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Review

Gram-negative and Gram-positive bacterial extracellular vesicles

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ABSTRACT

Like mammalian cells, Gram-negative and Gram-positive bacteria release nano-sized membrane vesicles into the extracellular environment either in a constitutive manner or in a regulated manner. These bacterial extracellular vesicles are spherical bilayered proteolipids enriched with bioactive proteins, lipids, nucleic acids, and virulence factors. Recent progress in this field supports the critical pathophysiological functions of these vesicles in both bacteria–bacteria and bacteria–host interactions. This review provides an overview of the current understanding on Gram-negative and Gram-positive bacterial extracellular vesicles, especially regarding the biogenesis, components, and functions in poly-species communities. We hope that this review will stimulate additional research in this emerging field of bacterial extracellular vesicles and contribute to the development of extracellular vesicle-based diagnostic tools and effective vaccines against pathogenic Gram-negative and Gram-positive bacteria.

Contents

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1. Introduction: extracellular vesicles as evolutionarily conserved intercellular communicasomes

Most cells in all domains of life on earth, including eukaryotes, Gram-negative and Gram-positive bacteria, and archaea, actively shed nano-sized membrane vesicles into the extracellular environment [1–4]. These evolutionarily conserved vesicles are spherical, bilayered proteolipids harboring specific subsets of bioactive proteins, lipids, nucleic acids, and metabolites [5–7]. Diverse terms have been employed to indicate extracellular vesicles: membrane vesicles for archaea and Gram-positive bacterial origin, outer membrane vesicles for Gram-negative bacterial origin, and exosomes and ectosomes (also known as microvesicles) for mammalian cell origin [8]. Here, in reflecting their presence outside the cells, the term "extracellular vesicles" will be used to refer to all these membrane vesicles. Recent progress in this field supports diverse physiological and pathological roles of extracellular vesicles in poly-species communities [3,7,9,10]. These observations suggest possible roles of extracellular vesicles as evolutionarily conserved intercellular communicasomes but not as long disregarded cellular debris [3,7,9–12].

This review will focus on the Gram-negative and Gram-positive bacterial extracellular vesicles, which have several physiological and pathological functions in both bacteria–bacteria and bacteria–host interactions.

2. Gram-negative bacterial extracellular vesicles: outer membrane vesicles

Gram-negative bacterial extracellular vesicles, known as outer membrane vesicles, were first observed in the 1960s, by electron microscopic studies of the bacterial structures [1,13]. Since then, Gram-negative bacterial extracellular vesicle-related studies have made steady progress, and the number of publications has increased rapidly in the last 5 years (Fig. 1). These extracellular vesicles have an average diameter of 20–200 nm, and are secreted from various Gram-negative bacteria [14–20]. Furthermore, Gram-negative bacterial extracellular vesicles have been found in various environmental conditions: (1) *in vitro* experimental conditions: planktonic and biofilm culture [21–23]; (2) natural environments: water drains, sewage, soil, and house dust [5,18,24–27]; and (3) tissues and biological fluids: cerebrospinal fluid and blood from clinically severe bacterial infections, and gastric biopsy specimens from *Helicobacter pylori*-infected patients [18,26,28].

Various studies on bacterial extracellular vesicles have contributed to identifying the biogenesis, components, and functions of the vesicles. In the following subsections, we will present the current understanding on the biogenesis and components of Gram-negative bacterial extracellular vesicles.

2.1. Biogenesis

Gram-negative bacterial extracellular vesicles, known as outer membrane vesicles mainly originate from the outer membrane of bacterial envelope [29]. To date, three potential biogenesis mechanisms have been suggested on the basis of genetic and biochemical studies. The vesiculation could occur by the loss of interaction between outer membrane and the underlying peptidoglycan layer caused by the different turnover rates. On the other hand, the vesicles could be released by the turgor pressure of outer membrane caused by the accumulation of periplasmic proteins or peptidoglycan fragments in the periplasmic space [5,30]. Recent studies on *Pseudomonas aeruginosa* suggested that Pseudomonas quinolone signal (PQS) incorporated into the lipopolysaccharides (LPS) in the outer leaflet of outer membrane, creates repulsion by the accumulation of negative charges, and therefore, leads to curvature formation on the outer membrane [31,32]. Recent reviews on Gram-negative bacterial extracellular vesicle biogenesis are available [5,9,11,33].

2.2. Components

Gram-negative bacterial extracellular vesicles harbor LPS, proteins, lipids, genetic materials, and other virulence factors [5,8,34]. In the following subsections, we will present the current understanding on these vesicular components.

2.2.1. Proteins

In early studies, the presence of abundant outer membrane proteins (OmpA, OmpC, and OmpF), periplasmic proteins (alkaline phosphatase and AcrA), and virulence factors (adhesins, invasins, and other enzymes) in Gram-negative bacterial extracellular vesicles have been found by biochemical analyses including polyacrylamide gel electrophoresis with protein staining methods and Western blotting with in-house antibodies [5,35–39]. During the last 8 years, however, mass spectrometry-based proteomic studies have enabled us to identify more than 3500 vesicular proteins [8]. The list of representative high-throughput proteomic studies having identified more than 100 vesicular proteins is presented in Table 1 and the entire list of mass spectrometry-based proteomic studies can be found in EVpedia public database [8]. EVpedia (http://evpedia.info) is a community web resource for prokaryotic and eukaryotic extracellular vesicle research, which deposit extracellular vesicle-related publications and high-throughput datasets on vesicular protein, mRNA, miRNA, lipid, and metabolite. Furthermore, for a systematic analysis on prokaryotic and eukaryotic vesicular components, EVpedia provides integrated bioinformatics tools (sequence search, set analysis, Gene Ontology enrichment analysis, and network analysis). Detailed description on EVpedia is addressed in another article of this issue.

2.2.2. Lipids

Although lipids are important structural components of Gram-negative bacterial extracellular vesicles, the analysis on lipid composition has been overlooked for a long time. By thin layer chromatography, Horstman and Kuehn first reported that glycerophospholipids, phosphatidylethanolamine, phosphatidylglycerol, and cardiolipin are the main lipid components of enterotoxigenic *Escherichia coli* extracellular vesicles [35]. More recently, Chowdhury and Jagannadham performed mass spectrometry-based lipidomic analysis. They found even-number carbon chained phosphatidylglycerol and phosphatidylethanolamine as the major membrane bilayers components of *Pseudomonas syringae* extracellular vesicles [40].

2.2.3. Genetic materials: DNAs and RNAs

Gram-negative bacterial extracellular vesicles carry both luminal and surface-associated DNAs [37]. Since their luminal DNAs are resistant to DNase treatment, DNase treatment is an effective way to distinguish these two types of vesicular DNAs [5]. Through this approach, several luminal DNAs have been identified in extracellular vesicles derived from *Neisseria gonorrhoeae*, *E. coli*, *Haemophilus influenzae*, and *P. aeruginosa* [5,41–43]. The presence of RNase-resistant RNAs within *N. gonorrhoeae* extracellular vesicles was also reported [41]. However, detailed mechanisms on how specific DNAs and RNAs are incorporated and sorted into the vesicles have not been elucidated.

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