Reviewer's report

Title: RNA expression patterns in serum microvesicles from patients with glioblastoma multiforme and controls

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Noerholm et al., isolated serum microvesicles from patients with de-novo primary glioblastoma multiforme (N=9) and performed microarray analysis to identify possible GBM biomarkers. GBM-ExoRNA signature was compared to that of serum exosomes from normal controls (N=7). The unsupervised clustering of microarray data from exoRNA perfectly separated the GBM patients from the controls. Surprisingly, the most significant differences between control and GBM exoRNA, are based on down-regulated genes in the glioblastoma multiforme patient microvesicle-RNA, validated by qRT-PCR on several genes.

On the contrary, the yields of microvesicle RNA from GBM patients was higher than from normal controls, but primarily based on RNA of size <500 nt as detected by Bioanalyzer profile.

Gene ontology of the down-regulated genes in GBM ExoRNA showed that they are transcripts coding for ribosomal proteins highly expressed in lymphocytes. The authors then speculate that this downregulation may be associated with a decrease of % of lymphocytes in GBMs, consistent with immunosuppression observed in most GBM patients in respect to control patients.

The overall results are interesting and well discussed. However, there are questions and information to provide:

1. Did the authors analyze the MV-pattern and surface molecules from GBM patients and controls to identify possible subpopulations?
   The authors should analyze the surface protein MVs and compare them with those of MVs derived from serum of controls. Is it possible to discriminate GBM-microvesicles from blood cell-derived microvesicles by FACS analysis?

2. Does the downregulation of lymphocytes-associated genes depend on a reduced number of MV released from lymphocytes (present in a small percentage in GBMs) or on a downregulation of the transcripts inside exoGBM?

3. The authors used 9 GBM patients and 7 controls, for microarray analysis, then they confirmed the data using 10 independent GBMs and 10 controls. Did the 10 GBM samples tested display the same depletion in % of lymphocytes detected in the 7 GBM patients used for the microarray analysis? Is there a correlation between the 2 GBM patients with normal lymphocyte counts and their ribosomal transcript expression?

4. Due to the lack of the possibility to measure % of lymphocytes in controls, it’s
impossible to completely correlate transcript downregulation with lymphocyte depletion in GBM.

5. In results the authors observed that the concentration of exoRNA used for qRT-PCR validation, ranged from 30.0+-8.3 ng/mL (for GBMs) to 8.8+-2.2 ng/mL (for controls). Are the measure units correct? The concentration seems not consistent.

6. The authors demonstrated an increase of exo-RNA isolated from GBM serum. Does this observation correlate with an enhancement of Exo/MVs isolated from serum of GBM patients? Protein quantification or nanosight analysis for exosome quantification have to be provide.

7. The authors should better explain the two clustering analysis described in Figure 1 and 2. What they mean with dendrogram in Figure 1A, which was prepared without the use of p-values? Does Figure 2 represent transcripts in GMB population with a p<0.05 in respect to controls? The authors should better explain their approach in Material and Methods.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

No competing interest.