Application Note

Squamous Cell Carcinoma (SCC) and **Adenocarcinoma** (AD) **Differentiation** in Non-Small Cell Lung Cancer **(NSCLC)** using New miRNA Biomarker Panel

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

Application Highlights:

- Subtyping of NSCLC, to identify adenocarcinoma and squamous-cell carcinoma histotypes, is clinically important for better patient management.
- BioGenex Xmatrx® automated systems and BioGenex miRNA ISH Lung panel miRNA probes were used to successfully classify NSCLC histotypes.

BioGenex Products Used:

- #HM205-100E: miR-205
- #HM196A-100E: miR-196a
- #HM375-100E: miR-375
- #DF400-YADE: XISH™ One-Step Polymer-HRP ISH Detection Kit (Automation)
- #DF400-25KE/50KE: Super Sensitive One-Step Polymer-HRP ISH Detection Kit (Manual)

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

Introduction:

Lung cancer is among the most frequently diagnosed cancer types and the leading cause of cancer associated death globally. A majority (85%) of the lung cancer cases are classified as non-small cell lung cancer (NSCLC), for which the 5-year survival rate is only 15%. Squamous-cell carcinoma (SCC) and adenocarcinoma (AD) are the common NSCLC histological subtypes. Subtyping of NSCLC to identify AD and SCC is clinically important, but can be inaccurate through routine histopathologic examination. Unlike AD, therapeutic progress in early diagnosis of SCC has been relatively slow due to the lack of molecular markers, genetic characterization, and specific and sensitive tools. Therefore, identification of novel diagnostic biomarkers is critical and essential for the management of lung cancer and increased patient care.

MicroRNAs (miRNAs), a group of small non-coding RNAs, are important modulators in cellular pathways and play a key role in carcinogenesis. Due to the characteristic nature of miRNAs, they have a potential to be used as a promising biomarker for the development of diagnostic, prognostic and targeted therapeutics. The Super Sensitive Nucleic Acid ISH-based microRNA (SSNA miRNA ISH) detection provides a reliable assessment of the physiologic function of miRNA at the tissue, cellular and subcellular level.

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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. These probes have high melting temperatures enabling stringent washes 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. Overall, SSNA miRNA probes aid in studying the lowly expressed miRNA populations to assess the physiological function of miRNA.

This Application Note highlights how BioGenex SSNA miRNA ISH probes can be used to analyze the differential expression pattern of miRNAs in NSCLC subtypes. Utilizing histotypic miRNA assays can aid the subtyping of NSCLC as AC or SCC. The original study and the results were presented as poster in AJCP (1) and AMP (2).

Study samples and detection methods:
miRNA expression profile was evaluated in 107 formalin-fixed paraffin-embedded (FFPE) lung cancers consisting of paired SCC, paired lymph node metastasis and normal lung tissue (1), and 40 FFPE lung cancer cases including SCC and AD (2). Lung cancer subtypes were classified and graded using the BioGenex Xmatrx® automated system and miRNA ISH Lung panel probes.

Experimental- In situ hybridization:
ISH miRNA probes were used for evaluating the expression pattern of miR-196a, miR-205, miR-375 and miR-205 in SCC, AD and lymph node. The hybridized probes were then visualized using the BioGenex Super Sensitive Polymer-HRP IHC detection system, wherein the bound fluorescein probes were scored as negative, weak, moderate, and strong based on intensity of the colored precipitate.

Results and conclusion:
miRNA-205 was overexpressed in majority (90%) of SCC than AC (55%) as analyzed by miRNA ISH system, indicating that miR-205 expression can be used as a biomarker for differentiating NSCLC subtypes (Figure 1) (1).

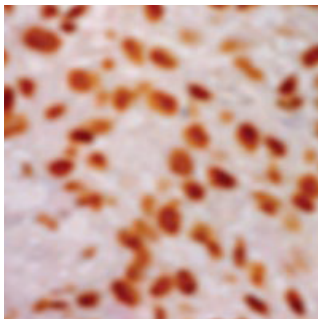

Figure 1. miRNA ISH results show overexpression of miRNA-205 in SCC.

In a similar study, miR-196a and miR-205 were upregulated and miR-375 was downregulated in SCC and invasive lymph node metastasis, respectively, as compared with normal lung cancer (2). Using the three miRNAs, undifferentiated NSCLC subtypes can be differentiated (Figure 2).

To conclude, miRNA ISH probes can be successfully used to classify NSCLC subsets and the expression pattern of miRNAs in lymph nodes can serve as a potential lead in diagnosis and prognosis of SCC. BioGenex SSNA miRNA ISH probes give consistent, reproducible, and reliable outcomes. Adaptation of automated processing using Xmatrx® in ISH procedure eliminates error-prone manual steps and greatly increases reproducibility, accuracy and sensitivity of the test results.

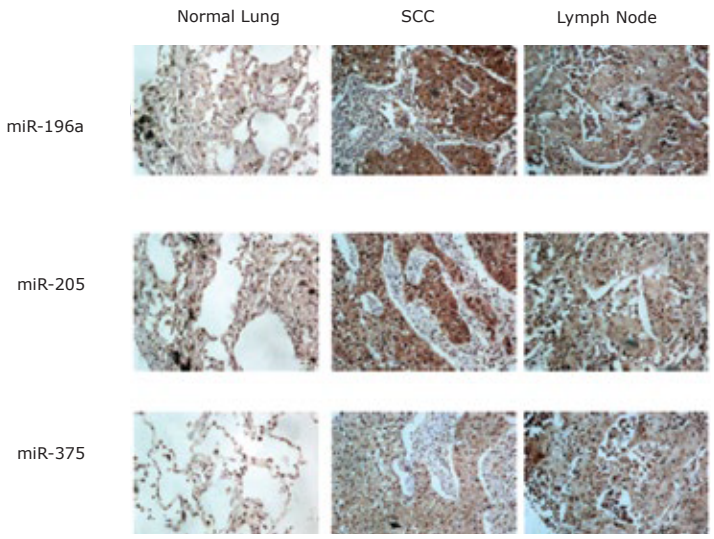

Figure 2. Upregulation and downregulation of marker miRNAs.

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

• HM205-100 • HM196A-100 • HM375-100 • DF400-YADE

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. Kalra K et al. Expression of microRNA miR-205 in pulmonary squamous cell carcinoma and adenocarcinoma. Poster presented at United States and Canadian Academy of Pathology, 2013.

2. Kalra K et al. Up-regulation of miR-196b and miR-205 and Down-regulation of miR-375 in lung squamous cell carcinoma and lymph node metastasis. Presented as poster in Annual Meeting of the Association for Molecular Patholgy, 2015.

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