Outer Membrane Vesicles

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OMVs are rounded nanostructures released during their growth by Gram-negative bacteria. Biologically active toxins and virulence factors are often entrapped within these vesicles that behave as molecular carriers. Recently, OMVs have been reported to contain DNA molecules, but little is known about the vesicle packaging, release, and transfer mechanisms.

outer membrane vesicles | horizontal gene transfer | gram-negative bacteria | DNA

1. Introduction

Many successful bacterial infections are consequences of bacterial virulence mechanisms associated with antimicrobial escape [1]. The transduction involves bacteriophages that transfer DNA to bacterial cells through infection. Plasmid transfer systems are strictly dependent on the characteristics of the genetic material to be transferred: conjugative plasmids contain genes necessary for pilus formation, while mobilizable plasmids carry mobility genes and transfer origin, lacking pilus coding genes [2][3]. Recent studies have revealed a novel mechanism for gene transfer via OMVs.

2. OMVs: An Overview from Structure to Function

OMVs (**Figure 2**) are spherical bi-layered membrane nanostructures (50–500 nm), secreted by Gram-negative bacteria through bulging and ‘pinching off’ of the outer membrane [4]. Purification techniques have revealed the specific proteins and lipid composition of these vesicles [5]. The lumen of the vesicles contains periplasmic proteins, cytosolic components, and nucleic acids [6]. The composition of OMVs is modulated by growth conditions and is also highly influenced from the interactions between host cells and bacteria [7][8].

Several studies have demonstrated the presence within OMVs of outer membrane proteins, periplasmic proteins, and different virulence factors, engaged in the adhesion and invasion of cell hosts [9]. The first group mainly includes outer membrane proteins such as porins, components of transport systems, adhesins, phospholipases, and proteases. InNeisseria meningitidisOMVs, porin A, factor H binding protein, and opacity-associated protein C represent the largest protein fraction [10]. OMVs fromTreponema denticolacontain active proteases that cause damage to host cells [11].

Lipids play an essential role in the structure of OMVs and consist of phospholipids and lipopolysaccharides. While phospholipids constitute the inner sheet of the outer membrane, LPS is exclusively located on the outside surface

of the outer membrane. InP. aeruginosa,the OMVs phosphatidylglycerol and stearic acid are abundantly detected, proving greater rigidity of the vesicles [12].Helicobacter pyloriOMVs have cardiolipin as the main lipid component [9]. The type of LPS band present on OMVs depends on the site of vesicle budding [4].

OMVs carry DNA and RNA on their surface or in the vesicular lumen. A clear difference can be observed by OMV treatment with DNase and RNase: luminal DNA and RNA are preserved even after the enzymatic treatment [13][14]. Furuse et al. have recently identified tRNA fragments in the OMVs ofChlamydiaandLegionellastrains, involved in direct subversion of host gene translation and mRNA stability [15]. Although novel information on this topic is piling up at a steady pace, nucleic acid incorporation mechanisms remain to be elucidated.

In the first model, Burdett et al. suggested that the absence or transfer of covalent links between the outer membrane and peptidoglycan layer promotes vesiculation [16]. The second model showed that the accumulation of peptidoglycan fragments or poorly folded proteins in periplasmic space exert pressure on the outer membrane, determining the curvature of the membrane and final budding [17]. Sequestration of positively charged compounds (Mg2+and Ca2+salt) by PQS causes anionic repulsion of lipopolysaccharides, increasing vesiculations [18]. These studies indicate that bacteria developed the OMV production mechanism as a part of stress response to ensure bacterial survival [19].

Several biological functions are attributed to the OMVs. These vesicles represent a long-distance delivery system of biomolecules, such as nucleic acids, enzymes, toxins, and virulence factors, protecting them from extracellular degradation and dilution.

Vesiculation allows intraspecies and interspecies communications and contributes to interaction with the host [20]. In addition, OMVs are involved in the acquisition of nutrients, stress responses, and the formation of a microenvironment necessary for the survival of pathogens [8][21][22]. The packaging of proteases, phosphatase, and glycosidases in OMVs plays an important role in the degradation of complex molecules, promoting nutrient availability [23]. Evans et al. have shown that alkaline phosphatase inMyxococcus xanthusOMVs causes the release of phosphate, contributing to the development of multicellular communities [24].

OMVs participate in the formation of the biofilm matrix by the release of exopolysaccharides, thus increasing cell co-aggregation [25][26].P. aeruginosais able to form biofilms and cause surgical site infections, orthopedic peri-implant bone infections, and lung infection in cystic fibrosis patients. They found that the addition of OMVs promoted biofilm formation in a dose-dependent manner (except for one strain, which turned out to be non-producer). The bacterium–host interactions trigger the release of OMVs, which carry toxins and adhesion and virulence factors [27]. The Shiga toxin inEscherichia coliOMVs efficiently inhibits eukaryotic protein synthesis compared to the soluble form [28].

In addition to the reported functions, OMVs have recently been recognized as gene transfer vectors [29]. Several studies have detected plasmids, chromosomal DNA fragments, bacteriophage DNA and RNA fragments in OMVs

[30][31][32][33]. Therefore, in the following section, recent evidence on the role of OMVs as carriers for horizontal gene transfer will be reported.

3. Horizontal Gene Transfer Mediated by OMVs

Gene transfer can occur via proven processes of transformation, conjugation, and transduction, as well as through recently identified OMV-mediated mechanisms [34]. Few studies have evaluated the gene transfer potential of OMVs.

were the first to identify OMVs as gene transfer vectors. Polymerase chain reaction (PCR) data revealed the presence of theeae,uidA,stx1,andstx2virulence genes in the luminal space. HGT was proved through PCR amplification of virulence genes in transformedEscherichia coliJM109 [35]. These first findings laid the foundations for other investigations, deepening the role of OMVs in gene transfer mechanisms.

proved that genetic exchanges through OMVs can also occur between bacteria of different species. Transformation experiments were performed, usingEscherichia coliJM109 andSalmonella entericaserovar Enteritidis as recipient cells, and target genes were determined by colony PCR amplification. The acquisition of virulence genes in the recipient cells resulted in an increase in pathogenicity; the latter was assessed by Vero cell assay. The transformed recipient strains induced a cytotoxicity six times higher than the unprocessed strains, indicating the expression of virulence factors only in transformed strains.

BesidesE. coli, other Gram-negative species exploit OMVs as HGT vectors. proved thatP. gingivalisOMVs mediated the transfer of virulence genes between members of the same species. Genes encoding the major subunit of long fimbriae (fimA) and superoxide dismutase (sod) were detected in the vesicular lumen by PCR analysis, suggesting possible preferential DNA packaging. OMV–HGT experiments were conducted using a mutantP. gingivalis49,417, obtained by introducing a 2.1 Kb segment of theermF-ermAMgene into thefimAgene, which conferred resistance to erythromycin.

The involvement of OMVs in the spread of resistance genes was only revealed 10 years later. identified for the first time OMVs as vectors of antibiotic resistance gene transfer. Chatterjee et al. also supported the transfer of antibiotic resistance genes through OMVs. The recipient cells were positive for theblaNDM-1gene and exhibited a broad profile of resistance to β-lactam antibiotics, recording higher MIC values, compared to untransformed strains.

DNA packaging into vesicular lumens protected it from adverse environmental conditions, demonstrating an additional bacterial survival advantage associated with OMV–HGT. After DNase treatment of OMVs, plasmid integrity in the vesicles was evaluated through agarose gel electrophoresis and HindIII plasmid digestion. Vesicular plasmid integrity was not compromised, which indicates that OMVs provide protection for the DNA within them. In the thermal environments and in the presence of DNase, OMVs increased the frequency of transformation, compared to free DNA.

Despite different studies that have shown a high gene transfer potential of OMVs, Renelli et al. suggested that vesicles carry the genetic material but do not promote an effective transformation. PCR amplification of plasmid (pAK1900) and chromosomal (oprL) sequences indicated the presence of only plasmid DNA within vesicles. The transformation experiments were performed usingP. aeruginosaPAO1 andEscherichia coliDH5a as recipient strains. The author speculated that p-OMVs transferred the plasmid into the periplasm of recipient cells, which does not by-pass the plasma membrane for efficient transformation [36].

Although previous studies have highlighted the role of OMVs in HGT, the mechanisms underlying the transfer and the factors influencing the process are not yet clear [31][37]. Few studies have been conducted to understand these aspects.

pLC291 and pUC19 are high-copy number plasmids, while pZS2501 is a low-copy plasmid. Furthermore, they investigated whether the OMVs released by different recipient strains were endowed with different characteristics. Purified vesicles from different recipient strains contained the same protein and plasmid amounts and had a similar size. The vesicles containing pLC291, isolated fromAeromonas veronii,Enterobacter cloacae,andEscherichia coli, were exploited to induce the transformation of five different recipient strains, i.e.,Aeromonas veronii,Enterobacter cloacae,Escherichia coli,Chromobacterium violaceum,andP. aeruginosa.

In a subsequent investigation, Tran et al. evaluated more closely the effect of plasmid features, such as plasmid copy number, size, and origin of replication on OMV-mediated gene transfer. In addition, this study assessed the impact of plasmid size on vesicle loading. Moreover, qPCR results showed that the plasmid size inversely affected the number of plasmid copies in the vesicle. To assess the impact of plasmid origin on OMV production, they constructed three plasmids based on plasmid pLC291, with the same size (3.5 kb) but different origins:

The vesicles purified by treated bacteria were characterized on the basis of protein and DNA contents. Treatment of bacteria with antibiotics and nutrient deprivation caused a significant increase in vesicular diameters. The zeta potential of the OMV produced in the presence of gentamicin showed more negative values compared to the other treatments. These results proved that stress factors can influence the vesicle release, DNA content, and vesicle size [38].

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