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小细胞外囊泡在实体器官移植中的应用进展

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摘　要

小细胞外囊泡(sEVs)作为细胞间通讯的关键载体，在实体器官移植的免疫调控中扮演着复杂而重要的角色。本文综述了sEVs在移植免疫中的作用机制、其作为无创生物标志物的应用潜力以及当前面临的临床转化挑战。sEVs可递送供体主要组织相容性复合体分子，参与同种异体免疫的“半直接识别”，从而启动针对移植物的排斥反应。另一方面，来源于调节性T细胞、间充质干细胞等特定细胞的sEVs又能参与诱导移植耐受。其最终效应取决于囊泡的细胞来源、内容物构成及所处的微环境。在临床诊断方面，体液(如血浆、尿液、支气管肺泡灌洗液)中的sEVs及其携带的蛋白质、RNA等分子，能够“镜像”反映移植物局部及全身的免疫状态。多项研究表明，sEVs及其内容物在肾、肝、心、肺等器官移植的排斥反应诊断、亚型鉴别及预后预测中具有重要潜力。未来，随着分离分析技术的进步和工程化改造sEVs技术的进步，sEVs有望成为推动实体器官移植精准诊疗的重要工具。

关键词

细胞外囊泡，器官移植，免疫排斥，免疫耐受，生物标志物

Advances in the Application of Small Extracellular Vesicles in Solid Organ Transplantation

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Abstract

Small extracellular vesicles (sEVs) serve as key mediators of intercellular communication and play complex and vital roles in the immune regulation of solid organ transplantation. This review summarizes the mechanistic roles of sEVs in transplant immunology, their potential as non-invasive biomarkers, and the current challenges in clinical translation. sEVs can deliver donor-derived major histocompatibility complex molecules and participate in the "semi-direct" pathway of allorecognition, thereby initiating immune rejection against the graft. Conversely, sEVs derived from specific cell types, such as regulatory T cells and mesenchymal stem cells, can contribute to the induction of transplant tolerance. Their ultimate biological effect depends on the cellular origin of the vesicles, their cargo composition, and the surrounding microenvironment. In clinical diagnostics, sEVs present in body fluids (e.g., plasma, urine, bronchoalveolar lavage fluid) and the proteins, RNAs, and other molecules they carry can "mirror" the local and systemic immune status of the transplanted organ. Numerous studies have demonstrated the significant potential of sEVs and their cargo for diagnosing rejection, differentiating its subtypes, and predicting prognosis in kidney, liver, heart, and lung transplantation. In the future, with advances in isolation and analysis technologies, as well as in engineering strategies for sEVs, they are expected to become important tools for advancing precision diagnosis and therapy in solid organ transplantation.

Keywords

Extracellular Vesicles, Organ Transplantation, Immune Rejection, Immune Tolerance, Biomarkers

1. 引言

器官移植是治疗终末期肾病、肝病、心脏病、肺病等不可逆器官衰竭的有效手段，据 2024 年统计，全球共完成实体器官移植超 17 万例，其中肾移植占比最高，超过 63%，肝移植与心脏移植则占比 24.5%和 5.9% [1]。移植排斥反应是影响移植物长期存活的主要障碍。虽然他克莫司等免疫抑制剂的应用显著改善了移植受者的近期存活率，但由其长期使用带来的肾毒性、代谢紊乱、机会性感染及慢性移植物失功等问题，始终是制约长期预后的主要临床挑战[2]-[4]。因此，深入理解移植排斥反应的免疫学机制，并探索新的诊疗策略具有重要的临床价值。

细胞外囊泡(Extracellular Vesicles, EVs)是由细胞释放的、具有磷脂双分子层结构的纳米至微米级囊泡，其内部携带蛋白质、脂质、核酸等来源细胞的生物活性分子，可作为重要的细胞间通讯载体[5]-[7]。早期研究曾根据其假定的生物发生途径，将细胞外囊泡分为外泌体(直径约 30~200 nm)、微囊泡(直径约 200~1000 nm)和凋亡小体(直径约 1000~5000 nm) [8] [9]。然而，为提升科学表述的严谨性，国际细胞外囊泡学会在最新指南中建议采用小细胞外囊泡(<200 nm; small EVs, sEVs)和大细胞外囊泡(>200 nm; large EVs, lEVs)进行描述[10]。因此，本文将以小细胞外囊泡(sEVs)来指代传统所指的外泌体。

移植免疫的核心是同种异体免疫识别与应答。移植物在缺血再灌注过程中释放的损伤相关分子模式(DAMPs)首先激活了受者的固有免疫，首先激活受者的固有免疫，形成局部炎症微环境[11]。在此基础上，供者与受者之间主要组织相容性复合体的差异被 T 细胞、B 细胞等识别，在炎症信号的协同下驱动强烈的

特异性适应性免疫应答，并可形成长期的固有免疫记忆，共同导致移植物损伤与排斥。近年来研究发现，小细胞外囊泡在免疫调节中发挥重要作用，参与抗原呈递、免疫细胞活化与分化等多种过程，在移植免疫中扮演关键角色[12]-[14]。在移植局部，来源于供者或受者细胞的囊泡携带 MHC 分子、自身抗原、免疫调节因子及非编码 RNA 等，能够进行远距离信号传递。有趣的是，这些囊泡的作用具有双向性，既可呈递同种抗原、启动免疫反应促进排斥，也能诱导免疫细胞失能或促进调节性细胞功能从而诱导耐受[15]-[18]。

2. 细胞外囊泡的来源

2.1. 按细胞来源分类

免疫细胞来源 sEVs 参与免疫调控，其效应受囊泡细胞来源、内容物构成及微环境共同影响[5]。ESCRT 复合体可识别并分选带有单泛素化或 K63 连接多聚泛素化标签的膜蛋白(如活化的免疫受体或信号衔接蛋白)，引导其进入多泡体内腔，最终以外泌体形式释放[9] [19]。膜筏与四跨膜蛋白(如 CD63、CD81)共同形成膜平台，富集特定信号分子与核酸[20]。细胞通过 RNA 结合蛋白(如 hnRNPA2B1)识别 miRNA 中的特异性序列基序，以此实现对细胞外囊泡的 RNA 货物进行主动分选。这种机制使母细胞能够程序化地组装 sEVs 的内容物，从而生成具有特定功能导向的分子信号包[21]。

间充质干细胞(mesenchymal stem cells, MSCs)以其免疫调节和多向分化能力受到广泛关注。有研究对比了不同细胞因子(TGF-β、IFN-γ 或其组合)刺激人脐带 MSCs 产生的 sEVs，均能以剂量依赖的方式抑制 PBMC 的增殖，且各组间效果相当，而在诱导 PBMCs 分化为 Tregs 方面，TGF-β 和 IFN-γ 联合刺激组的效果最佳[22]。sEVs 中高水平的 IDO 是增强这一诱导分化的关键因子[22] [23]。在异种移植模型中，MSCs 可引发显著的全身性抗体反应，而其分泌的囊泡在保持同等肾脏保护功能的同时，仅调控脾脏 B 细胞亚群且不产生抗体，显示出更低的免疫原性[24]。体外研究进一步表明，经 MSCs 或其 sEVs 处理后，DCs 的成熟标志物、共刺激分子和 MHC II 类分子表达下调，分泌模式向抗炎方向转变[25]。此外，脐带 MSCs 来源的 sEVs 可通过递送特定 miRNA 和蛋白质，激活 PI3K-Akt 通路，促进巨噬细胞向抗炎 M2 表型极化，从而在骨关节炎模型中减轻炎症、保护软骨[26]。综上所述，MSCs 来源的 sEVs 可通过靶向树突状细胞、巨噬细胞、B 细胞等多种免疫细胞，实现多层次的免疫调节。

作为关键的专职抗原呈递细胞，树突状细胞(Dendritic cells, DCs)分泌的囊泡携带有 MHC I/II 类分子、共刺激分子(如 CD80、CD86、CD40)及共抑制分子(如 CD273 和 CD274)。这些囊泡的功能与其亲代 DCs 的成熟与活化状态密切相关[27] [28]。与未成熟 DCs 来源的囊泡相比，成熟 DCs 所分泌的囊泡不仅表面免疫分子(如 MHC II、B7.2、ICAM-1、TNF、FasL 和 TRAIL)的表达水平显著更高，其诱导抗原特异性 T 细胞和 NK 细胞活化的能力也更强[28] [29]。

不同亚群 T 细胞来源的 sEVs，其免疫功能高度依赖于来源细胞的活化途径。例如，经抗原特异性识别活化的 $CD4^+$ T 细胞所分泌的囊泡，表面表达 CD25、T 细胞受体、LFA-1 及 FasL 等特征性分子，能够显著抑制 DCs 诱导的 $CD4^+$与 $CD8^+$ T 细胞增殖，并削弱 $CD8^+$ T 细胞的杀伤能力[30]。相比之下，由 IL-2 途径激活的 $CD4^+$ T 细胞释放的囊泡，则通过递送特定 miRNA 来激活 $CD8^+$ T 细胞，从而避免了直接应用 IL-2 可能激活调节性 T 细胞、抑制免疫效应的潜在副作用[31] [32]。细胞毒性 $CD8^+$ T 细胞来源的囊泡可携带穿孔素、颗粒酶 B、FasL 等效应分子，直接递送细胞毒性物质攻击靶细胞[33] [34]。调节性 T 细胞(Tregs)来源的囊泡则通过多重机制发挥免疫抑制作用。一方面，它们富含特定 miRNA，可转移至辅助性 T 细胞，实现远程功能抑制[35]。另一方面，其表面高表达的 CD73 蛋白能将细胞外的腺苷一磷酸转化为具有强效免疫抑制活性的腺苷，如同在局部微环境持续工作的“微型酶工厂”，有效抑制效应 T 细胞的增殖与细胞因子分泌[36]。此外，这些囊泡中富集的如 miR-142-3p、miR-150-5p 等 miRNA，在被 DCs 摄取后，可使其向抗炎、耐受性表型转化，表现为 IL-6 分泌减少和 IL-10 分泌增加，从而在抗原呈递的

起始环节抑制免疫反应[37]。

2.2. 按体液来源分类

细胞外囊泡广泛存在于血浆、尿液、肺泡灌洗液、胆汁、肠液等多种体液中，是反映器官生理与病理状态的重要生物标志物来源。然而，不同体液中复杂、特异的基质成分，为囊泡的分离富集带来了严峻挑战。准确、高效地克服特定体液中的分离难题，是推动囊泡在移植免疫临床应用的关键前提。

血浆是 sEVs 研究中最常用的样本之一，可反映全身免疫状态与移植物整体损伤。但其中高浓度的脂蛋白(如低密度脂蛋白)和可溶性蛋白(如白蛋白)在密度、尺寸上与囊泡高度重叠，在超速离心等常规分离步骤中易与囊泡共同沉淀，严重影响产物纯度与下游分析的信噪比[38] [39]。一项研究指出，经色谱柱分离的血浆样品中，超过 70%的纳米颗粒并非 sEVs [40]。因此，为获得高纯度囊泡，常需采用超速离心与尺寸排阻色谱等联用策略，以有效去除脂蛋白干扰[41]。

尿液 sEVs 主要来源于肾小管上皮细胞等部位，可直接反映移植肾局部病理状态[42]。然而，尿液中大量存在的 Tamm-Horsfall 蛋白可沉淀并包裹囊泡，导致其在离心分离中损失显著[43]-[45]。有研究显示，采用还原剂(如二硫苏糖醇)或盐沉淀法预处理尿液样本，可有效去除该蛋白，提高囊泡回收率[45] [46]。

支气管肺泡灌洗液是监测肺移植排斥反应的独特样本，但其获取的囊泡浓度极低。这主要是因为灌洗液样本本身具有高度稀释的特性。Dlugolecka 等人的研究表明，向肺部灌注 100 毫升生理盐水，平均回收体积约为 30 毫升，通过超速离心富集到的 sEVs 的平均浓度数量级在每毫升 10^8 到 10^9 个颗粒，这比血浆中每毫升 10^{11} 个颗粒的浓度低 2 到 3 个数量级[47]。虽然尿液中 sEVs 浓度也处于相似数量级[48]，但尿液样本易于大量获取。相比之下，灌洗液有限的回收体积严重限制了可分离的囊泡总量。

胆汁和肠液为肝、肠移植提供了“液体活检”窗口，但二者成分复杂且相关研究较为有限。胆汁中高浓度胆盐、胆固醇及肠液中混杂的消化酶、微生物外膜囊泡等，对囊泡稳定性和分离特异性构成严峻挑战[49]。利用宿主与细菌囊泡表面特异性标志物进行免疫亲和捕获，可能是实现高纯度分离的可行策略[50]。

当前，超速离心、尺寸排阻色谱、聚合物沉淀等是常用的分离方法。超速离心法步骤相对成熟，并因其设备普及且能获得较高产量而被广泛应用。一般的操作流程是：收集细胞上清或血浆后，依次经 300 g (10 min)去除活细胞、2000 g (10 min)和 10,000 g (30 min)去除细胞碎片和细胞器，接着在 100,000 g 下离心 70 min 沉淀 sEVs，并用 PBS 洗涤一次以去除杂蛋白，最后再次在 100,000 g 下离心 70 min 沉淀 sEVs [51]。值得注意的是，重复的洗涤与高速离心易导致囊泡膜破裂和回收率下降[52]。有研究评估发现，尿液经超速离心后，上清中仍可回收约 40%的囊泡[53]。因此，针对不同体液的特点，需进一步优化和标准化分离流程，以满足临床应用对囊泡纯度、产量及完整性的综合要求。

3. 小细胞外囊泡在免疫排斥和免疫耐受的作用

3.1. 免疫排斥

同种异体免疫识别是启动移植排斥反应的核心环节，其机制主要包括直接、间接与半直接三种途径。直接识别指受者 T 细胞直接识别移植物中供体抗原呈递细胞表面的完整同种异体 pMHC 复合物；间接识别指供体抗原被受者抗原呈递细胞摄取、加工后，由受者自身 MHC 分子呈递。目前尚无直接证据表明 sEVs 是这两种经典途径的必需组分，但临床观察提示其可能参与其中。例如，在肝移植受者外周血中可检测到携带供体 MHC 分子的 sEVs，而在肾移植受者中则未发现类似现象[54]。这种差异可能与肾脏体积较小、滤过功能强大，从而影响外源性囊泡在循环中的分布有关。基于此，可以猜想 sEVs 可能以辅助方式参与同种异体免疫识别。

半直接识别途径通常指受者 DCs 通过细胞间接触从供者细胞获取完整的 MHC-肽复合物，并展示于

自身细胞表面，称为“交叉修饰”。研究表明，供体来源的 sEVs 表面携带有完整的 MHC I 类和 II 类分子，可被受者抗原呈递细胞摄取并展示于其表面[54] [55]。这些囊泡部分来源于 T 细胞、DCs 或 B 细胞，另一部分来源尚不明确[55]。通过这一机制，囊泡能在无需细胞直接接触的情况下，激活受者 $CD8^+$细胞毒性 T 细胞与 $CD4^+$辅助 T 细胞，从而启动针对移植物的免疫攻击。值得注意的是，在皮肤与心脏移植模型中，移植物引流淋巴器官内几乎检测不到供体来源的“乘客”抗原呈递细胞[55] [56]，这与强烈的抗供体免疫反应形成鲜明对比。这一发现对“乘客白细胞主导排斥”的传统理论提出了挑战，提示 sEVs 介导的远程抗原呈递可能在移植免疫启动中扮演更为核心的角色。

3.2. 免疫耐受

小细胞外囊泡在特定条件下可发挥免疫抑制作用，参与移植免疫耐受的建立。研究表明，Tregs 分泌的 sEVs 表面高度富集 IL-35，邻近的淋巴细胞摄取这些囊泡后，可在其自身细胞膜上呈现 IL-35 [57]。这一过程使这些淋巴细胞获得调节功能：一方面，其自身会上调 PD-1、LAG-3、TIM-3 等免疫检查点分子，呈现类似耗竭性 T 细胞的表型；另一方面，这些被“重塑”的细胞能够进一步抑制周围免疫细胞的活性，从而实现“传染性耐受”。动物实验证实，IL-35 阳性的 EVs 可显著抑制迟发型超敏反应，并延长心脏移植物的存活时间。此外，Tregs 来源的细胞外囊泡还可通过向 DCs 递送 miR-150-5p、miR-142-3p 等 miRNA，诱导其呈现耐受性表型，表现为抑制 IL-6 并促进 IL-10 的产生，从而参与免疫耐受[37]。

MSCs 来源的 sEVs 在诱导耐受性 DCs 中扮演关键角色。这类 sEVs 可显著抑制 DCs 中 CCR7 的表达和 IL-12 的分泌，削弱其迁移及激活 Th1 反应的能力[58]。同时，经其处理的 DCs 可高表达 IL-10 和 TGF-β，进而更有效地抑制 T 细胞增殖[59]。有趣地是，经 TGF-β 和 IFN-γ 联合预处理的 MSCs 所分泌的 sEVs，在诱导人单核细胞向 Treg 分化方面表现更强，这可能与其富含 IDO、IL-10 和 IFN-γ 等耐受相关分子有关[22]。此外，MSCs 来源的 sEVs 还能影响 B 淋细胞功能，抑制其增殖和抗体分泌，提示其在调节体液免疫耐受中也具潜力[60]。

供体来源的 sEVs 同样参与耐受诱导。其携带的 MMP1a 可激活 PAR2 通路，将供体抗原特异性的 Th2 细胞转化为 Tregs，从而抑制同种异体移植心脏的免疫炎症，显著延长其存活时间[61] [62]。

4. 小细胞外囊泡作为生物标志物的临床应用

体液是细胞外囊泡在体内循环与交换信息的主要介质，为无创获取疾病及免疫状态信息提供了宝贵的窗口。由于细胞外囊泡的组成与功能在很大程度上受其来源细胞的类型及状态所决定，其携带的内容物能够相当程度地“镜像”反映特定器官或组织的病理生理变化[63]-[67]。因此，体液中的循环 sEVs 已成为监测移植后移植物状态与免疫反应的潜在生物标志物来源。

4.1. 肾脏移植

肾移植排斥反应的诊断目前仍依赖有创的穿刺活检。为寻求无创监测手段，尿液和血浆中的 sEVs 备受关注。蛋白质组学研究表明，尿液 sEVs 携带的分子谱可有效反映排斥病理，但不同研究报道的标志物存在显著异质性，这主要与免疫排斥类型、检测分子及技术平台的差异有关。抗体介导排斥反应(ABMR)患者尿液 sEVs 中 LBP 和 CST3 水平显著升高，与移植肾微循环炎症评分相关，提示参与补体激活与内皮损伤通路[68]。急性 T 细胞介导排斥反应(TCMR)患者则表现为 TSPAN1 和 HPX 等蛋白富集，反映细胞免疫浸润的独特机制[69]。此外，基于亚蛋白质组学的无偏筛选鉴定出 CLCA1、PROS1 等 11 种在排斥患者中上调的蛋白[70]。不同研究报道差异较大的原因，一方面在于 ABMR 与 TCMR 的生物学机制本身不同，另一方面也受限于单一蛋白在复杂尿液基质中的检测稳定性。相比之下，一项大型多中心研究开发的尿液 sEVs mRNA 多基因诊断标签，实现对全因排斥的高精度筛查(15-gene panel)及 TCMR/ABMR

亚型区分(5-gene panel)，临床转化潜力优于单一蛋白标志物[71]。

关于血浆的研究指出，供者来源 sEVs 的水平与稳定的移植物功能相关，可作为评估移植肾状态的指标[72]。在 HLA 致敏受者中，血浆 sEVs 的特定 mRNA 谱(如编码 gp130、SH2D1B 的 mRNA)可用于预测 ABMR [73]。

针对更复杂的慢性排斥，研究在慢性活动性抗体介导排斥反应(CAAMR)患者的尿液 sEVs 中发现了显著升高的 SYT17 蛋白，其与 IL-6 放大器通路激活相关[74]。另一项研究鉴定出一组在 CAAMR 患者中发生显著改变的尿液 sEVs 蛋白(APOA1, TTR, PIGR, HPX, AZGP1, CP)，其中 AZGP1 能有效区分 CAAMR 与移植功能稳定的患者[75]。

4.2. 肝移植

肝移植预后取决于供肝质量评估与术后免疫监测。研究表明，在肝脏冷保存期间，供肝细胞释放的囊泡及其携带的特定 miRNA (如 let-7d-5p、miR-200 家族)在心脏死亡捐献供肝中显著高于脑死亡捐献供肝，且与移植后生存率及早期功能障碍相关[76]。此外，在边缘供肝的低温氧合机器灌注过程中，实时监测灌注液中的黄素单核苷酸的水平，可有效预测移植后早期肝功能恢复与移植物丢失风险[77]。

术后免疫监测方面，发生急性排斥的受者循环 sEVs 中 Galectin-9 蛋白水平显著升高，且其在移植肝组织中的高表达是预后不良的独立危险因素[78]。对急性排斥患者血清 sEVs 的 miRNA 组学分析显示，特定 miRNA (如 miR-223)的表达谱发生显著改变，其预测靶基因富集于免疫相关通路[79]。

血清 sEVs 中的微小 RNA 还可用于预测肝细胞癌肝移植术后复发。术前受者血清 sEVs 中 miR-92b 水平结合甲胎蛋白检测，或术后一个月该 miRNA 水平，可预测移植后两年内的肿瘤复发[80]。其潜在机制在于，miR-92b 能够增强肝癌细胞(如 Hep3B、SK-Hep1)的迁移能力，并通过下调 NK 细胞表面的活化标志物 CD69 表达，抑制其杀伤功能，从而促进肿瘤免疫逃逸。

4.3. 心脏移植

心通过流式细胞术分析血浆 sEVs 表面特异性蛋白(HLA-I、CD3 等评估急性细胞性排斥，HLA-II、ROR1 等评估抗体介导的排斥)，并构建机器学习诊断模型，可高精度地区分这两种排斥反应[81]。另一项研究通过机器学习分析 14 种循环 sEVs 表面抗原(如 CD2、CD3、CD4、CD8、CD45、HLA-I 等)，构建的人工智能模型不仅能高精度诊断中重度急性细胞性排斥，还能在组织学确诊前提示排斥风险[82]。

此外，靶向供体 HLA 和 CD3 抗原分离循环中供体心脏来源与 T 细胞来源的 sEVs 发现，在中度急性细胞性排斥时，供体来源 sEVs 中的 cTnT 及其 mRNA 水平显著降低，而 T 细胞来源 sEVs 中 CD4、CD8、TCR 蛋白及 let-7i 等 miRNA 水平则显著升高[83]。这些变化在成功抗排斥治疗后发生显著的动态逆转，与临床进程高度相关。

4.4. 肺移植

慢性肺移植物失功是肺移植后影响长期生存的主要障碍，其定义为移植后 FEV1 较基线值持续下降超过 20%的综合征[84]。这并非单一疾病，主要包括以阻塞性通气功能障碍为特征的闭塞性细支气管炎综合征(BOS)，以及表现为限制性通气功能障碍伴肺部阴影的限制性移植物综合征(RAS)等[84] [85]。

在大鼠异基因肺移植模型中，循环内供体肺特异性 sEVs 水平在术后第一天即达峰值，早于组织学证实的急性排斥[86]。发生急性排斥时，BALF 来源 sEVs 的 RNA 谱发生显著重编程，先天性与适应性免疫通路被广泛激活[87]。临床研究进一步证实，肺移植受者血浆来源 sEVs 中供体 HLA 和肺自身抗原(如 V 型胶原和 Kα1 微管蛋白)的水平，在临床诊断急性排斥或 BOS 前数个月即显著升高，并表现出优异的预测性能[15] [88] [89]。这些 sEVs 还富含与内皮活化、炎症和 Th17 分化相关的 miRNA [89]。

在 CLAD 的发病机制与预后评估方面，sEVs 同样发挥关键作用。研究表明，CLAD 患者 BALF 中上皮细胞和红细胞来源的 sEVs 水平显著升高，且高水平的患者 4 年总体生存率较差的独立危险因素[90]。此外，这些囊泡能够激活气道上皮细胞的经典 NF-κB 信号通路，该通路的激活在疾病早期即可在活检组织中得到验证，且与不良预后相关[91]。对囊性纤维化肺移植受者的研究也发现，CLAD 临床诊断前 6~12 个月，患者血浆 sEVs 中肿瘤抑制因子 LKB1 的水平已显著降低[92]。此外，肺移植后 12 个月时血浆中囊泡结合型 HLA-G 的水平是预测远期预后的关键指标，低 HLA-GEV 是未来 3 年内发生 CLAD/BOS 和移植物失功的独立危险因素[93]。

供体来源的囊泡同样具有预后提示价值。一项前瞻性研究表明，脑死亡供体血浆 sEVs 的特定 mRNA 转录组特征，与受者术后发生原发性移植物功能障碍的风险显著相关[94]。

4.5. 小肠移植和角膜移植

在小肠移植领域，肠移植受者造口液中的肠道来源 sEVs 的表面标志物(如 HLA-II、CD326)在术后急性细胞排斥发生前即出现显著变化，提示其可作为早期预测标志物[95]。即使在免疫豁免的角膜移植模型中，排斥组小鼠血清 sEVs 数量在移植后第 7 天显著增加，且其蛋白组学特征(如供体 MHC-I 分子富集)可预测排斥反应，这为 sEVs 作为跨移植类型的通用生物标志物提供了证据[96]。

5. 结论与展望

小细胞外囊泡作为细胞间通讯的重要载体，在实体器官移植免疫中发挥着复杂而多样的作用。一方面，供体来源的 sEVs 通过直接和间接途径参与同种免疫识别，促进移植排斥反应的发生；另一方面，特定来源和组成的 sEVs 可发挥免疫调节功能，有助于诱导移植耐受。

作为非侵入性生物标志物，血浆中的 sEVs 在移植排斥诊断中显示出重要价值，尤其是在肾移植和心脏移植领域，多项研究已证实其可用于有效区分排斥与稳定状态[72] [73] [78] [79]。机器学习技术与 sEVs 分析的结合进一步提高了诊断的准确性和敏感度，为实现个性化监测提供了新思路[81] [82]。此外，通过基因编辑或体外修饰等技术对细胞外囊泡进行工程化改造，不仅实现靶向递送，还能增强其免疫调节能力，为精准免疫治疗开辟了新方向[97]。

尽管 sEVs 在移植领域展现出诊断与治疗的双重潜力，其临床转化仍面临挑战。首先，虽然已有研究尝试建立样本处理与分离纯化的标准[64] [98]，但由于临床样本的高度异质性，不同实验室采用的方法尚未统一，导致结果间可比性有限。其次，目前报道的生物标志物多源于单中心、小样本研究，尚缺乏大规模前瞻性验证。最后，sEVs 在体内的靶向分布、代谢动力学及长期安全性仍有待明确。尽管如此，随着技术的持续进步与研究的深入，细胞外囊泡有望成为实体器官移植精准诊疗的重要工具，推动移植医学迈向新的高度。

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