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UNIVERSITY OF LEEDS

Optimising the isolation of placental-derived extracellular vesicles from maternal plasma using immunomagnetic beads

Farrelly R[1], Spencer R[1]

Study Aims

1) To optimise the method of isolating placental-derived extracellular vesicles (pEVs) from maternal plasma
2) Determine whether pEVs isolated from this method contain sufficient quantities of genetic cargo for analysis.

Method

Pre-delivery maternal plasma samples were collected from 18 pregnancies diagnosed with early-onset FGR (estimated fetal weight <3rd centile at 20+0-20+6 weeks gestation). EVs were isolated using IZON size exclusion columns, pooled into fractions 7-12 and concentrated.

The isolated circulating EVs were then bound to immunomagnetic beads, conjugated with placental alkaline phosphatase (PLAP) antibodies to separate those o f placental origin (pEV's) from EVs originating from other maternal organs (Figure 1).

The PLAP bound pEVs were then lysed and their cargo analysed using qPCR for mRNA and microRNA content.

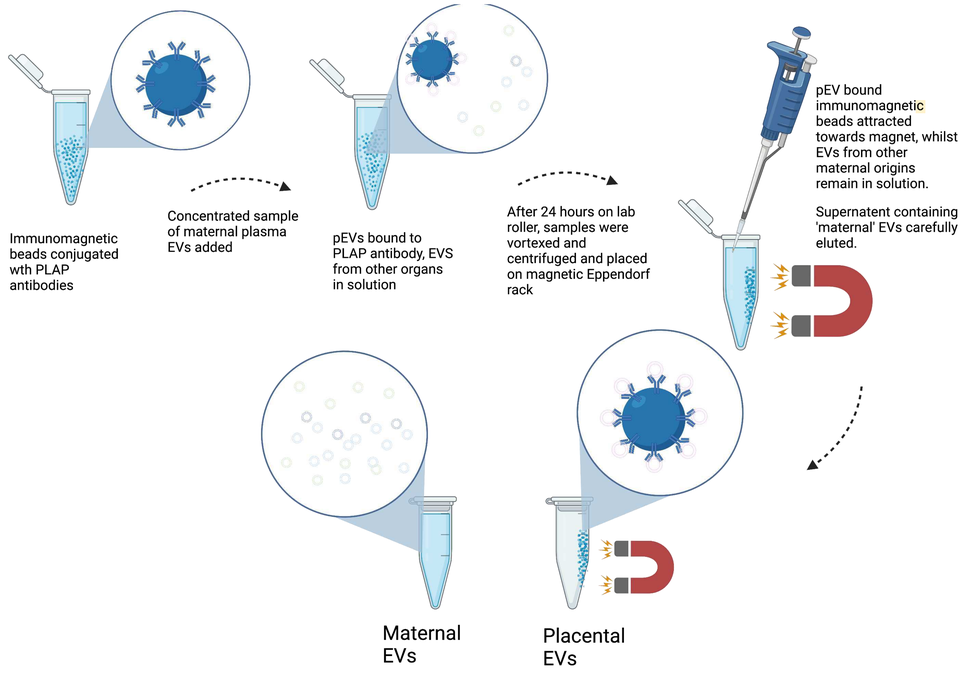

Figure 1. Schematic diagram describing the process of isolating pEVs. Once the immunomagnetic beads are conjugated with PLAP antibodies, concentrated samples of 20μl of EVs are combined with the beads. At this point PEVs bind tot the PLAP antibodies on the surface of immunomagnetic beads, whilst EVs which are not placental in origin, referred to as maternal EVs remain in solution. The samples are vortexed, centrifuged and left on a roller for 24 hours to ensure complete binding to all pEVs. Once 24 hours had surpassed, the samples are vortexed, centrifuged and placed onto a magnetic Eppendorf rack. The maternal EV's in solution were eluted leaving behind the immunomagnetic beads bound to pEVs. They were then washed with PBS and reconstituted in isolation buffer.

Conclusion

Whilst these are preliminary findings, it is believed that this method is successful at isolating pEVs from a pool of total EVs in maternal plasma. Since it is known that the mRNA and microRNA profile of placental tissue changes in pregnancy complications such as FGR, the translation of this method calls further research into whether these changes are also reflected in the cargo of placental derived EVs. This would aid the development of a non-invasive placental biopsy

Results

Whilst it was not possible to analyse. The mRNA profile of these EVs, it was found that microRNA's which have been identified in the literature to be released by the placenta in abundance were also found in the isolated pEVs and not in the remaining pool of EVs from other maternal organs (Figure 2).

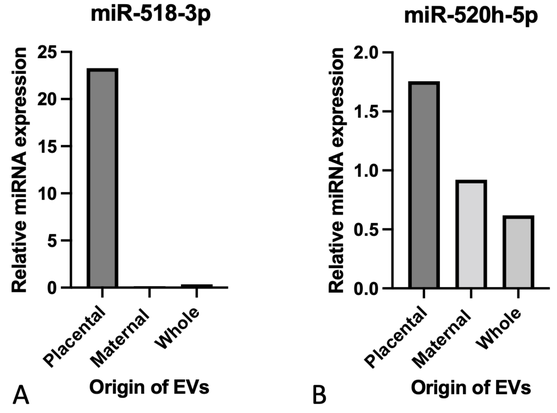

Figure 2. miRNA PCR assay of miR518e-3p (A) and miR-520h-5p (B) which are hypothesised to be released by the placenta within EVs. Raw expression in EVs from the placenta, the maternal organs and the whole EV population were measured in triplicate with the mean being taken from concordant results. This value was normalised using miR-16 expression and plotted. A. miR518e-3p shows complete expression within pEVs but not in maternal EVs. miR-520h-5p was expressed across all three populations but with the highest level of expression in pEVs.