环境下, 抗体间的交叉反应、非特异性结合等因素可能引入系统性误差。通过系统的抗体验证, 能有效提升数据的特异性、可靠性和可重复性, 为后续空间和功能分析奠定基础^[43,93-94]。

细胞分割是空间解析中的关键步骤。肿瘤组织中细胞异质性高且形态复杂, 因此分割精度将直接影响下游分析的准确性。当前常用的细胞分割方法主要有两类: 一类是基于监督学习的像素级分割算法, 例如 steinbock 利用随机森林对图像像素进行分类, 以实现细胞核与胞质的分割^[95]; 另一类是基于深度学习的自动分割方法, 如 DeepCell 中的 Mesmer 模型通过 TissueNet 等大规模标注数据集, 实现对复杂组织切片图像的高精度分割^[96]。商品化软件 VISIOPHARM 则整

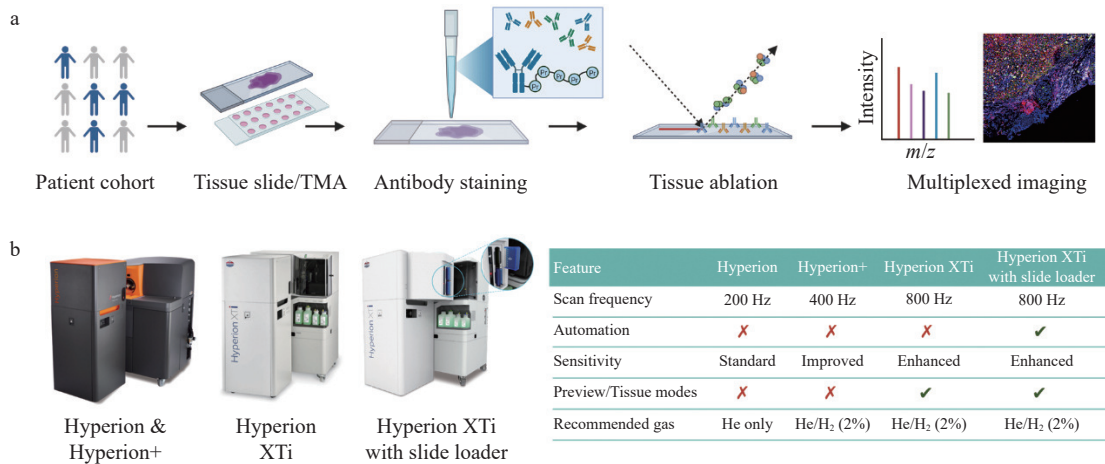


图 2 质谱成像的数据获取流程与 IMC 成像系统

Fig. 2 Data acquisition process of mass spectrometry imaging and the IMC imaging system

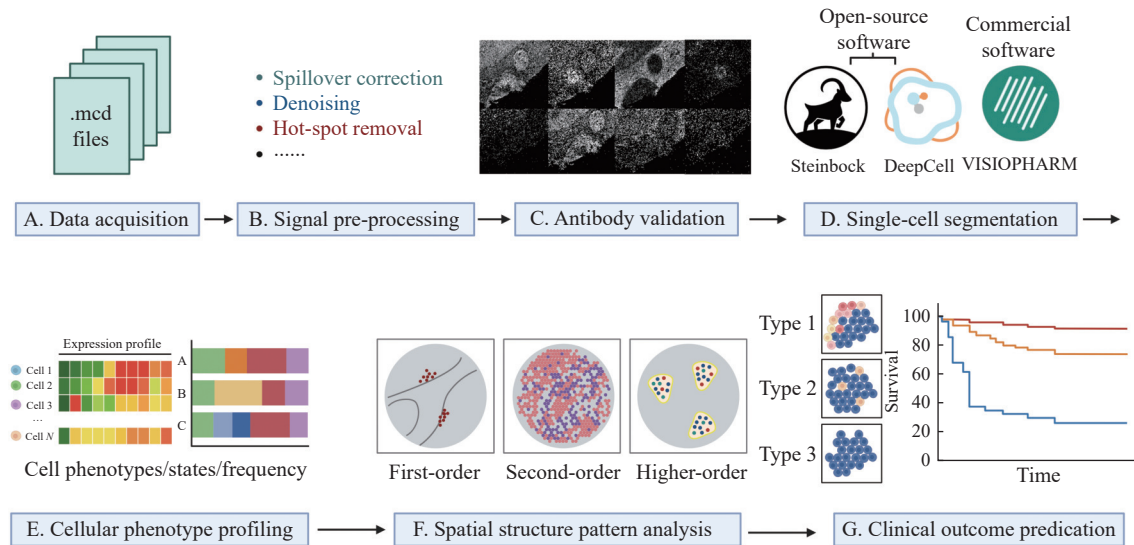


图3 质谱成像数据分析流程

Fig. 3 Analysis workflow of mass spectrometry imaging data

合机器学习与深度学习技术,支持多种成像平台与数据格式,具备强大的定量分析功能,广泛应用于大量临床场景^[97]。在完成细胞分割的基础上,细胞表型分析用于解析不同细胞类型的蛋白表达特征,明确其在肿瘤微环境中的组成比例、状态与功能,为后续空间建模奠定基础^[82,98]。这一过程依赖于优化且合理的抗体面板策略与单细胞分割精度,常结合无监督聚类、标记物组合等方法对免疫细胞、肿瘤细胞、成纤维细胞等主要类型进行注释^[10]。

空间结构与邻域分析进一步刻画细胞的空间分布格局及相互作用关系,根据解析维度的不同,可分为一阶空间分析(分析单一细胞类型的分布)、二阶空间分析(量化成对细胞间的空间互动)和高阶空间分析(识别多细胞群体构成的功能模块)^[34]。

一阶空间分析聚焦于特定细胞类型在组织内的空间分布,揭示其与关键解剖结构(如肿瘤边界、血管或坏死区)之间的空间关系,从而反映肿瘤微环境结构特征与空间分布偏好。例如, Keren等^[8]利用 MIBI 技术对三阴性乳腺癌组织成像,发现 B 细胞在肿瘤-免疫边界显著缺失,且该区域肿瘤细胞组蛋白修饰呈现空间梯度,提示肿瘤-免疫边界区域可能存在免疫细胞趋化倾向或表观遗传屏障;在结直肠癌研究中, Hartmann等^[79]应用 IMC 与 MIBI-TOF 成像发现 GLUT1⁺、PKM2⁺的代谢高活性 CD8⁺ T 细胞在肿瘤血管周

围及肿瘤边界呈双峰分布,提示免疫细胞定位可能受代谢资源和组织屏障共同调控。该类分析为理解肿瘤异质性与局部免疫微环境等提供了基础信息。

二阶空间分析关注成对细胞之间的空间邻近性,量化其协同或排斥模式,适用于探究细胞互动强度、免疫逃逸机制及治疗响应效果等问题^[99]。在三阴性乳腺癌中, Keren等^[8]通过量化肿瘤细胞与免疫细胞间的混杂程度对患者预后进行判断。结果显示,肿瘤-免疫细胞混合程度高的样本中免疫细胞与肿瘤细胞交错分布,呈现免疫耗竭状态且预后不良;而混合程度低的样本免疫结构清晰,生存结局更佳。在非小细胞肺癌中, Cords等^[77]发现不同 CAF 亚型与免疫细胞存在特异性邻近模式,如 ifnCAF 常与 IDO⁺ T 细胞共定位,而 mCAF 富集区域则排斥免疫细胞。除肿瘤与免疫细胞之间的关系外,二阶空间分析还可揭示不同谱系细胞之间的功能性共定位模式。在结直肠癌的多重成像研究中, Hartmann等^[79]发现,具备相似代谢标志物表达的不同类型细胞也倾向于空间上相互邻近,形成代谢高活性细胞群落,提示微环境中某些代谢资源或信号可能驱动了不同谱系细胞在空间上的共聚,构成特定的“代谢生态位”。总体而言,二阶空间分析能够定量刻画细胞间的空间关联和相互作用频率,对理解免疫排斥或免疫协同作用等肿瘤微环境特征及其对预后的影响具有重要意义。

高阶空间分析进一步识别由2种以上细胞类型组成的局部多细胞功能单元, 捕捉复杂的互作网络和空间生态结构。常见的高阶空间分析包括细胞邻域(cellular neighbourhood)、细胞群落(cellular community)、细胞微域(milieus)等, 为研究肿瘤微环境的异质性提供了独特视角和研究框架。细胞邻域分析是一种基于无监督聚类方法的高阶空间分析技术, 在预先设定的局部半径或相邻细胞数范围内, 如果某一区域富集某几种特定细胞类型, 则将该区域定义为“细胞邻域”^[100]。在肺腺癌与其转移灶的研究中, Cords等^[97]基于IMC数据, 通过K-means聚类定义细胞邻域, 识别出富含B细胞与CD4⁺T细胞的免疫活化型邻域, 这种免疫活化型邻域与免疫治疗反应密切相关, 提示其具备预测免疫治疗反应的潜力。高阶分析的另一种方法是将组织表示为细胞-细胞相邻关系网络, 通过算法寻找高度连接的“细胞群落”。Jackson等^[101]利用这种方法, 在乳腺癌成像质谱数据中发现了若干由肿瘤细胞、基质成纤维细胞、血管内皮细胞和T细胞等多种细胞紧密相互作用形成的群落结构。除基于细胞识别群落外, 高阶分析还可通过特定的功能性分子定义“细胞微域”。Hoch等^[76]在黑色素瘤中提出“趋化因子环境”概念, 发现不同趋化因子表达斑块(如CXCL9、CXCL13、CXCL12)可分别驱动CD8⁺T细胞、B细胞和成纤维细胞等在局部空间内的聚集, 形成具有特异组成和功能的微环境生态位。综上, 高阶空间分析通过挖掘多个细胞类型共同构成的空间模式, 揭示了肿瘤组织中更复杂的组织结构和细胞间互作网络。

总体而言, 基于质谱成像的空间结构分析体系, 从局部分布特征到细胞互作模式, 再到功能性空间单元的识别, 形成了一套层层递进的解析框架, 为揭示肿瘤组织的空间生物学规律提供了强有力的工具。未来, 随着成像精度与分析算法的不断提升, 空间蛋白质组学将持续推动肿瘤免疫微环境研究的深化, 并在精准诊疗与靶点开发中发挥更大作用。

2 质谱成像技术在肿瘤空间互作研究中的应用

肿瘤是由肿瘤细胞、异质性免疫亚群及动态变化的间质成分共同构建的三维生态系统, 其

空间架构对肿瘤演进、转移级联反应及免疫逃逸机制具有决定性调控作用。随着质谱成像等高通量空间技术的进步, 研究者能够在单细胞分辨率下, 精确探究肿瘤组织中不同细胞类型的空间分布及其相互作用关系。这些技术为深入研究免疫细胞和间质成分在肿瘤微环境中的作用提供了新视角, 特别是通过解析免疫细胞-肿瘤细胞-基质细胞的“空间对话”机制, 系统阐明其对肿瘤进展的影响, 示于图4。

2.1 免疫细胞间相互作用

骨髓源性免疫细胞与淋巴系细胞的跨谱系通讯构成了抗肿瘤免疫应答的重要调控网络, 质谱成像研究进一步揭示了这类跨谱系细胞空间分布及功能的关联性。研究发现, 免疫高浸润型肿瘤中常见髓系细胞与T细胞的紧密共定位。Xiao等^[102]在黑色素瘤研究中发现, 免疫治疗应答者的肿瘤中HLA-DR⁺巨噬细胞和树突状细胞富集, 并与CD8⁺T细胞共存; 而非应答者则富含IDO-1⁺/VEGF⁺的抑制性髓系细胞, 后者在空间上与T细胞缺乏相关性。在脑胶质瘤中, Karimi等^[78]利用IMC构建了细胞空间互动网络, 发现生存期较长的患者组织样本中M1型巨噬细胞与辅助性T细胞之间呈现高频共定位, 同时这些M1型巨噬细胞伴随CD40高表达, 提示其在诱导T细胞激活中的作用; 相比之下, 缺乏这种空间结构的患者多表现为免疫排斥表型, T细胞浸润稀少, 生存时间显著缩短。在非小细胞肺癌中, Enfield等^[103]观察到CD8⁺T细胞与CD163⁺巨噬细胞之间的高频共定位与新抗原负荷升高显著相关, 提示这些髓系细胞可能不仅介导T细胞的募集, 还通过空间邻近调控其功能活性, 从而共同塑造特异性抗肿瘤免疫反应。

同属髓系的不同细胞类型之间也存在复杂的空间关系, 这种髓系内的互作直接影响免疫微环境的功能状态。Keren等^[8]在三阴性乳腺癌中观察到, 树突状细胞与中性粒细胞可形成“先天免疫热点”, 展现出显著的同类聚集特征。在胶质母细胞瘤中, M1型巨噬细胞与M1小胶质细胞或中性粒细胞的共聚显著富集于长期生存者, 提示其可能增强局部免疫活性; 而在短期生存的胶质瘤患者中, M2型巨噬细胞富集, 并表达CD206⁺、CD39⁺等免疫抑制分子, 形成“冷”免疫微环境, 提示不良预后^[78]。此外, 髓系细胞之间

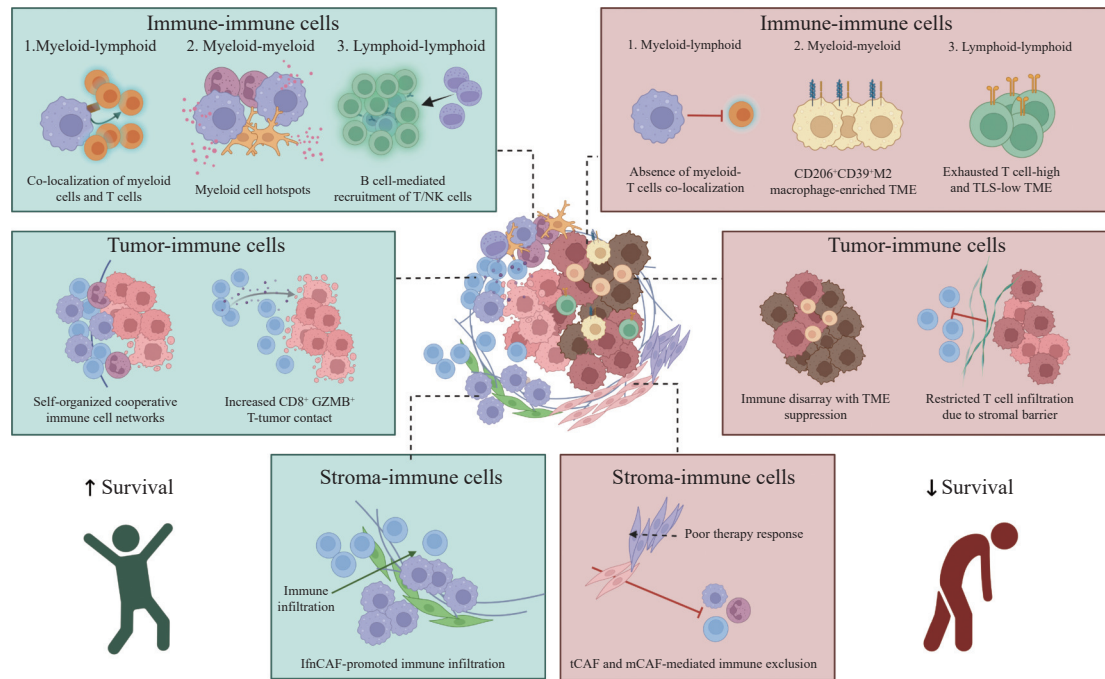


图4 质谱成像技术在肿瘤空间互作研究中的应用

Fig. 4 Applications of mass spectrometry imaging in tumor spatial interaction studies

也通过分泌因子相互影响;例如,肿瘤相关巨噬细胞分泌的CCL2可招募单核细胞,进一步补充髓系细胞数量^[76]。上述研究表明,髓系细胞既可通过物理聚集增强局部效应,也可通过分泌因子链式招募更多髓系细胞,从而协同行动以塑造肿瘤免疫微环境。

肿瘤内淋巴细胞之间的空间关系同样是决定抗肿瘤免疫反应强弱的重要因素。Danenberg等^[98]对乳腺癌的高通量IMC分析鉴定出部分肿瘤核心区域存在聚集的B细胞簇。在三阴性乳腺癌及黑色素瘤中,同样发现淋系细胞(B细胞、T细胞、NK细胞)可通过空间协同形成高度组织化的免疫网络。B细胞作为核心与CD4⁺T细胞互作,后者通过辅助信号激活B细胞功能,而活化的B细胞可招募T细胞及NK细胞,形成类二级淋巴器官的三级淋巴结构,以增强局部抗肿瘤应答。相对地,不良预后患者的组织中,三级淋巴结构数量显著减少,耗竭表型T细胞(PD-1⁺、TOX⁺)在空间上高度富集,且CD8⁺T细胞缺乏与其他淋系细胞的空间协同,形成功能孤立的免疫排斥表型^[8,76,78]。在肺腺癌中的研究进一步印证了该现象:Cords等^[104]利用IMC结合无监督K-means聚类发现,肾上腺转移灶中免疫活化型细胞邻域显著富集,表现为B细胞与CD4⁺

T细胞共定位及高密度三级淋巴结构,此类邻域与免疫治疗响应密切相关;而脑、肝转移灶中此类活化邻域显著缺失,提示肿瘤内B细胞与T细胞的空间共定位与抗肿瘤免疫应答增强、临床预后改善密切相关。淋巴细胞之间的另一重要互作是效应性T细胞与调节性T细胞(Treg)的空间关系。Cole等^[105]通过IMC对接受KRAS抑制剂治疗的肺癌患者组织进行解析,发现Treg往往聚集于肿瘤周边,削弱了治疗引发的免疫反应;而通过干预减少Treg后,CD8⁺T细胞对肿瘤细胞的杀伤功能明显增强。此外,一些特殊淋巴细胞亚群也通过空间协作放大免疫效应。Yi等^[106]对胰腺癌肝转移的研究表明,活化的iNKT细胞可促进NK细胞和CD8⁺T细胞在转移灶中的累积,从而改善抗肿瘤免疫微环境。基于IMC的多组学整合分析揭示,黑色素瘤中富集的大量增殖性效应记忆CD8⁺T细胞是免疫检查点抑制剂治疗应答的关键生物学特征^[107]。这些跨癌种证据表明,肿瘤组织中淋巴细胞之间的空间协同关系,特别是B细胞与CD4⁺T细胞形成的三级淋巴结构是驱动有效抗肿瘤免疫反应和预测免疫治疗响应的关键空间特征。

2.2 免疫细胞-肿瘤细胞相互作用

质谱成像技术同样能够在单细胞水平解析

肿瘤免疫细胞与肿瘤细胞的空间分布及相互作用,揭示免疫治疗应答机制的差异。研究表明,免疫细胞能否深入并贴近肿瘤细胞是影响疗效的关键因素,单纯计算浸润细胞数量不足以预测结果,必须将空间定位纳入考量^[101,108-109]。在三阴性乳腺癌研究中,Keren等^[8]利用MIBI系统性分析肿瘤免疫微环境,依据免疫细胞与肿瘤细胞的空间分布模式,将肿瘤组织划分为冷肿瘤(免疫细胞极度稀少)、混合型(免疫细胞与肿瘤细胞广泛混杂分布)和分隔型(免疫细胞聚集于肿瘤边缘,与肿瘤细胞形成清晰的空间分离)3类;在此基础上,进一步提出了“混合评分”(mixing score)以量化免疫细胞与肿瘤细胞间的空间接触程度。混合评分高的肿瘤被定义为高混合度,表现为免疫细胞与肿瘤细胞在组织中高度交错分布。然而,这种“接近”并不意味着有效免疫反应,高混合度肿瘤中常伴随PD-L1和IDO在肿瘤细胞中的高表达、CD4⁺T细胞耗竭标志物上调,提示免疫抑制微环境的形成,并与患者不良预后密切相关。相对而言,低混合度肿瘤中,免疫细胞倾向于形成自组织的协同网络结构,具有更强的免疫效应功能,通常反映更良好的生存预后。后续研究进一步验证了免疫细胞与肿瘤细胞空间邻近性的功能意义。Wang等^[45]通过IMC技术分析三阴性乳腺癌患者在接受免疫检查点抑制剂治疗前后的组织样本,发现治疗应答者中CD8⁺GZMB⁺T细胞与肿瘤细胞之间的直接空间接触显著增加,而非应答者则缺乏这一现象。这表明免疫细胞能否真正接触并作用于肿瘤细胞,是免疫治疗成功的关键前提。类似地,Enfield等^[103]基于非小细胞肺癌组织的空间图谱构建发现,免疫“热”型肿瘤中,T细胞和巨噬细胞可深入肿瘤巢,与肿瘤细胞直接接触形成免疫激活区域;而在“冷肿瘤”或“排斥型”中,CD8⁺T细胞往往被周围 α SMA⁺成纤维细胞形成的物理屏障阻隔,难以到达肿瘤细胞附近,显著削弱免疫杀伤效应。其他多癌种的成像质谱研究同样证明,肿瘤细胞与免疫细胞的空间邻近和接触程度显著影响免疫治疗的成败:当免疫细胞能够深入肿瘤并与肿瘤细胞直接接触时,可引发有效的免疫清除;反之,若免疫细胞被实体屏障隔离或肿瘤通过缺失抗原呈递等方式逃避免疫侦测,则疗效不佳^[110-111]。综上,质谱成像分析为解释

不同患者免疫治疗反应差异提供了重要依据,并提示通过消除物理屏障、促进有益的细胞邻近接触等手段,有望提高免疫治疗效果。

2.3 免疫细胞-基质细胞相互作用

肿瘤基质成纤维细胞(cancer associated fibroblasts, CAF)与肿瘤细胞的空间布局深刻影响免疫浸润格局、免疫逃逸以及患者对免疫治疗的反应。通过1000例非小细胞肺癌患者样本的质谱成像分析,Cords等^[77]鉴定出多种CAF表型,其中某些CAF亚群(如mCAF)与免疫排斥型微环境显著相关:当mCAF丰度低时,各类免疫细胞(包括CD4⁺T细胞、CD8⁺T细胞、B细胞、髓系细胞和中性粒细胞)更容易贴近肿瘤边缘,发挥免疫作用;相反,在mCAF高度富集的肿瘤中,免疫细胞与肿瘤细胞的接触减少,滞留于肿瘤周围的基质区域,形成典型的免疫隔离现象。类似方法也应用于乳腺癌的免疫微环境研究,Eng等^[112]发现高密度Vim⁺活化成纤维细胞包围肿瘤细胞可形成“排斥性”纤维母细胞壁垒,显著阻碍T细胞浸润,该特征在三阴性乳腺癌等患者队列中预示预后不良。相反,一些CAF与免疫细胞形成特定空间邻近关系可产生有利的免疫效应,当ifnCAF富集于肿瘤细胞簇周围时,可与CD8⁺T细胞或巨噬细胞形成稳定的空间共存模式,促进免疫浸润^[113]。这些空间偏好提示不同CAF亚型可能在肿瘤免疫逃逸、免疫浸润和血管生成等方面扮演着不同角色。值得一提的是,质谱成像技术还推动了其他肿瘤基质组分空间生物学的研究,例如:周细胞覆盖程度可提示肿瘤血管状态,影响免疫细胞进入肿瘤的能力^[113];细胞外基质中胶原纤维的高度有序化往往造成实体瘤的T细胞排斥,这类结构可用于预测组织免疫评分及患者生存等临床结局^[114]。各类高维质谱空间成像手段的应用为系统解析肿瘤-基质细胞空间互作及其免疫调控机制提供了强有力的工具。

质谱成像技术不仅解析了肿瘤微环境中细胞间互作的空间拓扑结构与功能协同网络,更奠定了基于空间组织结构驱动的免疫预后评分模型构建基础。未来,这类具备可视化与定量化的免疫图谱,有望转化为肿瘤分型与临床治疗决策的重要辅助工具,最终推动肿瘤免疫微环境研究向临床可转化工具的实质性跃迁。

3 总结与展望

质谱成像技术在肿瘤空间蛋白质组学研究中展现出巨大潜力,其不仅能够突破传统组学方法的局限,提供亚细胞级分辨率的空间蛋白质分析,还能揭示肿瘤微环境的细胞异质性与相互作用网络,为精准医学提供强有力的工具^[10,115]。然而,现有技术仍面临一些重要挑战。首先,灵敏度和通量的优化是当前研究的一大瓶颈^[91,116-117];利用高速激光扫描和人工智能预选区域的方法,有望提高数据采集效率,进而提升采集通量与速度^[117-118]。其次,当前空间蛋白质组学多依赖静态组织切片,未能全面捕获肿瘤组织的动态演化过程^[119-120];未来可通过结合活细胞追踪和多时间点采样技术,揭示肿瘤发展过程中的时空动态变化^[120-121]。此外,现有分析方法仍显零散,缺乏统一的标准化分析框架与集成化分析平台,需要进一步融合人工智能与大数据技术,开发更加高效、精准的分析工具,促进多维度数据的整合与深度挖掘^[122-125]。在临床转化方面,尽管质谱成像技术已取得初步进展,但其与具体病理场景的结合仍较为薄弱。肿瘤微环境的复杂性和多参数数据的解析难度,使该技术的广泛应用受到检测成本和操作复杂度的限制^[122]。为此,基于人工智能辅助的特异性标志物挖掘以及临床数据库的建立,将有助于推动这一技术的临床转化与普及^[42,126]。

展望未来,肿瘤空间蛋白质组学将朝着多模态技术平台整合的方向发展,通过深度结合不同的成像技术、分子分析手段以及临床诊疗数据,实现对组织样本的综合解析,更全面地剖析肿瘤的空间异质性与复杂性^[127-129]。随着技术的不断发展,可以预见质谱成像技术将在肿瘤免疫微环境、免疫逃逸机制和精准诊疗等领域发挥更大作用,为癌症治疗的个体化和靶向化提供坚实的科学依据。

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