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.

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).

Merged        Nucleus (DAPI)   CD10 (FITC)   Vimentin (EF615)   CD45 (CY5)

![Merged image of RCC cells](image1)

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.

Merged        Nucleus (DAPI)   CD10 (FITC)   Vimentin (EF615)   CD45 (CY5)

![Merged image of RCC cells](image2)

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

CONCLUSIONS

- CellSieve™ microfiltration assay is a straightforward 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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