微生物学通报
Microbiology China
http://journals.im.ac.cn/wswxtbcn

Sep. 20, 2023, 50(9): 4206−4219
DOI: 10.13344/j.microbiol.china.230262

专论与综述

肠道菌群来源细胞外囊泡在肝脏疾病中的作用研究进展

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贾文馨, 王丽蕊. 肠道菌群来源细胞外囊泡在肝脏疾病中的作用研究进展[J]. 微生物学通报, 2023, 50(9): 4206-4219.
JIA Wenxin, WANG Lirui. Research progress in the relationship between gut microbiota-derived extracellular vesicles and liver diseases[J]. Microbiology China, 2023, 50(9): 4206-4219.

摘　要：细胞外囊泡(extracellular vesicles, EVs)是一类具有脂质双分子层的膜性囊泡，可以被各种类型细胞分泌，是生物体通信的重要介质，参与原核生物和真核生物细胞之间的信号传输。在肠道微生态中，微生物-宿主的双向通信通常不需要细胞直接接触，微生物群来源 EVs 是这种“跨界”对话的关键参与者。肠-肝轴是连接肠道微生物与肝脏的桥梁，参与包含酒精性脂肪性肝病在内的多种肝脏疾病的发生与发展，近年研究发现肠道菌群来源的 EVs 在肝脏疾病的进程中具有重要的调控作用。本文概述了肠道菌群来源 EVs 的研究进展，特别是 EVs 的产生机制、包裹的内容物、在细菌-宿主互作以及在肝脏疾病中的作用。

关键词：细胞外囊泡；肠-肝轴；肠道微生物；肝脏疾病

Research progress in the relationship between gut microbiota-derived extracellular vesicles and liver diseases

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Abstract: Extracellular vesicles (EVs) are a type of lipid bilayer membrane vesicles, which can be secreted by a variety of cells. EVs as the key players of interkingdom crosstalk participate in the transmission of signals between prokaryotes and eukaryotes to regulate biological processes. In gut ecosystems, microbe-host communication usually does not involve direct cell

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Received: 2023-03-31; Accepted: 2023-05-22; Published online: 2023-06-16

contact. Microbiome-derived and host-derived EVs are key participants in such interkingdom crosstalk. The gut-liver axis plays a bridging role in the interaction between gut microbiota and the liver, that can modulate liver diseases including alcoholic fatty liver disease. Recent studies have demonstrated that gut microbiota-derived EVs play a key role in liver diseases. This article summarizes the research progress in gut microbiota-derived EVs, especially the mechanism of EVs production, the contents of EVs, bacteria-host interaction and its role in liver diseases.

Keywords: extracellular vesicles; gut-liver axis; gut microbiota; liver diseases

细胞外囊泡(extracellular vesicles, EVs)是一类由所有活细胞在不同产生途径下分泌的具有脂质双分子层的球形囊泡[1-2]，可以携带多种类型的分子，如蛋白质、RNA、DNA 和脂质[3]。自 Pan 等首次观察到网织红细胞可释放细胞外囊泡以来[4]，后续研究表明它们存在于几乎所有类型的哺乳动物细胞，包括各种癌细胞系[5]。1976 年，Hoekstra 等[6]在电子显微镜下观察到大肠杆菌(*Escherichia coli*)可以释放囊泡。之后，大部分革兰氏阴性菌和革兰氏阳性菌被证实可以分泌直径为 20−300 nm 的囊泡[7]。根据细菌分泌囊泡的结构和组成差异，人们将细菌来源的 EVs 分为革兰氏阴性菌的细菌外膜囊泡(outer membrane vesicles, OMVs)和外-内膜囊泡(outer-intimal membrane vesicles, OIMVs)，以及革兰氏阳性菌的膜囊泡(membrane vesicles, MVs)[8]。除细菌外，也有研究发现真菌可以产生囊泡，并且真菌可以通过囊泡传递信息参与维持调控机体的稳态[9-10]。

人体的皮肤和各种黏膜表面定居的大量微生物统称为微生物组[11]。其中肠道微生物群被认为是一个“重要器官”，结肠中每克湿重粪便中的微生物数量超过 10^{11} 个，它所包含的基因组大约是目前已发现人类基因组的 150 倍，远远超过并补充了人类基因组编码的遗传信息[12-13]。由于微生物进入肠上皮受到物理和化学上的限制，微生物与宿主的交流主要依赖于微生物分泌的因子，如代谢物、蛋白质和 EVs，它们可以穿过黏蛋白层被肠黏膜表面的宿主细胞内化。越来越多的证据表明，细菌分泌的装载着具有生物活性分子的囊泡被宿主细胞识别并整合入细胞内，能调控宿主细胞的信号通路，调节宿主的生理和病理过程[14-15]。肠道和肝脏在解剖学上接近，肝脏与肠道通过胆道、肝门静脉等通路进行双向交流。肠道菌群及其分泌的代谢物或囊泡与肝脏之间的信息通讯对维持宿主健康至关重要[16-18]。在酒精或者脂毒性影响下，肠道微生态系统发生紊乱，表现为菌群多样性改变，肠屏障破坏，并且肠道微生物及其代谢产物会通过被破坏的肠屏障易位至肝脏中，激活先天免疫系统受体并诱导肝脏脂质合成增加，促进肝脏疾病的发展。近年来，探究细胞外囊泡在介导慢性肝病发病中的作用以及作为慢性肝病的非侵入性诊断工具是国内外研究热点[19-21]。然而，目前研究大多集中在肝脏自身分泌的细胞外囊泡在肝脏疾病中的作用[22]，对于肠道来源的细胞外囊泡，尤其是微生物来源的细胞外囊泡在肝脏疾病中的调控作用及其背后的分子机制鲜有报道。本文关注细菌来源细胞外囊泡的产生过程、内容物及其在细菌-宿主互作的方式，最后探讨肠道菌群来源的细胞外囊泡在肝脏疾病中的作用。

1 细菌来源的细胞外囊泡：生物产生

在真核生物中，根据产生机制的不同，EVs被分为 3 种类型：外泌体(exosomes)、微囊泡(microvesicles)和凋亡小体[23]。外泌体是直径为50−150 nm 膜形囊泡，其产生过程涉及细胞质膜双重内陷和含腔内小泡(intraluminal vesicles, ILVs)的多泡内体(multivesicular bodies, MVBs)的形成[1,24-25]。与外泌体不同，微囊泡的产生不经过多泡内体过程，而是直接从质膜上出芽，释放一类直径为 0.1−1.0 μm 的囊泡；凋亡小体是在细胞发生凋亡时细胞骨架破裂和分解细胞碎片过程中释放的 EVs，其直径约 50−5 000 nm (图 1)[3]。

细菌正常生长和应激时可以释放直径为20–400 nm 不等的膜囊泡，影响多种生物过程包括细胞间通信和噬菌体感染[26-27]，革兰氏阴性菌来源的 OMVs 主要通过 3 种模型产生[7,26]：(1) 与膜交联调节相关(图 2A)。在细胞壁循环过程中，肽聚糖层(peptidoglycan, PG)和外膜之间的交联被破坏导致外膜出芽，OMVs 被释放到胞外空间[6,28-29]。相反地，若增加脂蛋白-PG交联则会使得 OMVs 分泌减少[30]。(2) 与脂质和脂质结合分子作用相关(图 2B)。喹诺酮假单胞菌信号(pseudomonas quinolone signal, PQS)中 2-烷基和 3-羟基与脂质 A 相互作用，刺激革兰氏阴性菌细胞膜出芽，从而细菌分泌 OMVs 增加[31-33]。(3) 与膜曲率诱导分子的聚集相关(图 2C)。肽聚糖碎片或错误折叠的蛋白质大量积累在周质(periplasm)，导致膨胀压力增加从而使得外膜膨出，OMVs 被释放到胞外[34-35]。另外，脂多糖(lipopolysaccharide, LPS)增加可以刺激细菌产生更多的 OMVs，其主要原因是 LPS 分子之间出现阴离子排斥，随后细菌细胞膜出现局部变形而分泌囊泡[33,36-37]。OMVs 的产生受多种因素的影响，其生物发生机制有待进一步研究。

相较于革兰氏阴性菌，对革兰氏阳性菌产生的 MVs 研究甚少。Lee 等在 2009 年研究中首次从金黄色葡萄球菌和枯草芽孢杆菌的培养上清中分离出革兰氏阳性细胞外囊泡[38]。革兰氏阳性菌含有内溶菌素，它是一种能降解肽聚糖的酶，能降解革兰氏阳性菌厚的肽聚糖壁并使细胞壁上出现小孔，继而导致细胞质膜从这些小孔中突出，并自发转化为细菌 MVs (图 2D)[8]。

2 细菌来源的细胞外囊泡：组成成分

EVs 具有囊形结构，可以携带不同类型的功能分子如蛋白质(膜蛋白、细胞质蛋白和核蛋白、细胞外基质蛋白)、RNA (mRNA、miRNA、IncRNA 和其他 RNA)、DNA (mtDNA、ssDNA 和 dsDNA)和脂质[39-41]，这些分子可以反映囊泡的起源及其供体细胞所处的病理生理状态[42]。

细菌分泌的 EVs 内容物主要包含三大类：蛋白质、脂质和核酸，有研究表明其中成分还会受环境因素，如温度和 pH 的影响而改变[26]。革兰氏阴性菌来源的 OMVs 中的脂质和蛋白质分布与供体外膜非常相似，其中 OMVs 的磷脂存在于膜内侧，LPS 存在于膜外侧，并与外膜蛋白和脂蛋白混合[43-44]。但 OMVs 的内容物含量与成分和供体细胞并不完全相同[45-47]，如 OMVs 中富集一些外膜蛋白包括 OmpA 和 AcrA 等周质蛋白。此外，研究者还发现 OMVs 中存在毒力因子和大量具有致病性的蛋白质[48-50]。

目前对革兰氏阳性菌来源 MVs 的内容物知之甚少。有 2 项脂质组学分析显示，革兰氏阳性细菌的 MVs 由多种脂肪酸组成，炭疽杆菌和肺炎链球菌来源的 MVs 富含肉豆蔻酸等短链饱和

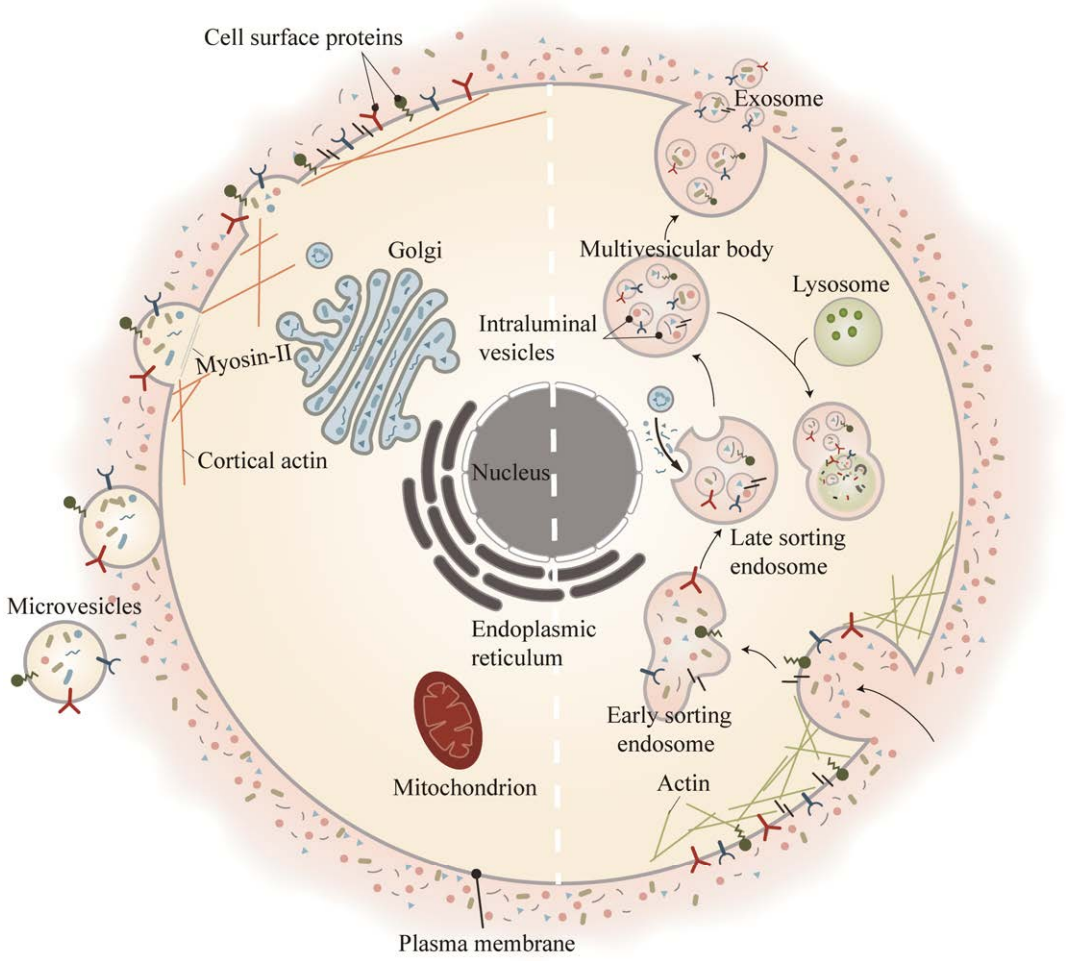

图 1　真核细胞细胞外囊泡产生过程　外泌体早期生物合成过程中，细胞外成分如蛋白质与细胞膜表面蛋白随细胞膜内陷所包裹起来，形成一个被称为早期分选核内体(early sorting endosome, ESE)的杯状结构. 随后，ESE 在内质网、高尔基体的作用下逐渐成熟成为晚期分选核内体(late sorting endosome, LSE)，LSE 膜内陷形成多个腔内小泡(intraluminal vesicles, ILVs)，最终形成多泡内体(multivesicular body, MVB). 细胞质膜和 MVB 相互融合之后，ILVs 就成为直径 50−150 nm 的外泌体被释放到细胞外，在外泌体形成过程中有细胞骨架蛋白如 actin 参与. 微囊泡则是在骨架蛋白 myosin-II 和 cortical actin 作用下直接从质膜上出芽，被释放到胞外

Figure 1　Generation and release of extracellular vesicle in eukaryotic cells. During the biosynthesis of exosomes, extracellular components such as proteins and cell membrane surface proteins are encapsulated by cell membrane invaginations, forming a cup-shaped structure called early sorting endosome (ESE). Subsequently, ESE gradually matures into late sorting endosomes (LSEs) under the action of endoplasmic reticulum and Golgi apparatus, and LSEs membrane invaginates to form multiple intraluminal vesicles (ILVs), and finally multivesicular bodies (MVBs). After the plasma membrane and MVBs fuse with each other, ILVs become exosomes with a diameter of 50−150 nm and are released outside the cell. Cytoskeletal proteins such as actin are involved in the formation of exosomes. Microvesicles directly bud from the plasma membrane under the action of the skeleton protein myosin-II and cortical actin, and are released to the outside of the cell.

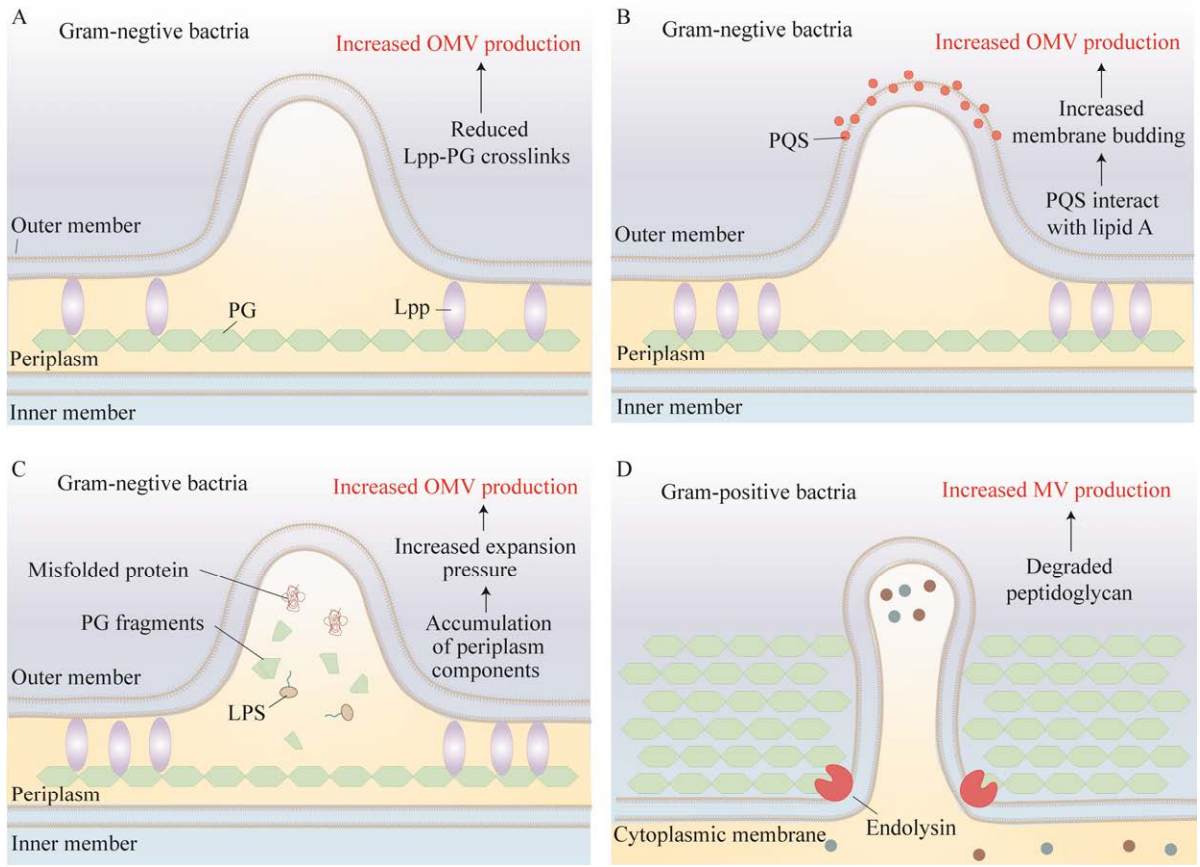

图 2 原核细胞中不同的细胞外囊泡产生过程 A、B、C 为革兰氏阴性菌产生细菌外膜囊泡(outer membrane vesicles, OMVs)的 3 种模型. A：革兰氏阴性菌外膜的脂蛋白(lipoprotein, Lpp)和肽聚糖层(peptidoglycan, PG)之间形成交联的能力受到肽聚糖内肽酶或其他因素影响，在低 Lpp-PG 交联区域，膜流动性增加从而使得 OMVs 产生增加. B：喹诺酮假单胞菌信号(pseudomonas quinolone signal, PQS)会与外膜的脂质 A 相互作用，增加膜曲率，导致 OMVs 的形成. C：错误折叠的蛋白质或包膜成分如脂多糖(lipopolysaccharide, LPS)或 PG 碎片所堆积的区域，使得交联被破坏膨胀压力增加，促进这外膜区域膨胀，导致 OMVs 产生增加. D：内溶菌素能降解革兰氏阳性菌厚的肽聚糖壁并使细胞壁上出现小孔，继而导致细胞质膜从这些小孔中突出，并自发转化为细菌细胞外囊泡，引发“气泡细胞死亡”，但其潜在机制尚不清楚

Figure 2 Different extracellular vesicle production processes in prokaryotic cells. A, B and C are three models of bacterial outer membrane vesicles (OMVs) produced by Gram-negative bacteria. A: Lipoprotein (Lpp) and the ability to form crosslinks between peptidoglycan (PG) is affected by peptidoglycan endopeptidases or other factors. In regions of low Lpp-PG crosslinking, increased membrane fluidity leads to increased OMVs production. B: Pseudomonas quinolone signal (PQS) interacts with lipid A of the outer leaflet of the outer membrane, increasing membrane curvature, resulting in Formation of OMVs. C: Areas where misfolded proteins or envelope components such as lipopolysaccharide (LPS) or PG fragments accumulate, causing cross-links to be destroyed and swelling pressure increased, which promotes the expansion of this outer membrane area, resulting in increased OMVs production. D: Endolysins degrade the thick peptidoglycan wall of Gram-positive bacteria and create pores in the cell wall, which in turn cause the plasma membrane to protrude from these pores and spontaneously transform into bacterial EVs, triggering “bubble cell death”, but the underlying mechanism remains unclear.

脂肪酸(C12–C16)[51-52]。*Clostridium perfringens* 产生的 MVs 中含有 16S 核糖体 RNA、编码 α 毒素的 DNA[31]。再者，有研究对金黄色葡萄球菌 MVs 进行蛋白质组学分析，鉴定出了 165 种蛋白质，其中富含细胞外膜的毒力相关蛋白，包括 IgG 结合蛋白 SbI 和 β-内酰胺酶，这些蛋白广泛参与了细菌-细菌和细菌-宿主相互作用的过程[38]。

如表 1 所示，不同来源的 EVs 内容物主要包括蛋白质、脂质和核酸三大类，但具体成分不尽相同，它们分布于 EVs 的膜上或腔内且发挥着不同的功能作用。这些功能分子可表征 EVs 的来源，如 CD9 和 TSG101 是真核生物来源 EVs 的生物标志物[53]。

表 1　各细胞外囊泡组成成分

Table 1　Composition of each extracelluar vesicles

Type	Position	Composition	Function	References
Exosomes	Member	Tetraspanins: CD9, CD81, CD63, CD53	Membrane organisers	[53]
		Annexin V, lactadherin	Cell adhesion	[3]
		RAB, GTPases, annexins	Intracellular trafficking	[54]
		A33, EpCAM, CD11c	Cell-type-specific proteins	[55-56]
	Lumen	Elongation factors, GAPDH	Enzymes	[3]
		Protein kinases, G proteins	Signal transduction	[3]
		ALIX, TSG101, syntenin-1	Biogenesis factors	[53]
		HSP70, HSP90	Chaperones	[57]
		microRNA and other non-coding RNAs, mRNA, DNA	Nucleic acids	[58]
Outer membrane vesicles	Member	β-lactamase, OprF, PonA	Antibiotic resistance	[50,59]
		LPS, PQS, peptidoglycan	Biogenesis factors	[59]
		Adhesin/Lnvasin, OmpA, OmpC, OmpF	Bacteria adhesion and invasion	[43]
		Cytolysin A, protease cholera toxin, shiga toxin	Host cell modulation	[60]
		EstA, FlgE, OprF, OprG	Host-bacteria interaction	[50]
	Lumen	Periplasmic proteins alkaline phosphatase, AcrA	Virulence factor delivery	[48]
		Endopeptidase L5, peptidoglycan hydrolase	Enzymes	[8]
		DNA, degP	Biofilm formation	[59]
Membrane vesicles	Member	Lipoteichoic acid (LTA)		[61]
		β-lactamase, penicillin-binding proteins: PBP1	Antibiotic resistance	[62-63]
		InlB, lgG-binding protein Sbl, protective antigen, lethal factor	Virulence factor Delivery	[38]
		Plasma-binding proteins, staphopain A	Bacteria adhesion and lnvasion	[38]
		α-hemolysin, proteolysin, β2 toxin, superantigens: SSaA1, SSaA2	Host cell modulation	[38]
	Lumen	DNA, RNA (including mRNA, rRNA, sRNA, and tRNA)	Bacterial communities	[60]
		Lipoprotein (MalX), transmembrane Protein (PspA), pneumolysin	Toxin	[52]
		Dehydrogenase, DNA polymerases, tRNA synthetases	Metabolism associated	[31,38]

GAPDH：甘油醛-3-磷酸脱氢酶；ALIX：ALG-2 相互作用蛋白 X；TSG101：肿瘤易感基因 101 蛋白；RAB：RAS-相关蛋白；LPS：脂多糖；PQS：喹诺酮类假单胞菌信号；OmpA：外膜蛋白 A

GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; ALIX: ALG-2 interacting protein X; TSG101: Tumour susceptibility gene 101 protein; RAB: RAS-related protein; LPS: Lipopolysaccharides; PQS: Pseudomonas quinolone signal; OmpA: Outer membrane protein A.

3 细菌来源的细胞外囊泡参与细菌-细菌或细菌-宿主的相互作用

细胞可以通过分泌 EVs 与邻近或远处细胞进行通信，细菌通过分泌 EVs 直接激活靶细胞或将 EVs 内的物质转移到受体细胞，参与细菌-细菌和细菌-宿主相互作用。

细菌来源的 EVs 参与细菌间互作有 3 种方式：群体感应、生物膜形成和相互竞争。据报道，铜绿假单胞菌(*Pseudomonas aeruginosa*)利用其 EVs 运输群体感应分子 PQS，PQS 结合 LPS 直接与细菌相互作用[32]；另外，变形链球菌(*Streptococcus mutans*)是人类龋齿的主要病原体，在细菌外膜产生过程中会大量释放含有细胞外 DNA 的 MVs，这些囊泡相关的细胞外 DNA 有助于生物膜的形成，并影响生物膜的结构完整性和稳定性，这可能是由于细胞外 DNA 与细胞外聚合物(extracellular polymeric substance)中的其他成分如多糖特异性结合导致；此外，EVs 可作为竞争武器，如铜绿假单胞菌 EVs 含有的水解酶可降解肽聚糖竞争性杀死其他细菌[64-66]。

细菌来源的 EVs 参与细菌-宿主相互作用的方式有 2 种：(1) EVs 被宿主细胞内化，从而内容物被传递到宿主细胞细胞质中。宿主细胞对细菌 EVs 的摄取主要通过内吞作用，这一过程的机制取决于囊泡的膜表面蛋白和内容物。最近的一项报道揭示了宿主细胞对常驻肠道菌多形拟杆菌(*Bacteroides thetaiotaomicron*)的 OMVs 摄取和运输机制[67]。研究者发现 *B. thetaiotaomicron* 来源的 OMVs 主要通过动力依赖的内吞作用被肠上皮细胞内化，并被运输到溶酶体内的囊泡；此外，体内成像研究表明，部分 *B. thetaotaomicron* OMVs 通过细胞旁转运穿过肠上皮并到达肝脏，这表明细菌来源的 EVs 可介导与肠外组织的远距离通信[67]。被宿主细胞内吞后，EVs 所携带的 sRNA、DNA、毒力因子诱导细胞炎症因子分泌，引发宿主炎症反应或细胞毒性反应[68-69]。(2) EVs 被宿主细胞膜表面受体识别。细菌来源 EVs 含有供体产生的生物活性成分，如病原体相关分子模式(pathogen-associated molecular patterns, PAMPs)可被宿主上皮细胞和免疫细胞表达的模式识别受体(pattern recognition receptors, PRRs)识别；Toll 样受体(Toll-like receptor, TLR)作为一种 PRRs，通常位于细胞质膜，可以与细菌来源 EVs 膜表面的 LPS 和脂蛋白结合，从而激活并参与调节免疫和防御反应的细胞信号通路[70-71]。此外，细胞质中还存在免疫受体，如核苷酸结合寡聚结构域蛋白 1 (nucleotide-binding oligomerization domain protein 1, NOD1)和 NOD2。革兰氏阴性菌侵染宿主时，OMVs 在细胞内运输过程中 NOD1 被招募，NOD1 与内吞囊泡中包含的肽聚糖相互作用[72]。结合肽聚糖后，这些受体通过激活 NF-κB 或丝裂原激活蛋白激酶途径启动炎症反应，最终上调炎症基因[72]。

4 肠道菌群来源细胞外囊泡在肝脏疾病中的作用

鉴于肝脏和肠道在解剖和功能上存在密切联系，肠腔中的囊泡极有可能会通过肠-肝轴进入肝脏，从而影响肝脏生理过程。有研究人员构建了表达 Cre 酶的工程大肠杆菌(*E. coli*^{Cre})，并定殖在 Rosa26.tdTomato 背景的小鼠中，导致 Cre 酶诱导的荧光报告基因在肠道上皮细胞中表达，包括肠道干细胞以及巨噬细胞等黏膜免疫细胞；在肠道外，OMVs 可越过肠道屏障转移到各种宿主组织中，包括心脏、肝脏、肾脏和大脑[73]。研究表明肝脏是 EVs 摄取的活跃部

位[74]，OMVs 主要集中在肝小叶汇管区，该区为肝动脉、肝门静脉和小胆管交汇处[73]。

近年来，随着对 EVs 的深入研究，细菌来源的 EVs 可作用于肝脏中各类型细胞，其在非酒精性脂肪性肝病、肝癌在内的各种肝脏疾病中的作用也被逐步发掘(图 3)。非酒精性脂肪性肝炎病人在循环血中细菌细胞外囊泡含量相较于健康人含量增多，而且粪便中的细胞外囊泡可通过抑制非肌球蛋白轻链激酶(nonmuscular myosin light chain kinase, nmMLCK)蛋白进而抑制肠上皮细胞紧密连接蛋白，如 ZO-1 的表达，增加肠道通透性，细胞外囊泡到达肝脏后增加肝脏内血管通透性以及肝脏内皮细胞炎症细胞因子和趋化因子的水平，激活 PS/TLR4 通路，从

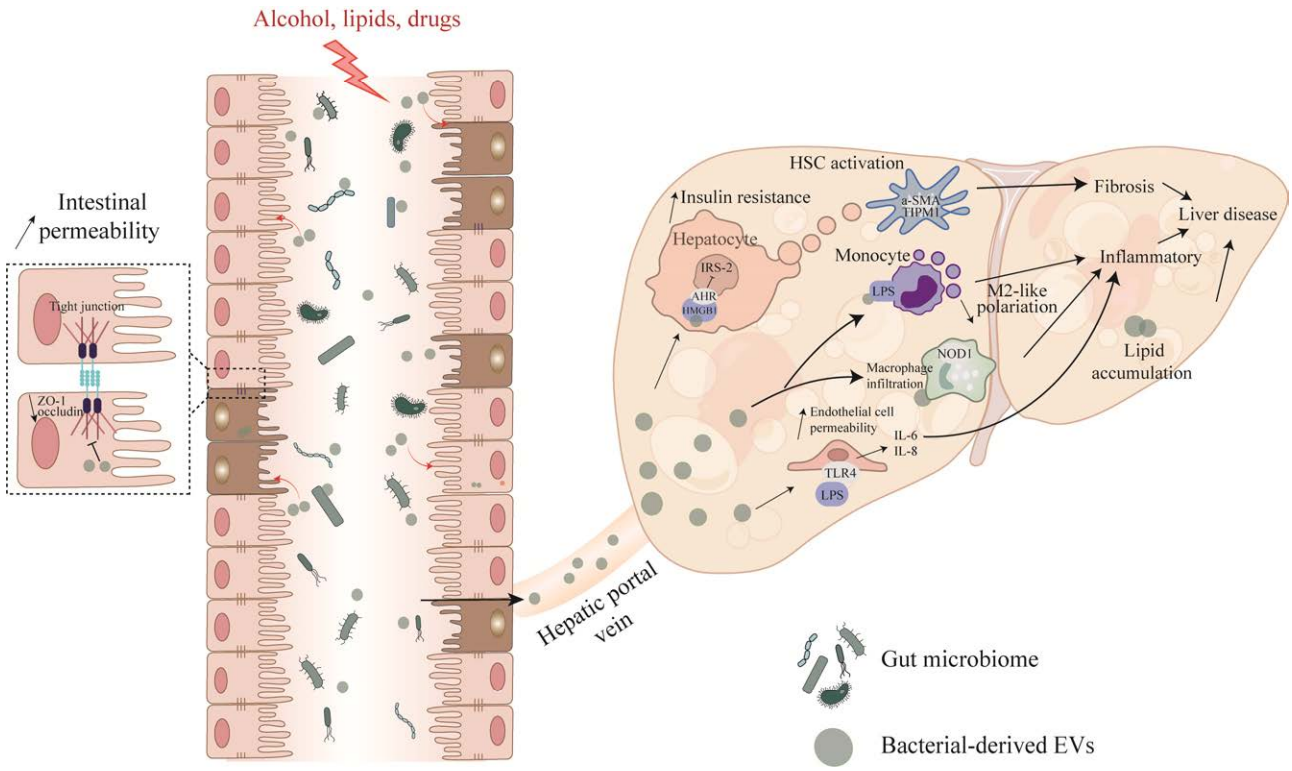

图 3　肠道菌群来源的细胞外囊泡在肝脏疾病的作用　酒精、各种脂毒性或者药物损伤刺激肠道细菌分泌细胞外囊泡，这些 EVs 可以抑制肠上皮细胞紧密连接蛋白如 ZO-1 的表达，增加肠道通透性. EVs 通过肠屏障及肝门静脉进入肝脏. 释放的 EVs 富含各种分子(miRNAs、蛋白质和 DNA)，并对肝脏中的几种肝细胞类型产生影响. 它们可以激活肝星状细胞并促进纤维化沉积. 它们被单核细胞、巨噬细胞和肝脏内皮细胞吸收，总体上导致肝脏炎症增加. 重要的是肝脏脂质积累也可以由肠道菌群衍生 EVs 引起

Figure 3　The role of gut microbiota-derived extracellular vesicles in liver disease. Alcohol, various lipotoxicity, or drug damage stimulate intestinal bacteria to secrete extracellular vesicles (EVs), which can inhibit the expression of intestinal epithelial cell tight junction proteins such as ZO-1 and increase intestinal permeability. EVs pass through the highly permeable intestinal lumen and enter the liver through the hepatic portal vein. The released EVs are enriched in various molecules (miRNAs, proteins, DNA) and exert effects on several hepatocyte types in the liver. They can activate hepatic stellate cells and promote fibrotic deposition. They are taken up by monocytes, macrophages, and liver endothelial cells, resulting in increased liver inflammation overall. Importantly, hepatic lipid accumulation can also be induced by gut-derived EVs.

而影响了 NASH 的发生发展[75]。高脂饮食小鼠或二型糖尿病人粪便中的 EVs 富含磷脂酰胆碱(phosphatidylcholine, PC)，PC 可以结合并激活肝脏中的芳香烃受体(aryl hydrocarbon receptor, AHR)，使得胰岛素信号通路相关基因包括 IRS-2 及其下游基因 PI3K 和 Akt 的表达受到抑制，从而引起胰岛素抵抗[76]。同时高脂饮食小鼠粪便中的 EVs 富含细菌 DNA，细菌 DNA 可以通过激活 cGAS/STING 引发肝细胞炎症和肝星状细胞(hepatic stellate cell, HSC)纤维化激活，这说明微生物 DNA 可能是肠道细胞外囊泡产生效应的关键货物[77-78]。近年来，幽门螺杆菌感染被认为在一些胃肠外的疾病特别是肝脏疾病中起着重要作用。OMVs 是幽门螺杆菌最重要的毒力因子之一。幽门螺杆菌来源的 OMVs 可能有助于肝实质细胞外泌体膜表面蛋白修饰，修饰后的外泌体可能在 HSC 激活和肝纤维化进展中发挥作用[79]。然而嗜黏蛋白阿克曼菌(*Akkermansia muciniphila*)被认为是新一代肠道益生菌，研究表明，*A. muciniphila* 来源的 EVs 能够改善高脂饮食合并肝纤维化小鼠模型的肠道通透性，同时，*A. muciniphila* 来源的 EVs 可减少肠道中的病原体，从而调节肝脏炎症反应，缓解肝纤维化，进而可预防肝损伤[80]。目前大部分的研究还停留在体外试验研究，更加深入的研究还有待探讨。

5 展望

肠道中含有庞大的微生物体系，并且肠道微生物稳态对维持人体健康具有重要意义。然而，关于微生物群作用的内在机制仍亟需探索，尤其是微生物如何将信息传递到靶细胞的有关机制，这对于了解疾病和将微生物群或微生物群衍生的 EVs 转化至临床有着重要意义。越来越多的证据表明，与疾病相关的微生物组变化可能反映在生物体液中细菌来源的 EVs 水平和组成上。因此，生物体液中特异性细菌来源的 EVs 可能与体内特定感染状态有关[81-82]。宏基因组学和代谢组学试验研究已经表明，细菌来源的 EVs 与阿尔兹海默病[83]、卵巢癌[82]和呼吸系统疾病[84-85]等疾病之间存在着紧密的关联。目前有临床研究发现，在肝脏疾病患者的粪便样本中发现肠道微生物 EVs[75]，并且 EVs 数量或者内容物含量与疾病损伤程度呈相关性。在这种情况下，肠道微生物 EVs 被认为是一种具有潜在价值的疾病诊断工具，主要优势在于：(1) 取样方便且无创，可以实现对患者疾病进程的实时监控；(2) EVs 携带特定的蛋白质、核酸等能提供丰富的生物学信息，特异性较高[1]；粪便中的 EVs 在不同的储存条件下具有较好的稳定性，能在−80 ℃长时间保存且内容物含量稳定[86]。尽管 EVs 在临床诊断上取得了一些令人鼓舞的成果，但仍然存在诸多挑战，比如需要特殊的仪器，如 MiSeq 系统和气相色谱以及生物信息学工具来分析获得的数据[87]。为了应对这些挑战，需要更多的研究来减少对技术平台的要求，并使数据分析更容易。预计在不久的将来，随着 EVs 基础研究和纯化手段的不断进步，EVs 有望在临床上成为疾病的特异性诊断工具，而且这一领域的研究工作将有助于理解复杂的微生物-宿主通信网络，这对保护人类健康至关重要。

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