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The Proteomic Analysis of Extracellular Vesicles Derived from *Plasmodium falciparum*-infected Red Blood Cells During Growth Development

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Introduction

Plasmodium falciparum (Pf) is the leading cause of severe malaria. During its intra-erythrocytic maturation, *Pf*-infected red blood cells (iRBC) release nano-sized extracellular vesicles (EVs) into the extracellular milieu. These EVs cargo both host and parasite proteins from iRBC and are important mediators in pathogenesis and host-parasite communication.

Objective

To study the proteomic profiling of *Pf*-derived EVs (Pf-EVs) during growth development which is significant in understanding their biological function in the course of infection.

Methodology

Four *Pf* strains were maintained in human RBC. The cultured media during parasite growth of early-stage (ring-to-trophozoite) and late-stage (trophozoite-to-ring) was daily collected to isolate EVs by multi-step centrifugation.

Two types of EVs, microvesicles (MV) and exosomes (Exo), were collected. Their proteomes were characterized by LC/MS-MS and comparisons were made using bioinformatics analysis.

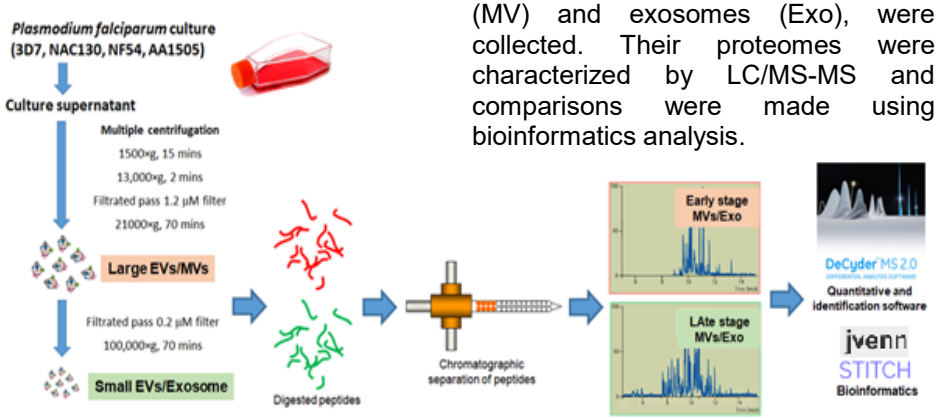

Fig 1 The schematic diagram of *Pf*-EV isolation from *Pf* culture supernatant and proteomics analysis

Results

The proteomic analysis revealed the 161 and 155 parasite proteins were found in MV and Exo, respectively. The numbers of common proteins found in each EVs from all 4 *Pf* strains are shown in fig 2.

The *Pf*-EVs contained the major virulence-associated parasite proteins such as merozoite surface protein 1 (MSP-1), elongation factor 1-alpha (EF-1α), knob-associated histidine-rich protein, apical membrane antigen 1 (AMA-1) and invasion ligands (EBA-175 and RESA).

Bioinformatics analysis revealed that MVs and exosomes during the early-stage were enriched in proteins that function in the ribosome pathway and DNA replication, respectively. In contrast, both MVs and exosomes released in the late- stage were enriched in proteins associated with metabolic pathways.

Conclusions

This proteomic analysis provided an insight into the *Pf*-EVs protein profile that may correlate with physiological activity and virulence during the host-parasite interaction.

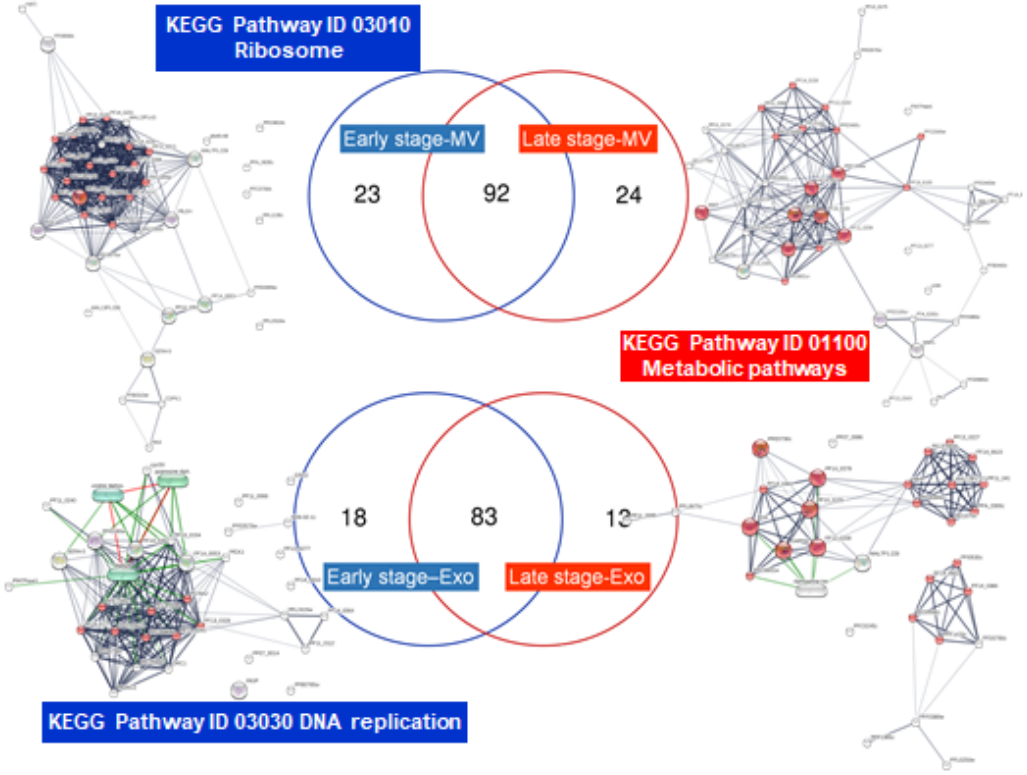

Fig 2 Venn diagrams and protein-protein interaction network of common *Pf* proteins in different type and stage of *Pf*-EVs derived from all 4 *Pf* strains

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