Application Note

Renal Cell Carcinoma (RCC) Classification - ccRCC pRCC, and chRCC using New miRNA Biomarker Panel

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

Application Highlights:

- MicroRNA expression profile was employed as a differential diagnostic marker to subclassify the renal cell carcinoma at molecular level.
- Renal cell carcinoma poses difficulty in differentiating subtypes using only morphological features.
- BioGenex miRNA ISH probes were used to differentiate all four subtypes by *in situ* hybridization.
- ISH steps were automated using the Xmatrx® automated system.

BioGenex Products Used:

- #HK873-5K: NAR-1
- #HM200B-100: Hsa-miR-200b probe
- #HM200C-100: Hsa-miR-200c probe
- #HM126-100: Hsa-miR-126 probe
- #HM222-100: Hsa-miR-222 probe
- #HM221-3p-100: Hsa-miR-221-3p probe
- #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:
Renal cell carcinoma, miRNA probe, Xmatrx®, *in situ* hybridization

Introduction:

MicroRNAs (miRNAs) are a family of non-coding, highly conserved, single-stranded RNA molecules that play an essential role in cellular regulation at the post-transcription level. miRNAs are known to affect cellular differentiation, proliferation, metabolism, and apoptosis. Several studies have substantiated miRNA dysregulation as a crucial contributor to cancer pathogenesis. The specific expression profile of each miRNA is associated with the cellular pathway, with unique changes being investigated for use as a biomarker. Over the years, miRNA expression profiles have been exploited as a tool for diagnosis and therapeutic targets for cancer treatment. MicroRNAs are multifaceted and can be used for differentiation of malignant and benign tumors, early-stage cancer detection marker, identification of cancer of unknown primary (CUP), and differentiation of cancer subtypes.

Renal cell carcinoma accounts for approximately 90% of all kidney cancers in adults. RCC tumors are a heterogeneous population with a distinct genetic makeup and three main sub-classes: clear cell RCC (ccRCC), papillary RCC (pRCC), and chromophobe RCC (chRCC). Apart from these subtypes, oncocytoma is a benign epithelial neoplasm condition of the renal tissue that shows no clinical attributes.

RCC subtypes exhibit specific clinical behavior and treatment outcomes. These subtypes are generally classified by histopathological observations, however, poor differentiation or overlapping morphological features create difficulty in determining the subtypes. Besides morphological evaluation, molecular techniques such as RT-qPCR are often used for accurate classification and effective prognosis.

BioGenex
Accelerating the pace of precision Oncology

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 explains how miRNAs can be used in differentiation of cancer subtypes in renal cell carcinoma (RCC). This collaborative study was completed by a group of academics and researchers from St. Michael's Hospital, University of Toronto, Mount Sinai Hospital, McGill University Health Centre, St. Joseph's Health Centre, and BioGenex Laboratories. The original study and results were published in Oncotarget (1).

Study samples and detection methods:

In this study, differential expression of six miRNAs: miR-221, miR-222, miR-126, miR-182, miR-200b, and miR-200c from a validated RT-qPCR study (Figure 1) were assessed for chromogenic-ISH (CISH) application.

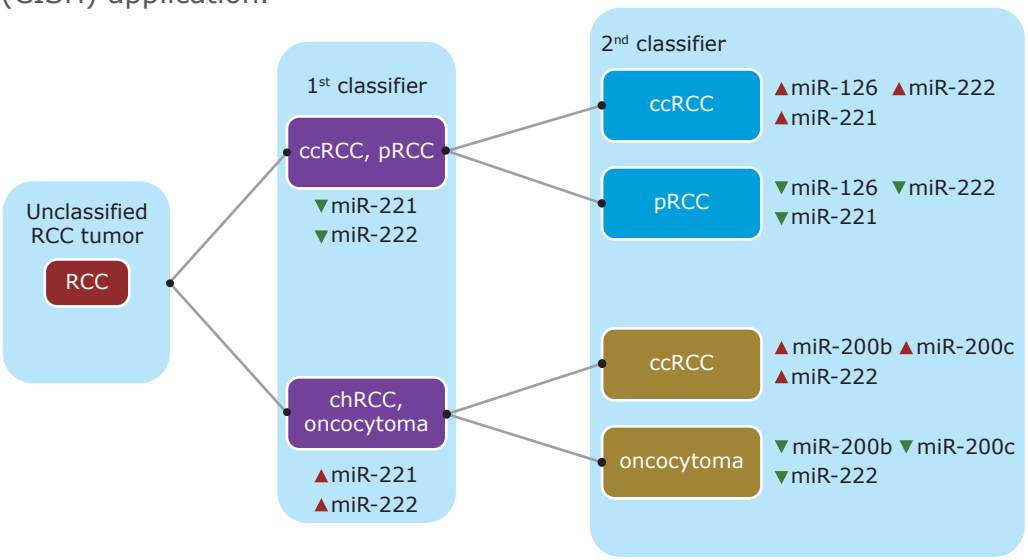

Figure 1: Classification scheme for renal cell carcinoma (RCC) by miRNA expression using RT-qPCR [ccRCC- clear cell RCC, pRCC - papillary RCC, chRCC - chromophobe RCC].

Experimental - *In situ* hybridization:

Tissue microarrays from 98 formalin fixed paraffin-embedded (FFPE) RCC tissues were processed into sections. The miRNA expression assessment was carried out using the BioGenex Xmatrx® automated system.

The tissues were baked and cross-linked nucleic acids were exposed by pre-treatment with Nucleic Acid Retrieval (NAR) buffer at 85 ^{0}C for 5 minutes and 100 ^{0}C for 20 minutes, followed by incubation in hybridization buffer at 40 ^{0}C for 20 minutes. ISH probes were applied and slides were incubated at 42 ^{0}C for 2 hours. Stringent washes at 42 ^{0}C were used to eliminate non-specific interactions of the probe. Anti-fluorescein secondary antibody reactions with the chromogenic substrate (DAB) and a hematoxylin counterstain were applied to visualize the hybridized probes.

Results and Conclusion:

The tissues that gave a weak or negative stain for miR-222 were either ccRCC or pRCC. Corresponding additional tissue sections were then stained with miR-126 as the dichotomous variable. The ccRCC tissues showed strong nuclear staining for miR-126 suggesting upregulation, while pRCC showed negative staining indicating downregulation. Similarly, tissue slides that demonstrated strong nuclear staining for miR-222 were then assessed for miR-200b and miR-200c expression. chRCC showed high expression of miR-200b and miR200c, while oncocytoma showed weak to negative staining for the same probes (Figure 2).

Application Note

The differential diagnosis of RCC subtypes can be classified using a limited panel of miRNA ISH probes. ISH-based differential diagnosis will aid in better patient management and treatment outcomes due to its higher specificity and ability to eliminate error-prone manual steps. By utilizing the BioGenex product line of miRNA ISH probes and automated Xmatrx® staining, clinical diagnosis time and reliability are significantly improved to better serve patients.

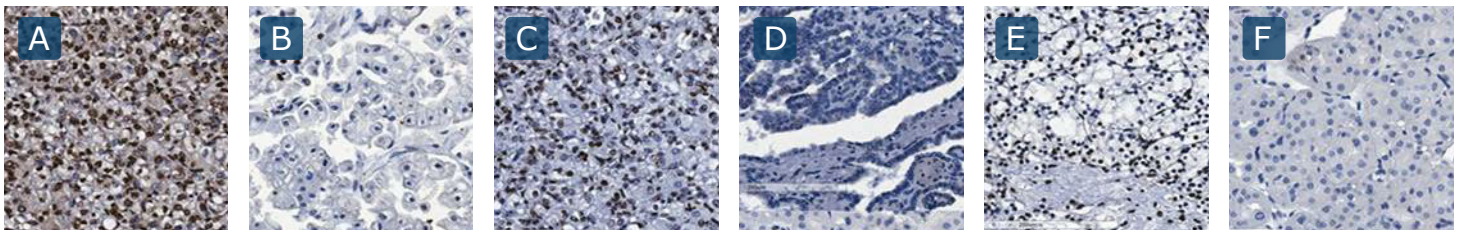

Figure 2: ISH results (A) strong miR-126 nuclear expression, (B) weak/negative miR-126 nuclear expression, (C) strong miR-222 nuclear expression, (D) negative miR-222 nuclear expression, (E) strong miR-200b nuclear expression and (F) negative miR-200b nuclear expression.

Datasheets:

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

• HM200B-100 • HM200C-100 • HM126-100 • HM222-100 • HK873-5K • DF400-YADE

References:

1. Di Meo, Ashley et al. A miRNA-based classification of renal cell carcinoma subtypes by PCR and in situ hybridization. Oncotarget 9.2 (2018): 2092–2104.

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.

For additional Application Notes, visit www.biogenex.com

BioGenex
Accelerating the pace of precision Oncology
In the U.S., call +1 (800) 421-4149
Outside the U.S., call +91-40-27185500
www.biogenex.com

Doc. No. 937-4099.0 Rev A