

Title: **Molecular Signatures of Serum Endothelial Exosomes in Pregnant Women with Placenta Accrete**  
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## **Abstract**

Placenta accreta (**PA**) describes an obstetric condition of deep myometrial invasion of trophoblast villi (1). With an incidence of ~0.9% of all pregnancies worldwide, PA is the most dangerous obstetric disease that threatens pregnant women. PA results in organ damage and massive hemorrhage; thus, cesarean hysterectomy is often required to control bleeding (2, 3). PA is associated with over-invasion of trophoblasts into the uterine wall on histology; based on the depth of trophoblast invasion, it is classified by pathologists into 3 categories: 1) creta when the villi adhere to uterine myometrium; 2) increta when the villi invade the myometrium; and 3) percreta when the villi penetrate the myometrium. In contrast, preeclampsia (**PE**) is a human-specific pregnancy disorder in which under-invasion of trophoblast is accepted to be a primary pathology (4, 5).

The pathogenesis of PA is currently totally unknown. We posited that causative substance(s) released from endothelial cells (**EC**) and/or trophoblasts in PA lesional sites may be circulated in maternal bloodstream where they can be isolated and identified because pathohistological studies have clearly shown that angiogenesis is involved in the formation of PA lesions along with trophoblast invasion. We speculate that the active factor(s) might be originated from exosomes secreted by the most active and abundant cells in PA lesional sites, including trophoblasts and ECs that express specific surface markers, placenta alkaline phosphase (PLAP<sup>+</sup>) and CD31<sup>+</sup>, respectively.

Exosomes are nanosized (50~160 nm) vesicles secreted by cells into the extracellular environment (6). They contain cell type-specific compositions of cellular contents such as mRNAs/microRNAs, proteins, and lipids, etc. They can be found in various body fluids such as blood, thus can be used as a novel class of biomarkers (7). Exosomes can be taken up by neighboring cells and through circulation by distant cells; they can facilitate intercellular communications by exchanging biological contents among cells thus to participate into various physiological and pathological processes (8). We quantified serum CD31<sup>+</sup> or PLAP<sup>+</sup> exosomes by using CD31<sup>+</sup> or PLAP<sup>+</sup> antibody conjugated magnetic beads and flow cytometry. We found significantly more ( $p < 0.01$ ) CD31<sup>+</sup> exosomes in sera from PA vs. normal and PE pregnant women, positively associated with PA severity. In contrast, serum PLAP<sup>+</sup> exosomes did not differ between PA and normal subjects, but significantly higher in PE subjects, consistent with previous studies (12, 13). These novel exciting findings clearly showed that: 1) exosomes derived from EC, but not trophoblast, play a crucial role in the pathogenesis of PA featured by trophoblast over-invasion and angiogenesis and 2) serum CD31<sup>+</sup> exosome may serve as a novel biomarker for PA.

**Hypothesis:** Circulating CD31<sup>+</sup> endothelial exosomes isolated from sera of PA possess unique molecular signatures (*i.e.*, mRNAs, miRNAs, and proteins) associated with the histological phenotypes of over-invasion of trophoblast and enhanced angiogenesis in PA vs. PE with histological phenotypes opposite to PA. This conjecture will be studied by the following three studies using omics approaches.

- **Aim 1:** to profile mRNA and miRNA signatures in CD31<sup>+</sup> exosomes isolated from sera collected from PA in comparison to normal and PE pregnant women in using RNA-seq.
- **Aim 2:** to profile protein signatures in CD31<sup>+</sup> exosomes isolated from sera collected from PA in comparison to normal and PE pregnant women in using quantitative proteomics.
- **Aim 3:** to perform gene ontology and functional studies of the RNA-seq and proteomics data for identifying PA-specific CD31<sup>+</sup> exosomal genes using bioinformatics tools.

The outcomes of this pilot project will be the PA-specific CD31<sup>+</sup> exosomal molecules associated with exosome secretion, angiogenesis, and trophoblast invasion. Based on these data, further studies will be performed to verify the function of the targets experimentally to generate additional preliminary data. Together, the goal of this pilot project is to generate “proof of concept” preliminary data for applying for a NIH RO1 grant to pursue our long-term goals: 1) to determine a causal role of CD31<sup>+</sup> exosomes in the pathogenesis of PA and 2) to utilize CD31<sup>+</sup> exosomes as a biomarker for developing noninvasive screening and/or diagnosis tests for PA. We target to apply for a RO1 grant specifically in response to the NIH/NICHD Human Placenta Project RFA [PAR-18-884](#): Novel approaches to safe, non-Invasive, real time assessment of human placenta development and function across pregnancy (R01) in early 2020, immediately after this pilot project is complete.