Isolation of DBA Positive Circulating Cancer Stem Cells in Glioblastoma Multiforme

Introduction
- Glioblastoma multiforme (GBM) is the most fatal primary malignant central nervous system neoplasm in adults, with a median overall survival of 14.6 months.
- Circulating tumor cell (CTC) detection offers a great potential to diagnose, guide therapy, monitor treatment response, relapse, and metastasis.
- GBM CTCs can be successfully captured by cell-surface vimentin antibody and detected by glial fibrillary acid protein (GFAP) and EGFRvIII variant antibodies.
- Dolichos Biflorus Agglutinin (DBA) lectin identifies alpha-N-acetylglucosamine (GlcNAc) glycan biomarkers on GBM-derived cancer stem cells in tissue.
- Isolation of DBA positive circulating cancer stem cells (CSCs) in GBM using positive or negative enrichment method has not been previously reported.
- We applied CytoQuest™ CR positive enrichment system to isolate DBA positive CSCs of GBM captured by cell-surface vimentin antibody.

Materials & Methods
- Peripheral blood of GBM patient was collected in Heparin Tube (02-689-6, BD).
- 4 mL blood was prepared to collect the peripheral blood mononuclear cells (PBMC) by Leucosep® (163290P, Greiner Bio-One) and Histopaque®-1077 (10771, Sigma-Aldrich) density gradient.
- The PBMC fraction was harvested and re-suspended in Wash Medium.
- Re-suspended PBMC was loaded into the CytoQuest™ CR System, and the GBM CSCs were captured by biotinylated CSV antibody (KA4908, Abnova) immobilized on the surface of CytoChipNano CR (U0096, Abnova).
- Immunofluorescence of GBM CSC was performed using DBA, CD45 (KA4908, Abnova) detection antibodies with Hoechst nuclear staining as the instruction protocol.
- Imaging was performed using Nikon Eclipse Ti-E fluorescent inverted microscope.

Results
- CSC Counts: In 4 mL blood of GBM patient, 6 cells counted as CSCs (DBA+, CD45-, Hoechst+).

![Merged DBA CD45 Nucleus](image)

Figure 1. Representative images of CSCs (red arrow) on CytoChipNano CR from GBM patient. CSCs were detected by using immunofluorescence staining for DBA (FITC, green), CD45 (PE, orange) and Nucleus (Hoechst, blue).

Discussions
- One of the main challenges in GBM is finding a suitable biomarker for identification of GBM cells. Conventional EpCAM/PanCK antibodies are insufficient to identify GBM CTCs and CSCs.
- Vimentin is a useful biomarker expressed in all mesenchymal tissues. Cell-surface vimentin is uniquely expressed during malignant transformation in wide range of normal tissues.
- In this study, surface-coated, biotinylated CSV antibody is used to capture GBM CSCs which are then validated by DBA fluorescent detection lectin.
- GBM CSC liquid biopsy is a powerful way to monitor metastasis, drug resistance, and relapse in the new era of precision medicine.
- Future studies should examine the molecular profiles of GBM and their corresponding CSCs to identify new treatment biomarkers to halt this fatal disease.
References


