CHIEF SCIENTIST OFFICE

CODE: TCS/18/13

RESEARCH PROJECT BRIEFING

Extracellular vesicles as novel therapeutics and biomarkers in stroke and cerebral small vessel disease

AIMS

Extracellular vesicles (EVs) are tiny particles that transport messages around the body and are important in health and disease. We have found that EVs in the blood from people with stroke differ from those in people without stroke and that this was most marked in those who had small vessel disease (SVD) in the brain. The main difference is in the types of microRNA (miRNA) they carry. MiRNAs are small pieces of a person's genetic make-up which control levels of other molecules in the body. Here, the aims were:

1. Assess the potential role of the miRNA-17 family (chosen based on our previous studies where this family of miRNAs changed after stroke) in people with SVD by profiling the contents of circulating EVs. We also looked at people with vascular cognitive impairment (VCI) or a combination of these conditions as they often occur together.
2. To determine how the miRNA-17 family might protect the brain using ‘simulated stroke’ in cells isolated from the rat brain and grown in dishes by loading EVs with miRNA(s) as therapeutic cargo.
3. Assess if alteration of miRNA-17 family, by intranasal delivery of EVs, affects outcome after experimental stroke in a rat with high blood pressure, a key risk factor for stroke.

By using a rat model we can determine how effective treatments are before considering a clinical trial. Our previous studies have shown the rat model was very good at replicating what was seen in people.

KEY FINDINGS

- Circulating EVs in blood from people with stroke, SVD or vascular cognitive impairment (VCI) carry different miRNAs than those found in healthy people.
- Using machine learning to help see patterns in large datasets (over 300 patients, more than 30 miRNAs profiled) we have identified miRNA signatures which reflect the disease load.
- EV loaded with specific miRNA(s) protect cells of the rat brain when grown in dishes and subjected to simulated stroke by removal of glucose and oxygen.
- Ongoing analysis will determine if therapeutically loaded EVs delivered intranasally after experimental stroke in rats with high blood pressure has a beneficial effect on how much brain is damaged and how they behave/move/can remember as we hypothesise it will.

WHAT DID THE STUDY INVOLVE?

We did 3 types of analysis:

- Clinical studies – we isolated EVs from blood samples from healthy people or those who had a stroke and either SVD, VCI or both (5 groups in total with over 300 people across these). We determined the level of over 30 miRNAs and assessed if they differed between groups. The study benefitted from extensive input from stroke survivors. The feedback to focus future stroke research on cognition came from the participants in an event hosted in partnership with NHS Research Scotland Stroke. The studies providing samples involved stroke survivors at all stages with some having a stroke survivor steering group.
- *In vitro* (in a dish) studies – we purchased cell lines from different parts of the brain (neurons and blood vessels), we assessed the effect of EVs loaded with micro~~cro~~RNAs (miRNA-17) when the cells were starved of oxygen and glucose like in a stroke.
- *In vivo* (rat) studies – EVs were isolated, loaded with therapeutic miRNA and administered to rats after experimental stroke. We gave the EVs through the nose as this can access the brain without being too invasive. Measures of the area of brain affected by the stroke along with associated measures of behavioural and cognitive changes were determined to the study endpoint. Analysis is ongoing to determine if the EVs were therapeutic.

WHAT WERE THE RESULTS AND WHAT DO THEY MEAN?

- EVs isolated from the blood of people with a stroke and either SVD, VCI or both show markers on their outside coat confirming they are EVs which is very important (Figure 1A).
- The amount of miRNA-17 members packaged inside circulating EVs decrease in patients with stroke (2), stroke with VCI (3), stroke with SVD (4) and stroke with both (5), compared to people who have not had a stroke (1) as can be seen in the bars with * in Figure 1B.
- EVs were successfully loaded with therapeutic contents and delivered to rat cells in dishes undergoing simulated stroke. This altered cell survival favourably demonstrating therapy.

Together, these results highlight the potential of EVs and miRNAs as both a biomarker of disease and as a novel therapeutic agent to treat stroke.

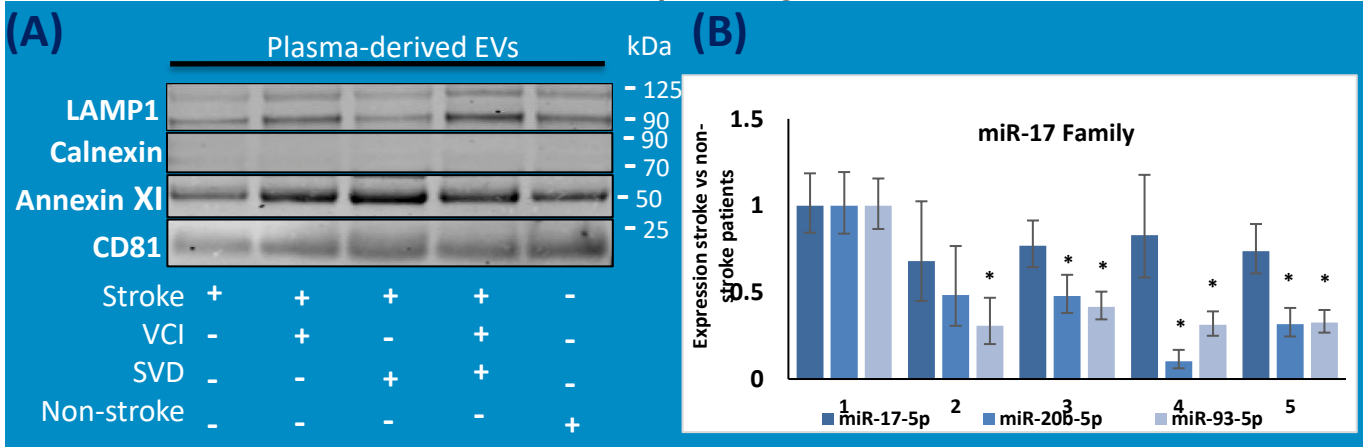

Figure 1: Characterisation & cargo of circulating EVs in those +/- stroke, SVD, VCI. (A) Western blot to confirm presence of EV markers and absence of potential contaminants (calnexin). The presence (or absence) of the grey/black band for each marker shows that these are present (or absent) in the isolated EVs. (B) Levels of miRNA-17 family expression in isolated EVs from people + stroke (2-5), + SVD (4 + 5), + VCI (3 + 5) or none of these (1). There are 3 members of the miRNA-17 family – miRNA-17, -20b and -93. This means they all have the same code which means they bind to and reduce the same target molecules.

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WHAT IMPACT COULD THE FINDINGS HAVE?

- Circulating EV contents can be used as a biomarker for SVD disease burden in the brain.
- Therapeutically loaded EVs can be used to improve outcome after stroke and so reduce associated disability and perhaps also the subsequent risk of developing dementia.
- This may lead to development of new treatments for this area of unmet clinical need. It opens the possibility of using miRNAs in other conditions in the brain or those affecting other organs in a similar way to stroke (e.g. heart attack).

HOW WILL THE OUTCOMES BE DISSEMINATED?

The findings from this project have been presented by Dr Fullerton through poster and oral communications at local, national and international conferences from both the stroke and EV research communities. Dr Work has presented some of the data in invited seminars at Institutions in the UK and at a national meeting. Three articles have been published, so far, with a further 2 in preparation - one from the clinical studies and one from the experimental, preclinical studies.

- Fullerton *et al (*2022) Systematic review: association between circulating microRNA expression stroke. *J Cereb Blood Flow & Metab* **42** 935-951 https://doi.org/10.1177/0271678X221085090
- Fullerton *et al* (2023) Extracellular vesicles and their microRNA cargo in ischaemic stroke. *J Physiol* **601** 4907- 4921 https://doi.org/10.1113/JP282050
- Lyon *et al* (2024) - Hypertension & dementia: Pathophysiology & potential utility of antihypertensives in reducing disease burden. *Pharmacol & Ther* **253** 108575 https://doi.org/10.1016/j.pharmthera.2023.108575

Public engagement events – Dr Work led a series of public engagement events to promote "Brain Health" and to discuss research across the UK (including this CSO funded study) in disorders of the brain (stroke, dementia) linked to an international conference held in Glasgow in 2022: https://brain2022.scot/ This included workshops in Primary Schools across Glasgow to encourage over 600 P5-7 children to "build a brain" and learn about their brain and how to look after it. A "Brain Health Awareness Day" was then held in May 2022 with researchers present in Buchanan Galleries and Central Station in Glasgow. Over 1400 people interacted across these sites. This combined outreach program resulted in international/national recognition through awards/nominations. The school workshops continue to be delivered. Additionally, Dr Work spoke about the EV/miRNA studies at "Pint of Science" https://pintofscience.co.uk/ (May 2022) through the "*Beautiful Minds*" theme.

CONCLUSION

EVs offer the potential to be used as a biomarker of cerebrovascular disease through profiling their contents and further can be exploited as a novel therapeutic when loaded with miRNA.

RESEARCH TEAM & CONTACT

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