Rapid and Specific Diagnosis of Astrocytomas with Fluorescent Aptamers

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Introduction

Neurosurgical tumor resections are routinely assisted by intraoperative histological assessment of biopsies. However, it can be difficult to differentiate, via gross appearance or frozen section histological analysis, high-grade astrocytomas from non-operative lesions, such as primary central nervous system lymphoma (PCNSL). Aptamers are an emerging class of polynucleotides that function as "chemical antibodies" with high binding affinity to specific cellular targets. They can diagnose samples in minutes compared to the typical 24-to-48-hour timeframe via immunohistochemistry (IHC). We have previously developed a PCNSL-specific aptamer (Figures 1 and 2) and aim to develop a fluorescent aptamer targeting glial fibrillary acid protein (GFAP), a common IHC target for astrocytomas, also known as gliomas.

Hypothesis: GFAP aptamers can accurately identify glioma cell lines in twenty minutes, a timeframe that can support intraoperative decision-making for best treatment practices.

Figure 1

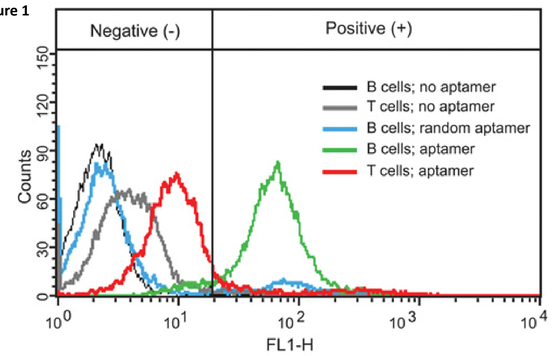


Figure 2

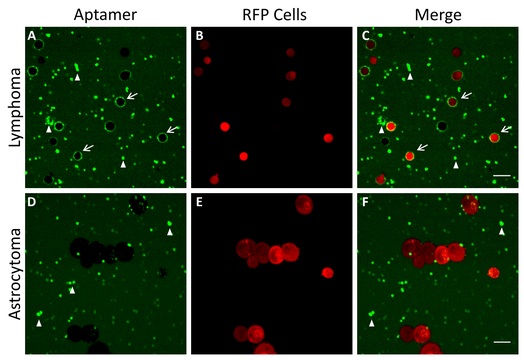


Figure 1: Aptamer Fluorescence intensity of the TD05 aptamer tested on negative control T cells and positive control B cells. Note increased fluorescence and number of labeled B cells versus T cells.
Figure 2: Aptamer staining of cultured human fluorescent lymphoma and astrocytoma cells. A-C lymphoma, D-F astrocytoma. A) B cell lymphoma cells incubated with the aptamer; note ring-like staining pattern (arrows) and fluorescent artifacts (arrowheads). B) Lymphoma cells expressing RFP (tumor cells were infected with a lentivirus to introduce expression of red fluorescent protein (RFP)) C) Merged image of RFP-lymphoma cells and aptamer staining. D) Astrocytoma cells incubated with the quenchable aptamer; note fluorescent artifacts (arrowheads) and lack of ring-like staining. E) Astrocytoma cells expressing RFP. F) Merged image of RFP-astrocytoma cells and aptamer staining.

Methods

Our data was obtained from human glioma cells (U251) and human PCNSL (Ramos) cell lines. Cell lines were cultured with their appropriate mediums and grown at 37C at 5% CO2. Aptamers had AF488 and Cy5 fluorophores for the CD20 (TD05) and GFAP aptamers, respectively. Annealed aptamers were incubated on ice for 20 min, treated with fixation/permeabilization, and tested at different concentrations. For comparison, we ran positive and negative control validation experiments for each aptamer individually and a mixed aptamer assay with analysis of fluorescence via flow cytometry.

Results

We found a concentration of 400nM or higher of both the TD05 and the GFAP aptamers was highly sensitive for labeling Ramos and U251 cells, respectively. When gating baseline fluorescence of non-stained cells, close to 100% of cells at all aptamer concentrations greater than 400nM showed Cy5 fluorescence for U251 cells stained with the GFAP aptamer (Figure 3a) and AF488 fluorescence for Ramos cells stained with TD05 aptamer (Figure 4a). We unexpectedly found Cy5 fluorescence with 87.4% of Ramos stained with GFAP aptamers (Figure 4b). AF488 fluorescence with 37.3% of U251 stained with TD05 aptamers.

We also found a significant difference when comparing the median fluorescence of GFAP aptamer with U251 cells vs Ramos cells ($p<0.001$). However, when looking at the TD05 fluorescence of U251 vs Ramos cells, the U251 cells trended towards higher fluorescence with a p-value of (0.10) (Figures 3b and 4c). Our previous experiments showed adequate labeling of lymphoma cells and not astrocytoma cells (Figures 5a & 5b).

With a mixed aptamer assay, we found Cy5 fluorescence in 100% of U251 stained with GFAP aptamers and AF488 fluorescence in 100% of Ramos stained with TD05 aptamers (Figures 6a and 7a). We found Cy5 fluorescence in 91% of Ramos stained with GFAP aptamers and AF488 fluorescence in 98.8% of U251 stained with TD05 aptamers (Figures 6b and 7b).

Figure 3a

	Sample Name	Subset Name	Count
	3.1 Unstained U251.fcs	U251 Single Cells	10116
	3.2 GFAP 400.fcs	U251 Single Cells	10258
	3.3 GFAP 800.fcs	U251 Single Cells	16277
	3.4 GFAP 1200.fcs	U251 Single Cells	14363
	3.5 GFAP 2400.fcs	U251 Single Cells	16930
	3.6 GFAP 4800.fcs	U251 Single Cells	16483
	3.7 OLD GFAP 400.fcs	U251 Single Cells	11771

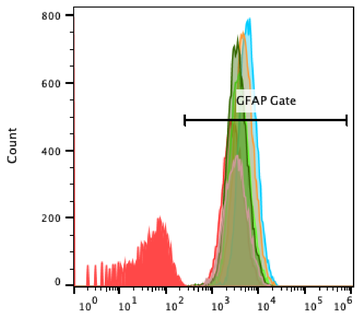


Figure 3b

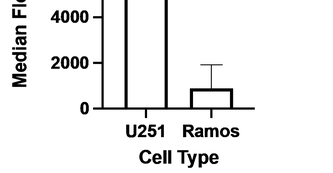


Figure 4a

	Sample Name	Subset Name	Count
	4.1 Unst Ramos.fcs	Ramos Single Cells	7857
	4.2 TD400.fcs	Ramos Single Cells	9063
	4.3 OLD TD400.fcs	Ramos Single Cells	8020
	4.4 GFAP400.fcs	Ramos Single Cells	11074
	4.5 OLD GFAP400.fcs	Ramos Single Cells	10474
	4.6 TD-GFAP 400-400.fcs	Ramos Single Cells	8144

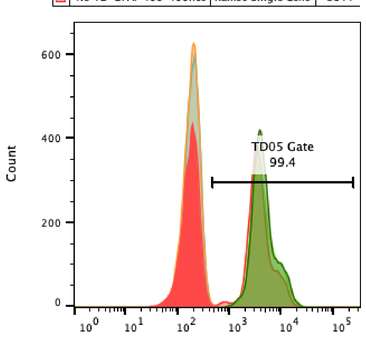


Figure 4b

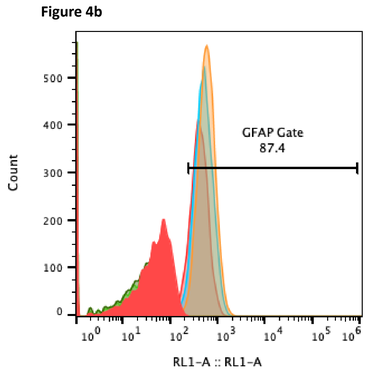
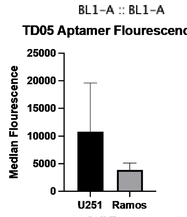


Figure 4c



Figures 5a & 5b

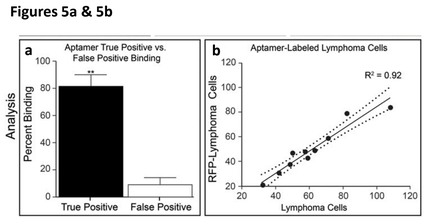


Figure 6a

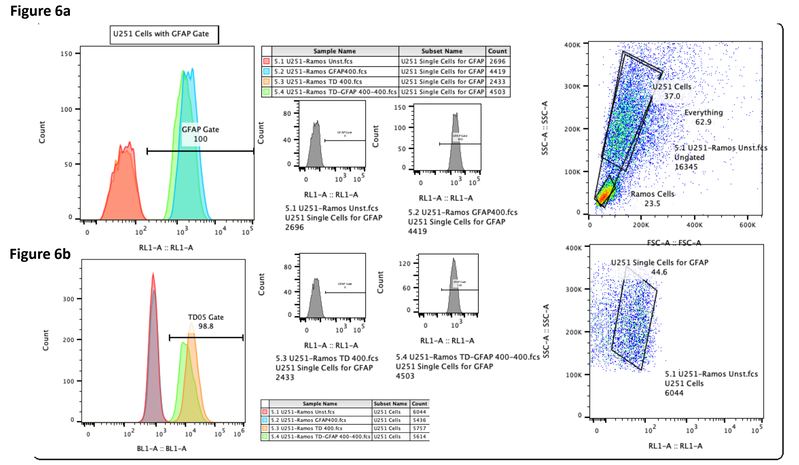


Figure 6b

Figure 7a

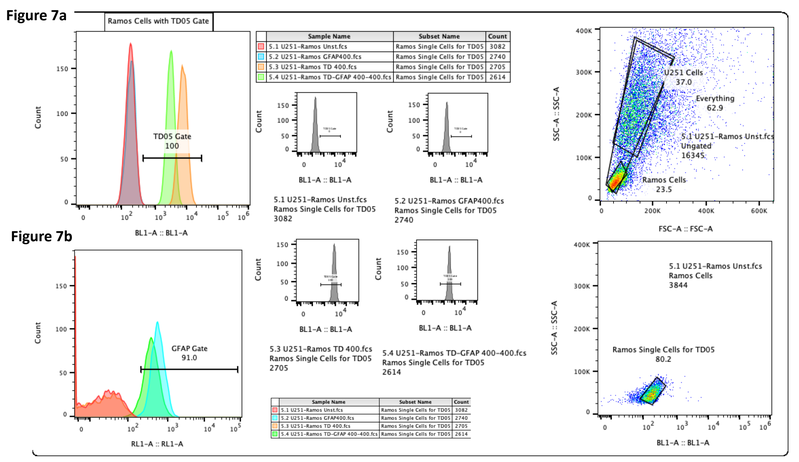


Figure 7b

Discussion/Conclusion

Our current aptamer protocols to label Ramos and U251 cells are highly sensitive in labeling the appropriate cells. However, we also encountered many false positives. Such results should not have occurred as CD20 is a surface receptor expressed only on lymphoma cells and GFAP is an intracellular protein specific to glia and glioma cells. This could be due to non-specific binding. Thus, future refinement of our staining protocol, and testing different concentrations may reduce false positives. Our results could also potentially be explained by a known phenomenon that Cy5 fluorophore can be taken up in mitochondria.

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