Application Note

Cholangiocarcinoma and **Pancreatic Ductal Adenocarcinoma**
Differentiation by New miRNA Biomarker Panel

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

Application Highlights:

- Cholangiocarcinoma and pancreatic ductal adenocarcinoma are highly aggressive cancer types and have poor prognosis.
- Owing to their overlapping immunohistochemical profiles and morphological similarity, they are virtually indistinguishable histologically.
- BioGenex Xmatrx® automated systems and BioGenex miRNA ISH Pancreatic panel miRNA probes were used to successfully differentiate cholangiocarcinomas from pancreatic ductal adenocarcinomas.
- The *in situ* experimental conditions for hybridization were optimized for both manual and automated systems.

BioGenex Products Used:

- #HM196A-100: miR-196a
- #HM216A-100: miR-216a
- #DF400-YADE: XISH™ One-Step Polymer-HRP ISH Detection Kit (Automation)
- #DF400-50KE: Super Sensitive One-Step Polymer-HRP ISH Detection Kit (Manual)

Keywords:
Cholangiocarcinoma, *In situ* hybridization, miRNA, Pancreatic cancer, Xmatrx®

Introduction:

Pancreatic ductal adenocarcinoma (PDAC) and cholangiocarcinoma (CC) are highly aggressive malignant tumor types, and are the leading cause of cancer-related death in the United States. PDAC and CC share a wide range of common features, have overlapping immunohistochemical profiles and are virtually indistinguishable histologically. Patients with PDAC and CC have poor prognosis, with a median survival rate of 3 to 6 months and poor quality of life. Given the rising incidence and high mortality, early differentiation between the two tumors is warranted, as they have important implications for better patient management with respect to surgical options, chemotherapy regimens, and prognosis assessments. Current methods of profile-based strategies using tissue homogenates may not adequately identify differential expression between normal and abnormal tissue in spatial context.

In recent years, microRNAs (miRNAs), a small, noncoding RNAs, that regulate multiple physiological and pathological processes at the posttranscriptional level, have emerged as biomarkers for prognosis and diagnosis of multiple cancers. Moreover, they have been identified as key players in carcinogenesis due to their role as both oncogenes and tumor suppressors. Molecular differentiation using Super Sensitive Nucleic Acid microRNA *in situ* hybridization (SSNA miRNA ISH) probes have demonstrated promising results in identifying the expression level and localize cell-specific miRNAs, leading to appropriate patient treatment.

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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 differentiate CC from PDAC using expression pattern of miRNAs. The original study and the results were presented as a poster in USCAP (1).

Study samples and detection methods:

miRNA expression profile was evaluated in 13 cases of intra/extrahepatic CCs and 18 cases of PDACs. PDACs and CCs were classified using the BioGenex Xmatrx® automated system and miRNA ISH panel probes.

Experimental- *In situ* hybridization:

BioGenex ISH miRNA probes were used for evaluating the expression pattern of miR-196a and miR-216a in CCs and PDACs. The *in situ* experimental conditions for hybridization were optimized for both manual and automated systems. The hybridized probes were then visualized using the BioGenex Super Sensitive Polymer-HRP IHC detection system, wherein the bound fluorescein probes were evaluated semi-quantitatively by intensity of the colored precipitate (low: no or weak stain; high: moderate or intense stain).

Results and conclusion:

miR-196a and miR-216a were downregulated in PDACs compared with CCs, (Figure 1, Table 1). High sensitivities and specificities of miR-196a and miR-216a were also reported (Table 2). Taken together, the study results emphasized the potential role of BioGenex SSNS miRNA ISH probes in differentiating CC from PDAC. 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. miRNAs as prognostic biomarkers have also been evaluated in the serum or plasma of patients with PDAC. It was demonstrated that serum miR-196a expression level had a potential value in predicting the median survival time of PDAC patients (high-level miR-196a, 6.1 months vs. low-level miR-196a, 12.0 months; p=0.007), indicating that miR-196a could be a potential noninvasive marker for PDAC prognosis (2).

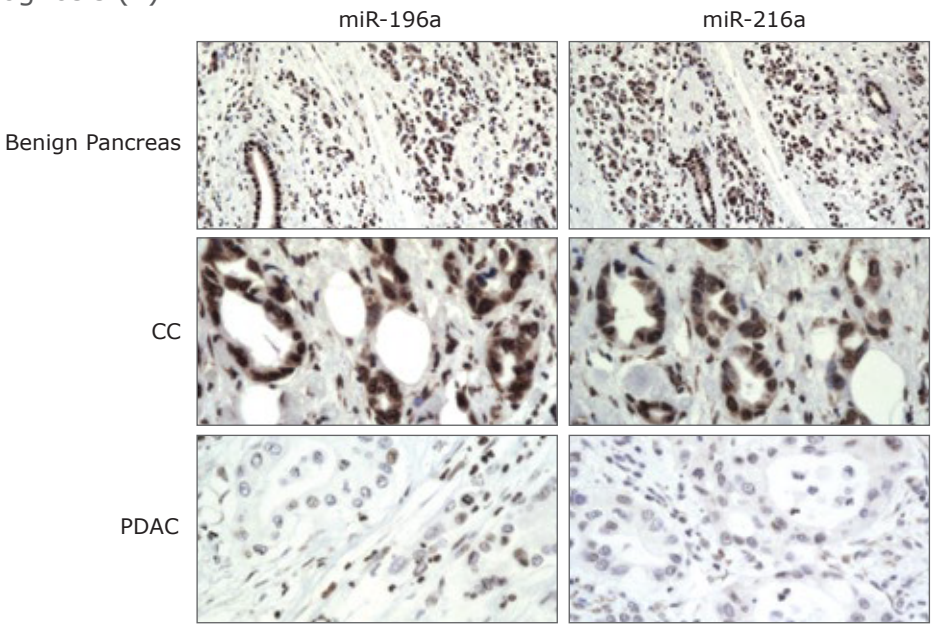

Figure 1. ISH results show differential expression of panel miRNAs in CC and PDAC.

Table 1. Comparison of expression levels of panel miRNAs between CC and PDAC.

miRNA levels	CC (n=13)	PDAC (n=18)	P value
miR-196a			
Low	2	14	0.001
High	11	4	
miR-216a			
Low	2	13	0.003
High	11	5	

Table 2. Sensitivities and specificities of miRNAs in differentiating CC from PDAC.

miR (high level)	Sensitivity (%), (95% CI)	Specificity (%), (95% CI)
miR-196a	85 (72-98)	78 (63-93)
miR-216a	85 (72-98)	72 (65-88)

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

• HM196A-100 • HM216A-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. Lai J et al. Differential expression of in cholangiocarcinoma and pancreatic ductal adenocarcinoma. Poster presented at Annual Meeting of the United States & Canadian Academy of Pathology (USCAP), 2014.

2. Kong X, et al. Detection of differentially expressed microRNAs in serum of pancreatic ductal adenocarcinoma patients: miR-196a could be a potential marker for poor prognosis. Dig Dis Sci. 2011;56:602-9.

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