Reviewer’s report

Title: Identification of biomarkers for amyotrophic lateral sclerosis by comprehensive analysis of exosomal mRNAs in human cerebrospinal fluid.

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Reviewer: ASHLEY JONES

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SYNOPSIS

The main aim of the paper is to establish a method to analyse CSF exosomal mRNA in a disease-related setting. The paper (a) successfully refines exosomal isolation and exoRNA-seq protocols, then using this refined protocol (b) compares CSF exosomal mRNA between sporadic ALS patients and controls.

Differential gene expression analysis highlights potential exosomal mRNA ALS biomarkers, and gene function ontology and pathway enrichment analyses identify putative ALS-related mechanisms (such as unfolded protein response).

GENERAL THOUGHTS

This paper is very well written; introducing exosomes nicely to the reader, precise aims and digestible results. Contextualising the need for biomarkers in relation to the failure of ALS clinic trials is written expertly. the gene ontology and pathway enrichment results are interesting. Although not wildly significant, it is a start.

There are several suggestions for the paper, which I will go into detail below.

INTRODUCTION

Introduction, paragraph 1: Introduction to exosomes

Introduction, paragraph 2: How they may help identify\be used as a ALS biomarker

(a) I am not sure this paragraph's structure is optimal, as it moves from "the reasons of harvesting CSF exosomes instead of tissue" straight into "ALS, what it is and the failure of DMT". Perhaps a new paragraph beginning when introducing ALS?
The purpose of the current study was to establish methodology for comprehensive analysis of exosomal mRNAs in CSF to identify disease-related biomarkers for ALS.

(a) If this is the main aim, and not to identify ALS biomarkers, then the results section in the Abstract need to reflect their findings with regards to this aim (i.e. they successfully implemented exoRNA-seq). For the abstract, can the authors prioritise a quality control metric to summarise why their method was successful or at minimum state its success? And append them to the findings regarding differential expression analyses etc.

(b) The reader has not been introduced to this subject (except in a potentially misplaced first paragraph in the Results). Why the need to develop a new method? Are there issues with current one or does not one exist? - Perhaps move the first paragraph in Results to the Introduction.

RESULTS

Line 142. Author states: "These genes were abundant in the exosomes as is the case with cellular RNA (Figure 3C)." Is there any other research to show that whole cell highly expressed genes are also highly expressed in exosomes? If so, recommend provide a reference.

DISCUSSION

Paragraph 1. I find it a little unclear. Did they achieve their aim of establishing 'a methodology for comprehensive analysis of exosomal mRNAs in CSF to identify disease-related biomarkers'? If so, how? Was it a success? In brief, did it find anything in relation to ALS?

Line 194: "In addition to our finding…", rewrite as "In addition to our previous finding…"

Line 199. "Compared with traditional proteomic or microRNA profiling analysis, our exoRNA-seq has the unique and novel approach aspect of biomarker identification." Not sure this is grammatically safe? Are the authors that the method is unique or that their exoRNA-seq protocol has a unique ability to identify biomarkers?

Line 203. "A problem in analysis of exosomal mRNAs lies in how the data should be normalized. DESeq2 can be applied to RNA-seq with normalization by logarithm of geometric mean of all genes." I am not sure there is an issue here with regards to the data generated in this paper? Or what problem the author is alluding to? The normalisation method is quite appropriate whereas the housekeeping approach may not be suitable here. Are the authors saying that further
investigation into whether ACTB and GAPDH can be used as housekeeping genes in exosome, warranted?

There needs to be a limitations paragraph, or at least more limitations integrated into the discussion. I strongly recommend that the authors recognise that the statistical power in this paper is low. This is the most likely reason there were many genes significant after FDR correction.

Are there ways to improve the isolation of exosomal mRNA in a disease-related setting? (given hypothetical infinite resources).

Given that ACTB and GAPDH resembles neuron expression, it may be worth highlighting the authors confidence that they are truly seeing this in exosomes and not other extracellular vesicles. Or if not confident, introduce the non-specificity of the mRNAs identified.

METHOD

I found this section excellent and, on the surface, highly replicable.

FIGURES

There is a peculiar font on the last figure. Would recommend consistent font for all figures.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes
Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
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I am able to assess the statistics

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