**Detection of Circulating Tumor Cells in Renal Cell Carcinoma**

Peixuan Zhu¹, Thai Ho², Erik P. Castle³, Richard W. Joseph³, Melissa L. Stanton², Shuhong Li¹, Daniel Adams⁴, Olga V. Makarova⁵, Patte T. Amstutz, III⁶, Cha-Mei Tang⁸


**ABSTRACT**

Detection and molecular characterization of circulating tumor cells (CTCs) can provide important information for cancer diagnosis and therapy. However, 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. The assay protocols were developed using three RCC cell lines and validated using blood samples from patients with metastatic RCC. The results demonstrated that the RCC tumor cells could be efficiently recovered from the blood by the CellSieve™ platform (97-98%).

**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). Twenty-eight blood samples were collected from RCC patients and processed with microfiltration and antibody staining. A cell population with CD10+/vimentin+/CD45- phenotype was detected in RCC patients, with enumeration ranging from 4 to 141 cells per 7.5 mL of blood. RCC CTC expression profiles were distinct from other previously observed cancer types. The cells occurred as both single cells and multicellular clusters, 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 (Fig. 3).

**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 mesenchymal 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, 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 Prefiltration 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. 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.

**CONCLUSIONS**

- CellSieve™ microfiltration assay is a straight-forward and efficient method to isolate CD10+/vimentin+/CD45- cells from patients with metastatic RCC.
- Single cells and clusters similar to RCC cell lines were identified.
- Cells with long rod shaped nucleus were common in some RCC patient samples.
- 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**