Introduction

- CD44 gene is alternatively spliced between exons 5 and 16 into hematopoietic or standard form (CD44s) and multiple CD44 variant isoforms (CD44v).
- CD44v splicing variant expression is associated with cancer stem cancers (CSC) in colorectal, gastric, breast, and prostate cancers.
- CD44v variant isoform is predominantly expressed in epithelial cancer cells, and CD44s standard isoform is mainly expressed in mesenchymal cancer cells.
- Detection of CD44v protein on the surface membrane of the circulating tumor cells (CTCs) of epithelial solid tumors has not been previously reported.
- Epithelial Cell Adhesion Molecule (EpCAM) is the principal antibody used in the capturing of prostate adenocarcinoma CTCs.
- We applied CytoQuest™ CR system to assess CD44v9 cancer stem cell protein expression in EpCAM/PanCK positive CTCs in a castration-resistant prostate cancer (CRPC) patient.

Materials & Methods

- Peripheral blood of castration-resistant prostate patient was collected in Heparin Tube (02-689-6, BD).
- 4 mL whole blood sample was prepared for peripheral blood mononuclear cell (PBMC) isolation by density gradient centrifugation using Leucosep® (163290P, Greiner Bio-One) and Histopaque®-1077 (10771, Sigma-Aldrich).
- The PBMC fraction was harvested and resuspended in Wash Medium.
- Resuspended PBMC was loaded into the CytoQuest™ CR System and CTC was captured by EpCAM (KA4897, Abnova) immobilized CytoChipNano (U0095, Abnova).
- Immunofluorescence staining for circulating epithelial cells detection was performed using CK, CD45, DAPI (KA4897, Abnova) and CD44v9(MAB12149, Abnova) as instructed in the protocol.
- Imaging was performed using Nikon Eclipse Ti-E fluorescent inverted microscope.

Results

- CTC Counts: In 4 mL blood of prostate cancer patient, 22 cells are counted as CTC (PanCK+, CD45-, CD44v9+, DAPI+).

![Image](image1.png)

Figure 1. Representative images of CTC (white arrow) from prostate cancer patient. CTC was detected by using immunofluorescence staining for PanCK (FITC, green), CD44v9 (APC, red), CD44s (APC, red), CD45 (PE, orange) and Nucleus (DAPI, blue).
Discussions

- Conventional EpCAM/PanCK antibodies are insufficient to identify CTCs related to epithelial-mesenchymal transition (EMT), metastasis, drug resistance, and relapse.
- CD44v acts as a critical regulator of EMT and cancer stemness by acting as a co-receptor for many growth factors and cytokines produced by cells in the tumor microenvironment.
- Moreover, CD44v regulates reactive oxygen species (ROS) metabolism by stabilizing xCT transporter, thereby protecting CSCs from high ROS in tumor microenvironment.
- In this study, CD44v9 protein staining of CRPC circulating tumor cells is a powerful and specific biomarker of cancer, EMT CTC and CSC detection and diagnosis.
- CTC, EMT CTC, CSC liquid biopsy is an advanced, non-invasive, and interrogative technique for detection of epithelial tumors.
- Future studies should include both localized and metastatic prostate cancer to further expand the clinical applications of CD44v9.

References