CfDNA in the archived low-quality, low-volume serum samples: rate of concordance with mutations in tumor

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Concept of liquid biopsy

- Non-invasive approach
- Potential replacement of tissue
- In oncology:
  - Broad range of the malignancy’s properties
  - Reflecting the intra-tumor heterogeneity
Background data

TP53 Mutation rate of 90%

➢ The most common mutation type: G:C to A:T transitions (38.3%)

➢ G:C to T:A transversions as the second common type (16.7%)
Main Study

• Identification of tumor mutations in ESCC cases occurring during follow up of Golestan Cohort

• Searching the same mutations in CfDNA in plasma at the time of diagnosis of malignancy

• Searching the same mutations in CfDNA in plasma at the time of recruitment in cohort
Proof-of-Principle

- To examine if known TP53 mutations in the ESCC cases can be detected in cfDNA from the serum of the same patients
Comparison of pre-analytical recommendations and our archived samples

<table>
<thead>
<tr>
<th>Recommended</th>
<th>Status of our study samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma – 1 ml</td>
<td>Serum &lt;0.8 ml (mean 0.5 ml)</td>
</tr>
<tr>
<td>Single freeze-thaw cycle</td>
<td>Multiple freeze-thaw cycles &gt;3</td>
</tr>
<tr>
<td>Proceed within 6-hours after phlebotomy</td>
<td>Varied</td>
</tr>
<tr>
<td>Storage at -80</td>
<td>A year of storage at -20</td>
</tr>
</tbody>
</table>

CfDNA concentration of 2.1 to 2.2 ng/ul
Study design

• Unavailable data on the allelic fraction of tissue mutations
• In-silico selection: positions with low error in sequencing
  • 40 ESCC cases
  • 39 matched controls (age, gender, and residence)
• To help improving pipeline’s calculation of estimates
Sequencing method

- 27 primers covering TP53 exons and splicing sites were designed and pooled.
- Each sample in duplicate
- Modified GeneRead to amplify TP53 coding areas
- One pool instead of 4 pools (due to low cfDNA level)
- Libraries at the size of 200-300 bp, Ion-Torrent platform
Needlestack pipeline- variant caller

- For each position/alteration, regression line of coverage and number of alternative determine outliers

- Duplicates to distinguish between sequencing errors and real variants

https://github.com/IARCbioinfo/needlestack
## All mutations

<table>
<thead>
<tr>
<th>CtDNA Status</th>
<th>Case</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>cfDNA mutation</td>
<td>23/40</td>
<td>25/39</td>
</tr>
<tr>
<td>No of mutations</td>
<td>49</td>
<td>55</td>
</tr>
<tr>
<td>Putative effect scoring</td>
<td>1.47</td>
<td>1.23</td>
</tr>
</tbody>
</table>

**Graph:**

![Graph showing allelic fraction comparison between Case and Control](image)
## Comparison with other studies

<table>
<thead>
<tr>
<th>Published in</th>
<th>Year</th>
<th>Tumor type</th>
<th>Sample type</th>
<th>Sample volume (ml)</th>
<th>mutation in TP53 cfDNA / tumor (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Science</td>
<td>2018</td>
<td>ESCC</td>
<td>Plasma</td>
<td>7 – 7.5</td>
<td>9/40 (22.5%)</td>
</tr>
<tr>
<td>Oncotarget</td>
<td>2017</td>
<td>HNSCC</td>
<td>Plasma</td>
<td>0.6 - 2.1</td>
<td>13/45 (29%)</td>
</tr>
<tr>
<td>Current study</td>
<td>------</td>
<td>ESCC</td>
<td>Serum</td>
<td>0.5 - 1</td>
<td>9/50 (20%)</td>
</tr>
</tbody>
</table>

*Using duplicate filters will decrease number of reads from 8390 libraries to 220.
Conclusion and future plan

- *Low-volume, low-quality* archived *serum* samples can be used for CfDNA extraction and mutation detection
- Our laboratory method in combination of IARC call variant pipeline rendered comparable results to highly cited recent publication
- Combination of different body fluids might improve the mutation detection rate
  - Capsule sponge wash and supernatants
  - Compare with concordant plasma
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