Application Note

Prostate Cancer (PC) and **Benign Prostatic Hyperplasia (BPH) Differentiation** by New miRNA Biomarker Panel

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

Application Highlights:

- Both benign prostatic hyperplasia (BPH) and prostate cancer remain the most prevalent urologic health concerns affecting elderly men in their lifetime, frequently coexist and therefore share common symptoms.
- Distinguishing nonaggressive BPH from aggressive prostate cancer is crucial for effective treatment and better clinical outcome.
- Several techniques for diagnosing prostate cancer have evolved over the past decades; however there are concerns on spatial resolution of these diagnostic tools.
- BioGenex Xmatrx® automated systems and BioGenex miRNA ISH Prostate panel probes were used to successfully differentiate prostate cancer and BPH.
- Downregulation of miRNAs involved in cellular regulation and upregulation of miRNA in disease progression were observed.
- The *in situ* experimental conditions for hybridization were optimized for both BioGenex manual and automated systems.

BioGenex Products Used:

- #HM125B-100: miR-125b
- #HM017-100: miR-17
- #DF400-YADE: XISH™ One-Step Polymer-HRP ISH Detection Kit (Automation)
- #DF400-50KE: Super Sensitive One-Step Polymer-HRP ISH Detection Kit (Manual)

Keywords:
Benign prostatic hyperplasia, *In situ* hybridization, miRNA, Prostate cancer, Xmatrx®

Introduction:

Prostate cancer and benign prostatic hyperplasia (BPH) are the most frequent pathologies of the prostate gland that are responsible for morbidity in men. Unlike prostate cancer, BPH is nonmalignant and nonfatal. BPH and prostate cancer frequently coexist and therefore share common symptoms; over 20% of men with prostate cancer have BPH. There remains an important clinical challenge in prostate oncology to distinguish the aggressive from the nonaggressive form, without requiring all patients to undergo a painful tissue biopsy. In the United States, prostate cancer is the second leading cause of cancer death affecting one in nine men. This year, an estimated 164,690 men in the United States will be diagnosed with prostate cancer and 29,430 will die from it. Therefore, early diagnosis and timely detection of disease progression is a very important step for effective treatment and a beneficial clinical outcome. Although several techniques for diagnosing prostate cancer have evolved over the past decades, lack of specificity and sensitivity of these diagnostic tools hinder their application in clinical practice. Additionally, the existing methods to utilize serum prostate-specific antigen (PSA) does not provide enhanced survival rates nor do these techniques offer absolute results for the differentiation of prostate cancer and BPH. Elevated levels of PSA have also been reported in non-malignant conditions of the prostate, including BPH, which is commonly misdiagnosed as prostate cancer, leading to unnecessary biopsies. Furthermore, metastatic and advanced prostate tumors types respond very poorly to chemotherapy. Accumulating data suggest that small noncoding RNAs such as microRNAs (miRNAs) can be utilized as potential biomarkers for differentiation of prostate cancer and BPH.

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miRNAs are small, evolutionarily conserved, noncoding RNAs that negatively regulate gene expression in divergent cellular processes including development, differentiation, proliferation, cell cycle control, and apoptosis. Owing to their stability in biological fluids and resistance to various storage conditions, miRNAs are considered as useful biomarkers for cancer diagnosis, prognosis, and prediction of treatment efficacy. Previous reports have shown that miRNAs have the potential to improve current clinical practice to distinguish aggressive from the nonaggressive prostate cancer. Adopting BioGenex Super Sensitive Nucleic Acid ISH-based microRNA (SSNA miRNA ISH) detection, the expression pattern of miRNAs in prostate cancer and BPH can be successfully differentiated.

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 addresses the feasibility of using BioGenex SSNA miRNA ISH probes for differentiation of prostate cancer and BPH. *In situ* visualization of miRNA provides a benefit of localization of miRNA inside a tumor cell, which is essentially lost during miRNA isolation used in the high-throughput screening methods. The original study and the results were presented as posters in USCAP (1).

Study samples and detection methods:

miRNA expression profiles were evaluated in 166 formalin-fixed paraffin-embedded (FFPE) cases of different grades of prostate cancer, including paired normal prostate and BPH (1). Prostate cancer tissues and BPH were differentiated using the BioGenex Xmatrx® automated system and miRNA ISH Prostate panel probes.

Results and conclusion:

As shown in figure 1, miR-17 expression levels were strongly overexpressed in 50% (13/26) of prostate cancer IV compared with normal cases, while miR-125b was strongly upregulated in over 77% (20/26) of cases of prostate cancer IV and III (Table 1, Figure 2). However, miR-17 and miR-125b did not show any significant variation in expression pattern in BPH cases, suggesting oncogenic role of these miRNAs in prostate cancer tumorigenesis and differentiation (1).

miR-17							
Pattern	**BPH (n=20)**	**P Normal (n=18)**	**PCa II (n=18)**	**P Normal (n=29)**	**PCa III (n=29)**	**P Normal (n=26)**	**PCa IV (n=26)**
Negative	9	6	3	10	4	10	2
Weak	3	2	1	4	1	4	0
Moderate	4	8	7	13	14	10	11
Strong	4	2	7	2	10	2	13
miR-125b							
Negative	3	3	1	1	0	1	0
Weak	3	5	3	17	5	14	2
Moderate	7	6	3	3	3	6	4
Strong	7	4	11	8	21	5	20

Table 1: *In situ* expression profile of miRNAs in normal, BPH and different grade of prostate cancer (PCa).

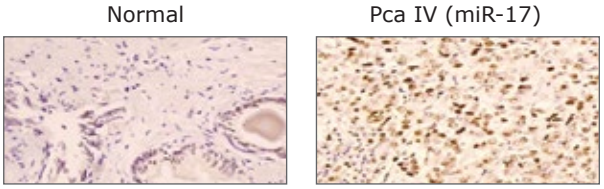

Figure 1. ISH expression profile of miR-17 in normal and high-grade prostate cancer.

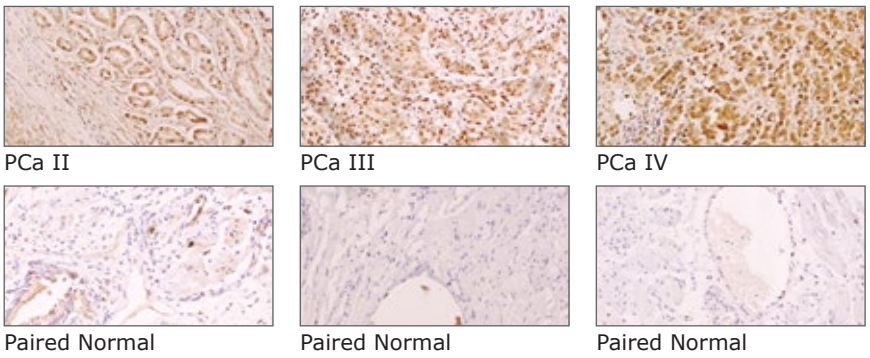

Figure 2. miR-125b ISH expression profile in different grade of prostate cancer.

These findings underscore the importance of visualizing miRNA expression profile *in situ* on a cellular level. Previous studies have indicated that miR-125b is usually downregulated in prostate cancer and its low expression is associated with poor survival (2-4), In another study, overexpression of miR-17 in prostate cancer biopsies provided evidence for a stem-cell-like miRNA profile in high grade/high stage tumors (5).

In summary, miRNA ISH probes can be successfully used for differentiation of prostate cancer and BPH, thereby providing the feasibility of using these miRNAs in the diagnosis, prognosis, and therapy regime of prostate cancer patients. 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.

Datasheets:

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

• HM125B-100 • HM017-100

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:

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4. Wang N et al. miR-205 is frequently downregulated in prostate cancer and acts as a tumor suppressor by inhibiting tumor growth. Asian J Androl. 2013;15:735-41.

5. Gaston SM et al. Overexpression of miR-17 family miRNAs in prostate cancer biopsies: Evidence for a stem-cell-like miRNA profile in high grade/ high stage tumors. Cancer Research 2010;70:3049.

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