

Application Note

Squamous Cell Carcinoma Differentiation of Cervical Cancer using New miRNA Biomarker Panel

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

Application Highlights:

- There are usually no signs or symptoms of early cervical cancer.
- Abnormal expression of miRNAs has been reported in a number of cervical cancer tissues.
- BioGenex Xmatrx® automated systems and BioGenex miRNA ISH Cervical panel (miR-146a probe) was used to successfully to differentiate moderate and poorly differentiated cervical squamous cell carcinoma.
- The *in situ* experimental conditions for hybridization were optimized for both BioGenex manual and automated systems.

BioGenex Products Used:

- #HM146A-100: miR-146a
- #DF400-YADE: XISH™ One-Step Polymer-HRP ISH Detection Kit (Automation)
- #DF400-50KE: Super Sensitive One-Step Polymer-HRP ISH Detection Kit (Manual)

Keywords:

Cervical cancer, *In situ* hybridization, miRNA, Xmatrx®

Introduction:

Cervical cancer is the second most common cancer affecting women globally, with an estimated global incidence of 530,000 new cases and 270,000 deaths annually. Human papillomavirus (HPV) infection is the major cause of almost all the cervical cancer cases. Histologically, cervical cancer is divided into two major subtypes: squamous cell carcinoma (SCC; ~80%) and adenocarcinoma (AD; ~5–20%). With no early signs or symptoms, cervical cancer often remains undetected until cancer becomes more advanced. Currently, surgery resection, radiotherapy and chemotherapy are the standard treatment options for patients with cervical cancer. Despite advances in the treatment of cervical cancer, many women continue to suffer and die of this preventable disease each year. Molecular mechanisms underlying carcinogenesis in the cervix has opened up the potential for molecularly targeted therapies, among which microRNAs (miRNAs) are playing a vital role in cancer research.

The miRNAs are a novel group of endogenous, non-coding, short RNA molecules that function as posttranscriptional gene regulators. Abnormal expression of miRNAs has been reported in a number of cervical cancer tissues and cell lines. To better understand the molecular mechanisms that regulate miRNA gene expression, BioGenex Super Sensitive Nucleic Acid microRNA *in situ* hybridization (SSNA miRNA ISH) probes can help identify the best diagnostic and prognostic biomarker for cervical cancer.



Application Note

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 probe can be used to analyze the miR-146a expression levels in patients with cervical SCC (SCC-Cx) and use the expression levels to differentiate moderate and poorly differentiated carcinomas. The original study and the results were presented as a poster in USCAP (1), and published as an abstract in the FASEB journal (2).

Study samples and detection methods:

miR-146a expression profile was evaluated in 60 cases of SCC-Cx and 10 normal cervix biopsies (1) and 33 cervical cancer on tissue microarray (TMA) samples. SCC-Cxs were classified using the BioGenex Xmatrix[®] automated system and miRNA ISH panel probes.

Experimental- *In situ* hybridization

BioGenex ISH miRNA probes were used for evaluating the expression pattern of miR-146a in SCC-Cxs. Briefly, following dewaxing and rehydration, formalin-fixed paraffin-embedded (FFPE) tissue slides were heated in BioGenex Nucleic Acid Retrieval Solution I (NAR-I) for 10 min at 90 °C. The *in situ* experimental conditions for hybridization were optimized for both manual and automated systems. After incubation with 40 nM of miRNA probe for 60 min at 50 °C, the signal was amplified with anti-fluorescein antibody. The hybridized fluorescein-labeled probes were developed as a colored precipitate.

Results and Conclusion

miR-146a was upregulated in the majority (66.7%) of SCC-Cx tissues compared with normal cervical tissues (Figure 1), and showed a trend towards increased expression in moderate to poorly differentiated cases (1).

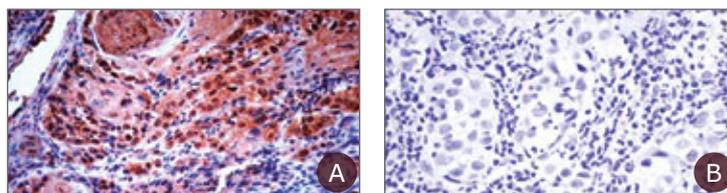


Figure 1. Expression of A) miR-146a and B) Scramble probe (negative control) in SCC-Cx tissue microarray.

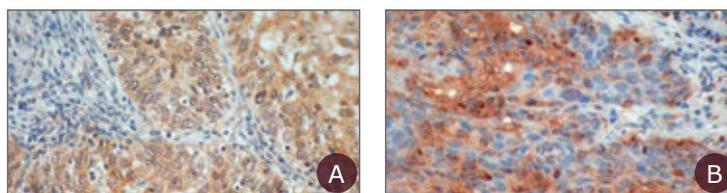


Figure 2. Upregulation of miR-146a in cervical cancer TMA samples.

Application Note

Similarly, miR-146a was also found to be upregulated in majority (66.7%) of cervical cancer TMA samples (figure 2) (2), suggesting miR-146a role as potential biomarker for diagnosis of cervical cancer. Increased expression level of miR-146a was also reported in cervical cancer compared with normal tissues (3). The study results emphasized the potential role of BioGenex SSNA miRNA ISH probes in analyzing the miRNA expression profile in patients with SCC-Cx. Adaptation of automated processing using Xmatrix® in ISH procedure eliminates error-prone manual steps and greatly increases reproducibility, accuracy and sensitivity of the test results.

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

- [HM146A-100](#)

Refer to the user manual for the automated detection kit and manual kit

1. [DF400-YADE](#): XISH™ One-Step Polymer-HRP ISH Detection Kit (Automation)
2. [DF400-50KE](#): Super Sensitive One-Step Polymer-HRP ISH Detection Kit (Manual)

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. Xue L, et al. miR-146a Expression in cervical squamous cell carcinoma. Presented as a poster in Annual Meeting of the United States & Canadian Academy of Pathology (USCAP), 2013.
2. Wang HW, et al. Investigation of microRNA-146a and microRNA-218 expression in cervical cancer. Abstract Published in The FASEB Journal, 2011.
3. Wang X, et al. Aberrant expression of oncogenic and tumor-suppressive microRNAs in cervical cancer is required for cancer cell growth. PLoS One. 2008;3:e2557.

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