

Diagnosis of Gliomas Using Circulating Glial Cells.

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Conflict of Interest :

Datar Cancer Genetics offers commercial services in the area of Oncology.

BACKGROUND

Diagnosis of CNS malignancies is reliant on histopathological evaluation (HPE) of biopsied tumor tissue. However, invasive biopsies carry inherent risks of high morbidity and mortality in CNS malignancies. Cell free DNA (cfDNA) and microvesicle (MV) borne nucleic acids have limited stand-alone diagnostic utility in CNS malignancies. Though Circulating Glial Cells (CGCs) in peripheral blood could offer a viable alternative to invasive biopsies for diagnosis of CNS malignancies, their detection and isolation in sufficient numbers have presented a challenge.

DEMOGRAPHICS OF STUDY POPULATION

15 mL peripheral blood was collected from 23 patients with various CNS malignancies and a case of atypical meningioma. The population included 17 Males and 6 Females with a median age of 43 years (range 17 - 77 years).

Cancer Type	#
Glioblastoma	11
Oligodendroglioma	5
Anaplastic Astrocytoma	3
Medulloblastoma	2
Gliosarcoma	1
Atypical Meningioma	1

CellWizard™ PROCESS FOR ENRICHMENT OF CGCs

CellWizard™ epigenetically active media induces lethality in normal cells (with functional apoptosis machinery) and simultaneously confers survival privilege on apoptosis resistant cells of tumorigenic origin (such as CGCs). This paradoxical cytotoxicity selectively eliminates most leukocytes and facilitates a label free negative enrichment of CGCs, which can be harvested and further characterized.

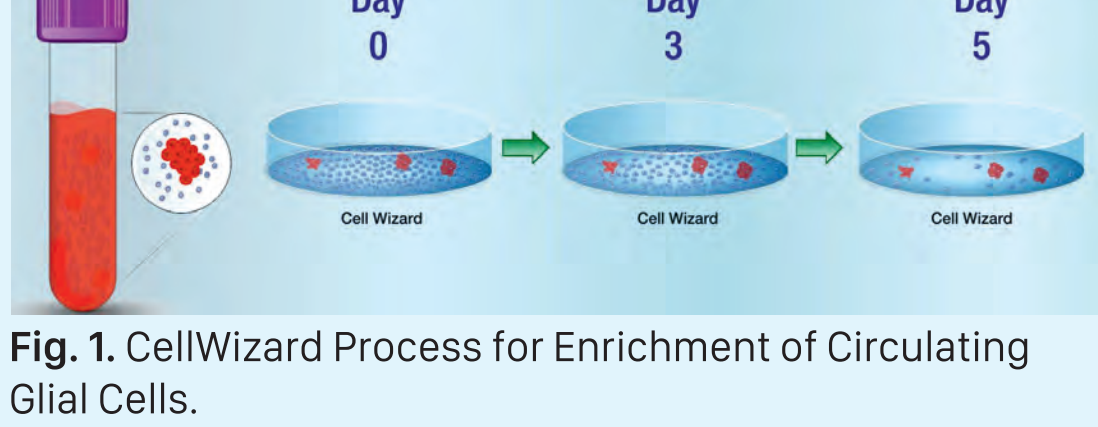


Fig. 1. CellWizard Process for Enrichment of Circulating Glial Cells.

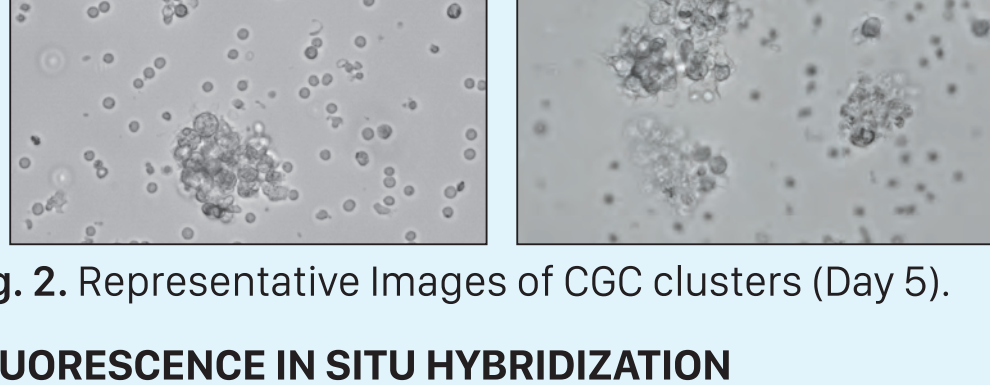


Fig. 2. Representative Images of CGC clusters (Day 5).

FLUORESCENCE IN SITU HYBRIDIZATION

Viable CGCs enriched and harvested using the CellWizard™ process were characterized by FISH to detect EGFR amplification which is common in high grade Gliomas.

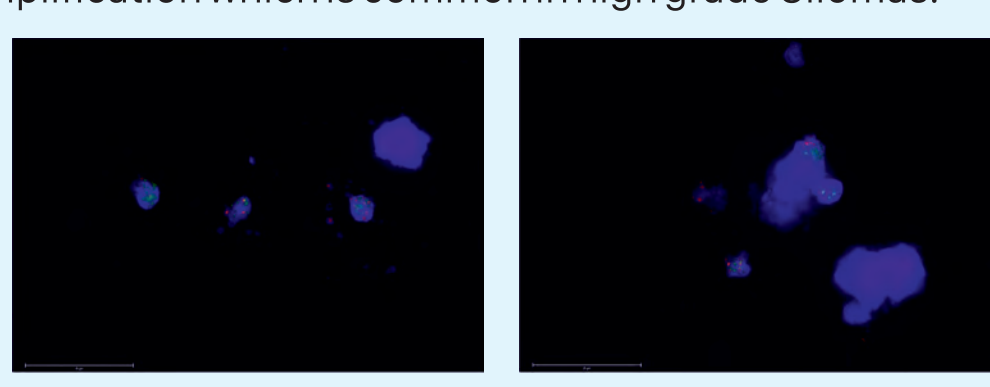


Fig. 3. FISH of CGCs for EGFR amplification (EGFR/CEN7). Images depict CGCs with multiple copies of Chromosome 7 (red) and increased EGFR signal (green).

IMMUNOFLUORESCENT STAINING OF CGCs (GLIOBLASTOMA)

Fig. 4. ICC of CGCs for EMA and GFAP.

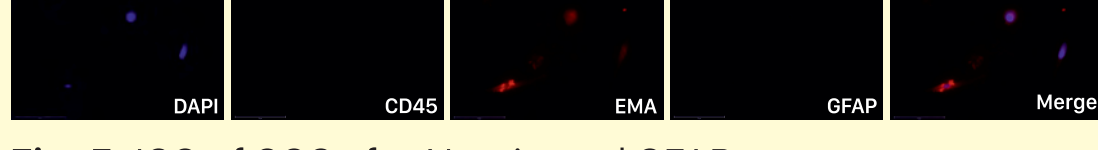


Fig. 5. ICC of CGCs for Nestin and GFAP.



Fig. 6. ICC of CGCs for panCK and GFAP.

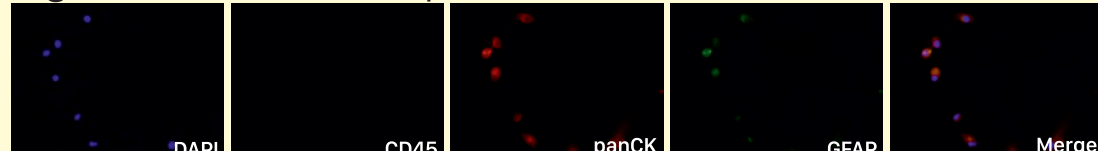
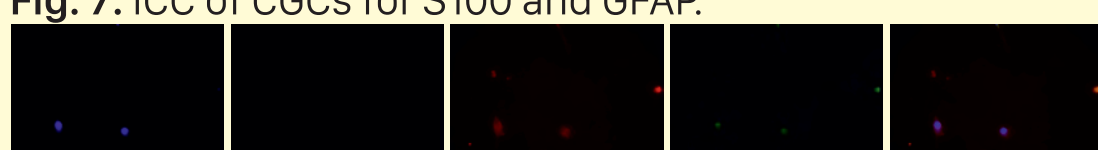


Fig. 7. ICC of CGCs for S100 and GFAP.



IMMUNOFLUORESCENT STAINING OF CGCs (ATYPICAL MENINGIOMA)

Fig. 8. ICC of CGCs for EMA and GFAP.

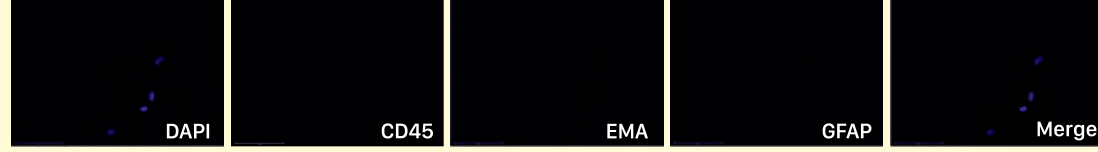


Fig. 9. ICC of CGCs for Nestin and GFAP.



Fig. 10. ICC of CGCs for panCK and GFAP.

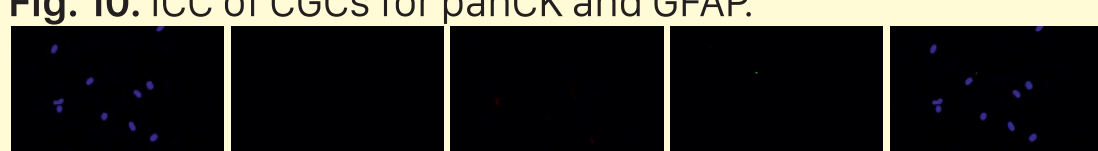
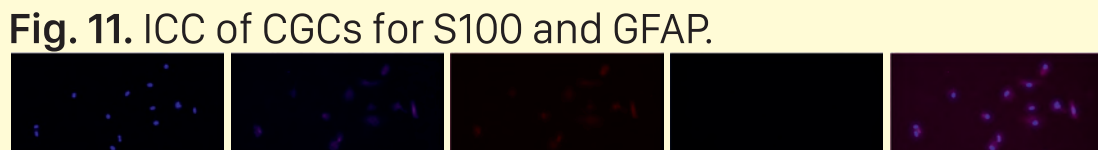


Fig. 11. ICC of CGCs for S100 and GFAP.



FINDINGS

- CGCs could be enriched and harvested in all samples evaluated,
- FISH showed EGFR gain in CGC from Glioblastoma patients,
- ICC profiling discerned CGCs from Glioblastoma and Atypical Meningioma.

CONCLUSION

ICC profiling and FISH of CGCs can provide necessary diagnostic information non-invasively to substitute conventional procedures dependent on tissue extraction for diagnosis of Glioblastoma.