Extracellular vesicles identify novel prostate cancer biomarkers

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Introduction

Prostate Cancer is a worldwide problem and more specific biomarkers are needed. Biomarkers are needed to distinguish:

- **indolent prostate cancer from more aggressive forms**
- **metastatic prostate cancer from localised prostate cancer**
- **localised prostate cancer from benign prostatic hyperplasia (BPH)**

Extracellular vesicles (EVs) are a new avenue for cancer biomarkers.

Non-invasive cancer biomarker discovery

Aim

To identify new prognostic biomarkers in prostate cancer, that can be tested for in non-invasive ways.

Methods

EVs from a panel of 13 prostate cell lines (Divided into Normal (3 cell lines), Prostate Cancer (5), Metastasis (4) and BPH (1)) were investigated to identify prognostic and or diagnostic biomarkers. EVs were collected after 48h by ultrafiltration from supplement-free cell culture media. Total RNA was extracted with Trizol and analysed using HTA 2.0 arrays. Samples were run in triplicates.

![Figure 2: Heatmap showing differential expression between the four groups. Using Transcriptome Analysis Console (TAC), identified 5502 transcripts. Normal (group 1), prostate cancer (group 2), metastatic (group 3) and BPH (group 4) EVs. N = 3 per cell line.](image)

Results

Analysis showed 5502 transcripts with different abundance in EVs when classified into the four groups of normal, prostate cancer, metastasis and benign prostatic hyperplasia. A panel of 11 biomarkers was identified for validation. 2 targets did not validate.

Figure 5: Venn diagram* highlighting unique incorporation of RNAs into EVs released from normal prostate, prostate cancer and metastatic prostate cancer cell lines. comparison of transcripts with greater than 2-fold increase and decrease and p<0.05. Interestingly only 1 transcript was common, VTRNA1-3.

Table 1: Panel of 11 potential biomarkers based on fold changes from arrays. 9 were able to be validated by qPCR. N-Normal, P- Prostate Cancer, M-Metastasis, B-BPH.

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>VTRNA1-3</th>
<th>VTRNA1-2</th>
<th>VTRNA1-1</th>
<th>HIST1H4E</th>
<th>SNORA79</th>
<th>SNORA78</th>
<th>SNORA77</th>
<th>SCARNA4</th>
<th>SCARNA8</th>
<th>SCARNA8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Significant fold changes from arrays</td>
<td>MvN - 70</td>
<td>MvP - 13</td>
<td>MvB - 14</td>
<td>MvL - 27</td>
<td>MvL - 12</td>
<td>MvL - 101</td>
<td>MvL - 137</td>
<td>MvB - 71</td>
<td>MvB - 12</td>
<td>MvB - 9</td>
</tr>
<tr>
<td>Not validated</td>
<td>MvN - 60</td>
<td>P - 60</td>
<td>P - 60</td>
<td>MvL - 60</td>
<td>P - 60</td>
<td>P - 60</td>
<td>P - 60</td>
<td>P - 60</td>
<td>P - 60</td>
<td>P - 60</td>
</tr>
</tbody>
</table>

![Figure 4: qPCR Delta Ct graphs of the 9 genes for validation. Transcript Delta Ct values in EVs. High expression is represented by low Delta Ct values.](image)

Conclusion

We have demonstrated that prostate cancer biomarkers can be found in EVs using HTA 2.0 arrays. In particular, the VTRNAs were all downregulated in the metastatic group and upregulated in prostate cancer in EVs. This family of genes has been shown to be involved in apoptosis protection and drug resistance. Notably the abundance in the EVs appears enriched when compared to the parent cell. Further work involving these genes functions, relating to metastasis, will have the potential to identify novel therapeutic targets.

*In prostate EVs, Vault RNAs can distinguish metastatic prostate cancer from localised prostate cancer. SCARNA4, SNORA54, and VTRNA1-2 can distinguish metastatic prostate cancer from normal.