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Characteristics of exosomes in the cerebrospinal fluid of preterm infants

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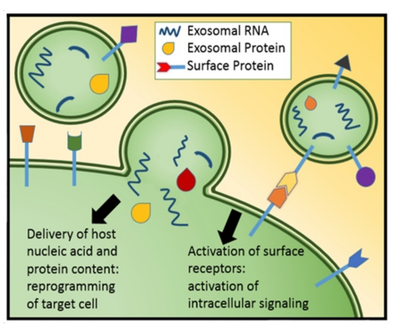
INTRODUCTION

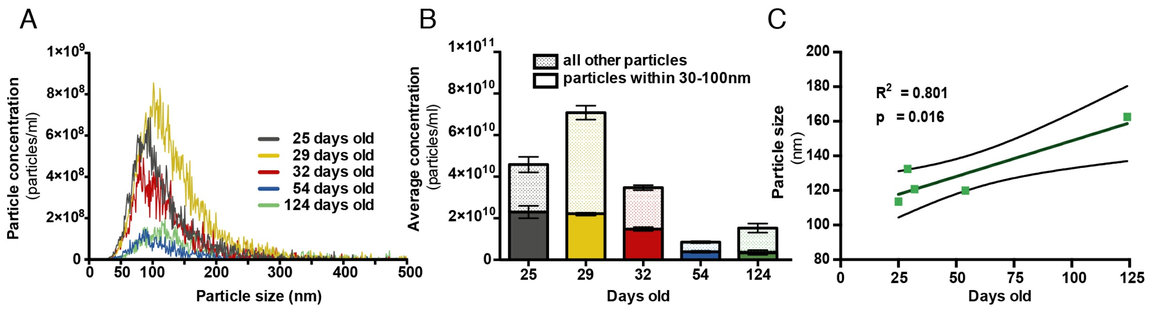
Extracellular vesicle (EV) signalling has been recognised to play a key role in cellular interaction both in neurogenesis and neuroregeneration.



Exosomes are 30-100nm lipid vesicles, containing RNA, DNA and protein. Micro RNAs are selectively enriched and mediate signalling in recipient cells.

Exosomes are actively released via the endosomal pathway from a variety of cells contributing to intercellular communication and are crucial for maintaining neuronal integrity.

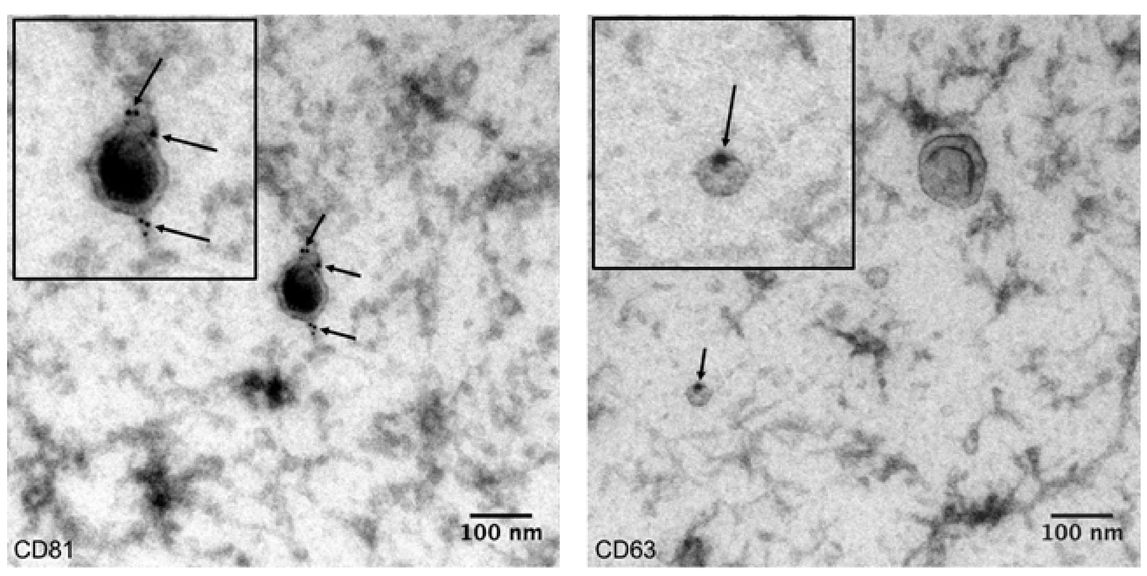
Nanotracking analysis shows CSF nanoparticles



Nanotracking analysis (NTA) of CSF nanoparticles shows within unprocessed CSF: (A) The concentration of particles against plotted against size. Vertical bars represent standard error.
(B) Exosomal fraction (full colour) relative to days after intraventricular haemorrhage.
(C) Modal particle size increases with gestation and time after injury.

TEM shows CD63 and CD81+ exosomes

Transmission Electron Micrographs using uranyl-acetate negative staining of extracellular vesicles isolated from ultra-centrifuged CSF.
Immunogold labelling (arrows) of CD81 (left) and CD63 (right), exosomal surface markers.

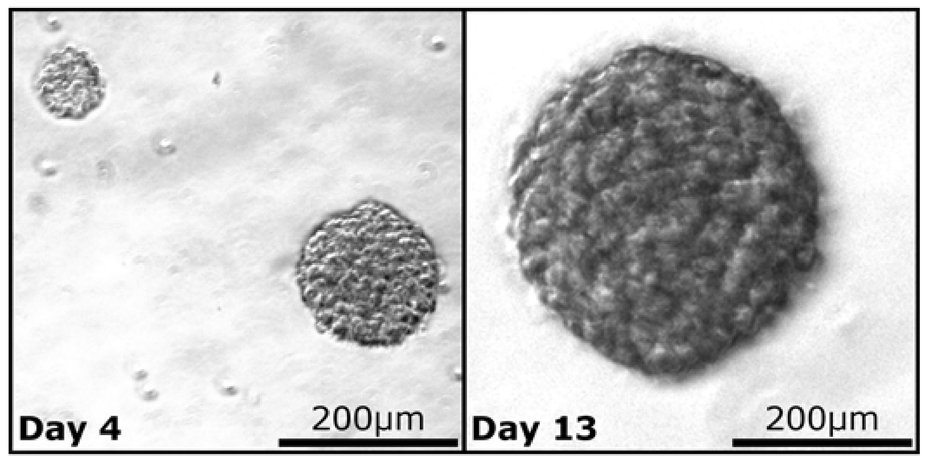


METHODS

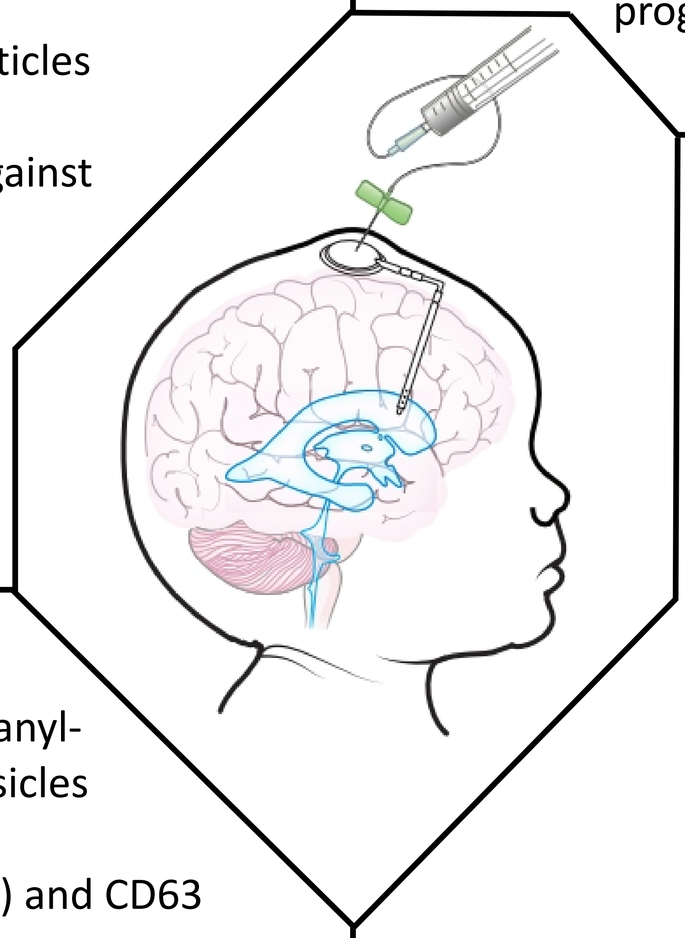
Cerebrospinal fluid (CSF) was taken from infants with evolving post-haemorrhagic hydrocephalus (Ethics:NHS-REC:15-YH-0251). Concentration of EVs was determined using a NanoSight NS300.

EVs were isolated using differential ultracentrifugation, and characterised using transmission electron microscopy (TEM) and gold immunolabelling. Candidate microRNA expression were analysed from lysed EVs.

Isolation and culture of neural progenitor cells



Light microscopy showing spheres of neural progenitor cells grown from cells isolated from preterm infant CSF.

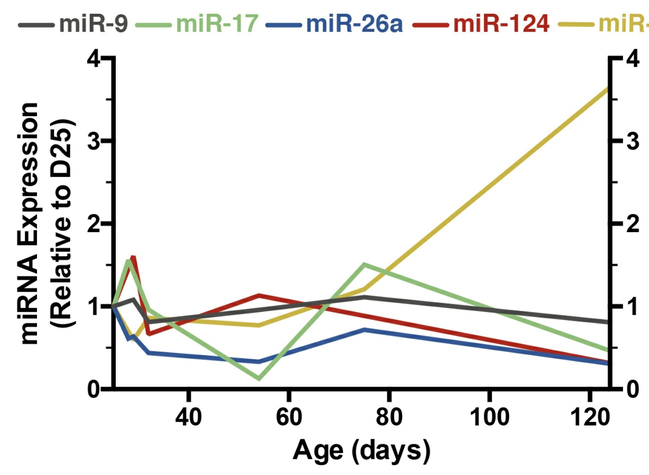


Micro RNAs enrich the EV fraction

A

miR-base ID	Expression	Mature miRNA Sequence
hsa-miR-9-5p	Brain/CSF (S+E)	UCUUUGGUUAUCUAGCUGUAUGA
hsa-miR-17-5p	Serum (S+E)	CAAAGUGCUUACAGUGCAGGUAG
hsa-miR-26a-5p	CSF (S)	UUCAAGUAAUCCAGGAUAGGCU
hsa-miR-124	Brain/CSF (S+E)	CGUGUUCACAGCGGACCUUGAU
hsa-miR-1911-5p	Brain (E)	UGAGUACCGCCAUGUCUGUUGGG

B



Relative concentration of micro RNA from the EV fraction. All 5 miRNAs could be detected in all samples. miRNAs analysed (A), including tissue specificity.

CONCLUSIONS

- This is the first reported characterisation of exosomes from the CSF of preterm infants
- Our results describe a time course of microRNA expression and support the hypothesis that exosome signalling is involved in early brain development
- Such exosomes may represent ideal sources of biomarkers for injury and neurodevelopment

ACKNOWLEDGEMENTS

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This work has previously been presented in 2017 at the joint European Neonatal Societies Congress in Venice and is under review for publication.

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