Accurate Identification of Glial Malignancies from Peripheral Blood

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

BACKGROUND

Symptomatic presentation of patients with intra-cranial malignancy is frequently non-specific and differentiating such patients from those with benign conditions is challenging. Accordingly, GBM presents as a medical emergency more frequently than any other common cancer, implying that effective strategies for rapid and simple diagnostic stratification of such patients are urgently required. Here we describe the detection of glial malignancies based on the enrichment and identification of Circulating Glial Cells (CGCs) in peripheral blood.

METHODS AND FINDINGS

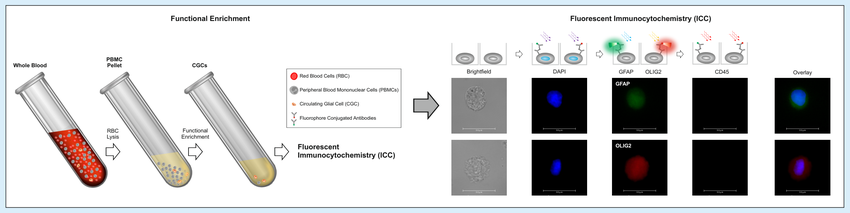

Figure 1 (left) is a schema of the test. Peripheral blood mononuclear cells (PBMC) isolated from blood samples are treated with a proprietary high efficiency CGC enrichment medium (CEM). The CEM exerts greater cytotoxicity on all non-malignant cells which are eliminated by cell death resulting from apoptosis whereas majority of the apoptosis reluctant malignant cells survive.

Table 1. Decision Matrix

GFAP	OLIG2	PanCK	CD45	Classification
+	+	-	-	GLI-M
+	-	-	-	
-	+	-	-	
-	-	+	-	CAR-M
-	-	-	±	CNS-B / HDI
(any other findings)				Indeterminate

GLI-M: glial malignancy; CAR-M: carcinoma with brain metastases; CNS-B: benign CNS conditions; HDI: healthy donor individual

The surviving cells are harvested and immunostained to determine the cellular expression status of GFAP, OLIG2, CD45 and PanCK. As indicated in the Decision Matrix (Table 1), CGCs are identified as cells that are positive for GFAP and /or OLIG2 and negative for PanCK and CD45, whereas circulating tumor cells (CTCs) from an epithelial malignancy (carcinomas) are identified as cells that are positive for PanCK and negative for GFAP, OLIG2 and CD45.

Table 2. GLI-M samples	Number
Total samples	101 + 44
Gender Female	41 + 16
Male	60 + 28
Age Median (Range)	46 (2 – 83)
Subtype Astrocytoma, Gr II	20 + 9
Astrocytoma, Gr III	11 + 5
Ependymoma, Gr II	5 + 1
Ependymoma, Gr III	1 + 0
Glioblastoma, Gr IV	44 + 18
Glioma, mixed, Gr II	1 + 1
Glioma, NOS, Gr II	10 + 4
Glioma, NOS, Gr III	5 + 3
Oligodendroglioma, Gr II	3 + 2
Oligodendroglioma, Gr III	1 + 1

"a + b" indicates the number of samples assigned to "Training + Test" sets.

Table 3. CNS-B samples	Number
Total samples	31 + 13
Gender Female	20 + 7
Male	11 + 6
Age Median (Range)	46 (20 – 67)
Subtype Arachnoid cyst	1 + 0
Choroid Plexus Papilloma	0 + 1
CP Angle Epidermoid Cyst	0 + 1
CP Angle Schwannoma	5 + 1
Craniopharyngioma	1 + 1
Hemangioblastoma	1 + 0
Meningioma (Gr I)	19 + 6
Pituitary Adenoma	2 + 1
Trigeminal Schwannoma	0 + 1
Tuberculoma	1 + 0
Ventricular Epidermoid Cyst	0 + 1
Ventricular Colloid Cyst	1 + 0

In the first (case-control) study, blood samples were obtained from 189 participants [145 cases of glial malignancies (GLI-M) and 44 cases of benign CNS conditions (CNS-B)]. The samples were split into discrete Training and Test Sets (Tables 2 and 3). Identities of all samples were masked and processed for CGC enrichment. The clinical status of the Training Set samples was first unmasked to the analysts to review the marker expression profiles and status of CGCs. In the Training Set (Table 4, N = 132), 1 CNS-B sample was positive for CGCs and 30 samples were negative, while 100 GLI-M samples were positive for CGCs and 1 sample was negative. The findings in the identity masked samples in the Test Set (Table 5, n = 57) were then evaluated to predict the clinical status of the samples. Of the 57 samples in the Test Set (Table 4), all 13 CGC negative samples were determined to be CNS-B and all 44 CGC positive samples were GLI-M. The test was determined to have 100% Sensitivity and 100% Specificity in this Study.

Table 4. Training Set Findings

Sample Type	CNS-B	GLI-M
Samples	31	101
Negative	30 (96.8%) (Specificity)	1
Positive	1 (3.2%)	100 (99.0%) (Sensitivity)

Table 5. Test Set Findings (Reported Performance)

Pre-unmasking status		Post unmasking	
		CNS-B	GLI-M
Samples	57	13	44
Negative	13	13 (100.0%) (Specificity)	-
Positive	44	-	44 (100.0%) (Sensitivity)

The second (case-control) study evaluated identity masked blood samples from 40 cases of GLI-M, 22 cases of CNS-B, 24 cases of carcinomas with brain metastases (CAR-M), and 500 healthy donor individuals (HDI) with no prior diagnosis or current suspicion of cancer.

Table 6. Study Cohort	Cohorts			
	GLI-M	CNS-B	CAR-M	HDI
Total samples	**40**	**22**	**24**	**500**
Age Median (Range)	48 (2-73)	47 (17-65)	52 (25-87)	43 (11-86)
Gender Male	22	11	12	305
Female	18	11	12	195

Table 7. Study Findings

Test Findings		Clinical Status				
CGC / CTC Status	Prediction	GLI-M (n=40)	CNS-B (n=22)	CAR-M (n=24)	HDI (n=500)	Concordance
CGC+, CTC-	**GLI-M (n = 40)**	40	-	-	-	100%
CGC-, CTC+	**CAR-M (n = 24)**	-	-	24	-	100%
CGC-, CTC-	**CNS-B/HDI (n = 522)**	-	22	-	500	100%
CGC+, CTC+	**-**	-	-	-	-	-

GLI-M : Astrocytoma, Gr II (n = 4), Astrocytoma, Gr III (n = 8), Ependymoma, Gr II (n = 2), Ependymoma, Gr III (n = 1), Glioblastoma, Gr IV (n = 13), Glioma, NOS, Gr II (n = 5), Glioma, NOS, Gr III (n = 3), Oligodendroglioma, Gr II (n = 3) and Oligodendroglioma, Gr III (n = 1).

CNS-B: Arachnoid cyst (n = 1), Choroid Plexus Papilloma (n = 1), Benign Neuroparenchyma (n = 1), CP Angle Epidermoid cyst (n = 1), CP Angle Schawnnoma (n = 2), Meningioma (n = 13), Pituitary Adenoma (n = 1), Trigeminal Schwannoma (n = 1) and Ventricular Epidermoid cyst (n = 1).

CAR-M: breast (n = 7), colorectum (n = 2), esophagus (n = 2), esophagus + breast (n = 1), stomach (n = 1), head and neck (n = 2), kidney (n = 1), lung (n = 6), testes (n =1) and unknown primary (n = 1).

The test accurately classified all samples with 100% Sensitivity and 100% Specificity in this Study.

The third (prospective) study evaluated blood samples from 68 patients presenting with radiologically evident intracranial space-occupying lesion (ICSOL) suspected of GLI-M. All samples were processed for CGC enrichment and ICC followed by prediction of the sample status (GLI-M or CNS-B) based on the GFAP, OLIG2 and CD45 expression profiles.

Table 8. Study Cohort	GLI-M	CNS-B
Total samples	**56**	**12**
Age Median (Range)	52 (6-77)	58 (17-75)
Gender Female	36	6
Male	20	6
Subtype Astrocytoma, Gr II	6	-
Astrocytoma, Gr III	9	-
Glioblastoma, Gr IV	30	-
Glioma, NOS, Gr II	1	-
Glioma, NOS, Gr III	1	-
Oligodendroglioma, Gr II	1	-
Oligodendroglioma, Gr III	8	-
CP Angle Schwannoma	-	3
Craniopharyngioma	-	1
Meningiomas (Gr I)	-	5
Benign Neuroparenchyma	-	2
Benign Cystic lesion	-	1

The concordance of the prediction with the actual clinical status was used to determine the performance characteristics. Of the 68 cases, 56 were positive and 12 were negative. After unblinding, it was revealed that all positive samples were GLI-M (100% sensitivity) while all negative samples were CNS-B (100% specificity).

Table 9. Study Findings

Pre-unmasking status		Post unmasking	
		CNS-B	GLI-M
Samples	68	12	56
Negative	12	12 (100.0%) (Specificity)	-
Positive	56	-	56 (100.0%) (Sensitivity)

SUMMARY OF FINDINGS

The test showed 100% sensitivity and 100% specificity in both case control studies to detect and differentiate GLI-M from CNS-B, CAR-M and HDI based on detection of CGCs. The test also showed 100% sensitivity and 100% specificity in the prospective study to differentiate GLI-M and CNS-B on detection of CGCs.

CONCLUSION:

We present a non-invasive approach for the diagnostic stratification of patients presenting with suspicious but non-specific neurocognitive symptoms and hence early detection of glial malignancies. Moreover, immunocytochemistry facilitates the differentiation of GBM from metastatic intra-cranial epithelial cancers.