细菌外膜囊泡: 疾病治疗的新途径

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摘要: 细菌外膜囊泡 (outer membrane vesicles, OMVs) 是由革兰阴性菌分泌的球形纳米囊泡, 粒径为20～250 nm, 由脂质双分子层和多种来源于亲本细菌的成分组成。基于表面的细菌抗原、病原体相关分子模式、多种蛋白质及囊泡结构, OMVs可作为疾病治疗的一种新途径, 开发用于多种生物医学应用, 如肿瘤治疗、抗感染疫苗等。本文介绍了OMVs的结构和组成, 总结了分离、提取以及鉴定OMVs的方法, 对OMVs最新的研究进展和应用前景进行了概述。

关键词: 细菌外膜囊泡; 纳米囊泡; 疫苗; 肿瘤免疫; 药物递送载体
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Bacterial outer membrane vesicles: a new approach to diseases therapy

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Abstract: Outer membrane vesicles (OMVs) are nano-sized spherical vehicles, with a size range between 20−250 nm. OMVs are spontaneously secreted from Gram-negative bacteria and formed by lipid bilayer membranes, comprising multiple parent bacteria-derived components including bacterial antigens, pathogen-associated molecular patterns, proteins and lipids. OMVs have shown multiple potentials for the treatment of various diseases, including cancer therapy and bacterial infection. In this review, the structure, composition and methods for isolating and characterizing of OMVs were introduced. The applications of OMVs for diseases therapy were summarized and future perspectives were discussed.

Key words: outer membrane vesicle; nanoparticle; vaccine; cancer immunotherapy; drug delivery vehicle

细菌是自然界广泛存在的一类微生物, 种类繁多、可塑性强, 具有天然的侵袭能力、肿瘤靶向能力和细胞毒性。在生物医学领域中, 有着广泛的应用。然而, 由于细菌疗法作用机制不明、可控性差和安全性低等问题, 限制了其作为治疗药物的开发和应用[1]。细菌外膜囊泡 (outer membrane vesicles, OMVs) 是革兰阴性菌在生长过程中自然分泌的球形纳米囊泡, 与细菌相比, 不具备复制的能力, 但拥有与细菌相似的功能, 可控性和安全性大大提高, 可作为疾病治疗的一种新途径[2]。

OMVs最早于1967年由Chatterjee和Das[3]在体外研究霍乱弧菌细胞壁结构时发现, 随后在越来越多的革

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兰阴性菌中发现OMVs的存在。多项研究证明, OMVs是细菌与宿主细胞间的一种交流方式, 细菌分泌OMVs向宿主递送活性物质来调节细胞功能[4-6]。基于OMVs独特的结构和功能, 可设计用于多种疾病治疗。来源于细菌外膜的OMVs, 含有与亲本细菌相同的抗原以及多种病原体相关分子模式 (pathogen-associated molecular patterns, PAMPs)[7,8], 抗原蛋白和PAMPs被细胞摄取后呈递至抗原提呈细胞 (antigen-presenting cells, APCs), 刺激机体产生抗原特异性免疫反应。此外, 多种抗原决定簇的存在使OMVs具有疫苗佐剂特性, 能够调节或增强机体对抗原的特异性免疫应答[9], 天然OMVs或工程化OMVs可开发作为疫苗[10-12]。纳米粒径的OMVs具有高渗透长滞留 (enhanced permeability and retention, EPR) 效应, 可渗透进入肿瘤组织并在肿瘤部位富集[13], 多种抗原蛋白和PAMPs同时诱发强大的抗肿瘤免疫反应, 显示出抗肿瘤治疗的潜力[14]。此外, OMVs的囊泡结构可开发成药物递送载体, 药物可以共价连接到OMVs的表面或封装到OMVs的内部[15]。直接对OMVs进行修饰或对亲代细菌基因工程改造, 可以获得靶向配体修饰后的OMVs, 实现细胞特异性靶向并增加靶向部位的药物积累[16]。

1 OMVs的结构和组成

OMVs是具有脂质双分子层的球形纳米囊泡, 粒径为20～250 nm[17,18], 主要由脂质、蛋白质和PAMPs组成 (图1), PAMPs主要包括脂多糖 (lipopolysaccharide, LPS)、肽聚糖、DNA和RNA等[18]。OMVs的脂质主要是磷脂和LPS, 磷脂来源于革兰阴性菌的外膜, 而LPS来源于革兰阴性菌的细胞壁, 是PAMPs的重要组成部分[19]。OMVs的蛋白大多数都是毒力相关蛋白, 也包含一些运输糖、氨基酸和离子等的孔蛋白和跨膜通道蛋白[20]。

2 OMVs的分离和提取

2.1 OMVs分离和提取的条件

一般来说, 细菌在液体培养基培养适当时间后会收集到天然的OMVs。但OMVs会受到细菌培养时间和条件的影响, 根据细菌生长曲线确定最佳收集时间至关重要。当细菌培养至非常晚的稳定期时, 能够收获到大量的OMVs, 但同时细菌死亡数量增加, 细胞溶解, 破裂的膜和细胞质蛋白会污染OMVs; 当细菌处于对数生长期时, 活菌数以稳定的几何级数快速增长, 该时期是收集OMVs的最佳时期。因此, 不同的细菌生长阶段在数量上和质量上都影响着OMVs的形成, 在分离和提取OMVs前, 首先应测定细菌的生长曲线, 选择最合适的对数生长期收集OMVs[21]。

影响OMVs质量的另一重要因素就是培养基。培养基为细菌的生长提供氮源、维生素和氨基酸。不同培养基培养的细菌分泌的OMVs蛋白质含量和免疫原性有所区别。MH肉汤培养基 (Mueller-Hinton broth) 主要用于抗生素敏感实验, LB肉汤培养基 (Luria-Bertani broth) 在生化分子实验中常用来培养菌株, 使菌株成倍扩增。提取和分离OMVs的常用培养基为LB肉汤培养基[22]。

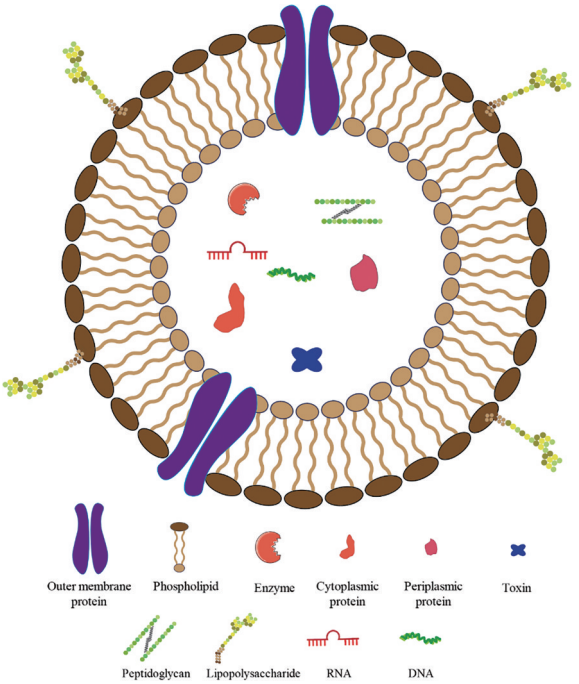

Figure 1 Schematic structure of outer membrane vesicles (OMVs)

2.2 OMVs分离和提取的方法

OMVs的分离和提取主要包括以下几个步骤: 培养细菌、除去细菌菌体、从培养基上清中分离和提取OMVs。分离OMVs首先要除去培养基中的细菌菌体, 一般采用中高速离心, 除去绝大部分的菌体、细胞碎片、大的蛋白质和膜聚集物等。收集培养基上清液, 用微孔滤膜 (0.22或0.45 μm) 过滤去除残留的细菌, 微孔滤膜的孔径应该与细菌的大小相适应。值得注意的是, OMVs的大小为20～250 nm, 使用0.22 μm的微孔滤膜时粒径较大的OMVs有可能会被截留。由于OMVs的产量较低, 需要采取超滤和沉淀的方法对OMVs进行浓缩。

2.2.1 超滤 离心后的细菌培养基上清通过50～100 kDa的滤膜超滤, 可以选择性过滤大部分的非OMVs蛋白质组分[23]。

2.2.2 超速离心 超速离心是分离和提取OMVs最常用的方法, 根据培养基中不同粒子的大小和密度设定不同的离心力, 利用平衡沉降实现纯化。首先, 使用中高速离心力 (4 000～10 000 ×*g*) 离心, 除去大的杂质, 如细菌和细菌碎片。然后将上清液通过0.45或0.22 μm

滤膜过滤, 再将过滤后的滤液通过50～100 kDa的滤膜超滤, 将浓缩后的超滤液进行超速离心 (100 000～200 000 ×*g*), 最终可得到OMVs[24,25]。可以采用密度梯度离心法进一步纯化OMVs, 分离介质可选用碘克沙醇或蔗糖[26,27]。

2.3 OMVs的分析方法

2.3.1 粒径 OMVs的平均粒径和分散系数可以采用动态光散射 (dynamic light scattering, DLS) 和纳米粒子跟踪分析 (nanoparticle tracking analysis, NTA) 技术来确定。DLS技术测量粒子粒径, 具有准确、快速和可重复性好等优点。DLS仪器可广泛用于表征OMVs的粒径、分散系数及zeta电势[28]。采用NTA技术分析OMVs, 能够更全面、准确地测定多分散样品中不同大小的粒子, 也能完成对纳米颗粒的精确计数, 并最终得到样品浓度[29]。

2.3.2 形态和结构 OMVs的形态和结构可通过光学显微镜来观察, 如透射电子显微镜 (transmission electron microscopy, TEM)、扫描电子显微镜 (scanning electron microscopy, SEM)、冷冻电子显微镜 (cryo-electron microscopy, cryo-EM) 和原子力显微镜 (atomic force microscopy, AFM)[30-33]。TEM是分析OMVs形态和结构最常用的方法, 可以提供高分辨率的形态、大小和结构信息。SEM可显示OMVs的三维结构, 但是分辨率较TEM低。与需要大量样品进行固定和染色的TEM或SEM不同, cryo-EM能够分析冷冻形式的OMVs, 避免由脱水和化学固定引起的形态变化。AFM在测量纳米粒子尺寸方面具有独特的优势, 可空气或流体中实时可视化OMVs, 不需要样品操作过程, 能够获得纳米粒子表面的细微结构信息[34]。

2.3.3 蛋白组成 OMVs的组成可采用多种方法进行分析, 如BCA测定法、SDS-PAGE凝胶电泳、蛋白质印迹法 (Western blot, WB)、酶联免疫吸附测定 (enzyme-linked immunosorbent assay, ELISA) 及质谱 (mass spectrometry, MS) 分析等[35]。BCA测定法可定量计算OMVs的总蛋白浓度。SDS-PAGE凝胶电泳对OMVs的蛋白进行定性分析, WB和ELISA可验证目标蛋白的存在。此外, 基于MS的高通量蛋白质组学分析已经鉴定了数千个蛋白质, 为OMVs的生物起源和功能提供证据[20,36]。

3 OMVs在肿瘤治疗中的应用研究

3.1 OMVs作为肿瘤免疫治疗药物

3.1.1 OMVs作为肿瘤免疫治疗药物的优势 19世纪晚期以来, 细菌和细菌产物已被用于抗肿瘤研究。细菌具有运载基因和治疗药物的能力, 并且对低氧肿瘤环境有固有趋向性, 可特异性分散到肿瘤组织中, 抑制肿瘤生长[37-39]。但细菌、炎症反应及宿主免疫三者之间具有复杂的关系, 难以达到最大化疗效果, 也存在安全隐患[40]。OMVs与细菌相比, 不具备复制的能力, 但拥有与细菌相似的功能, 安全性大大提高。同时, OMVs含有多种免疫刺激因子, 能够被免疫细胞识别和摄取, 激活免疫系统[41]。OMVs还是纳米尺寸的粒子, 可以通过EPR效应在肿瘤组织中积累, 诱导局部免疫反应, 减少治疗产生的不良反应[14]。

3.1.2 OMVs作为肿瘤免疫治疗药物 基于独特的免疫学和结构特征, OMVs的抗肿瘤作用受到越来越多的关注。Kim等[42]研究了OMVs的抗肿瘤活性, 结果显示OMVs能显著有效地诱导长期抗肿瘤免疫反应, 完全根除肿瘤而没有明显的不良反应。OMVs静脉给药后, 可以特异性靶向肿瘤组织并且在肿瘤组织中积累, 诱导抗肿瘤细胞因子CXCL10和IFN-γ的产生。由于OMVs的结构中含有脂多糖, 可能会导致致命性脓毒血症休克, 用于肿瘤治疗的主要问题是安全性。在该研究中, 研究者比较了野生型 (wild-type) 和基因修饰型 (ΔmsbB) *E. coli*产生的OMVs对人胚胎肾HEK293细胞的影响, 结果发现基因修饰后OMVs安全性更高。在未来, 应该对OMVs进行更加深入的安全性评估, 以确保OMVs临床应用的安全性。

3.1.3 OMVs杂交膜作为肿瘤免疫治疗药物 在肿瘤治疗中, 细胞膜衍生的纳米平台是一种有效的仿生策略。Chen等[43]将黑色素瘤细胞膜 (cytomembrane vesicles, CMVs) 与减毒沙门氏菌OMVs融合, 构建真核-原核囊泡 (eukaryotic-prokaryotic vesicle, EPV) 纳米平台。融合的EPV整合了肿瘤特异性抗原和OMVs天然佐剂特性, 表现出强大的刺激免疫系统和诱发肿瘤特异性免疫反应的能力。体内疫苗接种实验表明, 接种EPV后能刺激免疫系统并引发抗肿瘤免疫反应, 保护小鼠免受黑色素瘤的攻击, 突出了作为预防性癌症疫苗的潜力。在黑色素瘤模型中, 研究者还设计用EPV包载聚乳酸-羟基乙酸共聚物 [poly (lactic-*co*-glycolic acid), PLGA] 和吲哚菁绿 (indocyanine green, ICG) (PI@EPV), ICG吸收近红外光, 将其转化为癌细胞的细胞毒性热, 诱导免疫原性细胞死亡, 产生免疫系统的补充肿瘤抗原。经黑色素瘤模型验证, 混合纳米疫苗可有效地破坏实体肿瘤, 显示出作为治疗性疫苗协同抗肿瘤的作用。Wang等[44]将OMVs与B16-F10黑色素瘤细胞 (cancer cell, CC) 膜组成OMV-CC杂交膜, 将其涂覆在中空聚多巴胺 (hollow polydopamine, HPDA) 纳米粒上, 利用OMVs免疫治疗和HPDA介导的光热治疗 (photothermal therapy, PTT) 的优势来提高对黑色素瘤的抗肿瘤疗效。

3.1.4 OMVs联合化疗药物作为肿瘤免疫治疗药物 多项研究显示, OMVs可诱导有效的抗肿瘤免疫反应, 与化疗药物联合治疗可进一步增强肿瘤的免疫抑制状态, 从而彻底根除肿瘤并防止肿瘤复发和转移[45]。Kuerban等[46]将广谱抗肿瘤药物多柔比星 (doxorubicin, DOX) 与OMVs 37 ℃共孵育4 h, 得到具有双重抗肿瘤作用的DOX-OMVs。经过MTT法、WB和流式细胞术分析, DOX-OMVs在体外可引起强烈的细胞毒作用和细胞凋亡。在荷瘤BALB/c裸鼠中, DOX-OMVs比DOX对肿瘤的生长抑制、凋亡和坏死作用更强。研究结果显示, OMVs能够高效地将化疗药物DOX转运至非小细胞肺癌A549细胞, DOX-OMVs在肿瘤部位聚集后, 由于OMVs的免疫原性, 巨噬细胞在肿瘤微环境中募集, 诱导适应性免疫反应, 与DOX产生协同作用。

肿瘤特别是实体瘤具有复杂的免疫微环境和很强的免疫逃逸能力[47], 其中一个重要机制是免疫检查点程序性死亡1配体1 (programmed death 1 ligand 1, PD-L1) 在持续IFN-γ暴露的肿瘤细胞表面表达[48]。OMVs在小鼠体内会诱导强烈的IFN-γ和T细胞介导的抗肿瘤作用。IFN-γ上调肿瘤微环境中的免疫抑制因子, 特别是PD-L1, 可能阻碍T细胞功能并限制免疫治疗效果。Li等[49]通过工程化*E.coli*获得稳定表达与表面蛋白ClyA融合的小鼠PD1外域的OMV-PD1, 这种基因修饰不会影响OMVs触发免疫激活的能力, 而且OMV-PD1在肿瘤部位的积累可以增加免疫细胞如DC细胞和NK细胞的浸润, 并激活体内的免疫反应。同时, OMV-PD1表面上的PD1外域阻断了PD1/PD-L1免疫抑制轴的相互作用并保护$CD8^+$ T细胞, $CD8^+$ T细胞可攻击肿瘤细胞。在该研究中, 工程化的OMVs驱动肿瘤中效应T细胞的积累, 抑制肿瘤生长, 效果比单用天然OMVs或PD-L1抗体更好。

OMVs介导的免疫疗法与基因工程或与其他肿瘤治疗方法结合, 可进一步提高肿瘤治疗效果。Grandi等[50]用人表皮生长因子受体变异III型 (epidermal growth factor receptor variant III, EGFRvIII) 的14个氨基酸B细胞表位和kif18b基因的$CD4^+$ T细胞新表位 (B16-M30) 单独或联合修饰OMVs, 基因工程修饰后的EGFRvIII-OMVs免疫可显著抑制肿瘤生长。Zhuang等[51]将PTT和OMVs免疫疗法相结合, 显著降低了OMVs的给药剂量, 避免了OMVs静脉注射给药引起的不良反应和毒性问题, 协同增强抗肿瘤治疗效果。

3.2 OMVs作为肿瘤药物递送载体

3.2.1 OMVs作为肿瘤药物递送载体的优势 OMVs作为肿瘤药物递送载体具有很多优势。首先, OMVs可以靶向递送药物到定点部位, 通过对亲代细菌进行基因工程改造, 得到靶向配体修饰的OMVs, 促进药物在靶向部位的积累[49]; 其二, 纳米粒径的OMVs能够通过EPR在肿瘤中被动积累, 有助于将药物输送至肿瘤部位[52]; 其三, OMVs具有与其他载体相似的优势, 能够保护药物在到达靶点之前不被降解和变性, 减少药物在非靶向部位的释放[53]; 其四, OMVs具有免疫原性, 可以诱导免疫反应, 但免疫系统过度反应会对宿主细胞造成损伤[54]。所以, OMVs需要进行减毒处理, 提高OMVs作为肿瘤药物递送载体在临床应用的安全性。

3.2.2 OMVs载药的方法 OMVs载药通常可以采取两种方法, 除了可以直接将药物装载到OMVs中外, 还可以在亲本细菌分泌OMVs的生物发生过程中装载药物[55]。第一种方法是基于OMVs的脂质双分子层结构, 具有装载亲水性或疏水性化合物的潜力。一些疏水性化合物可以通过共孵育被动地装载到OMVs, 如DOX[46]。对于一些难以通过膜被动扩散的亲水性药物, 可以采用超声[56]、电穿孔[31,57]或挤压[58,59]的方式装载; 第二种方法是在OMVs生物合成过程中装载药物, Huang等[60]在细菌培养基中加入药物, 细菌会通过将药物装载到OMVs的形式排出药物, 从而获得载有药物的OMVs。近年来, 研究者们成功地使用生物工程方法载药, 利用基因工程修饰亲本细菌, 在其生长过程中通过出芽自然分泌载有修饰分子的OMVs[61,62]。工程化的OMVs载药效率高, 还可批量生产, 有助于实现临床转化[63]。

3.2.3 OMVs的肿瘤靶向治疗 目前的肿瘤化疗方案不良反应严重且持久, 特异性不高, 化疗药物在体内会被快速清除, 循环半衰期短, 需要更高剂量的药物才能达到治疗效果[64]。OMVs可作为有效的肿瘤靶向递送载体, 减少化疗药物的不良反应, 优化肿瘤治疗方案。

黑色素瘤是一种侵袭性癌症, 具有快速进展、复发和转移的特点, 黑色素瘤的全身疗法毒性高、治疗效果不佳。Peng等[65]用pDNA-TRAIL转化*E. coli*, 获得含有肿瘤坏死因子相关凋亡诱导配体 (tumor necrosis factor related apoptosis-inducing ligand, TRAIL) 的OMVs, 用合成$\alpha_v\beta_3$整合素靶向配体 (tumor-targeting ligand Arg-Gly-Asp, RGP) 和ICG修饰OMVs, 获得同时含有TRAIL和ICG的肿瘤靶向制剂I-P-OMVs。当I-P-OMVs用于皮肤黑色素瘤部位后, 通过黑色素瘤表面的RGP和$\alpha_v\beta_3$整合素之间的特异性结合, 在近红外刺激下, I-P-OMVs不仅可以诱导针对原发性黑色素瘤球体的光热−光动力反应, 还激活了TRAIL诱导的播散性肿瘤细胞凋亡, 根除黑色素瘤。化疗是结肠癌的重要治疗手段, 但存在生物利用度差、全身毒性等严重不良反应。

Shi等[58]构建了一个新的基于OMVs的纳米平台用于结肠癌的治疗, 用介孔二氧化硅纳米粒 (mesoporous silica nanoparticles, MSNs) 修饰OMVs并负载5-氟尿嘧啶 (5-fluorouracil, 5-FU), 制备OMVs-MSNs-5-FU, 结合了纳米载药系统的高载药量和生物载体的肠道吸附性, 显著增强了细胞毒性和对肿瘤细胞的细胞摄取。人乳头瘤病毒 (human papilloma virus, HPV) 高危基因型的持续感染是宫颈癌的致病原因, 预防性HPV疫苗可以防止大多数高危HPV病毒的感染, 但不能治疗已确定的感染和相关病变。Wang等[66]以工程化的OMVs为疫苗载体, 将HPV16E7通过基因重组技术嵌入到OMVs中, 皮下免疫诱导E7抗原特异性细胞免疫应答, 有效地传递了肿瘤抗原并激发强大的抗肿瘤免疫反应, 显著抑制了小鼠宫颈癌细胞生长。

3.2.4 OMVs载体存在的问题及解决方法 OMVs作为药物递送载体存在的最大问题就是安全性, 由于OMVs的免疫原性会激活机体免疫反应甚至引起免疫风暴, 导致不良反应甚至死亡[67]。Qing等[68]通过给健康BALB/c小鼠单剂量或多剂量静脉注射OMVs评估OMVs的体内毒性。研究发现, 在实验结束时, 单次高剂量注射组有50%的小鼠死亡, 单次低剂量组和多剂量组也有死亡病例, BALB/c小鼠不能耐受OMVs疗法。因此, 设计用磷酸钙 (calcium phosphate, CaP) 壳包被OMVs, 降低OMVs在体循环时的炎症反应。实验结果证明, OMV@CaPs克服了OMVs严重全身炎症反应, 通过EPR到达肿瘤后, CaPs帽壳在微酸性的免疫抑制肿瘤微环境 (tumor microenvironment, TME) 中溶解, 不仅有助于中和酸性TME, 而且将肿瘤暴露于TME免疫调节, 引发显著的肿瘤抑制作用。CaPs溶解引起的肿瘤内酸碱度增加还可诱导巨噬细胞M2向M1高度极化, 以提高抗肿瘤效果。此外, CaP外壳可以与功能性成分如叶酸或光敏剂结合, 有助于在联合治疗中获得协同治疗效果。

OMVs的免疫原性是一把双刃剑, 由于OMVs的“非自身”特性, 易于被吞噬细胞吞噬和清除, 降低了疗效。与此同时, OMVs的免疫原性也是抗肿瘤作用的基础, 合理利用OMVs的免疫原性可增强抗肿瘤治疗效果。Li等[69]设计了一种纳米病原体系统 (nano-pathogenoids, NPN), 用OMVs包裹PEG-b-PLGA胶束, 中性粒细胞能够有效识别和内化NPN, 随着中性粒细胞向炎症肿瘤的迁移, NPN被迅速释放, 随后被肿瘤细胞吸收, 发挥抗肿瘤作用。使用天然高分子材料包裹OMVs[70,71]或采用仿生策略, 模拟生物系统, 制备仿生纳米囊泡, 可帮助OMVs逃避免疫系统识别和攻击[72]。Go等[72]开发了装载地塞米松的OMVs模拟纳米囊泡, 靶向激活内皮细胞, 减轻OMVs导致的全身炎症综合反应。用来自宿主的细胞膜 (红细胞膜、白细胞膜和巨噬细胞膜等) 包裹OMVs, 或用OMVs与其他类型细胞膜融合, 制备杂化纳米粒, 在降低OMVs免疫原性的同时, 还可以延长循环时间, 提高治疗效果[43,44,73]。

4 OMVs在抗感染疫苗中的应用研究

4.1 OMVs作为抗感染疫苗的优势

OMVs含有多种PAMPs, 能被上皮细胞和免疫细胞上的模式识别受体 (pattern recognition receptors, PRRs) 识别并激活免疫反应。抗原递呈细胞 (APCs) 对OMVs的识别和摄取, 促进抗原呈递、共刺激分子的表达和促炎细胞因子的分泌。这三个信号同时引发抗原特异性T细胞的激活, 活化的$CD8^+$ T细胞特异性杀死细菌感染的细胞, $CD4^+$ T细胞增强$CD8^+$ T细胞的细胞毒性, 激活B细胞产生抗体[74-76]。

与传统疫苗相比, OMVs具有以下优点: 20～250 nm的大小使其容易被抗原呈递细胞处理; 含有多种刺激机体免疫反应的成分, 具有更强的诱导主动免疫的能力, 克服了单一抗原的缺陷; OMVs疫苗比活细胞疫苗安全性更高。基于以上优势, OMVs为开发新一代抗病原微生物感染的疫苗开辟了新的途径[11,54]。

4.2 OMVs作为抗感染疫苗

耐药细菌的快速出现和传播仍然是一个难以解决的问题, 随着抗菌药物大量广泛使用, 细菌在抗菌药物的选择压力下, 耐药率越来越高, 临床亟需新型细菌病原体疫苗[12,77,78]。耐碳青霉烯类肺炎克雷伯菌 (carbapenem-resistant *Klebsiella pneumoniae*, CRKP) 感染是一个棘手的临床问题, Wu等[59]设计用OMVs包裹牛血清白蛋白 (bovine serum albumin, BSA), 制备了结构稳定、大小均一的BSA-OMVs纳米疫苗。经BN-OMV免疫后, 致死剂量CRKP感染的小鼠存活率显著提高。鲍曼不动杆菌 (*Acinetobacter baumannii*, *A. baumannii*) 经常引起严重的医院感染。由于其耐药问题严重, 耐药机制复杂, 对抗生素的适应迅速, 常表现为泛耐药和高病死率, 给临床治疗带来巨大挑战。Huang等[79]给小鼠接种*A. baumannii* OMVs (AbOMVs), 在体内产生了高水平的IgG抗体。在败血症模型中, 主动和被动免疫联合应用显著提高了泛耐药*A. baumannii*对喹诺酮类抗菌药物的敏感性。在肺炎模型中, AbOMVs与左氧氟沙星联合应用, 提高了左氧氟沙星的敏感性, 显著降低了炎症细胞浸润和炎症细胞因子聚集, 保护了小鼠免受*A. baumannii*菌株的攻击。在Choi等[80]的研究中, 接种金葡菌 (*Staphylococcus aureus*, *S. aureus*) OMVs疫苗能够诱导小鼠T细胞应

答, 上调共刺激分子和T细胞极化因子在APCs中的表达, 预防致死剂量*S. aureus*感染和亚致死剂量*S. aureus*引起的肺炎。寨卡病毒 (Zika virus, ZIKV) 感染会造成神经和自身免疫系统并发症以及新生儿畸形, 迫切需要开发针对ZIKV的疫苗。Martins等[81]利用脑膜炎奈瑟菌外膜囊泡 (*Neisseria meningitidis* OMV) 融合感染细胞释放的ZIKV, 得到ZIKV-OMV囊泡。小鼠免疫结果显示, 与未接种的小鼠相比, 抗体产生的效价大于1∶160, 免疫应答还激活了TH1和TH2细胞免疫应答。此外, 血清中和能够预防神经胶质肿瘤细胞模型 (M059J) 中病毒颗粒的感染。

然而, 单抗原OMVs疫苗受到诸如效力有限和单一抗原等因素的限制。Chen等[82]采用OMVs包被ICG负载MSN, 构建多抗原疫苗 (EV/ICG/MSN), 将EV/ICG/MSNs暴露于激光, 促进了树突状细胞 (dendritic cells, DC) 成熟和溶酶体逃逸, 提高了蛋白酶体活性和MHC-I表达, 增强了蛋白酶体依赖性抗原呈递途径, 可预防耐药金葡菌感染。

5 总结与展望

OMVs是具有脂质双分子层的纳米球形囊泡, 通过对OMVs的设计, 如基因工程、膜修饰或膜包裹等, 可开发成疫苗和药物递送载体等, 在肿瘤治疗和抗感染等疾病治疗领域中显示出巨大的潜力 (表1[42-46,49-51,58,59,65,66,68,79-82]), 但OMVs的临床应用仍然存在许多挑战。首先就是OMVs的安全性问题, 虽然对OMVs的修饰可以降低免疫原性和毒性, 但也限制了相应的治疗效果。此外, OMVs是细菌的产物, 在复杂的人体环境中, OMVs有可能会扰乱靶器官的微环境, 导致并发症。在未来, 需要对OMVs的纯化和减毒进行更进一步的研究, 以达到既具有治疗效果, 又能满足

Table 1 Application of OMVs for diseases therapy. ICG: Indocyanine green; PLGA: Poly(lactic-*co*-glycolic acid); NPs: Nanoparticles; PEG: Polyethylene glycol; RGD: Tumor-targeting ligand Arg-Gly-Asp; CTLs: Cytotoxic T lymphocytes; DOX: Doxorubicin; PD1/PD-L1: Programmed death 1/programmed death 1 ligand 1; EGFRvIII: Epidermal growth factor receptor variant III; NIR: Near-infrared; TRAIL: Tumor necrosis factor related apoptosis-inducing ligand; HPV16E7: Human papillomavirus type 16 early protein E7; 5-FU: 5-Fluorouracil; CaP: Calcium phosphate; BSA: Bovine serum albumin; CRKP: Carbapenem-resistant *Klebsiella pneumoniae*; ZIKV: Zika virus; MSN: Mesoporous silica nanoparticle

Application	Bacteria	Disease	Design	Key advance	Ref.
Tumor immunotherapy	*Escherichia coli*	Colon cancer	OMVs: induce antitumor immune responses	Eradicate established tumors without notable adverse effects	[42]
	Salmonella	Melanoma	OMVs envelope with melanoma cytomembrane vesicles: co-delivery of tumor antigens and immune stimulants ICG load with PLGA: induce immunogenic cell death and generate supplementary tumor antigen to immune system	Evoke the tumor-specific immune responses, protect mice from melanoma challenge	[43]
	Escherichia coli	Melanoma	OMVs fused with the cancer cell membrane: cancer targeting and immunotherapy ability Hollow polydopamine NPs: mediate photothermal therapy	Potentiate the therapeutic ability, eradicate melanoma without notable adverse effects	[44]
	Salmonella	Melanoma	OMVs fused with DSPE-PEG-RGD: activate cancer immunotherapy Tegafur-loaded micelles: sensitize cancer cells to CTLs and kill cancer cells directly	Provide effective protective immunity against melanoma occurrence, inhibite tumor growth and tumor metastasis to the lung, extende the survival rate of melanoma mice	[45]
	Klebsiella pneumonia	Non-small cell lung cancer	Encapsulate DOX in OMVs: facilitate the accumulation of DOX in tumor tissue, induce appropriate anti-tumor immune responses	Lead to a much more substantial tumor growth inhibition, apoptosis and necrosis	[46]
	Escherichia coli	Colon cancer Melanoma	Surface-modified OMVs with PD1: combination of immune activation and blockage of the PD1/PD-L1 axis	Lead to a significant reduction in tumor growth and no apparent adverse effects in non-tumor tissues	[49]
	Escherichia coli	Melanoma	Decorate OMVs with EGFRvIII: elicit antigen-specific, protective antitumor responses	Induced a strong inhibition of tumor growth, protect mice from tumor challenge	[50]
	Salmonella typhimurium	Colon cancer Breast cancer	OMVs combined with photothermal therapy: activate the immune system and effective photothermal ablation	Effective elimination of tumors with no significant toxic side effect	[51]

Continued

Application	Bacteria	Disease	Design	Key advance	Ref.
Antitumor drug delivery	*Escherichia coli*	Melanoma	NIR irritation combined with TRAIL: switch TRAIL-resistant tumor cells into a sensitive state OMVs modified with $\alpha_v\beta_3$ integrin and ICG: penetrate stratum corneum and target melanoma	Complete eradication of melanoma	[65]
	Escherichia coli	Cervical cancer	Decorate OMVs with HPV16E7: delivery the components to antigen-presenting cells	Stimulate specific cellular immune response and intervene the growth of Tc-1 tumor	[66]
	Escherichia coli	Colon cancer	Encapsulate MSN and 5-FU in OMVs: combine high drug loading capacity of the nano drug-loading system and the intestinal adsorption of biological carrier	Enhance cytotoxicity and cellular uptake toward tumor cells	[58]
	Escherichia coli	Colon cancer	CaP shells covered OMVs: decrease antibody-dependent clearance and high toxicity	Enable potent OMV-based tumor microenvironment reprograming without side effects	[68]
Anti-infective vaccine	Carbapenem-resistant *Klebsiella pneumoniae*	Carbapenem-resistant *Klebsiella pneumoniae* infection	BSA: reinforce the OMVs structure and uniform the size OMVs: stimulate the immune system	Mediate higher CRKP specific antibody titers, increase the survival rate of the mice infected with a lethal dose of CRKP	[59]
	Acinetobacter baumannii	Sepsis and pneumonia caused by *Acinetobacter baumannii*	OMVs: induce a prolonged IgG antibody response	Improve antibiotics sensitivity, reduce lung inflammatory, reverse bacterial resistance	[79]
	Staphylococcus aureus	Pneumonia caused by *Staphylococcus aureus*	OMVs: induce immune responses	Protect against staphylococcal lung infections *via* Th1 cell-mediated immunity	[80]
	Neisseria meningitidis	ZIKV infections	OMVs: induce immune responses and enhance the immune stimulation induced by ZIKV vaccine	The serum neutralization prevents infection of virus particles in the glial tumor cell model	[81]
	Staphylococcus aureus	Skin infection caused by *Staphylococcus aureus*	ICG: induce lysosome escape MSN: facilitate the binding of negatively charged ICG to MSN OMVs: activate T cells responses	Modulate antigen presentation pathways in dendritic cells, prevent and treat superficial infection, decrease bacterial invasiveness	[82]

临床试验的安全性。在肿瘤治疗中, 尽管OMVs可普遍诱导抗肿瘤活性, 但不同肿瘤类型定植独特的微生物群[83], 不同细菌来源的OMVs是否会对某些特定类型的肿瘤表现出最佳抗肿瘤活性, 值得更加深入的研究。

基于OMVs的生物功能, 临床应用前景可期。进一步的研究和发展可从以下几个方面着手: ① OMVs的规模化提取。OMVs的临床应用迫切需要大规模提取OMVs的技术; ② 制备减毒OMVs。目前对OMVs减毒的研究还很少, 需要更进一步探索降低OMVs免疫原性的方法和策略; ③ 开发仿生OMVs。OMVs的PAMPs组成限制了其应用, 结合天然生物材料的独特功能及人工纳米材料的多功能特点, 构建仿生OMVs, 以规避OMVs所面临的问题; ④ 研究不同来源OMVs对不同肿瘤的适用性。由于OMVs免疫刺激分子的多样性和肿瘤的异质性, 不同来源OMVs对不同肿瘤的适用性有待进一步的研究探讨。

综上所述, OMVs在疾病治疗领域显示出多方面的潜力, 尽管面临着诸多挑战, 但OMVs在疾病治疗方面的优势将为生物医学领域的研究带来新的前景。

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