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New Assay Offers Sensitive, Rapid, Noninvasive Classification of Gliomas

Robust isolation protocol allows for analysis of extracellular vesicle RNA from blood samples.

Challenge

In adults, the most common type of abnormal growth in the central nervous system is glioma. With more than 20,000 cases diagnosed in the United States each year, gliomas present in a variety of ways: glioblastomas, astrocytomas, oligodendroglial tumors, and ependymomas are the most common subtypes.[1] The prognosis for these subtypes varies significantly. Low-grade oligodendrogliomas, for example, have five-year survival rates better than 50%, while the same measure for glioblastomas is just 5%.[2,3]

That's why it is imperative to classify each glioma subtype correctly. Scientists have identified a number of useful molecular changes, including single nucleotide variants, that help with this classification process. But gaining access to the mass is difficult, and sometimes even dangerous. Standard tissue biopsy procedures are rarely welcome for growths in the brain. Even when they can be performed, sampling just a tiny piece of the glioma introduces the risk of missing important clonal heterogeneity. Direct tissue biopsies are also not conducive for repeat testing to monitor disease progression and treatment response.

One of the more interesting molecular changes associated with gliomas comes from the IDH1 gene. According to the 2021 WHO classification of central nervous system tumors, diffuse adult type gliomas are now categorized into three distinct groups based on morphological and molecular features, including IDH mutation status. These groups include astrocytoma IDH-mutant (grades 2-4), oligodendroglioma IDH-mutant and 1p/19q-codeleted (grades 2-3), and glioblastoma IDH-wildtype (grade 4).[4-5] A specific IDH1 mutation in codon 132 has been linked to improved prognosis and survival.[6] The traditional approach for identifying this mutation is to combine neuroimaging with a tissue biopsy procedure, with a typical diagnostic turnaround time of two to three weeks.

For glioma patients, there is a clear need for a better approach that is less invasive and more comprehensive with faster generation of results. Developing better techniques now could one day make a real difference for people who are diagnosed with these masses.

Solution

Fortunately, the era of minimally invasive liquid biopsies may bring a better solution for classifying gliomas. In many types of cancer, liquid biopsy methods have already been successful for diagnosis, prognosis, and routine surveillance. The most common techniques rely on interrogating circulating tumor DNA (ctDNA).

For brain cancer, however, a different approach may be more effective. Scientists from Massachusetts General Hospital and Harvard Medical School recently published a paper in Nature Communications describing the development and

validation of a liquid biopsy that queries RNA from extracellular vesicles to classify glioma subtypes using the IDH1.R132H mutation.[7]

Extracellular vesicles (EVs) are membrane-bound structures released by cells that contribute to the cell-to-cell communications network. Unlike ctDNA, which is only shed by tumors when a cell dies, EVs are released by all living cells, all the time. These vesicles contain molecular cargo such as DNA, RNA, and proteins. Capturing EVs via liquid biopsy can offer a snapshot of the biological state of the cell of origin. EVs also protect more labile molecules with remarkable stability in RNase-rich environments such as plasma.[8]

EV RNA has been shown to offer clear benefits for some applications over circulating tumor cells and ctDNA as a cancer biomarker.[7] This is due to the high abundance of EV-derived RNA in the blood, combined with low background noise, which significantly amplifies their diagnostic potential.[9]

In this study, scientists developed a blood-based assay from which they analyzed EV RNA found in just 2 mL of plasma. The assay, known as *mt*-IDH1$_{dx}$, incorporates droplet digital PCR for detection of the IDH1.R132H mutation. Using EV RNA instead of DNA as a target allowed the assay to achieve sensitivity of 75% and specificity of nearly 89%. Importantly, the entire workflow takes less than four hours from the time a sample is collected.

To isolate EV RNA for analysis, the scientists used the ExoLution™ kit from Exosome Diagnostics, a Bio-Techne brand. This kit enables users to extract and enrich for target RNA from EVs including exosomes in a simple, streamlined workflow. For the analysis of EV RNA versus EV DNA to determine which offered a more robust signal, the team used the ExoLutionPlus™ kit, also from Exosome Diagnostics, to isolate EV DNA and RNA as well as cell-free DNA in a single step.

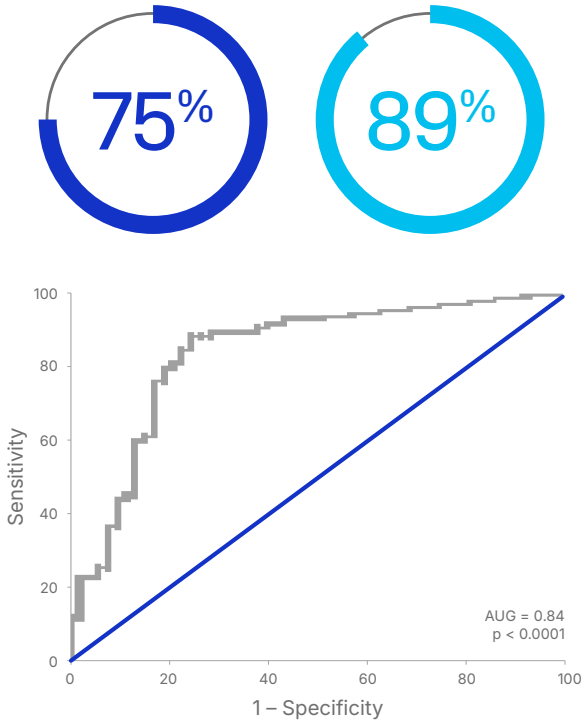
Evaluation

In an initial assessment of the assay's performance, the team tested 48 tumor tissue samples that had already been graded and classified with standard histopathology techniques. Scientists found 100% concordance between the EV RNA assay and samples already found to have the IDH1 mutation.

In addition, the assay identified the mutation in astrocytoma samples that had originally been classified as wild type during conventional analysis.

For validation, the assay was tested in a blinded study including 80 individuals with the IDH1.R132H mutation, 44 who had gliomas but without the mutation, and nine healthy controls. Compared to the conventional tissue biopsy approach, the assay demonstrated positive predictive value of nearly 91% and negative predictive value better than 70%.

Overall Sensitivity and Specificity



Sensitivity and specificity calculated for the individual cohorts and the combined study population. Area under curve analysis performed to measure assay efficiency. Figure reproduced from Nature Communications paper with author permission.[7]

Deeper analysis showed that the assay was helpful for more than simply identifying the presence or absence of the IDH1 mutation. In addition to sensitive detection of the target variant, the assay "can also be used in conjunction with the radiological imaging as a companion diagnostic to accurately differentiate between the common IDH1mt glioma subtypes," the scientists report in their paper.[7]

The team also looked at how the assay performed for prognostic use. Results indicated that the minor allele frequency identified by the test aligned with previous studies showing that higher frequencies were associated with improved survival rates.

"Considering the challenges encountered in standard clinical testing, our tissue and blood-based *mt*-IDH1$_{dx}$ assay emerges as a promising, feasible platform for rapid and sensitive diagnostics, overcoming the limitations posed by disease heterogeneity and technical limitations," the team notes. "Overall, the proposed *mt*-IDH1$_{dx}$ assay can be utilized for blood-based diagnosis, prognostication, and longitudinal monitoring."[7]

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