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Update of syncytiotrophoblast derived extracellular vesicles in normal pregnancy and preeclampsia

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ABSTRACT

The release of extracellular vesicles (EV) by the syncytiotrophoblast (STB) may be an important mechanism by which the placenta signals to the mother. STB derived EV (STBEV) are comprised predominantly of exosomes (50–150 nm) and microvesicles (100–1000 nm) that contain bioactive mediators such as proteins, nucleic acids and lipids. They, along with larger syncytial nuclear aggregates are released by the STB into the maternal circulation throughout gestation in normal pregnancy where they appear to have an immunoregulatory role, inhibiting T cell and NK cell responses. In pre-eclampsia (PE) STBEV are released in significantly increased numbers and have pro-inflammatory, anti-angiogenic and procoagulant activity, implicating them in the maternal systemic inflammation, endothelial dysfunction and activation of the clotting system which typifies the disorder. Research has focused on understanding the biological significance of STBEV by measuring their size and repertoire of molecules carried and how they differ in normal pregnancy and PE, using techniques such as Nanoparticle Tracking Analysis, flow cytometry and mass spectrometry. We have also found alterations in STBEV surface glycans associated with PE. The goal is to better understand the role STBEV play in normal pregnancy and PE and whether they are potential biomarkers of placental pathology and therapeutic targets in PE.

Contents

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1. Introduction

The syncytiotrophoblast (STB); a multinucleated, terminally differentiated, polarised epithelium that covers the entire surface of human placental villi, is one of the largest cell types in human biology (Burton and Fowden, 2015). It forms during the early stages of embryo development by the initial fusion of mononuclear cytotrophoblasts and then is maintained throughout gestation by a process of turnover, with spent material shed from the apical surface being replenished by incorporation of underlying cytotrophoblast cells (Huppertz et al., 2006). It is the largest and most critical fetal-maternal interface, responsible for nutrient uptake, gas exchange, waste removal, protein and steroid hormone production and modulation of maternal physiology. The STB is also specially adapted to shield the fetus from the maternal immune system. The semi-allogeneic STB is unique in being human leukocyte antigen (HLA) null and therefore immunologically inert to prevent allorecognition and rejection by maternal T cells (Nancy and Erlebacher, 2014). While this lack of HLA expression might be expected to render the STB open to attack by maternal natural killer cells (NK cells; which are programmed to destroy HLA negative tumour cells), there is no evidence that this occurs. This may be due to the presence of a glycocalyx on the STB membrane which could prevent interactions with NK cells (Arkwright et al., 1994). The STB communicates with the maternal immune system using both soluble factors, such as chemokines, cytokines and steroid and protein hormones, and factors carried by extracellular vesicles (EV).

EV are cell-derived membranous vesicles and potent mediators of both physiological and pathological processes (Colombo et al., 2014). The term EV encompasses three main vesicle types: exosomes, microvesicles (also known as ectosomes and microparticles) and apoptotic bodies. EV biology has been extensively reviewed for comprehensive reviews see (Colombo et al., 2014; Kalra et al., 2016), so briefly, exosomes are the smallest vesicle type (~50–150 nm) and are produced in a constitutive manner, using machinery of the endocytic pathway, in structures called multivesicular bodies (MVB), which enable loading with a targeted cargo followed by release of exosomes into the extracellular environment by fusion with the plasma membrane and exocytosis. Microvesicles (~100 nm–1 μm) are released directly from the plasma membrane in response to stimuli that cause a rise in intracellular calcium levels and cytoskeletal remodeling such as cellular activation or stress. Also included under the umbrella of EV are apoptotic bodies which overlap in size with microvesicles (~200 nm–5 μm) and are released from apoptosing cells, but only once in the life of a cell as it is a terminal event in the apoptotic pathway. As such, research into the role of EV in cell–cell communication has tended to focus on microvesicles and exosomes. EV carry proteins, lipids and RNAs (such as mRNA, miRNA, vaultRNA and tRNAs) and are thought to signal to their target cells via surface interactions including protein or lipid ligand-receptor binding, by fusing and releasing their contents into the cytosol of the target cell and finally via endocytosis and subsequent fusion with endosomes (Raposo and Stoorvogel, 2013).

EV release appears to be evolutionarily conserved, involving the coordinated activity of numerous proteins (comprehensively reviewed by (Colombo et al., 2014)). The transmembrane proteins tetraspanins, including CD9, CD81, CD82 and CD63, clustered in tetraspanin enriched domains (TEMs) in the plasma membrane and the endosomal sorting complex required for transport (ESCRT) complex, made up of around 30 different proteins including ALIX, TSG101, syntenin and multiple RAB proteins, are major components of EV biogenesis, involved at multiple stages in a cell specific manner (Henne et al., 2011; Friand et al., 2015; Stuffers et al., 2009). TEMs act as specialized scaffolds, enabling the compartmentalization of proteins from the plasma membrane into EVs and the downstream sorting of proteins and possibly RNA and lipids into EV cargo (Villarroya-Beltri et al., 2014; Mazurov et al., 2013). Components of the ESCRT complex are required for endocytosis of the endosomal membrane wall to form exosomes, targeting of MVB for fusion with the plasma membrane for exosome exocytosis and EV release (Hanson and Cashikar, 2012). The selective recruitment of proteins, such as adhesion molecules, glycoproteins and externalization of phosphatidylserine (PS) also enables the targeting of EV to particular recipient cells following their release, while pathological cellular changes lead to characteristic alterations in EV cargo (Colombo et al., 2014; Andreu and Yanez-Mo, 2014).

The STB is the primary source of placenta derived EV, that may constitute a major signaling mechanism between fetus and mother, augmenting maternal physiology to allow the presence and meet the demands of the developing fetus. Pregnancy is an ideal system to study EV as the entire process has a definite start and end point, with specific STB markers, principally placental alkaline phosphatase (PLAP), distinguishing STB derived EV (STBEV) from those produced by other cell types, and availability at the end of pregnancy of the STBEV source, the placenta. This is particularly relevant to the investigation of pregnancy disorders driven by placental dysfunction, such as preeclampsia (PE); a problem of human pregnancy and leading cause of maternal mortality (Tannetta and Sargent, 2013; Redman et al., 2012). PE affects 2–5% of women worldwide and carries a substantial risk of long-term cardiovascular health for both the mother and baby. It is characterized by the maternal signs of hypertension, proteinuria and hypercoagulation, triggered by the release of placental proinflammatory, antiangiogenic and procoagulant factors in response to ischemia reperfusion and downstream inflammatory and endoplasmic reticulum (ER) stresses (Redman and Sargent, 2005; Burton and Yung, 2011).

This review will outline our current understanding of STBEV subtypes, interactions of STBEV with maternal cells, potential novel mediators of these interactions including altered STBEV surface glycan groups and their possible role in pregnancy and PE.

2. STBEV release and composition in normal pregnancy and PE

It has been known for many years that release of membranous material into the maternal circulation by the STB is a feature of normal pregnancy (Burton and Jones, 2009). This material, ranging from multinucleated syncytial sprouts and knots (known as syncytial nuclear aggregates (SNA)) and viable trophoblast cells to STBEV has, until relatively recently, been regarded as inert STB debris of little consequence. However, the demonstration of their immunomodulatory activities has increased interest in their role in both normal and pathological pregnancies, particularly PE.

Given their size, SNA can easily be isolated using low speed centrifugation (Abumaree et al., 2006b). However, the subcellular nature of STBEV means that specialised isolation protocols are required. There is no consensus in the field of EV research on protocols for the isolation of specific EV subtypes. Efforts to standardise isolation procedures may also not be practical given the range of biological fluids that contain EV (e.g. plasma, urine, saliva, cerebrospinal fluid, breast milk) and *in vitro* culture systems used in EV research (Witwer et al., 2013). Methods routinely used to isolate EV include precipitation, differential centrifugation, density gradient ultracentrifugation, filtration, size exclusion chromatography and immunocapture on beads or chips. Sample type (ranging from complex biological fluids such as plasma to *in vitro* derived samples such as conditioned media) and downstream analyses (e.g. cargo determination using transcriptomic and proteomic approaches or EV characterization techniques such as electron microscopy, flow cytometry and nanoparticle tracking analysis (NTA)) will