Isolation and in-chip characterization of lung cancer circulating tumour cells (CTCs) using a microfluidics device

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Introduction

Lung cancer is the world’s largest cause of mortality related to cancer disease [1]. There are two main types of lung cancer, non-small cell lung cancer (NSCLC), and small cell lung cancer (SCLC), the latter less common but associated to worse prognosis [2]. CTCs are responsible for tumor dissemination and metastasis formation. Thus, enumeration of CTCs may serve as a biomarker for cancer stage diagnosis, monitoring during and after treatment of either early, locally advanced, or advanced state of lung cancer [3].

Aim

In this work, a microfluidic system was used for CTCs isolation based on size. Cells were characterized by surface markers of epithelial and mesenchymal state, through immunofluorescence. The main goal was to isolate CTCs from plasma samples of lung cancer patients, perform CTCs characterization and assess a correlation with the tumour type and stage.

Methodology

Fig.1 System design. Cells pass under laminar flow through a micro patterned microfluidic chamber and get trapped within the system.

Fig.2 Sequence of steps performed. The plasma sample is diluted at 1:1 with NaCl and 0.5% EDTA. The plasma is processed through the microfluidic device and fixed with 4% PFA. The isolated CTCs are then stained with fluorescence markers (EpCAM/CD133, VIM, CD45, DAPI) and the sample is analysed under a fluorescence microscope.

Results

Fig.3 CTCs were stained for EpCAM/Vim/CD45/DAPI for NSCLC samples and CD133/Vim/CD45/DAPI for SCLC samples. Cells were identified as CTCs if DAPI+ EpCAM+ and/or VIM+ and CD45+ for NSCLC and DAPI−, CD133+ and/or VIM+ and CD45+ for SCLC. CD133, EpCAM and Vimentin were used for CTCs identification in lung cancer, CD45 for leukocytes and DAPI for cells nuclei.

Fig.4 Distribution of CTC levels in advanced stage NSCLC and SCLC samples analysed.

Fig.5 Distribution of CTC levels in early and advanced stages of NSCLC samples analysed.

Conclusion

- The microfluidic chip proved to be efficient in the capture of CTCs, as well as the immunoaffinity characterization.
- From the advanced stage 14 SCLC and 13 NSCLC samples analysed, the concentrations of CTCs were similar in both types.
- Regarding the NSCLC type analysis, a higher enumeration of CTCs mostly represented more advanced stages of tumour (13 samples analysed), in comparison to lower CTCs count in early-stage ones (11 samples analysed).
- This type of studies may contribute to the improvement of early diagnosis, patient monitoring, and more effective methods to improve lung cancer patients outcome.

References