Application Note

Glioma Prognostic Subtyping - **Astrocytoma, Oligodendroglioma, Meningioma,** and **Glioblastoma** using New miRNA Biomarker Panel

Ready-to-Use fully optimized **SSNA** miRNA *in situ* hybridization (ISH) Kit

Application Highlights:

- Brain tumors are the leading cause of cancer-related morbidity and mortality in the United States. Malignant gliomas, specifically, are the most aggressive form of brain cancer and difficult to treat.
- BioGenex Xmatrx® automated systems and BioGenex miRNA ISH Brain panel miRNA probes were used to successfully classify complex glioma subtypes

BioGenex Products Used:

- #HM021-3P-100: miR-21
- #HM010B-100: miR-10b
- #HM096-100: miR-96
- #HM146B-100: miR-146b
- #DF400-YADE: XISH™ One-Step Polymer-HRP ISH Detection Kit (Automation)
- #DF400-25KE/50KE: Super Sensitive One-Step Polymer-HRP ISH Detection Kit (Manual)
- #HK873-5K: Nucleic Acid Retrieval Solution 1 (NAR-1)

Keywords:
miRNA, Brain cancer, *In situ* hybridization, Xmatrx®

Introduction:

Brain tumors represent one of the leading causes of cancer-related morbidity and mortality in the United States, with a 5-year survival rate of 33.4%. Among brain tumor types, gliomas constitute about 80% of all cerebral tumors, showing varying degrees of malignancy. They are the most aggressive forms of brain cancer and are difficult to treat due to dismal prognosis and limited therapeutic options. Astrocytes, oligodendrocytes and ependymal cells are subtypes of gliomas that give rise to high-grade astrocytomas, oligodendrogliomas, and ependymomas, respectively.

Among many novel approaches used to distinguish glioma subtypes, in situ hybridization (ISH) has shown to efficiently detect microRNA (miRNA) expression levels in formalin-fixed paraffin embedded (FFPE) tissue. MicroRNAs are a class of highly conserved, single-stranded RNA molecules that negatively regulate gene expression at the posttranscriptional level in a sequence-specific manner. Differential expressions of miRNAs including miR-10b, miR-96, miR-146b, and miR-21 have been associated with brain tumors, allowing them to be a prominent biomarker for prognosis. Specifically, high miR-21 expression correlates with poor patient survival due to higher tumor grade in gliomas.

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Super Sensitive Nucleic Acid (SSNA) miRNA probes:
BioGenex has developed proprietary SSNA miRNA probes that are specially designed to enhance signals from the intrinsically low populated miRNAs. The probes have high melting temperatures enabling stringent wash assays at elevated temperatures to remove non-specific binding. BioGenex miRNA probes are dual-end labeled with a fluorophore that amplifies the signal, giving intense stains. SSNA miRNA probes successfully help in studying the low expressed miRNA populations.

This Application Note highlights how BioGenex SSNA miRNA ISH probes can be used to detect and localize altered miRNA expression level in glioma subtypes. Importantly, defining unique expression patterns of miRNAs in brain cancer would provide molecular biomarkers for early detection and prognosis, and prediction of responses to specific treatment. The original study and the results were published in AJCP (1) and presented in AMP (2).

Study samples and detection methods:
Study samples consisted of 30 low- and high-grade gliomas, 13 cases of normal autopsy brain cortex (1) and 35 FFPE tissues of varying grades and types (astrocytoma, oligodendroglioma, meningioma, and glioblastoma) (2). Glioma subtypes were classified and graded using the BioGenex Xmatrx® automated system and miRNA ISH Brain panel probes.

Experimental- *In situ* hybridization:
Formalin-fixed paraffin-embedded tissues were mounted onto glass slides. ISH miRNA probes were hybridized and amplified using anti-FAM IHC system. The hybridized probes were then visualized using the BioGenex Super Sensitive Polymer-HRP IHC detection system, wherein the bound fluorescein probes were developed as a colored precipitate.

Results and conclusion:
miR-21 was overexpressed in high-grade gliomas compared with cells of control brain and non-brain tissues, indicating poor patient survival outcome (Figure 1). Endothelial cells within both low-and high-grade glioma also demonstrated moderate to intense miR-21 expression (1). This study results demonstrated that miR-21 expression correlated well with glioma grade by SSN miRNA ISH probe in FFPE tumor tissue, and miR-21 can serve as a potential therapeutic target for interventions in high-grade gliomas.

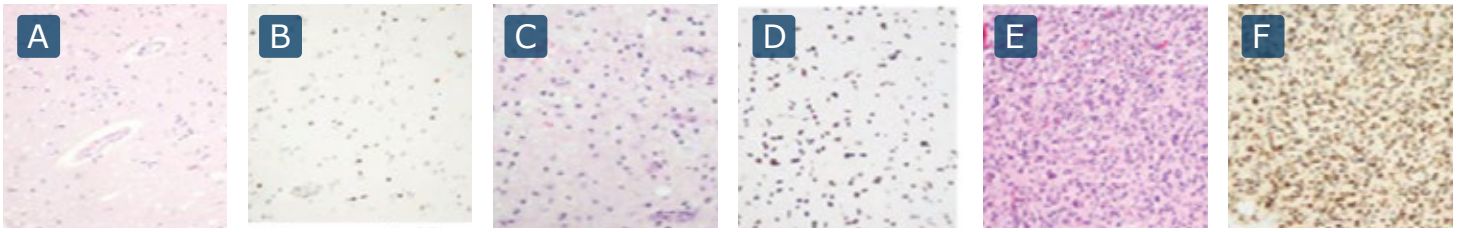

Figure 1. ISH results show (A and B) low to absent miR-21 nuclear expression, (C, D) moderate to intense miR-21 nuclear expression, (E, F) intense miR-21 nuclear expression.

In a similar study, variable hybridization patterns were seen with miR-10b, miR-96, and miR-146b (2). All three miRNAs were down-regulated in paired normal cerebrum, oligodendroglioma and meningioma; miR-146b was up-regulated in astrocytoma, while miR-10b was down-regulated in astrocytoma and glioblastoma. miR-146b demonstrated moderate and strong staining in 2 cases of oligodendroglioma, while miR-10b demonstrated strong staining in one case of meningioma (Figure 2). Using a panel of three miRNA panels, the unclassified gliomas were successfully classified.

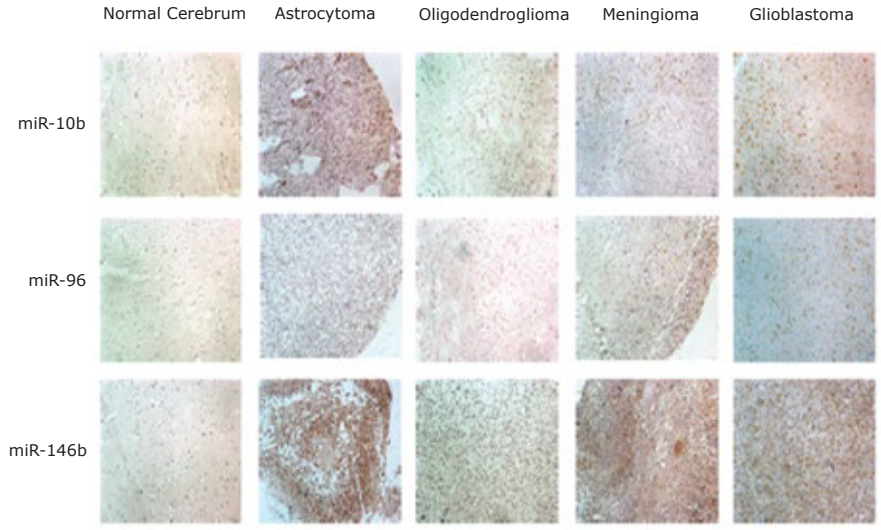

Figure 2. Differential expression pattern of miR-10b, miR-96 and miR-146b.

	Paired N Cerebrum	Astrocytoma	Oligodendroglioma	Meningioma	Glioblastoma
miR-10b	Negative/weak	Moderate/strong	Negative/weak	Negative/weak	Negative/weak
miR-96	Negative/weak	Negative/weak	Negative/weak	Negative/weak	Negative/weak
miR-146b	Negative/weak	Moderate/strong	Weak	Moderate	Weak

In conclusion, miRNA ISH probes can be successfully used to classify and grade glioma subtypes using a selected miRNA brain panel. BioGenex SSNA miRNA ISH probes and automated Xmatrx® staining device, allow rapid, sensitive detection of miRNA with high specificity while preserving tissue morphology. Adaptation of automated processing using Xmatrx® in ISH procedure eliminates error-prone manual steps and greatly increases reproducibility, accuracy and sensitivity of the testing result.

Datasheets:
The BioGenex miRNA probe datasheets provide additional information on the recommended usage guidelines and storage. Refer to the datasheets below before usage:

• HM021-3P-100 • HM010B-100 • HM096-100 • HM146B-100 • DF400-YADE • HK873-5K

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Disclaimer:

The research group and authors have expressed no conflict of interest. BioGenex has optimized the protocols for optimal staining results using positive tissue controls. Due to complex ISH procedures care should be taken in each step. Variations in tissue embedding and fixation and tissue nature should be taken into account for variation in results. Reagents and probes must be prepared and handled according to the manufacturer's instructions.

References:

1. Cheng J et al. In situ hybridization confirms overexpression of novel potential therapeutic target (Hsa-miR-21) in high grade gliomas. AJCP 2018 (manuscript under review).

2. Kalra K et al. In situ hybridization profiling of brain tumors showed differential expression of miR-10b, miR-96 and miR-146b. Poster presented at Annual Meeting of the Association for Molecular Patholgy 2015.

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