Application Note

Triple Negative Breast Cancer - Differentiation of **TNBC** (ER-, PR-, and HER2/neu-) using New miRNA Biomarker Panel

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

Application Highlights:

- Abnormal expression of miRNAs has been reported in various types of cancer, including many breast cancer subtypes
- Triple-negative breast cancer (TNBC) is a poorly differentiated, highly malignant, aggressive form of breast cancer
- TNBC is characterized by the absence of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2/neu (HER-2/neu)
- TNBC cells are insensitive to current targeted therapies due to their lack of common biomarkers, causing poor prognosis in clinical settings
- BioGenex Xmatrx® automated systems and BioGenex SSNA TNBC panel probes were used to successfully differentiate triple-negative breast cancer from other subtypes.
- Differentiation of TNBC can create targeted treatment regimens for patients

BioGenex Products Used:

- #HM021-100: miR-21
- #HM205-100: miR-205
- #HM211-100: miR-211
- #HM222-100: miR-222
- #DF400-YADE: XISH™ One-Step Polymer-HRP ISH Detection Kit (Automation)
- #DF400-50KE: Super Sensitive One-Step Polymer-HRP ISH Detection Kit (Manual)

Keywords:
In Situ hybridization, miRNA, Triple-Negative breast cancer, Xmatrx®

Introduction:

Breast cancer (BC) is a complex disease and specifically, triple-negative breast cancer (TNBC) is extremely aggressive and currently accounts for 10-20% of all diagnosed breast cancer cases. TNBC is characterized by the absence of estrogen receptors (ERs), progesterone receptors (PRs) and human epidermal growth factor receptor-2/neu (HER-2/neu). Patients with TNBC often have an increased risk for recurrence and metastasis, as well as a decreased 5-year survival. Therefore, identifying cases of TNBC is important for selecting an effective therapy for these patients. Numerous studies have demonstrated that aberrantly expressed microRNAs (miRNAs) are involved in the pathogenesis of the aggressive TNBC phenotype.

miRNAs are small, noncoding RNA molecules that are involved in many critical cellular processes, including oncogenesis. Different cancer types and subtypes at different stages of progression display unique miRNA profiles that may be used as diagnostic, prognostic, and therapeutic tools. The detection of miRNA in clinical samples has been difficult, requiring total RNA extracts which lack critical spatial information. However, *In situ* hybridization (ISH) assays have enabled the direct assessment of miRNA expression levels in formalin-fixed paraffin-embedded (FFPE) malignant tissues. Evaluation of miRNA profiles using BioGenex Super Sensitive Nucleic Acid (SSNA) probes holds promise for improving the current understanding of pathogenesis and therapeutic outcome in patients with TNBC.

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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-copy-number miRNAs. These probes have high melting temperatures enabling stringent washes to remove non-specific binding. BioGenex miRNA probes are dual-end labeled with an anti-fluorescein reporter to amplify the signal, yielding intense stains. Overall, the BioGenex SSNA probes aid in studying the regulatory functions of miRNA.

This Application Note highlights the potential of BioGenex SSNA miRNA ISH probes in the differentiation of TNBC from other types of breast cancer. The original study and the results were presented as a poster in the Annual Meeting of the United States & Canadian Academy of Pathology (USCAP) (1).

Study samples and detection methods:

The miRNA expression profile was evaluated in 10 normal and 18 FFPE breast cancer tissues. The breast cancer tissues were subtyped using immunostains for ER, PR, and HER-2/neu. Sub-categorization of the breast cancer tissues resulted in 5 ER/PR+, 5 Her2+, and 8 TNBC. Differential expression of the miRNAs was documented using the super sensitive BioGenex Xmatrx® automated system and miRNA ISH TNBC panel probes.

Experimental- *In situ* hybridization:

miRNA ISH probes were used for differentiation of TNBC from other breast cancer subtypes. The tissues were hybridized with BioGenex ISH probes targeting miR-21, miR-205, miR-221, and miR-222. The hybridized probes were visualized using the BioGenex Super Sensitive Polymer-HRP IHC detection system, wherein the bound anti-fluorescein reporters were developed as a colored precipitate. Scramble probes were used as negative control. The *in situ* experimental conditions for hybridization were optimized for both manual and automated systems.

Results and conclusion:

In the TNBC samples, miR-21, miR-221, and miR-222 were strongly upregulated, while miR-205 was downregulated (1) (Figure 1), suggesting differential miRNA expression patterns between the TNBC cells and other breast tumor tissues (Figure 2). miR-21 was upregulated in 50% (2/4), miR-221 in 50% (4/8) and miR-222 in 63% (5/8) of cases, while miR-205 was downregulated in 37.5% (3/8) of cases. All four miRNAs are not essential for the differentiation of TNBC, and any one SSNA miRNA probe can be used independently to differentiate TNBC from other subtypes.

Additional research has indicated that the overexpression of miR-221 and miR-222 is related to clinicopathological factors and prognosis of TNBC (2). While, previous studies have also noted the downregulation of miR-205 in TNBC (3, 4).

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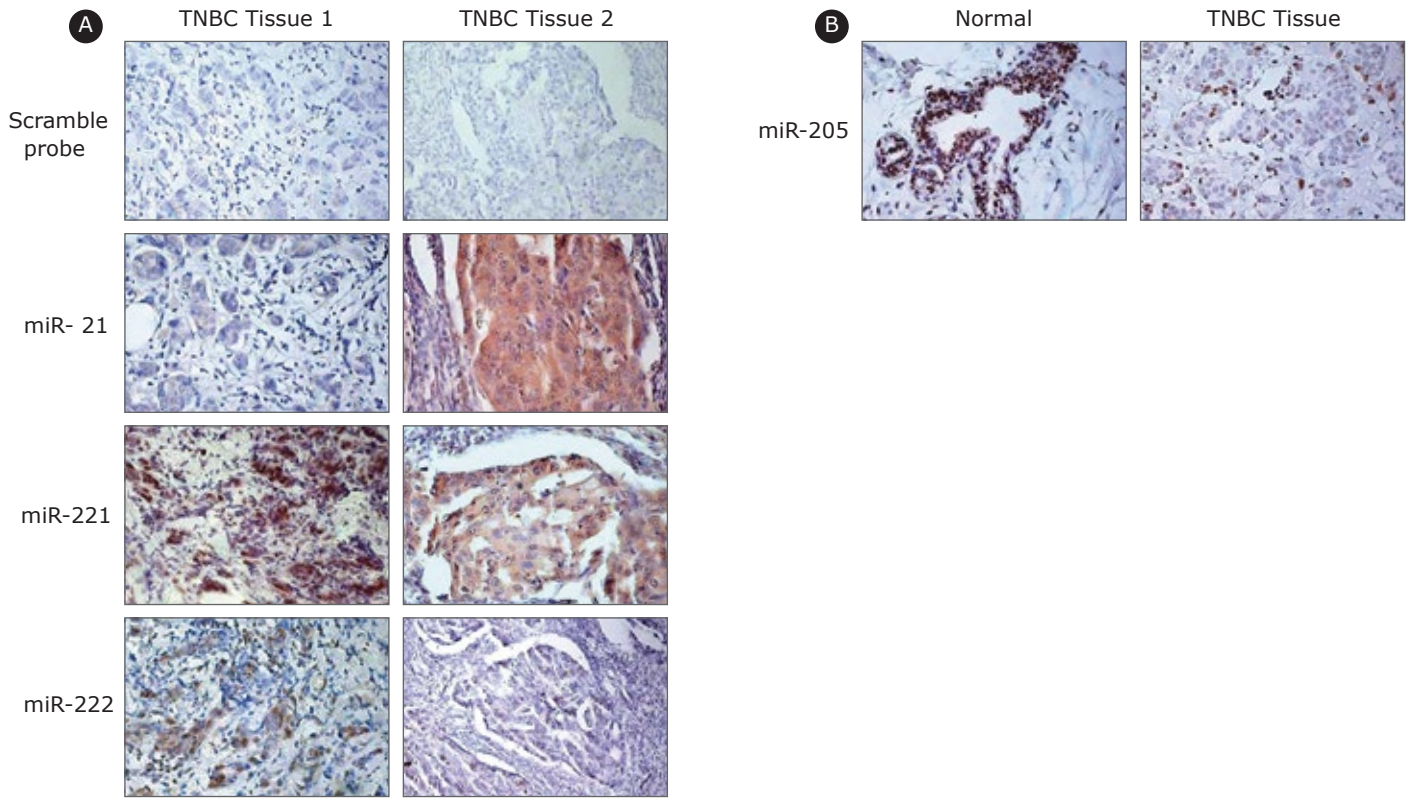

Figure 1. A. Differential expression of miRNA in TNBC tissues B. Differential expression of mir-205 in normal control tissue and TNBC using BioGenex miRNA probes.

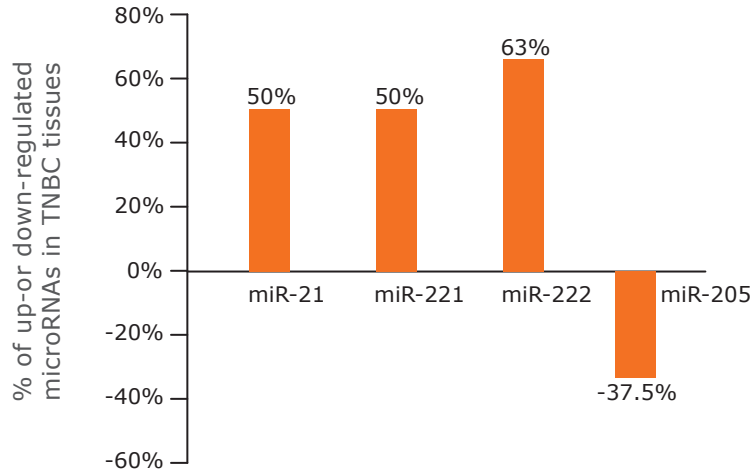

Figure 2. Differential expression of miRNA in TNBC tissues.

Triple Negative Breast Cancer is a poorly differentiated, highly malignant form of breast cancer that is currently insensitive to targeted therapies. BioGenex SSNA miRNA ISH probes have successfully differentiated TNBC from other molecular subtypes, alluding to their potential in diagnostic assays. This study showcases that the dysregulated miRNA expression patterns may hold promise as potential biomarkers and therapeutic targets for patients with TNBC. Compared to other methods for miRNA detection, miRNA ISH retains critical spatial information inside the tissue. BioGenex SSNA miRNA ISH probes along with Super Sensitive™ Detection system and Xmatrx® automated system give consistent, reproducible, and reliable test results that are ideal for clinical and research laboratories.

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Datasheets:

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

• HM021-100 • HM205-100 • HM211-100 • HM222-100

Disclaimer:

The research group and authors have expressed no conflict of interest. BioGenex has used positive tissue controls to optimize protocols for optimal staining results. Due to complex ISH procedures, care should be taken in each step. Variations in tissue embedding, 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. Poongothai AR et al. Differential Expression of miR-21, miR-205, miR-221, miR-222, and miR-150 in Molecular Subtypes of Breast Cancer. Presented as Poster in Annual Meeting of the United States & Canadian Academy of Pathology (USCAP) 2013.

2. Li Y et al. miR-221/222 promotes S-phase entry and cellular migration in control of basal-like breast cancer. Molecules. 2014;19:7122-37.

3. Huo L et al. MicroRNA expression profiling identifies decreased expression of miR-205 in inflammatory breast cancer. Mod Pathol. 2016;29:330-46.

4. Piovan C et al. Oncosuppressive role of p53-induced miR-205 in triple negative breast cancer. Mol Oncol. 2012;6:458-72.

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Doc. No. 937-4112.0 Rev A