The novel association of circulating tumour cells and circulating megakaryocytes with prostate cancer prognosis

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BACKGROUND

• Circulating tumour cell (CTC) is rare in blood.
• Previous CTC studies have mainly focused on epithelial CTCs, but not CTCs undergoing/undergone epithelial-mesenchymal transition (EMT).
• EMT has been recognised to play an important role in cancer progression and metastasis.
• Size and deformability based CTC isolation system can detect different subtypes of CTCs, including epithelial, EMTing and EMTed CTCs.

AIMS

To develop an approach for the investigation of different subtypes of circulating tumour cells (CTCs) and other cells to evaluate their potential prognostic value of prostate cancer.

MATERIALS & METHODS

• Study population: 81 prostate cancer patients, including 38 untreated localised and 43 progressive castration-resistant prostate cancers (CRPCs).
• CTC isolation: From 7.5 mL of whole blood using the Parsortix™ system from ANGLE plc (Fig. 1).
• Immunofluorescence (IF): anti-CK, VIM, and CD45 antibodies with different fluorescence detection.
• Fluorescence in situ hybridisation (FISH): 5 rounds of FISH including 10 Probes were applied on cells after immunofluorescence.
• Statistics: Kendall’s rank correlation was used to assess the association between CTCs and concurrent PSA level. Wilcoxon rank-sum test was applied to assess the distribution of CTCs in sub-groups divided by specific clinical features.

RESULTS

1. CTC isolation and identification in prostate cancer and healthy men.

Fig. 2. Different types of detected cells with epithelial and mesenchymal properties. (A) Representative images of CTCs with epithelial (CK+/VIM-+CD45-) feature and CTC cluster. (B) Detected rate of each subtype in untreated localised, progressive CRPC and healthy men.

2. CTCs were confirmed by repeated FISH.

Table 1. Percentage of detected genetic changes in sub-population of cells

<table>
<thead>
<tr>
<th>Sub-type</th>
<th>Prostate cancer</th>
<th>Healthy men</th>
</tr>
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<tbody>
<tr>
<td>Epithelial type</td>
<td>10%</td>
<td>5%</td>
</tr>
<tr>
<td>EMTing type</td>
<td>30%</td>
<td>15%</td>
</tr>
<tr>
<td>EMTed type</td>
<td>5%</td>
<td>2%</td>
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3. Subgroups of CTCs are associated with serum PSA level, primary biopsy GS, the risk of localised tumour and metastases.

4. Circulating megakaryocytes identification and association with better survival.

CONCLUSIONS

• The size-based system, Parsortix™, can efficiently harvest both CK+ CTCs and CK-/Vimentin+/CD45- cells.
• Genetic alterations were detected in a large proportion of VIM+/CD45- circulating cells, indicating that Parsortix™ captures CTCs under EMT.
• EMTing CTC counting correlated the best with metastases. Combination of EMTing CTCs and PSA greatly increase the accuracy of metastasis prediction.
• Combination of circulating megakaryocytes and mesenchymal CTCs has great power to predict CRPC patient survival.