Insights and applications of bacterial outer membrane vesicles (OMV)

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Outline

- Introduction
- Insights into bacterial OMV
  - Mechanism of OMV formation
  - OMV in pathogenesis
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- Application of OMVs
  - OMVs as vaccines
  - OMVs as specialised drug delivery vehicles
- Summary

Introduction

- Membrane bound materials are produced and released by all domains of life
- Outer membrane vesicles (OMVs) - derived from cell envelopes of Gram negative bacteria
  - Variety of environments-planktonic cultures, fresh and salt water, biofilms, inside eukaryotic cells and within mammalian hosts
- Early studies of OMVs- function in relation to pathogenesis
- Recent studies- function of OMVs from non-pathogenic bacteria
- OMVs as tools- vaccines and specialized drug delivery vehicles

What are OMVs

- Spherical portions of outer membrane of Gram negative bacteria
- Produced by growing cells- not products of cell lysis
- Approximately 50-250nm in diameter
- OMVs host a large repertoire of cargoes
  - Nucleic acids, enzymes, toxins, fragments of peptidoglycans, and proteins and fluid from the periplasmic space

Gram negative bacterial cell envelope

- Two lipid bilayers-outer membrane (OM) and inner membrane (IM)
- IM: symmetrical bilayer
- OM-asymmetrical bilayer
- Periplasm – contains peptidoglycan (PG)
- Braun's lipoprotein (Lpp)- membrane protein covalently linked to peptidoglycan- provides integrity to outer membrane

Mechanisms of OMV formation

Alteration in PG and OM cross-linking

- Areas of reduced Lpp - ↑OMV formation
    - Lpp links OM to PG-envelop stability
    - Lack of Lpp-more fluid OM- ↑propensity to bulge
    - Deletion of *Lpp* enhanced production of OMVs (*E.coli*) (Moon et al. (2012))

Enrichment of lipid microdomains

- Enrichment of certain areas of outer membrane
  - Particular types of LPS, phospholipid and/or LPS-associated protein
  - Distinct from rest of outer membrane
  - Increased membrane fluidity, ↑propensity to bulge

OMV promoting molecules

- Pseudomonas quinolone signal (PQS)
  - Produced by *P. aeruginosa*
  - Leads to OMV formation in variety of Gram negative bacteria
- Fuse with LPS
  - Anionic repulsion in OM surface- OMV formation (Tashiro Y. et al., 2010)
- Incorporation into OM-inducing curvature

Function of OMVs in pathogenesis

Antimicrobial/phage Resistance

Serve as decoy targets

- **Manning AJ, et al. (2011)**- In *E. coli*, addition of OMV or hypervesiculating mutant - resistance to colistin and polymixin B
- Addition of OMV or hypervesciulating mutant - increased viability of *E. coli* cultured with lytic T4 phage
  - Irreversible binding to phage

Antimicrobial resistance

Transfer of resistance genes/antibiotic degrading enzymes

- **Schaar V, et al. (2011) –** OMVs from amoxicillin resistant *M. catarrhalis* carried active β-lactamases
  - Protected amoxicillin sensitive *M. catarrhalis*
  - Improved amoxicillin resistance in non-typable *H. influenzae* and *S. pneumoniae*

Delivery of virulence factors

Transport of immunomodulatory molecules

Vidakovics ML, et al. (2010)

- ***M. catarrhalis* OMV**: the outer-membrane-bound superantigen: Moraxella immunoglobulin D-binding protein (MID)
- Binds to immunoglobulin D → B cell activation
- B cell activation → polyclonal immunoglobulin M (IgM) production → Delay of specific antibody production → increased survival of *M. catarrhalis*

Delivery of virulence factors

Transport of genotoxins

Chitcholtan K, et al. (2008)

- ***H. pylori* OMVs transport genotoxins to gastric epithelial cells**
  - Micronuclei formation
  - Alterations in iron metabolism and oxidative stress→ genomic damage
  - Dependant on OMV associated cytotoxin Vacuolating toxin A (VacA)
  - Purified VacA- ↓ glutathione- used by glutathione peroxidase to break down H_2O_2, ↑ H_2O_2 associated DNA damage

Function of OMVs in bacterial physiology

OMVs in stress response

Means to dispose envelope ‘garbage’

Schwechheimer C and Kuehn MJ (2013)

- *E. coli* Δ*degP* strain- Misfolded proteins are not degraded because it lacks the chaperone–protease DegP- can be lethal
- *E. coli* Δ*degP* strain produced higher levels of OMVs compared to wild type
- Lumen of OMVs produced by the Δ*degP* strain contained misfolded outer-membrane proteins, which are DegP substrates

OMVs in nutrient acquisition

Berleman JE, et al. (2014)

- *M. xanthus*- OMVs contain hydrolytic and proteolytic enzymes
- Secondary metabolites with antibiotic activities (cittilin A, myxovirescin A, myxochelins and myxalamids)
- Killing of microbial prey within a community

Biller SJ, et al. (2014)

- OMVs from the marine cyanobacterium *Prochlorococcus*
- Support growth of heterotrophs *Alteromonas* and *Halomonas* - sole carbon source

OMVs in Iron/Zinc acquisition

- **Lappann M, et al. (2013)**: OMVs from *N. meningitidis* enriched in iron acquisition proteins, such as the iron-transporter components FetA and FetB
- OMVs from *N. meningitidis* are also enriched in the zinc acquisition proteins ZnuA and ZnuD
- Findings imply OMVs capture iron/zinc and transport it back to the cells (not yet shown in Gram negative bacteria)

Applications of OMVs

OMVs as vaccines

- OMVs make ideal vaccine candidates
  - Stable
  - Non-replicative, safe
  - Contain many of the immunogenic surface-associated and membrane-associated components of their parent bacterium
  - Ability to activate the innate immune system
  - Provide their own adjuvant activity- able to enhance T cell and antibody response
  - Versatility- bioengineered to carry any chosen antigen
  - Manipulated to reduce endotoxicity (associated with LPS)

M.Kaparakis-Liaskos and RL Ferrero (2015)

OMVs as vaccines

Meningococcal OMV vaccine

- *N. meningitidis*- Meningococcal disease (meningitis/sepsis)- groups A,B,C,W-135 and Y
- Conjugate vaccines (capsular polysaccharide) available for groups A, C, W-135, and Y
- Group B- safety concerns about cross-reactivities of anticapsular antibodies with glycoproteins in human tissues
- Early MenB OMV vaccines- elicit immune response against PorA
  - Highly variable- used to control epidemic by clonal populations of isolates (New Zealand, **Oster, P. et al. (2005)**)

OMVs as vaccines

- **Breakthrough-** OMV-based 4CMenB vaccine (**Vesikari, T. et al. (2013)**)
  - Contains three highly immunogenic proteins incorporated into MenB OMV
  - Induces protective antibody responses against *N. meningitidis* including group B isolates
  - Approved by the European Commission for use (2 months of age and above)
- OMV vaccines against other Gram negatives in development
- Challenges: ability to elicit broad protection, inclusion of more antigens in OMVs and reducing LPS-mediated toxicity

OMVs as specialised drug delivery vehicles

- OMV with low immunogenicity-generated from mutant *E. coli*
- *E. coli* K12 strain W3110-msbB mutation
  - Produces under-acetylated LPS, ↓ toxicity to human cells
- Carry a human epidermal growth factor receptor 2 (HER-2) specific affibody- targeting cancer cells
  - HER-2 overexpressed in 18-25% of breast cancers, ovarian cancers, gastric carcinoma and salivary gland tumors
- OMVs also loaded with small interfering RNAs-causes gene silencing
  - Targeting Kinesin spindle protein (critical role in mitosis)
- Lead to tumour regression in a mouse model
- Potential to be developed in human therapeutics

Gujrati V, et al. (2014)

Summary

- OMVs- Spherical portions of outer membrane of Gram negative bacteria (50-250nm)
  - OMVs host a large repertoire of cargoes- proteins, nucleic acids and periplasmic fluid
- Mechanisms of OMV formation
  - Alterations in PG-OM crosslinks
  - Enrichment of envelop with special lipid microdomains
  - OMV promoting molecules- PQS
- Role in bacterial pathogenesis
  - Antimicrobial/phage resistance
  - Delivery of virulence factors
- Role in bacterial physiology
  - Disposal of undesired components
  - Nutrient acquisition
  - Iron/Zinc acquisition
- OMVs as tools
  - Vaccines – 4CMenB
  - Specialised drug delivery vehicles- anti-cancer drugs

Thank you!