# 细菌来源的细胞外囊泡在病原微生物与宿主细胞相互作用中的研究

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**摘要** 细胞外囊泡指所有类型的细胞包括免疫细胞通过不同的生物发生途径释放的具有双层膜结构, 包含脂类、蛋白质、糖类、遗传物质的球形纳米级膜结合小泡, 其也可以从许多生物液体中分离出来。细胞外囊泡在原核生物和真核生物中都被认为是细胞间通讯的有力载体, 可以参与广泛的生物过程。该文从细胞外囊泡、细菌来源的细胞外囊泡、细菌来源的细胞外囊泡与宿主细胞相互作用等方面介绍了细胞外囊泡大小和组成, 进一步阐述了细菌来源的细胞外囊泡的特点、功能、发生及其引发的炎症反应和细胞死亡, 最后对细菌来源的细胞外囊泡作为疫苗和载体的应用进行了展望。

**关键词** 细胞外囊泡; 细菌外膜囊泡; 炎症反应; 细胞死亡; 疫苗

# The Role of Bacterial Outer Membrane Vesicles in the Interaction between Pathogenic Microorganisms and Host Cells

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**Abstract** Extracellular vesicles refer to spherical nano-scale membrane vesicles with a double-layered membrane structure released by all types of cells, including immune cells through different biogenesis pathways, containing lipids, proteins, carbohydrates, genetic material, enzymes and various virulence factors. They can be separated from many biological fluids. Extracellular vesicles are considered to be powerful carriers of intercellular communication in both prokaryotes and eukaryotes, and can participate in a wide range of biological processes. The paper describes the extracellular vesicle size and composition in terms of extracellular vesicles, bacterial-derived extracellular vesicles, and bacterial-derived extracellular vesicle-host cell interactions, further elaboration on the characteristics, functions, and occurrence of extracellular vesicles of bacterial origin and the inflammatory response and cell death they trigger. Finally, the application of bacterial-derived extracellular vesicles as vaccines and carriers has prospected.

**Keywords** extracellular vesicles; bacterial outer membrane vesicles; inflammatory response; cell death; vaccines

细胞外囊泡(extracellular vesicles, EVs)泛指由细胞释放的膜结合小泡[1]。EVs是一个总称, 指所有类型的细胞通过不同的生物发生途径释放的具有双层膜结构, 包含脂类、蛋白质、糖类、遗传物质的球形纳米级膜结合小泡[1-2]。EVs也可以从许多生物液体(如唾液、尿液、鼻部和支气管灌洗液、羊水、

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母乳、血浆、血清和精液等)中分离出来, 其包含移植物抗原、共刺激/抑制分子、细胞因子、生长因子和可能调节受体细胞基因表达的功能性miRNA等生物活性物质[2-3]。EVs在原核生物和真核生物中都被认为是细胞间通讯的有力载体, 可以参与广泛的生物过程[3]。EVs包括真核细胞来源的外泌体和微生物来源的多种EVs, 本文就细菌来源的EVs的特点、发生及在感染宿主细胞等功能方面进行总结和描述。

## 1 微生物来源的EVs

微生物来源的EVs包括由细菌、真菌和寄生虫本身释放的EVs, 也包括在病毒、细菌、寄生虫和真菌感染过程中宿主细胞所产生和释放的EVs, 如表1所示, 主要包括: (1) 真核微生物释放的EVs, (2) 革兰氏阳性菌释放的EVs, (3) 革兰氏阴性菌释放的EVs, (4) 由病原微生物感染宿主细胞所产生的EVs。第一类: 真核微生物释放的EVs来源于真菌和寄生虫直接从细胞表面出芽后脱落的微囊泡, 这些囊泡可能发挥免疫调节作用[4]; 第二类: 革兰氏阳性菌释放的EVs, 由于革兰氏阳性菌细胞壁不具有外膜结构, 所以它们释放的EVs被称为细胞质膜囊泡(cytoplasmic membrane vesicles, CMVs)[5-7]; 第三类: 革兰氏阴性菌释放的EVs, 由于革兰氏阴性菌细胞壁具有外膜结构, 所以由革兰氏阴性菌释放的EVs被称为外膜囊泡(outer membrane vesicles, OMVs)[6,8]; 第四类: 由病原微生物感染过程中宿主细胞所产生和释放的外泌体, 其携带细胞内蛋白质和RNA等宿主成分, 可调节免

**表1 微生物来源的EVs**

**Table 1 Microbial-derived EVs**

| 分类<br>Classification | 来源<br>Source | | 直径/nm<br>Diameter /nm | 组成<br>Composition | 产生机制<br>Production mechanism | 功能<br>Features |
|---|---|---|---|---|---|---|
| Microvesicles | Eukaryotic microorganisms | Fungus | 20-<br>1 000 | Proteins, lipids, glycans, polysaccharides, nucleic acids and pigments | (1) the multivesicular bodies are released after fusion with the plasma membrane;<br>(2) sprouting from the plasma membrane;<br>(3) produced by cytoplasmic subtraction through invagination of the plasma membrane | (1) protection and defense;<br>(2) onset;<br>(3) immune regulation;<br>(4) perceive nutrition and environment, etc |
| | | Parasites | 30-100 | Lipids, proteins, DNA, RNA and metabolites | ESCRT mechanism | (1) cell communication;<br>(2) regulate immune response;<br>(3) new vaccines, etc |
| Cytoplasmic membrane vesicles (CMVs) | $G^+$ | Gram-positive bacteria | 10-400 | Enzymes, toxins, hemolysin and IgG binding proteins | Unknown | (1) inflammation;<br>(2) cell death;<br>(3) immune to escape;<br>(4) intercellular communication, etc |
| Outer membrane vesicles (OMVs) | $G^-$ | Gram-negative bacteria | 20-250 | DNA, RNA, lipopolysaccharide, enzyme, peptidoglycan | (1) protein accumulation;<br>(2) charge repulsion;<br>(3) SOS reaction | (1) inflammation;<br>(2) cell death;<br>(3) immune to escape;<br>(4) intercellular communication, etc |
| Exosomes | Pathogenic microorganisms infect host cells | Eukaryotic cell | 30-100 | Protein, carbohydrate, lipid, nucleic acid | (1) ESCRT mechanism;<br>(2) ceramide drive;<br>(3) four transmembrane protein CD63 | (1) antigen presentation;<br>(2) immune regulation;<br>(3) carrier;<br>(4) intercellular communication, etc |

疫反应[1]。革兰氏阳性菌释放的CMVs和革兰氏阴性菌释放的OMVs, 被统称为膜囊泡(membrane vesicles, MVs), MVs最近被确定为零型分泌系统[9-10]。

## 2 细菌来源的EVs

### 2.1 细菌来源的EVs的特点

细菌来源的EVs包括革兰氏阴性菌释放的OMVs和革兰氏阳性菌释放的CMVs。如表2所示, 多种革兰氏阳性菌, 包括金黄色葡萄球菌、单核增生李斯特菌、肺炎链球菌、B族链球菌等[6,10-11]可以释放CMVs。CMVs的大小处于10~400 nm, 含有酶、毒素、溶血素和IgG结合蛋白等成分[12-13]。如表2所示, 多种革兰氏阴性菌(包括铜绿假单胞菌、鲍曼不动杆菌、肺炎克雷伯氏菌、大肠杆菌等)也可以产生OMVs。OMVs大小并不是一致的, 直径大小为20至250 nm不等, 它们起源于细菌的外表面, 由母细菌外膜释放出来, 因此它们的组成通常与母体细菌的外膜相似[14-16], OMVs含有脂类、蛋白质、脂多糖和遗传物质等成分[17]。此外, 研究表明, 相同的细菌在不同的环境中产生的EVs的组成和含量也是不同的[18]。

### 2.2 细菌来源的EVs的功能

研究发现细菌来源的EVs可调节多种功能, 如可以调节细菌与环境之间的相互作用, 也可以调节细菌群落内微生物的之间的相互作用等。(1) 细菌来源的EVs参与了广泛的生物过程, 包括毒力传递、抗生素耐药性、水平基因转移、细胞通讯、铁清除、营养物质获取、宿主免疫系统调节和噬菌体感染抑制等[5,9,16-17,33]。研究表明, 致病性或非致病性的细菌来源的EVs在生物活性上可能表现出很大的差异[10,34]。(2) 细菌来源的EVs还可以为微生物群落提供保护功能, 对抗活性氧、抗生素、抗菌肽等有害物质[15]。(3) 细菌来源的EVs也可以传递抗原, 诱导保护性免疫反应, 从而作为一种潜在的疫苗[35-38]。

### 2.3 细菌来源的EVs发生的因素

研究发现许多因素都会导致细菌来源的EVs的生物发生, 对于革兰氏阴性菌而言: (1) 细菌表面的某些抗原的丢失和细菌中的一些毒力因子可能调节OMVs的生物发生[39]; (2) Vac J/Yrb ABC传输系统参与革兰氏阴性菌中OMVs的形成[40]; (3) 某些物质如环丙沙星、美罗培南、磷霉素、多粘菌素B等物质也会使OMVs产生增加, 有证据表明创伤弧菌OMVs

**表2 不同种类细菌释放的EVs**

**Table 2 EVs released by different types of bacteria**

| 分类<br>Classification | 种类<br>Species | 生物学特征<br>Characteristics | 参考文献<br>References |
|---|---|---|---|
| Gram-negetive bacteria | *Pseudomonas aeruginosa* | Inflammation/ cell death/ autophagy | [5,19-20] |
| | *Acinetobacter baumannii* | Inflammation/ cell death | [21] |
| | *Porphyromonas gingivalis* | Inflammation | [22-23] |
| | *Klebsiella pneumoniae* | Inflammation/ cell death | [24] |
| | *Escherichia coli* | Inflammation/ cell death | [25] |
| | *Helicobacter pylori* | Inflammation/ cell death/ autophagy | [20,26] |
| Gram-positive bacteria | *Staphylococcus aureus* | Inflammation/ cell death/ autophagy | [27] |
| | *Streptococcal* | Inflammation | [28] |
| | *Group B Streptococcus* | Promote Infection | [29-30] |
| | *Listeria monocytogenes* | Inhibit autophagy/ inhibit cell death | [31] |
| | *Bacillus subtilis* | Cell death | [32] |

的形成与包膜多糖的表达有关, 肠出血性大肠杆菌OmpT对OMVs的生物发生、组成和大小都会产生影响[14,41]; (4) 生长条件可能会对OMVs的释放产生巨大的影响, 如营养物质、温度、抗生素等的不同会使OMVs的组成、产量和含量不同; (5) 细菌经紫外线处理后, 产生的OMVs数量可能增加; (6) 细菌成分和OMVs之间的密切关系可能会调节OMVs的产生, 如LPS重塑会导致沙门氏菌中OMVs的形成[42-43]; (7) PQS是扩大外膜形成OMVs的必要条件, 通过增加膜曲率而导致小泡的形成[37]。

然而, 在革兰氏阳性菌中, 激活CMVs产生和释放的调节因子尚不清楚, 只有少数参与CMVs产生的因素被报道, 如(1) 细菌所处的环境会控制CMVs的数量和局部浓度[10]; (2) 基因毒性胁迫也可能诱导CMVs的形成; (3) 产生CMVs的细胞诱导其邻近细胞中CMVs的产生, 并且在丝裂霉素C(MMC)的存在下, CMVs的形成以浓度依赖的方式增加[32]; (4) 噬菌体内溶素触发的细胞裂解也可以导致CMVs的释放[5], (5) 研究表明, 用抗生素处理细菌, 是产生CMVs的一个触发因素, 但其潜在机制尚不清楚[44]。

### 2.4 细菌来源的EVs的发生

革兰氏阴性菌被膜包含外膜(由磷脂的内部小叶和LPS组成)和细胞质膜(由磷脂双分子层组成)两部分, 二者中间的周质间隙, 包含一层肽聚糖(peptidoglycan, PG), 周质是一种氧化环境, 可以促进蛋白质折叠[18]。要形成OMVs, 外膜必须从下面的肽聚糖层中释放出来并向外膨胀, 直到出芽的囊泡与细菌细胞膜发生分裂和分离[33]。如图1所示(由Biorender.APP绘制, https://biorender.com/), OMVs生物发生这一过程可能涉及到两个关键方面: (1) 外膜膨胀, OMVs生物发生的第一步是外膜的向外膨胀形成芽, 这个过程一般发生在外膜与肽聚糖层连接而缺少蛋白质的区域, 蛋白质的错误折叠和过表达也会导致外膜膨胀出芽; (2) 膜分裂释放囊状小泡, 当芽生长到膜曲率迫使芽状小泡与膜分离的点时, OMVs从细菌细胞中分离释放[42]。

导致外膜出芽的确切机制尚不清楚, 但有研究强调了脂蛋白、LPS和肽聚糖在OMVs形成过程中的重要性[45]。目前更多的研究集中在以下三个OMVs生物发生的模型: (1) 附着在肽聚糖层上的脂蛋白含量影响囊泡的产生, 低含量的脂蛋白导致外膜膨胀从而导致OMVs的形成[46]; (2) 由于物理或化学压力诱导的膜破损, 导致肽聚糖片段或错误折叠蛋白的积累, 在肽聚糖浓度较高的位点, 容易导致外膜向外突出[43]; (3) LPS所带电荷影响会诱导LPS的结构变化, 随后LPS之间的排斥力导致膜结构局部

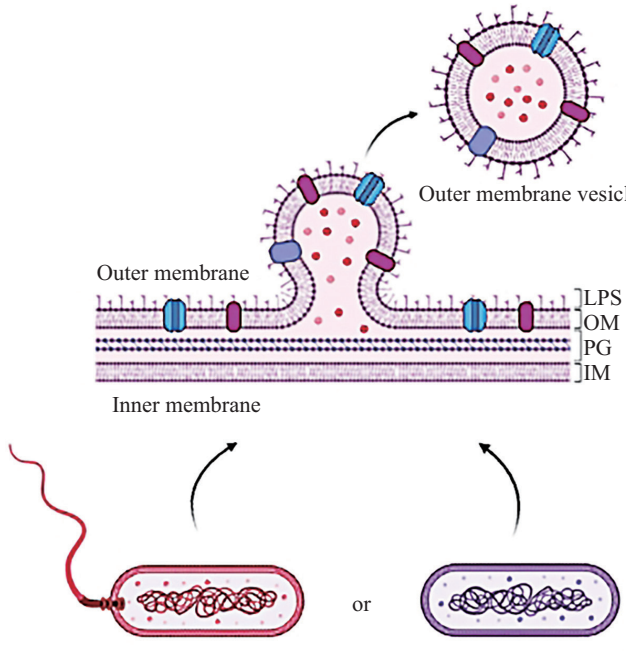


**图1 OMVs生物发生模式图(根据参考文献[42]修改)**

**Fig.1 The pattern diagram of biogenesis of OMVs (modified from reference [42])**

变形[43,47]。这些OMVs生物发生模型是否有协同作用仍然未知[45]。

在革兰氏阳性细菌中, CMVs形成的机制尚不清楚, 因为这些细胞拥有一个单一的细胞质膜, 被一个厚厚的细胞壁所包围, 目前支持CMVs形成机制的假说涉及细胞壁降解酶的作用, 细胞壁降解酶削弱肽聚糖层, 促进CMVs的释放[3,16,32]。

## 3 细菌来源的EVs与宿主细胞相互作用

细菌来源的EVs可以促进细菌–细菌和细菌–宿主之间的相互作用, 调节宿主先天免疫应答, 具体包括[25,48]: (1) 细菌来源的EVs可以向宿主传递sRNA或DNA, 以促进宿主与病原体之间的相互作用[27,49-50]; (2) 细菌来源的EVs可以通过其所携带的病原体相关分子模式(pathogen-associated molecular patterns, PAMPs)被宿主细胞的模式识别受体(pattern recognition receptors, PRRs)感知, 诱导细胞炎症因子的分泌, 引发炎症反应[25]; (3) 细菌来源的EVs可以诱导细胞发生细胞凋亡和细胞焦亡[14]; (4) 细菌来源的EVs还可以通过将抗生素抗性基因、染色体β-内酰胺酶或毒力因子等传递给其他细菌来增加细菌的致病性或促进细菌的存活[5,10,17,51]。

### 3.1 细菌来源的EVs引发的炎症反应

来源于病原微生物的蛋白质、脂质、多聚糖和核酸等PAMPs可以被宿主细胞表达的不同家族的PRRs感知, 并引发炎症反应。细菌来源的EVs所携带的PAMPs, 例如LPS、外膜蛋白、孔蛋白、鞭毛蛋白和核酸等, 如表3所示, 同样可被宿主细胞膜表面和胞质中的PRRs感知, 激活促炎细胞因子的表达和分泌[35]。

3.1.1 细菌来源的EVs被细胞膜表面PRRs感知而引发的炎症反应 如图2所示, 机体的巨噬细胞和中性粒细胞膜表面的PRRs可以感知细菌来源的EVs携带的PAMPs, 激活NF-λB和MAPK通路, 促进炎症因子的表达, 引发炎症反应[53]。OMVs不仅可被免疫细胞膜表面的PRRs感知, 也可以被肺上皮细胞、肠上皮细胞等非免疫细胞膜表面的PRRs感知。例如, 铜绿假单胞菌OMVs携带的LPS需要通过MyD88依赖的TLR4信号通路在上皮细胞中引发强烈的免疫反应, 还能够促进肺上皮细胞的IL-8的表达而引发炎症反应[5,19]; 鲍曼不动杆菌OMVs携带的外膜蛋白、孔蛋白等可通过TLR信号通路介导细胞因子和趋化因子的表达分泌而引发炎症反应, 它还可与宿主细胞的细胞质膜结合, 并向宿主细胞传递细菌效应物[5,21]; 肺炎克雷伯菌OMVs中包装的毒力因子和其他PAMPs如LPS、外膜孔蛋白、鞭毛蛋白和肽聚糖等靶向宿主细胞, 与宿主细胞相互作用并促进促炎活性[24]; 产肠毒素大肠杆菌OMVs的表面具有不热毒素(heat-labile enterotoxin, LT), 使OMVs通过PRRs直接与宿主细胞相互作用, 并激活调节细胞因子和趋化因子的信号通路[25,48]。产气荚膜梭菌CMVs携带的蛋白质和核酸等成分激活宿主细胞的先天免疫反应, 通过TLR2信号通路诱导J774.1和RAW264.7小鼠巨噬细胞中促炎细胞因子IL-6的释放[10]; 金黄色葡萄球菌CMVs携带的DNA、RNA和肽聚糖可以激活TLR2、TLR7、TLR8、TLR9及NOD2信号通路, 最终激活NF-κB通路而诱导促炎反应并释放细胞因子[27]。

3.1.2 细菌来源的EVs被胞质内PRRs感知引发的炎

**表3 引起炎症反应的细菌来源的EVs所携带的PAMPs**

**Table 3 PAMPs carried by bacterial-derived EVs that cause inflammation**

| 病原体相关分子模式<br>PAMPs | 细菌<br>Bacteria | 模式识别受体<br>PRRs | 参考文献<br>References |
|---|---|---|---|
| Lipopolysaccharide (LPS) | *Pseudomonas aeruginosa, Klebsiella pneumoniae* | Cell membrane surface/cytoplasmic | [19,24] |
| Out membrane protein (Omp) | *Acinetobacter baumannii* | Cell membrane surface | [52] |
| Porin | *Acinetobacter baumannii* | Cell membrane surface | [5] |
| Flagellin/<br>Peptidoglycan | *Klebsiella pneumoniae* | Cell membrane surface/cytoplasmic | [24] |
| ETEC heat-labile enterotoxin (LT) | *Enterotoxigenic Escherichia coli* (ETEC) | Cell membrane surface | [25] |
| Protein,<br>Nucleic acid | *Clostridium perfringens* | Cell membrane surface | [10] |
| Nucleic acid | *Staphylococcus aureus* | Cell membrane surface/cytoplasmic | [27] |

症反应　除了细胞表面的PRRs, 细菌来源的EVs所携带的PAMPs也可以被递送至宿主细胞的细胞质中, 被胞质内PRRs感知识别[54-55]。PAMPs被胞质内的PRRs感知识别后组装炎性小体, 现主要有NLRP1、NLRP3、NLRC4、AIM2四类炎性小体, 他们最终都可激活caspase-1, 并激活参与炎症反应的细胞因子IL-1β和IL-18[56]。如图2所示, 炎性小体可以感知OMVs所携带的PAMPs激活炎性caspase-1, 触发IL-1β和IL-18前体的加工随后将其释放到细胞外环境, 这种依赖于capase-1的过程被称为“经典的”炎症小体激活。例如, (1) 在小鼠树突状细胞中, OMVs携带的百日咳杆菌毒力因子通过NLRP3传感器激活的caspase-1触发IL-1β的产生[55]。(2) 鼠伤寒沙门氏菌和铜绿假单胞菌等鞭毛细菌释放的OMVs以依赖内吞作用的方式在巨噬细胞中引起强烈的NLRC4介导的caspase-1激活和IL-1β分泌[51]。(3) OMVs也可以将细菌DNA运送到宿主细胞质并触发AIM2, 随后与ASC一起形成炎性小体复合物以激活caspase-1并介导促炎细胞因子IL-1β和IL-18的释放[54-55]。“非经典”炎症小体的激活依赖于caspase-11(小鼠)或caspase-4/5(人类)[55]。例如, (1) 百日咳OMVs可携带LPS进入细胞质激活caspase-11, 诱导caspase-11依赖的非典型炎症小体激活, 促进炎性细胞因子的表达和分泌[14,51,55]。(2) 非鞭毛细菌如非鞭毛大肠杆菌释放的OMVs携带的LPS通过激活caspase-11非典型炎症小体间接激活NLRP3依赖的IL-1β的表达和分泌[51]。

革兰氏阳性菌如金黄色葡萄球菌产生的CMVs, 可以将核酸和肽聚糖递送到上皮细胞以激活细胞内先天免疫, 可以在巨噬细胞中激活NLRP3炎性小体并诱导IL-1β的产生, 也可以激活人巨噬细胞中的

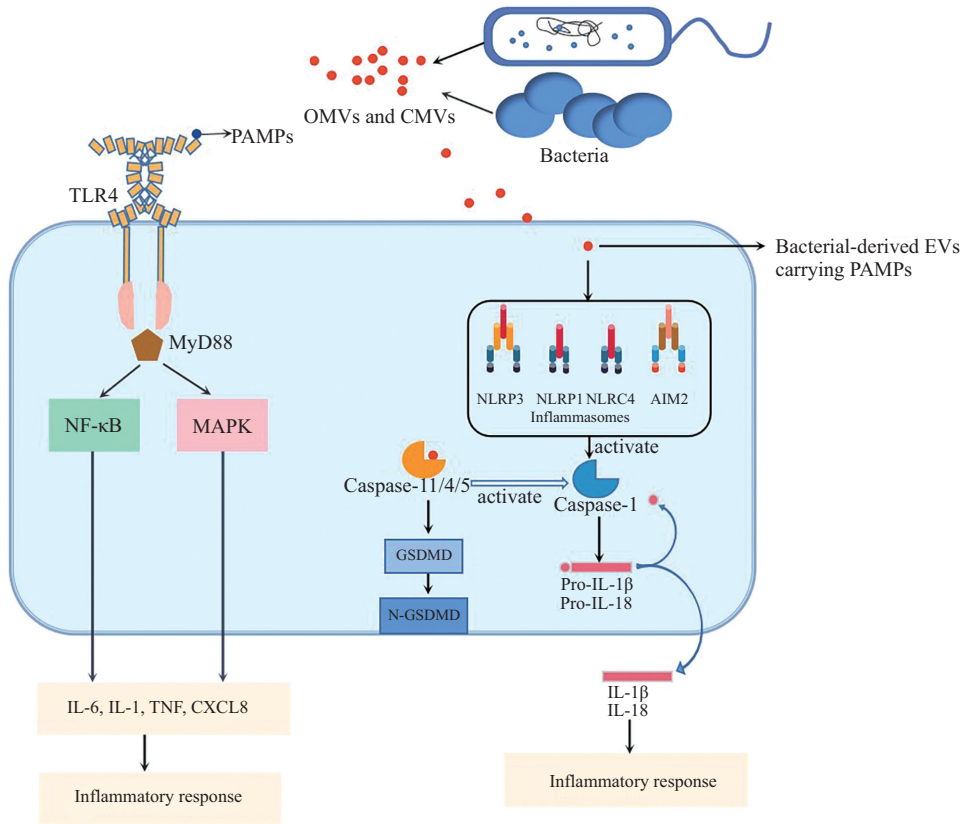


图2左: 细菌来源的EVs所携带的PAMPs, 被细胞膜表面模式识别受体TLR4识别, 通过MyD88, 激活NF-κB或MAPK通路, 释放炎症因子, 引起炎症反应; 图2右: 细菌来源的EVs携带的PAMPs如LPS、核酸, 激活胞质内炎性小体, 最终激活caspase-1, 切割Pro-IL-1β、Pro-IL-18为活性IL-1β、IL-18, 引发炎症反应, 同时, LPS激活caspase-11导致的$K^+$外流也会激活caspase-1。

Fig.2 left: PAMPs carried by bacterial-derived EVs, which are recognized by the cell membrane surface pattern recognition receptor TLR4, activate NF-κB or MAPK pathways through MyD88, release inflammatory factors, and cause inflammation; Fig.2 right: bacterial-derived EVs carrying PAMPs, such as LPS and nucleic acids, activate intracytoplasmic inflammasomes, and finally activate caspase-1, cleave Pro-IL-1β and Pro-IL-18 into active IL-1β and IL-18, trigger inflammation, and at the same time, the $K^+$ outflow caused by LPS activation of caspase-11 will also activate caspase-1.

**图2　细菌来源的EVs引发的炎症反应(根据参考文献[53-56]修改)**

**Fig.2　Inflammation caused by bacterial-derived EVs (modified from references [53-56])**

caspase-1, 并诱导IL-1β和IL-18的释放, CMVs也可以被NOD2样受体检测, 以剂量依赖的方式激活肺上皮细胞产生细胞因子和趋化因子[27,57-59], CMVs也可将其携带的脂蛋白传递到宿主细胞并通过激活TLR2和NLRP3炎症小体来驱动炎症反应[27]; 肺炎链球菌CMVs携带的脂蛋白或DNA等成分被内化到A549肺上皮细胞和人单核细胞来源的树突状细胞中, 并导致促炎细胞因子表达产生[5,60-61]。

### 3.2 细菌来源的EVs诱导的细胞死亡

除了引起炎症反应, OMVs和CMVs携带的PAMPs还可以诱导宿主细胞死亡, 包括细胞凋亡和细胞焦亡[62-63], 目前所报道的引起细胞死亡的PAMPs如表4所示, 详述如下。

3.2.1 细菌来源的EVs诱导的细胞凋亡 如图3所示, 从细菌中脱落的OMVs可以引发线粒体应激, 并激活凋亡细胞死亡因子, 并针对线粒体诱导程序性细胞死亡[14,51]。例如, (1) 爆发株大肠杆菌O104:H4释放的OMVs携带的毒力因子志贺毒素(Stx2a)是导致细胞凋亡的主要OMVs成分, Stx2a由OMVs携带至细胞内通过激活caspase-9和caspase-3而导致人肠上皮细胞凋亡[64]; (2) 鲍曼不动杆菌OMVs的ompA可以通过线粒体和核靶向诱导宿主细胞凋亡, omp33-36孔蛋白也可以在HeLa和HEp-2细胞中诱导细胞凋亡并调节自噬[52,65]; (3) 致病性大肠杆菌OMVs携带蛋白毒素, 定位到线粒体并诱导细胞色素c的释放, 释放的细胞色素c诱导凋亡小体的形成, 激活介导凋亡的caspase-9和caspase-3, 从而导致细胞凋亡的发生[62]; (4) 淋病奈瑟球菌利用OMVs将PorB运输到宿主细胞细胞质中, 随后将其转运至线粒体膜, PorB在线粒体中诱导细胞色素c释放、caspase-3激活、质膜起泡和细胞凋亡[66], 但PorB本身并不足以诱导细胞凋亡, 并且有证据表明并非所有的PorB同源物都会诱导细胞死亡, 如脑膜炎奈瑟球菌PorB可靶向线粒体以防止细胞凋亡[66,70]。(5) 肠出血性大肠杆菌(enterohemorrhagic *E. coli*, EHEC)的毒素之一EHEC溶血素(EHEC-Hly), 以OMVs为载体通过动力蛋白依赖性内吞作用内化到溶酶体中, 在内涵体酸化过程中, EHEC-Hly与OMVs分离, 从溶酶体中逸出并靶向线粒体, 线粒体中EHEC-Hly的存在导致线粒体跨膜电位降低和细胞色素c释放到细胞质中, 随后caspase-9的激活触发了细胞凋亡[71]。研究发现革兰氏阳性菌金黄色葡萄球菌CMVs以剂量依赖的方式诱导HEp-2细胞凋亡[5]。

3.2.2 细菌来源的EVs诱导的细胞焦亡 炎性小体的激活除了引发炎症反应还会引发一种称为细胞焦亡的炎症性细胞死亡形式, 如图3所示, OMVs携带的LPS被胞质内PRRs识别, 从而使激活的caspase-11/caspase-4/caspase-5裂解GSDMD(gasdermin D)的氨基末端成为片段, 导致细胞焦亡[62]。例如大肠杆菌OMVs已被证明可作为细胞溶质LPS的传递系统, 它结合并激活细胞溶质caspase-11(caspase-4/caspase-5)以通过形成孔的gasdermin D的裂解引起细胞焦亡[53,55,67,72]。铜绿假单胞菌、鼠伤寒沙门氏菌(野生型和orgA/fliC/fljAB缺陷型)和弗氏志贺氏菌释放的OMVs, 也通过激活caspase-11而诱导细胞焦亡和IL-1β释放[67]。各种肠杆菌中产生的OMVs携带的溶血素可以显著促进人肠上皮细胞系中caspase-4依赖性细胞焦亡和IL-18的分泌[68]; 金黄色葡萄球菌CMVs被人巨噬细胞通过TLR2信号通路响应, 并通过$K^+$外排激活NLRP3炎症小体, 导致ASC的招募和caspase-1的激活。通过活化的caspase-1切割Pro-IL-1β、Pro-IL-18和

**表4 细菌来源的EVs相关PAMPs及生物学特征**

**Table 4 Bacterial-derived EVs related PAMPs and their biological characteristics**

| 病原体相关分子模式<br>PAMPs | 生物学功能<br>Biological characteristics | 参考文献<br>References |
|---|---|---|
| Stx2a (escherichia coli O104:H4 outbreak strain) | Apoptosis | [64] |
| ompA (acinetobacter baumannii) | Apoptosis/<br>autophagy | [52,65] |
| PorB (neisseria gonorrhoeae) | Apoptosis | [66] |
| HIa (staphylococcus aureus) | Apoptosis | [5] |
| LPS (gram-negetive bacteria) | Pyroptosis | [67] |
| Hemolysin (enterobacter) | Pyroptosis | [68] |
| VCC (Vibrio cholerae) | Autophagy | [69] |

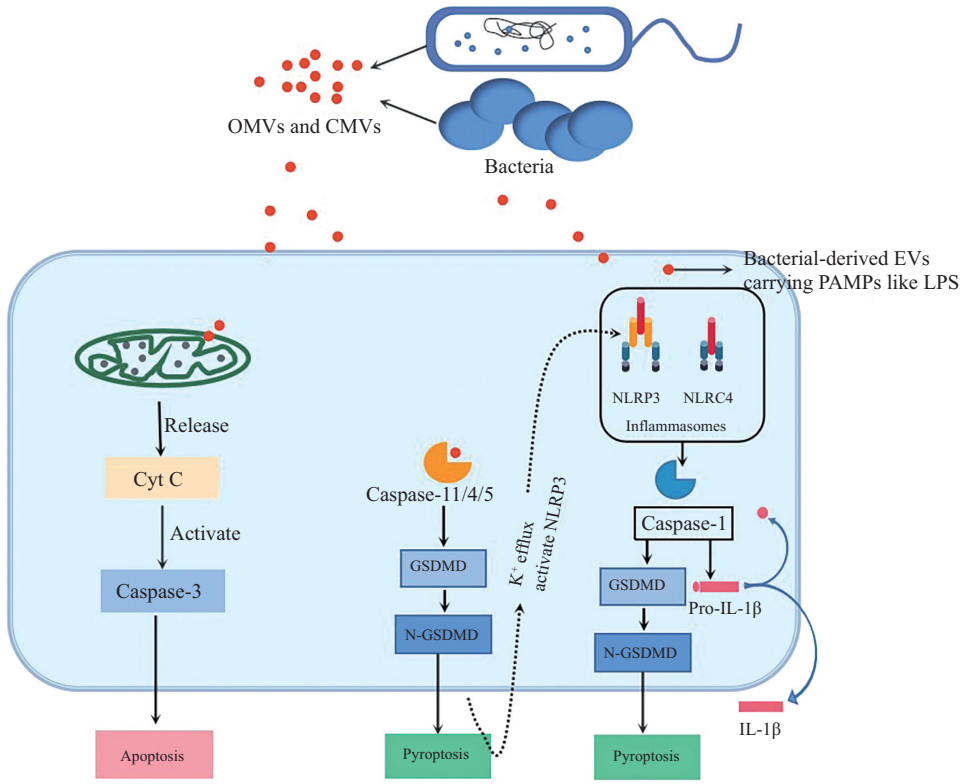


图3左: 细菌来源的EVs携带的一些毒力因子在线粒体内膜上诱导细胞色素c的释放, 激活caspase-3而诱导细胞凋亡; 图3右: 细菌来源的EVs携带的PAMPs如LPS进入胞质后, 可以通过激活炎性小体介导的caspase-1经典途径切割GSDMD诱导细胞焦亡, 可以通过激活caspase-11(小鼠)或caspase4/5(人)非经典途径切割GSDMD诱导细胞焦亡; 同时, caspase-11非经典途径中导致的$K^+$外流也会激活NLRP3介导的caspase-1途径。

Fig.3 left: some virulence factors carried by bacterial-derived EVs induce the release of cytochrome c on the inner mitochondrial membrane and activate caspase-3 to induce apoptosis; Fig.3 right: the entry of PAMPs such as LPS carried by bacterial-derived EVs After cytoplasm, GSDMD can be cleaved by the classical pathway of caspase-1 mediated by activating inflammasome to induce pyrolysis, and GSDMD can be cleaved by activating caspase-11 (mouse) or caspase4/5 (human) by non-classical pathway. At the same time, the $K^+$ efflux caused by the non-classical pathway of caspase-11 can also activate the caspase-1 pathway mediated by NLRP3.

**图3 细菌来源的EVs引发的细胞死亡(根据参考文献[51,53,55,67,72]修改)**

**Fig.3 Cell death caused by bacterial-derived EVs (modified from references [51,53,55,67,72])**

GSDMD, 导致细胞因子IL-1β和IL-18释放, 并诱导焦亡[59]。

## 4 总结和展望

除了激活炎症反应和细胞死亡, 细菌来源的EVs还可以诱导自噬, 自噬是一种受到严格调控的降解过程, 由一系列细胞受到的胁迫激活[73]。例如, OMVs携带的霍乱弧菌溶细胞素(vibrio cholerae cytolysin, VCC)可以在CHO细胞中导致AMPK和mTORC1活性的变化, 以及自噬激酶复合物的激活而诱导自噬[69]; 革兰氏阳性细菌产生的CMVs有能力在宿主细胞内诱导自噬降解途径, 从而促进它们从宿主中被清除[27]。此外, OMVs可作为诱饵, 吸收宿主产生的抗微生物化合物, 并允许细菌在定殖过程中逃避免疫检测[19]。研究表明, 牙龈卟啉单胞菌(*P.gingivalis*) OMVs通过阻碍宿主反应来促进牙龈卟啉单胞菌的局部免疫逃避[14,74]。同时有蛋白质组学分析表明, 这些OMVs包含的一些毒力因子有助于这些细菌逃避宿主防御系统, 在肺炎链球菌的CMVs中发现了一种细胞外DNase, 它可以阻断中性粒细胞活性, 并有助于其逃避宿主的先天免疫反应[5]。此外, 由于(1) OMVs在不同温度和处理下保持完整和稳定; (2) OMVs含有大量与亲本细菌相关的免疫原性成分等特征, 其可以作为疫苗、佐剂或载体, 用于药物治疗新出现的疾病[14,75-76]。另外进一步了解OMVs生物发生将有助于生产富含理想抗原的OMVs, 从而创造更有效的疫苗并改善人类健康[42]。

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