Development of Outer Membrane Vesicles as Delivery carriers for Cancer Treatment

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Background of research

Outer membrane vesicles (OMVs), ranging from 50 to 250nm in diameter, are spherical outer membrane products secreted from both gram-negative and gram-positive bacteria[1]. They can carry different types of cargos including DNA, RNA, toxin, signaling molecules, small metabolites and many different proteins from the bacterial periplasm, cytoplasm and outer membrane, and transfer to other cells for intra-kingdom and inter-kingdom communication and functions[2]. Outer membrane vesicle was firstly discovered by observing *V. cholera* under the microscope from late 1960s[3] and since then, the mechanism of OMV formation and their functional relevance to the bacterial physiology and pathogenesis have been studied. In the last two decades, OMVs have been shown to play roles in bacterial intercellular communication, virulence, protein degradation and host immune response, which opened up a new avenue to our understanding of bacterial pathogenesis[1]. Moreover, due to the small size, immunogenic properties and non-replicative properties, OMV has been utilized in vaccine development[4]. Moreover, the usage of OMV to deliver molecules such as drugs or nucleic acids to the cells has emerged as the new approach for disease treatment. Therefore, in this project, we set out to develop the OMV as the safe and manipulating carriers to deliver drugs to target cells.

Previous related work by the team: Previously, we purified OMVs from different UPEC strains isolated from Hong Kong hospitals including 2D2[5], SPISO[6], KWH, PWH, and QMH, and characterized their sizes distribution and production level in comparison with nonpathogenic lab strain *E. coli* J53 (Figure 1). We found that all pathogenic strains produced larger size of OMVs than J53 and the production rate of UPEC-specific OMVs were higher than that of J53, suggesting the host specificity of OMVs. Moreover, the UPEC-specific OMVs may contain consensus factors that provide similar function to the pathogenesis of bacteria, as previous studies showed that OMVs from pathogenic strains carried virulent genes, toxin and drug resistant proteins. In order to determine the consensus factors of UPEC-specific OMVs, we performed the mass spectroscopic studies to identify the protein components from all the UPEC-specific OMVs and compared with each other as well as with the non-pathogenic strains. The proteins isolated from OMVs were firstly analyzed by SDS-polyacrylamide electrophoresis (Figure 2a), and then subjected to protein identification using mass spectrometry. The total number of proteins identified from different OMVs ranged from 250 to 750 (Table 1) and interestingly, the OMVs produced from two clinical strains, PWH and QMH, contained over 700 proteins. In order to identify the UPEC-specific proteins, we performed protein clustering among different UPEC-OMVs. As shown in Figure 2b and 2c, 79 proteins were shared among each other and most

of them were outer membrane, periplasm and plasma membrane proteins, and ribosomal subunits. Moreover, we also compared these proteins with non-pathogenic strains *E. coli* J53-OMV and found 36 unique proteins that only found in UPEC-OMVs. In addition, we performed network analysis and interestingly, we found that there is numerous overlapping of the pathways among OMVs from different bacteria (Figure 3). These observations strongly suggested these proteins play pathogenic role in UPEC such as enhancing virulence, colonization, invasion and fitness as they were only presented in UPEC-OMVs [7-9].

In addition to the characterization of the OMVs, our team member, Prof. BL Khoo's group has developed the microwell-based assays which enabled the co-culture of bacteria-tumor clusters under defined conditions, providing the new approach to investigate the interaction between bacteria and cells. Moreover, the tumor clusters can be characterized by live/dead staining and immunostaining for the study of the response of cells, which provide an in-situ platform to evaluate the cellular response upon the drug delivery.

Provided the accumulated experience in purifying and characterizing OMVs, the knowledge of the difference between OMVs secreted from pathogenic and non-pathogenic bacteria and evaluation platform for drug delivery, we set out to develop the non-immunogenic OMVs as a cargo to deliver the molecules to the target cell. We firstly generated and purified the OMVs that have no or very low immunogenic effect to mammalian cells. As shown in Figure 4, the purified OMVs possess very low immunogenic effect to the cells as IL6 is the pro-inflammatory cytokines induced by lipopolysaccharide (LPS), the surface antigen of all bacterial cells. This result suggested that the purified OMVs can be potentially utilized as the carrier to deliver the molecules to the cells without raising the immune response to the cells. Indeed, further investigation has to be done to ensure the immunogenic effect of these OMVs. Moreover, in this project, we will try to inject the molecules such as anti-cancer drugs to investigate the delivery efficiency as well as the subsequent effects of the cells. In collaboration with Prof. B.L. Khoo, we are able to test the delivery system in the 3D cancer microfluidic model. Moreover, we can further investigate the survival of cancer cell after drug delivery and and their immune responses with OMVs.

Figures and Tables:

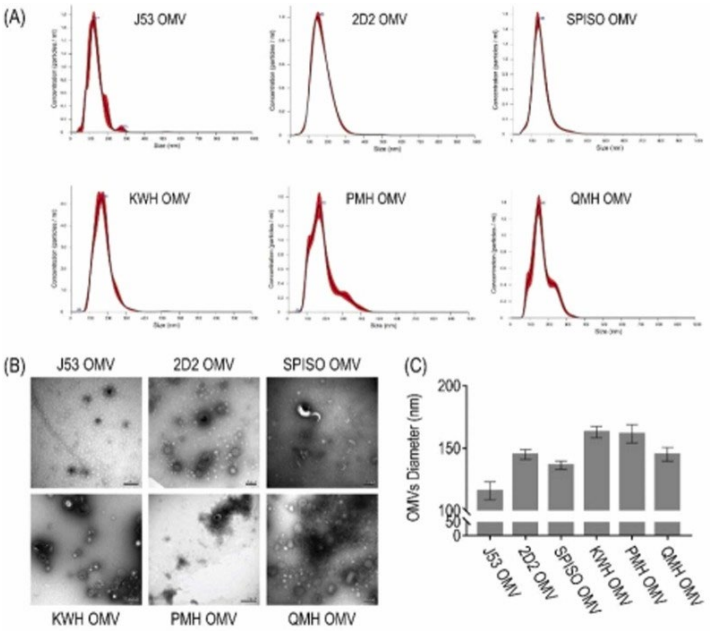

Fig. 1. Purification and characterization of outer membrane vesicle (OMV) from different *Escherichia coli* strains. *E. coli* strains were grown in terrific broth for 20 h and the OMVs were harvested by filtration through 0.45 µm membrane followed by ultracentrifugation. (A) The average concentration and diameter of OMVs were determined using Malvern NanoSight NS300. (B) Transmission electron microscopy images of the OMVs isolated from each *E. coli* strain. (C) Mode of the OMVs diameter in each *E coli* strain.

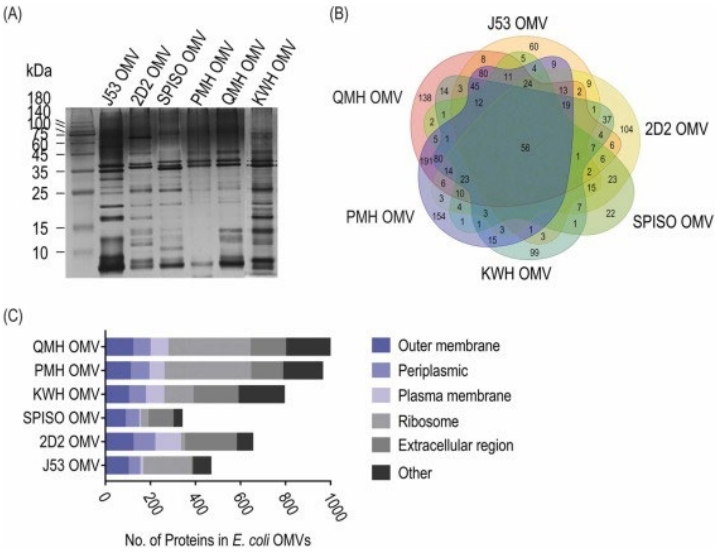

Fig. 2. Proteomic analysis of OMVs. (A) The protein compositions of OMVs isolated from different E. coli strains are visualized and compared using SDS-polyacrylamide electrophoresis. (B) The Venn diagram illustrates the number of common and unique proteins identified by mass spectrometry in different OMVs. (C) Functional enrichment analysis of the OMVs protein expressed in each E. coli strain. The quantified OMVs proteins were identified to be involved in five main categories including cellular components, including outer member, periplasmic, plasma membrane, ribosome, and extracellular.

Table 1. Number of Proteins identified by LC-MS/MS from *E. coli* OMVs.

No.	Samples	Parental bacteria (Sequence type)	MDR Plasmid	Numbers of identified proteins
1	2D2 OMV	2D2 (ST38)	pHK01	402
2	J53 OMV	J53 (ST10)	Null	384
3	KWH OMV	KWH (ST131)	pKWH	452
4	PMH OMV	PMH (ST131)	pPMH	788
5	QMH OMV	QMH (ST131)	pQMH	785
6	SPISO OMV	SPISO (unknown)	pNDM-HK	248

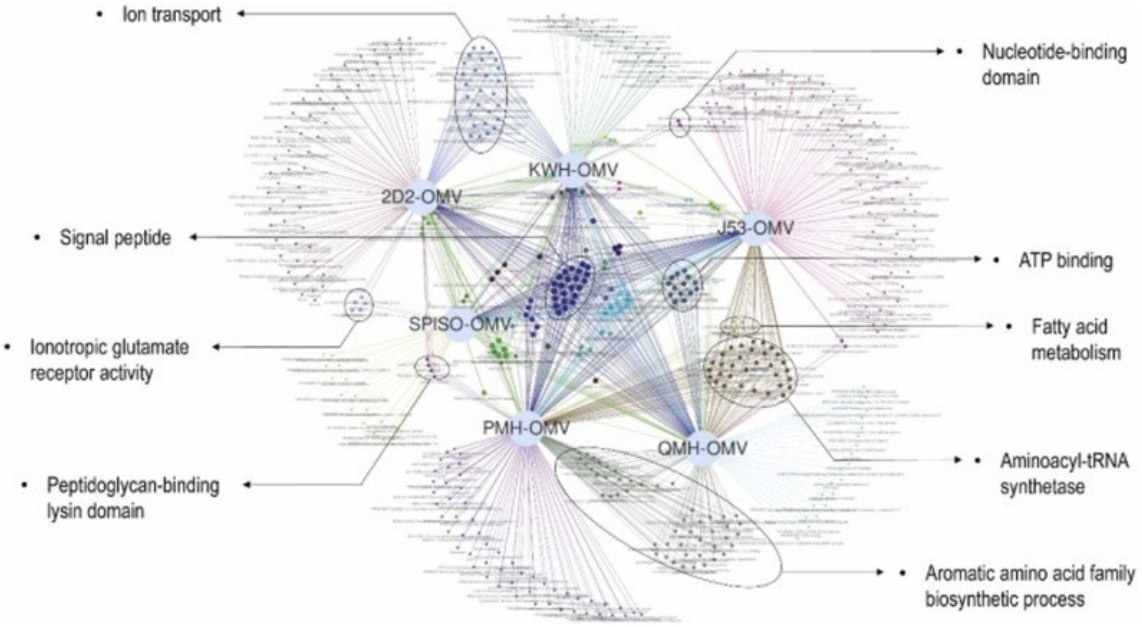

Fig. 3. Network analysis for the OMV proteins produced from different strains. Venn network shows gene ontology (GO) enrichment information and InterPro (IPR) domain mapping. Nodes in the middle were shared among six strains. The nodes were connected by 2–4 edges considered to be shared with 2–4 strains. Outer nodes with only one connected edge specially belong to each E. coli strain. The network was algorithmically constructed using EVENN software.

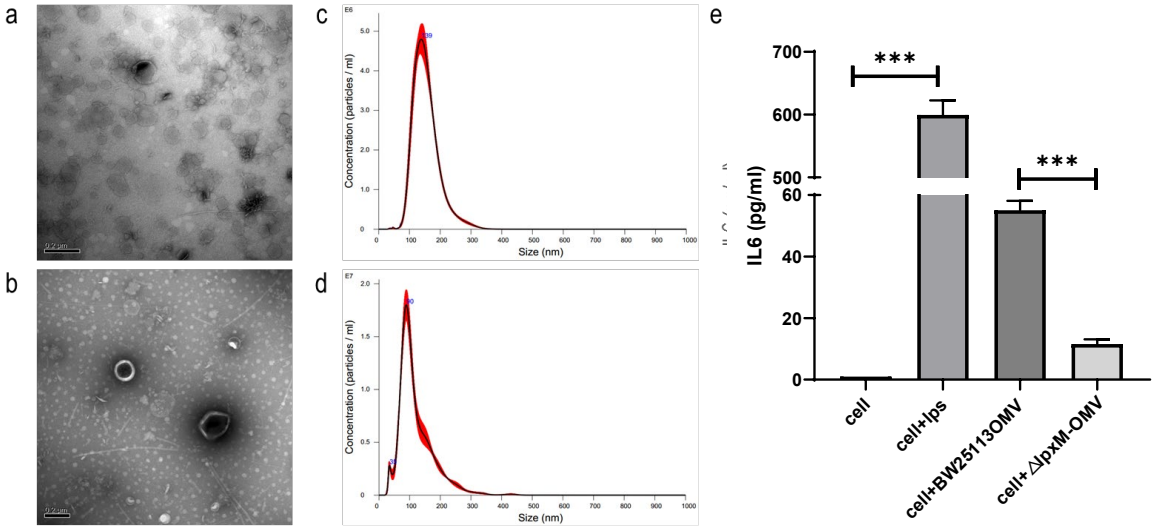

Fig. 4. Purification and characterization of outer membrane vesicle with low immunogenic effects to human cells. (a-d) Purification and characterization of OMVs (e) Immunogenic responses of cells incubated with OMVs. IL6 is the immunogenic markers; LPS: lipopolysaccharide.

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