Serum exosomes: A liquid biopsy for neurological disease

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• Tiny membrane-bound nano-sized vesicles
  • **Exosomes (50-100 nm)**
  • **Microparticles (100-1000 nm)**
• Exosomes are released constitutively by all normal cells; secretion is upregulated in neoplasia
• Exosomes can be found in all biological fluids (e.g. CSF, urine, saliva, breast milk, blood), and can move b/w anatomical compartments
• **Exosomes from multiple cell types circulate in the blood**
Glioblastoma extracellular vesicles

Exosomes can traverse biological barriers, including the blood-brain barrier

DNA sequences within glioma-derived extracellular vesicles can cross the intact blood-brain barrier and be detected in peripheral blood of patients

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Exosomes are an attractive option as a vehicle for delivery of drugs or gene therapy. Dendritic cells engineered to express a neural surface peptide on exosomes. Exosomes electroporated with siRNA for GAPDH. Loaded exosomes injected into the tail vein of mice.
Exosomes carry a complex cargo of their own

Lipids
Proteins
RNA
that reflect the cell of origin

Many molecules are ‘selectively packaged’ into exosomes

GBM cells

GBM exosomes

GBM exosomes are rich in small RNA

microRNA are dominant small RNA
miRNA in GBM exosomes are selectively packaged
Exposure of normal brain EC to GBM exosomes alters EC gene expression

U251 glioma cells

U251 exosomes

Primary brain microvascular endothelial cells

Affy expression microarray

D

<table>
<thead>
<tr>
<th>Gene</th>
<th>Log$_2$ fold change</th>
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<tr>
<td>DKK1</td>
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<tr>
<td>TGFBI</td>
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<tr>
<td>GDF6</td>
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<tr>
<td>KRT7</td>
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<td>SC4MOL</td>
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<td>LDLR</td>
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miRs in MV targeting gene

- miR-5095
- miR-29a, -25, -23a, -92b, -21
- let-7b, miR-448, -5096
- miR-221, miR-222
- miR-29a
- miR-29a, -25, -92b
- miR-23a, -4301
- miR-30a, miR-30b

Li et al. RNA Biology 2013
Exosomes function as natural transporters of intercellular messages.

- **Microparticles** produced by direct budding from the cellular membrane.
- **RNA and proteins**.
- **Exosomes** produced inside endosomes and released after endosomal fusion with the cell membrane.
- **EVs releasing their contents to the extracellular space**.
- **Surface binding to trigger signalling cascades**.
- **Direct fusion with cells and the transfer of cargo**.
- **Endocytosis of EVs for future fusion or release**.
- **Altered recipient cell phenotype/behaviour**.

CM Suter, supplied
Monitoring GBM is difficult, expensive, and sometimes unreliable

- MRI imaging is central to preoperative diagnosis and tumour monitoring but can lack specificity, and sensitivity.
- Serial surgical biopsy for monitoring is infeasible
- Modern clinical trials often rely on MRI as a ‘surrogate endpoint’, rather than a real endpoint of overall survival.
- This can speed up clinical trial results, however there are limitations, such as pseudo-progression and pseudo-response

A blood-based biomarker, or ‘liquid biopsy’ could be transformative for GBM diagnosis and management
Blood-based biomarkers for brain tumours

• There is currently no blood test for GBM diagnosis or monitoring

• Attempts
  • Circulating tumour cells
  • Circulating/‘cell free’ tumour DNA/RNA
  • Circulating proteins

Extracellular vesicles as a platform for ‘liquid biopsy’ in glioblastoma patients


Extracellular vesicles (EVs) are cell-secreted vesicles that range from 30–2000 nm in size. These vesicles are secreted by both normal and neoplastic cells. Physiologically, EVs serve multiple critical biologic functions, including cellular remodeling, intracellular communication, modulation of the tumour microenvironment and regulation of immune function. Packaging physiological.
GBM exosomes

Contain selectively packaged miRNAs that are likely delivered to local cells to modulate the recipient cell’s behaviour, perhaps to induce a tumour-permissive environment.

But exosomes can also cross the blood-brain-barrier (in both directions), and GBM-derived exosomes can be detected in the blood.

Might there be a signature of GBM exosome miRNA in the blood that can be used as a biomarker for GBM?
Discovery cohort

Table 1A: Overview of cohorts used for discovery

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<tr>
<th></th>
<th>GBM, IDH WT</th>
<th>GBM-matched HC</th>
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<tr>
<td>Sample n</td>
<td>12</td>
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<tr>
<td>Age (mean ±SD)</td>
<td>63.3 ± 11.5</td>
<td>56.2 ± 12.4</td>
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<tr>
<td>Gender</td>
<td>7M, 5F</td>
<td>7M, 5F</td>
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All IDH wild type GBM
10 ml blood collected pre-operatively
Serum separated immediately

Strategy

- Size-exclusion chromatography
- RNAse treatment, RNA extraction
- Exosome validation
- Library prep and Illumina sequencing @2M reads/sample

BIOINFORMATICS ANALYSIS
A broad signature of GBM in serum exosomes

- **26 miRNAs** were differentially packaged into serum exosomes of GBM patients relative to matched healthy controls ($q < 0.01$)
Functional analysis of miRNA in serum of GBM patients identifies GBM pathways

- These data suggest that at least some of the exosomes captured from the serum are derived from the tumours themselves
- Exosomes are pretreated with RNAse prior to RNA extraction so these are not free circulating miRNA

<table>
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<tr>
<th>Top canonical pathways</th>
<th>p-value</th>
<th>Overlap</th>
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<tr>
<td>Molecular mechanisms of cancer</td>
<td>2.16E-12</td>
<td>39.2% (152/388)</td>
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<tr>
<td>Glioblastoma multiforme signaling</td>
<td>3.36E-12</td>
<td>48.4% (78/161)</td>
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<tr>
<td>Pancreatic adenocarcinoma signaling</td>
<td>6.07E-11</td>
<td>50.8% (61/120)</td>
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<tr>
<td>Role of macrophages, fibroblasts &amp; endothelial cells</td>
<td>4.37E-10</td>
<td>39.3% (119/303)</td>
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<tr>
<td>Glioma signaling</td>
<td>1.25E-09</td>
<td>49.6% (56/113)</td>
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<tr>
<th>Diseases and disorders</th>
<th>p-value</th>
<th>#Molecules</th>
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<tr>
<td>Cancer</td>
<td>1.96E-06 - 1.52E-16</td>
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<td>Organismal injury and abnormalities</td>
<td>1.97E-06 - 2.97E-13</td>
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<tr>
<td>Neurological disease</td>
<td>1.72E-06 - 8.76E-13</td>
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<td>Tumor morphology</td>
<td>1.96E-06 - 2.81E-12</td>
<td>366</td>
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<tr>
<td>Developmental disorder</td>
<td>1.39E-05 - 3.49E-12</td>
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<th>Molecular and cellular functions</th>
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<th>#Molecules</th>
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<td>Cell death and survival</td>
<td>2.13E-05 - 8.28E-17</td>
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<td>Gene expression</td>
<td>8.34E-07 - 1.67E-15</td>
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<td>Cellular growth and proliferation</td>
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<td>Cell cycle</td>
<td>2.01E-06 - 6.25E-15</td>
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<tr>
<td>Cellular development</td>
<td>1.92E-08 - 1.51E-14</td>
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Machine learning focusses the signature

Random forest method
RF algorithm trained to pick out best classifiers of GBM
70% data in training sets; 30% test
Multiple iterations

Q: Which miRNA differences most accurately predict GBM?
A combination of 7 miRNAs predicts GBM with 92% accuracy

C/w CEA ~50 – 80% accuracy
Left, CEA in OR to chemotherapy in NSCLC patients
Combinations of just a few individual miRNAs classifies GBM with high accuracy.
Serum EV miRNA can also distinguish between GBM and GII-III IDH\textsuperscript{MUT}
Summary and remaining questions

- There is a strong miRNA signal of GBM in exosomes derived from the peripheral blood.
- Combinations of just a few serum exosome miRNA can predict GBM with >90% accuracy.
- Provides platform for establishment of a liquid biopsy for brain tumours.

What are the limits of sensitivity for burden of disease?
How early might serum exosome signatures reflect recurrence?
Can exosome miRNA profile reflect tumour molecular evolution after Rx?

Large longitudinal cohort from VERTU
60 patients sampled at 4 time points.
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