Activatable Fluorescent Nanoprobes for Rapid and Specific Identification of Central Nervous System Lymphoma

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Introduction

Brain tumors remain a significant clinical problem, affecting over 60,000 newly diagnosed Americans each year. During surgery, frozen section diagnoses frequently determine surgical excision versus medical management. However, in some cases, neither gross morphology nor frozen sections can distinguish non-operative versus operative lesions, such as lymphoma versus astrocytoma. In such cases, immunohistochemistry is ordered, which requires 1-2 days to yield a specific diagnosis. Initial diagnostic uncertainty may lead to lengthened hospital admissions and additional surgeries for patients, or even an inappropriate treatment strategy. Improved tools for specific intraoperative brain tumor diagnoses are desperately needed. Here, we develop a novel class of nanoprobes for rapid and specific intraoperative diagnoses.

Methods

Nanoprobes were designed targeting an immunoglobulin on CD20-positive human lymphoma cells, and fluorescence dynamics were evaluated by spectro-fluorometry. Utilizing a one-step staining protocol, labeling affinity was interrogated by flow cytometry and confocal imaging of human cell culture and fresh xenograft brain tumor biopsies harboring human B-cell lymphoma or astrocytoma.

Results

Nanoprobes produced minimal fluorescence in unbound conformations and generated 8-fold increased fluorescence once bound to CD20-positive lymphoma cells. Flow cytometry and confocal imaging demonstrated strong binding to B-cell lymphoma cells within 15 minutes of incubation. Within one hour, specific histopathological differentiation of lymphoma from astrocytoma was routinely possible from biopsies (80.75 ± 2.52% lymphoma cells vs. 8.25 ± 1.51% astrocytoma cells, $p < 0.001$).

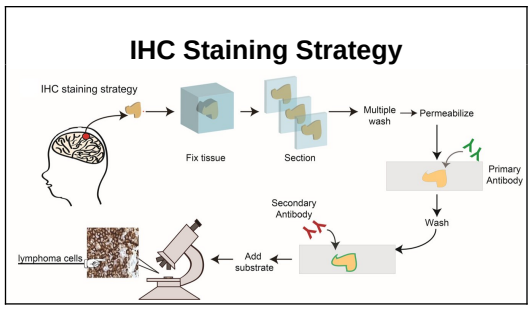

Figure 1. Conventional IHC with multiple steps requires 24-48 hours.

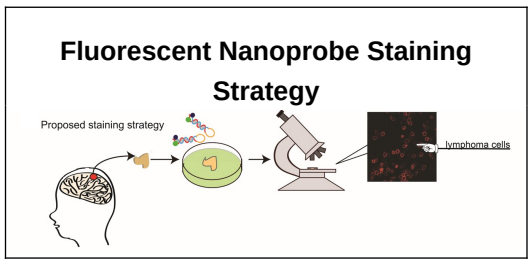

Figure 2. Fluorescent staining strategy with nanoprobe "switching on" with target-specific binding requires 20 minutes or less.

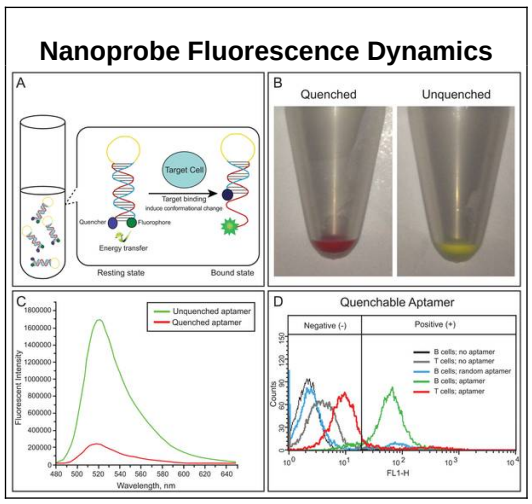

Figure 3. A) Illustration. B) Macrofluorescence comparison of quenched and unquenched. C) Spectrofluoremetry of unquenched versus quenched. D) Fluorescence intensity of quenchable nanoprobe tested on negative control T cells and positive control B cells.

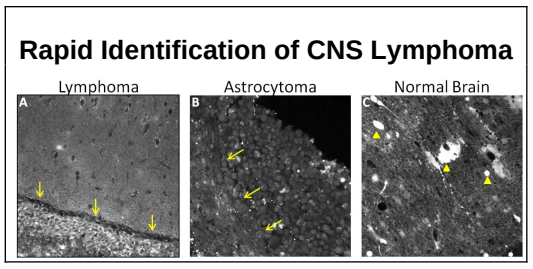

Figure4. Xenograft Biopsies. A) Tumor region of B cell lymphoma biopsy incubated with the quenchable aptamer; note ring-like staining pattern. B) Tumor region of astrocytoma acute slice, note lack of ring-like staining pattern. C) Contralateral normal brain from lymphoma acute slice.

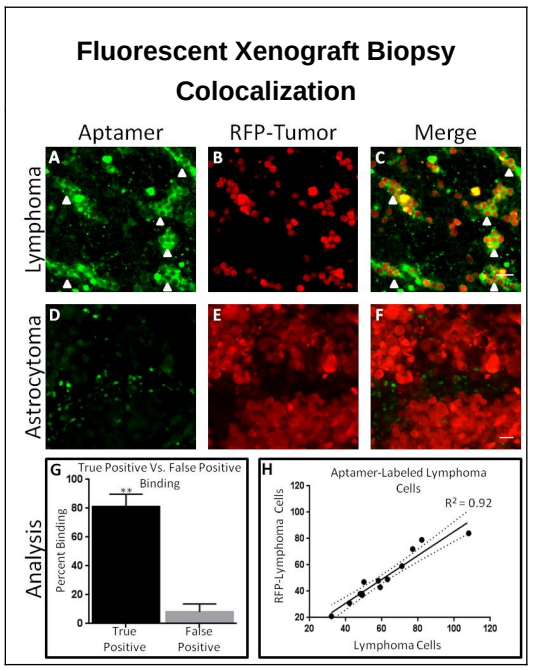

Figure 5. A) Aptamer staining of lymphoma biopsies with positive ring-like staining regions (arrowheads) B) RFP-expressing lymphoma cells within tissue slice. C). Merge. D) Aptamer staining of astrocytoma biopsy; note lack of positively stained cells. E) RFP-expressing astrocytoma cells in tissue slice. F) Merge. Scale bar=20µm.

Learning Objectives

By the conclusion of this session, participants should be able to: 1) Describe the advantages of intraoperative fluorescence imaging with novel contrast agents compared to current intraoperative diagnostics, 2) Discuss in small groups the advantages/disadvantages of fluorescence ex vivo imaging compared to frozen section imaging, 3) Identify clinical scenarios which would benefit from rapid and specific intra-operative diagnostics.

Conclusions

This activatable fluorescent nanoprobe provides rapid and specific labeling of B-cell lymphoma. Our one-step ex vivo labeling approach simplifies tissue staining and drastically reduces time to histopathological diagnoses relative to IHC-based methods. This may improve both speed and accuracy of brain tumor diagnoses, allowing for proper goal-oriented surgery and enrollment in the appropriate treatment strategy.

References

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