Detection and molecular characterization of EpCAM positive and EpCAM negative circulating tumour cells isolated from SCLC patients using an epitope independent platform

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Background

Personalised medicine relies on a detailed understanding of the molecular basis of disease in an individual patient that can subsequently be used to follow-up with a tailored course of treatment based on the presence of specific disease biomarkers.

Small cell lung cancer (SCLC), an extremely aggressive disease, due to the small size and localisation of the tumors, as well as the co-morbidity of the methods of tissue collection, intravenous biopsy sampling is rarely tolerable and not feasible.

Liquid biopsies, such as circulating tumour cells (CTCs), can be an alternative for standard procedures, and provide an option to determine the genetic profile of cancer patients.

The gold standard for enrichment of these rare cells is CellSearch®, an epidep dependent system that positively selects EpCAM expressing tumour cells.

To fully realise the clinical potential of CTCs as a liquid biopsy there is a requirement to establish a robust pipeline of isolation and storage of CTCs that will facilitate retrospective as well as prospective analysis.

The evaluation of the cells enriched by epitope independent devices, such as Parsor® (Chudziak, 2015), is important to identify and characterise the range of CTC phenotypes present in SCLC patients.

CTC Isolation and Storage

Blood samples from SCLC patients have been collected and CTC enriched by CellSearch® followed by isolation and/or storage.

A pipeline has been developed to maximise the availability of CTCs for molecular analysis based on the CTC burden of patients.

Cells showing positive staining for pan-pan cytokeratin (CK), undetectable CD45 labelling and positive nuclear staining are classified as CTCs; corresponding white blood cells (WBCs), i.e. cells staining as CK-/CD45+ and positive nuclear staining were also isolated as germline controls.

Molecular analysis of CTCs

Individual CTCs, pools of CTCs and control WBCs were subjected to whole genome amplification (WGA) and its efficacy was evaluated by multiplex PCR to determine the Genome Integrity Index (GII) of each sample.

Low pass whole genome sequencing (WGS) was used to establish the genome wide copy number alteration (CNA) (0.1 to 0.2x coverage).

Molecular evaluation of CTCs following epitope independent enrichment

Blood samples from eight SCLC patients were enriched by Parsor® followed by DEPArray™ single cell isolation (poster presented by Chudziak et al. and Chudziak et al. 2015).

Sample stability was evaluated from enriched CTCs from patient 4 following storage at -20°C in glycerol for 6 months: with no detrimental effect seen following WGA.

Molecular analysis of single CTCs and WBCs was performed from cells enriched by Parsor® and isolated by DEPArray™ from patient 4 (Table 2 and Figure 9).

CNA profiles generated were consistent with tumour associated aberrations seen only in CTCs and a normal karyotype seen in WBC controls.

Conclusions

Development of a robust protocol for the isolation and storage of CTCs compatible with downstream molecular analysis.

Demonstrated reliable whole genome amplification of single cells following storage of CellSearch® enriched samples for more than 2 years.

DEPArray™ isolated CTCs can be stored for long periods (+2 years) and maintain a good genomic integrity index.

Possible to isolate single cells from samples enriched with an epitope independent device (Parsor®) following long term storage.

Preliminary results of tumour CNA profiles identified from cells isolated following Parsor® enriched samples.

These results broaden the scope of SCLC analysis and describe a novel approach for isolating EpCAM positive and EpCAM negative CTCs in SCLC.

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