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Detection of Circulating Tumor Cells in Renal Cell Carcinoma

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ABSTRACT

Most current CTC methods that primarily rely on affinity capture of the epithelial surface marker EpCAM to enrich CTCs, fail to recognize the CTCs in renal cell carcinoma (RCC), because they often lack or express low levels of epithelial markers. The objective of this study was to develop a new platform to enable a more reliable detection of CTCs, including those with a mesenchymal phenotype in metastatic RCC patients. By using three RCC cell lines in spiking experiments, we demonstrated that RCC tumor cells could be efficiently recovered from the blood by the CellSieve™ platform (97-98%). The filter-captured tumor cells could be further characterized by fluorescence antibody staining within a specially-designed cartridge. Forty-two peripheral blood samples were collected from 29 patients with metastatic RCC and analyzed by using the CellSieve™ platform. A cell population with CD10+/vimentin+/CD45- phenotype was detected in the RCC patients with enumeration ranging from 3 to 171 cells per 7.5 mL of blood. These cells occurred as both single cells and multiple-cellular clusters, and displayed morphological heterogeneity. In conclusion, the CellSieve™ microfiltration platform is highly effective for detection of CTCs in metastatic RCC.

INTRODUCTION

Clear cell renal cell carcinoma (ccRCC) is the most common type of kidney cancer and is responsible for approximately 80% of kidney cancer cases. CTCs in ccRCC are of mensenchymal origin and therefore may not be detected by EpCAM-based capture methods. Microfiltration is an EpCAM-independent method for isolating CTCs from the peripheral blood of cancer patients with solid tumors. The microfiltration approach can be used as a non-invasive liquid biopsy for cancer detection and subtyping.

MATERIALS & METHODS

Peripheral blood samples were collected from patients with metastatic RCC by Mayo Clinic Arizona and analyzed by Creatv MicroTech. Clear CellSieve™ microfilters[1], with 160,000 pores in a 9-mm diameter area (Fig. 1), were used for isolation of the CTCs. 7.5 mL of whole blood was diluted in the Prefixation Buffer and filtered through the microfilter in 3 min using a syringe pump. Clinical immunohistochemical markers for RCC, such as CD10 and vimentin, were used to identify candidate CTCs.

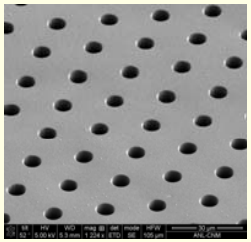

Figure 1. SEM image of CellSieve™ microfilters with 7-µm diameter pores in a uniform array.

The cells collected on the filter were further fixed, permeabilized, and stained with DAPI and fluorescent antibodies specific to CD10 (FITC), vimentin (EF615), and CD45 (CY5). The assay was performed inside a filter holder, providing for a clean and straight-forward protocol. After the assay, the filter was placed on a glass slide for imaging.

RESULTS

The capture efficiency for 786-O, Caki-1, and Caki-2 cell lines was determined to be 98%, 98% and 97%, respectively. On-filter antibody staining revealed heterogeneous expression of vimentin and CD10 in RCC cells (Fig. 2).

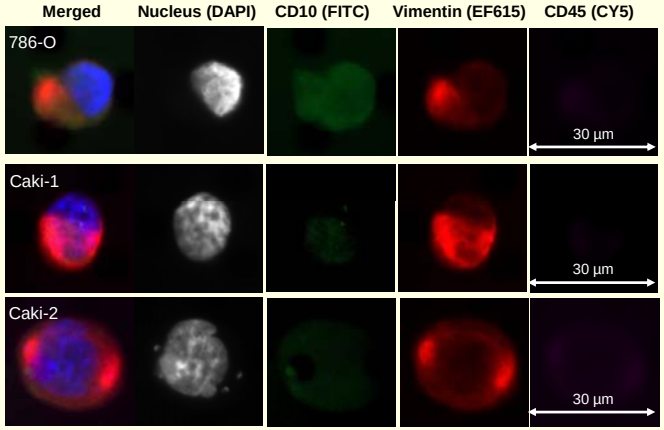

Figure 2. Microfiltration and antibody staining of RCC cell lines. RCC cells were spiked in healthy blood and filtered through CellSieve™ filter. The filter-captured cells were stained with DAPI, CD10, vimentin and CD45. The DAPI staining is shown as blue in the merged images.

Forty-two blood samples were collected from 29 RCC patients and processed with microfiltration and antibody staining. A cell population with CD10+/vimentin+/CD45- phenotype was detected in the RCC patients, with enumeration ranging from 3 to 171 cells per 7.5 mL of blood.

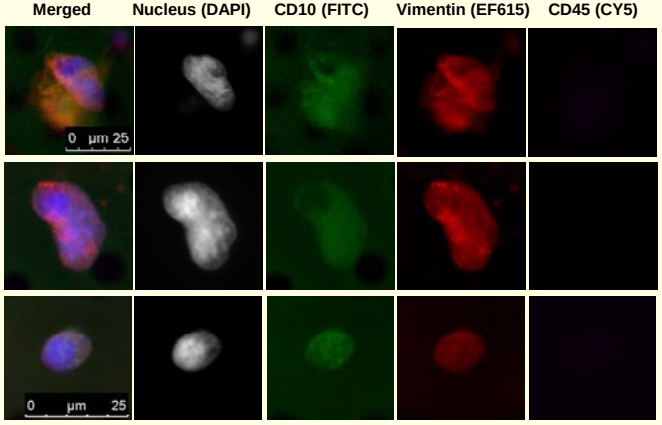

Figure 3. Individual CTCs in the blood samples from patients with metastatic RCC. The DAPI staining is shown as blue in the merged images.

The CTCs occurred as both single cells (Fig. 3) and multicellular clusters (Fig. 4A,B), expressing vimentin and CD10, and lacking CD45. The typical CTCs display abnormal morphology, including large nuclei (typically 15-30 µm in size), irregular cell size and shape, and high nucleus-to-cytoplasm ratio. Although CD10 and vimentin antibodies have cross-reactivity with white blood cells, the CTCs could be identified based on morphology, cell size and CD45- staining. Expression of vimentin was significantly increased in a subtype of RCC CTCs, which usually formed multiple cellular clusters (Fig. 4A,B). We also observed megakaryocyte-like cell (Fig. 4C) and atypical large, rod-shaped, naked nuclei (Fig. 4D), which need to be further characterized.

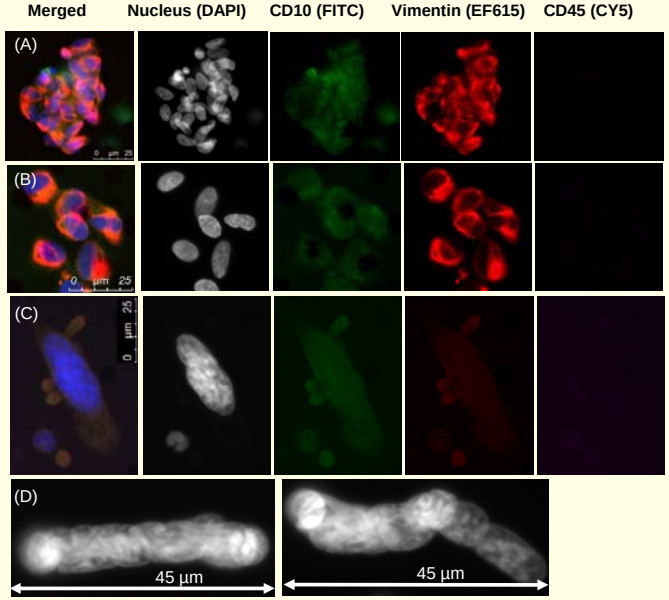

Figure 4. Morphological and phenotypical variations of cells captured on the filter membrane. (A) Larger CTC cluster; (B) Small CTC cluster; (C) Megakaryocyte-like cell; (D) Rod-shaped nuclei in cells with very little cytoplasm. The DAPI staining is shown as blue in the merged images.

CONCLUSIONS

- CellSieve™ microfiltration assay is a straight-forward and efficient method to isolate CTCs from patients with metastatic RCC.
- Single cells and clusters similar to RCC cell lines were identified.
- Morphologies of cancer associated cells are diverse.
- CellSieve™ microfiltration facilitates detection of mesenchymal CTCs to improve prediction of therapy response and monitoring, especially in metastatic RCC.

REFERENCES

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