Diagnostic Non-Invasive Biopsy Can Substitute Conventional Tissue Dependent Procedures in Suspected Cases of Renal Cell Carcinoma

BACKGROUND
- Diagnosis of RCC is based on HPE of tumor tissue obtained by invasion biopsies, which are associated with pain and complications.
- There are presently no sensitive non-invasive means for diagnosis of RCC - low yields of Circulating Tumor Cells (CTCs) restrict meaningful follow-up.
- We have developed a blood based liquid biopsy diagnosis for RCC based on immunochromocytochemistry (ICC) of Circulating Tumor Associated Cells (CT-Ac).

RATIONALE
- We used an epigenetically activated medium that is cytotoxic to normal cells (PBMCs) which have an intact apoptotic machinery, but confers survival privilege on apoptosis resistant cells of tumorigenic origin (Circulating Tumor Associated Cells - CT-Ac).
- C-TACs enriched and harvested from PBMCs are identified by ICC.
- Deep ICC profiling of CT-Ac was performed with RCC and subtype specific markers.

APPROACH
- 15 ml blood obtained from 133 confirmed RCC cases and 1090 asymptomatic individuals.
- C-TACs enriched and harvested from PBMCs and identified by ICC.
- Deep ICC profiling of CT-Ac was performed and subtypes markers.

Table 1: Age RCC Healthy
<table>
<thead>
<tr>
<th>Age</th>
<th>RCC</th>
<th>Healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>90</td>
<td>77</td>
</tr>
<tr>
<td>58</td>
<td>Unavailable</td>
<td>50</td>
</tr>
</tbody>
</table>

Table 2: Metastases RCC
<table>
<thead>
<tr>
<th>Metastases</th>
<th>RCC</th>
<th>Healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-metastatic</td>
<td>67</td>
<td>77</td>
</tr>
<tr>
<td>Unavailable</td>
<td>50</td>
<td>57</td>
</tr>
</tbody>
</table>

IDENCRIFICATION OF CT-Ac BY IMMUNOCYTOCHEMISTRY (ICC)
- Fig. 1a: Representative images of immunostained (ESCam, panCK) CT-Ac cells obtained from a known case of Clear Cell RCC.
- Fig. 1b: Representative images of immunostained (ESCam, panCK) CT-Ac cells obtained from a known case of Clear Cell RCC.

DEEP ICC PROFILING C-TACs FOR ORGAN SPECIFICITY
- Fig. 3: Representative images of C-TACs from a known case of Clear Cell RCC immunostained for PAX-8 (Positive in Clear Cell, Papillary and Chromophobe).
- Fig. 4: Representative images of C-TACs from a known case of High Grade Clear Cell RCC immunostained for Vimentin (Negative in Papillary and Chromophobe).
- Fig. 5: Representative images of C-TACs from a known case of Papillary RCC immunostained for CK-19 (Negative in Clear Cell and Papillary, Positive in Chromophobe).

FINDINGS
- C-TACs obtained in 133 samples out of 133 (100%).
- Organ specificity could be determined in 128/133 samples.
- C-TACs were detected in 128/133 asymptomatic individuals.
- C-TACs in asymptomatic individuals were negative for RCC and subtypes markers.

CONCLUSION
Non-invasive (blood based) ICC profiling of C-TACs can provide necessary diagnostic information for RCC and can substitute conventional procedures dependent on tissue extraction.