Antibodies Targeting ASGPR and CPS1 for Capture and Identification of Circulating Hepatocellular Carcinoma Cells

Introduction

- Hepatocellular carcinoma is the fifth most common cancer in the world and has the highest incidence in Asia countries.
- Biomarkers for capture and identification of circulating tumor cells (CTCs) in patients with hepatocellular carcinoma (HCC) are not well known.
- Although HCC cells are epithelial cells amenable to CTC capture, only about 35% of the HCC cases express EpCAM biomarker.
- Sialoglycoprotein receptor (ASGPR), found exclusively on the surfaces of hepatocytes, binds and internalizes molecules with terminal galactose or N-acetylgalactosamine.
- CPS1 is a known cytoplasmic antigen recognized by hepatocyte paraffin 1 (Hep Par 1) human hepatocyte-specific antibody used in pathology to determine hepatocellular origin.
- In this Application Note, we applied CytoQuest™ CR system, ASGPR and CPS1 monoclonal antibodies to capture and identify circulating HCC cells.

Materials & Methods

- Peripheral blood of HCC patient with solitary tumor nodule was collected in Heparin Tube (02-689-6, BD).
- Two mL of blood were prepared for collecting the peripheral blood mononuclear cell (PBMC) by density gradient centrifugation using Leucosep® (163290P, Greiner Bio-One) and Histopaque®-1077 (10771, Sigma-Aldrich).
- PBMC fraction was harvested and resuspended in Wash Medium.
- Resuspended PBMC was loaded into the CytoQuest™ CR System and HCC CTCs were captured by ASGPR (KAxxxx, Abnova) immobilized CytoChipNano (U0095, Abnova).
- Immunofluorescence staining for detecting HCC CTCs was performed using CPS1, CD45, DAPI (KAxxxx, Abnova) as the instruction protocol.
- Imaging was performed using Nikon Eclipse Ti-E fluorescent inverted microscope.

Results

- HCC CTC Counts: In 2ml blood of HCC patient, 1 cell counts as HCC CTC (CPS1+, CD45-, DAPI+)

![Figure 1. Representative images of HCC CTC (white arrow) and WBCs (yellow arrow) from HCC patient. HCC CTC was detected by using immunofluorescence staining for CPS1 (FITC, green), CD45 (PE, red) and Nucleus (DAPI, blue).](image)

Discussions

- Clinical management of HCC is mostly hampered by the lack of effective therapies and inability to monitor early metastasis and recurrence.
- Conventional CTCs are detected based on patient’s preexisting tumor and EpCAM and PanCK epithelial biomarkers which are not hepatocyte-specific.
- A new ASGPR and CPS1 biomarker cocktail successfully captures and identifies HCC cells using CytoQuest™ CR microfluidic system.
- Use of CPS1 hepatocyte-specific biomarker has advantage over PanCK epithelial biomarker for hepatocyte-specific, circulating HCC cell detection.
- Therefore, hepatocyte-specific CTC detection and monitoring of HCC can provide clinically insights into treatment attempts, metastasis, and prediction of prognosis.

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References


