Epigenetic Imprinting as a Novel Precision Biomarker for Early Breast Precancer and Cancer Diagnosis and Detection

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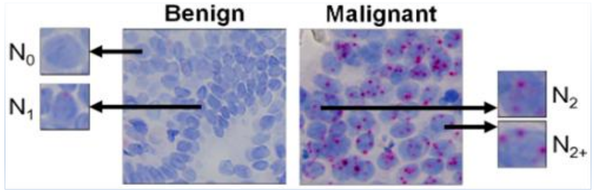
Abstract

Accurate classification of atypical mammary lesions is limited by subjective histomorphologic evaluation, with **error rates up to 35%[1].** We investigate the potential of Loss of imprinting (LOI), an early epigenetic alteration, as an objective biomarker in breast cancer diagnostics via **Q**uantitative **C**hromogenic **I**mprinted **G**ene **I**n-**S**itu **H**ybridization (**QCIGISH**).

Methods: We retrospectively identified 20 benign, atypical, and malignant breast core biopsies from 2021-2023. QCIGISH was performed using RNA scope–based probes targeting HM13 and GRB10. Gene-expression signals were visualized as chromogenic dots and quantified as bi-allelic (BAE), multi-allelic (MAE), and total expression (TE). A decision-tree model was developed using these metrics.

Results: QCIGISH demonstrated distinct expression patterns across diagnostic categories. High MAE strongly predicted malignancy. The decision-tree model achieved 100% sensitivity and specificity compared to histopathologic ground truth. One morphologically ambiguous atypical lesion was LOI-positive and correctly classified as DCIS, later confirmed on excision.

Conclusion: QCIGISH reliably detects LOI at single-cell resolution and shows strong potential as an objective, reproducible diagnostic tool for atypical breast lesions.



Introduction

There is a wide-spread urgent need for an objective reproducible diagnostic tool capable of precisely classifying atypical mammary lesions into cancer vs. benign. Current diagnostic methods in pathology rely on routine and **highly subjective** histomorphologic and immunophenotypic features. Diagnostic variability can drop to **the flip-of-the-coin 50%,** even amongst expert breast pathologists [2,3], and is largely attributed to the lack of innovative tools to adequately distinguish biologic differences[4], resulting in diagnoses that are described as **"murky" and "arbitrary**"[5]. Genomic imprinting, a vital epigenetic regulatory mechanism, is critical in mammalian development and tumorigenesis[6,7]. Typically, imprinted genes exhibit parent-specific allele silencing through differential methylation. However, in cancer, this regulation often breaks down, resulting in the aberrant activation of the typically silenced allele—a phenomenon known as loss of imprinting (LOI)[8]. LOI is considered one of the earliest molecular changes in cancer development, occurs early in tumorigenesis, making it ideal for identifying precursor lesions.

Results

A decision tree model based on BAE, MAE, and TE metrics showed differential signal expression. High MAE was a strong predictor for malignancy. We achieved 100% sensitivity and specificity with the ground truth, thereby initiating proof of concept (Figure 3). Furthermore, **one histomorphologically challenging atypical lesion** (Figure 3, upper right corner), for which the **positive LOI finding accurately classified as ductal carcinoma in situ** (DCIS), was confirmed indeed DCIS by ground truth validation on the excision specimen.

Figure 1. Representative images illustrating nuclear signal patterns used to distinguish benign from malignant mammary lesions. Benign cells predominantly exhibit low or absent signal (N_0–N_1), whereas malignant cells demonstrate progressively increased punctate nuclear signals (N_2–N_2^+), indicating higher levels of aberrant gene expression.

Figure 2. Comparison of QCIGISH performance with other DNA methylation methods

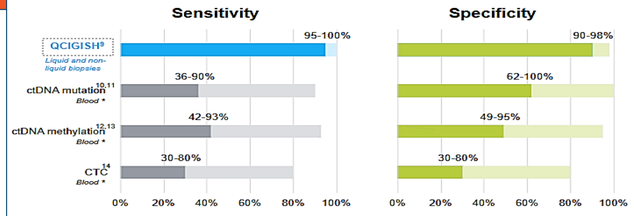


Figure 3. Comparison of QCIGISH performance with gold standard (histomorphologic diagnosis by breast pathologist)

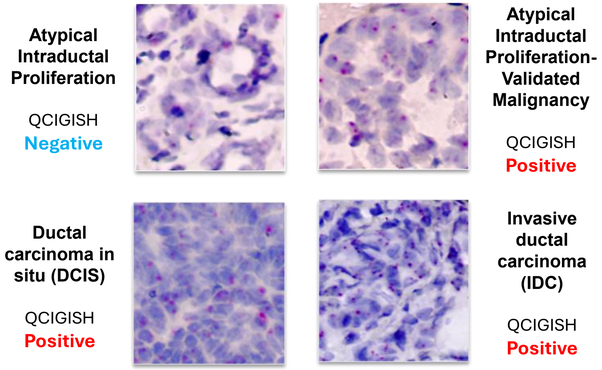


Table 1. Area under the receiver operating characteristic curve (AUROC) values for differential analysis of imprinted genes GNAS, GRB10, and SNRPN across three analytic models: BAE (Biallelic Expression), MAE (Monoallelic Expression), and TE (Total Expression).

Differential Analysis	Area Under the Receiver Operating Characteristics Curve (AUROC)		
Imprinted Genes	BAE	MAE	TE
GNAS	0.87	0.87	0.86
GRB10	0.86	0.94	0.81
SNRPN	0.95	0.95	0.87

Methods and Materials

We retrospectively identified benign, atypical, and carcinoma core needle specimens from year 2021-2022 in the Department of Pathology at Mayo Clinic Florida. Inclusion criteria included female patients, ages 18-99, with archived breast material obtained through standard of care (n=20). All diagnoses were ascertained by a breast pathologist (MKK). Following a thorough literature review, we processed the material for in situ hybridization, and pretreated with RNA scope preparation procedure using probes targeting noncoding intronic regions of nascent RNAs (QCIGISH). Based on high Area Under the Receiver Operating Characteristics Curve (AUROC) from prior studies[9,10], 2 genes – HM13 and GRB10 were identified as the most promising candidates for classification with QCIGISH[9,10]. Upon test completion, the detected gene-expression site appeared as a distinct red dot on the slide under the common bright-field microscope. Next, the gene expression signals were counted and further classified as bi-allelic (BAE), multi-allelic (MAE) and total expression (TE) (Table 1).

Conclusions

1. We have successfully completed **proof of concept**
2. We have demonstrated a breakthrough molecular biomarker that detects the **earliest step in carcinogenesis**
3. QCIGISH is **objective and reproducible**, with **highest sensitivity and specificity**
4. First of its kind, the innovative QCIGISH allows **for single-cell resolution** of epigenetic changes
5. QCIGISH is **easy to perform** and utilizes established laboratory techniques and **light microscopy**
6. **Correlation with H&E stain** is easy; importantly, the test is AI-compatible and results **can be fully automated**
7. Currently, we are undergoing **retrospective model validation** (supported by DeSantis Florida Fund, 2025)
8. In 2026, we will **begin prospective model validation**

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