Liquid biopsy-based detection of triple negative breast cancer using DNA methylation biomarkers

Jocelyn Charlton, Shiva Farashahi, Kade Pettie, Elie Massaad, Josh Hubbell, Feras Hantash, Kieran I Chacko
Harbinger Health, Cambridge, MA USA

AACR Liquid Biopsy, November 2024 | Poster A053

BACKGROUND

Triple-negative breast cancer (TNBC) presents significant clinical challenges due to its aggressive phenotype, rapid progression, and limited targeted treatment options. TNBC often mimics benign lesions on ultrasound and distinct imaging features can be missed in routine mammography screening leading to misdiagnosis and delayed treatment. These limitations led us to investigate whether a blood-based liquid biopsy could provide a non-invasive, sensitive method for early TNBC detection, potentially overcoming the limitations of conventional imaging techniques.

METHODS

To discover biomarkers for TNBC, we performed targeted bisulfite sequencing on a cohort of 46 breast cancer solid tissue biopsy samples (19 TNBC and 27 non-TNBC) and identified regions with difference in methylation >0.2. To ascertain whether these biomarkers could be useful in a liquid biopsy approach, we performed targeted bisulfite-sequencing on cell-free DNA samples derived from prospectively collected patients with breast cancer. All samples had independent tissue-based assessment of ER, PR and HER2 using immunohistochemistry or in situ hybridization.

RESULTS

Biomarker identification

We identified 150 differentially methylated regions (mean length 703bp) separating TNBC and non-TNBC samples (**Figure 1**). Upon linking each region to its nearest gene, we observed several genes previously associated with prognosis and survival (HOXB3, PAX9, SOX9) substantiating the clinical relevance of these biomarkers (**Table 1**). Using public TCGA data (369 breast cancer samples), we integrated expression and methylation data and observed directionally consistent changes. For example, HOXB3 showed increased methylation at its associated differential regions and decreased expression in TNBC cases (**Figure 2**).

Gene	Clinical finding
ACSL1	Up-regulated in triple negative breast cancer: high expression is linked to unfavorable prognosis[1]
AQP	Expression is significantly associated with overall survival and relapse free survival[2,3]
DNALI1	Downregulated in breast cancer and has a positive correlation with overall survival[4]
HOXB3	Loss correlates with the development of triple negative breast cancer; lower expression associated with poor prognosis[5]
ITPKA	Expression is associated with overall survival[6]
NFIX	Upregulation associated with poor prognosis[7]
PAX9	Downregulated expression causes cancer malignancies and is associated with the poor prognosis of breast cancer patients[8]
SOX9	Essential for triple negative breast cancer cell survival and metastasis[9]
SPDEF	Upregulated in luminal breast cancer and positively associated with tumor progression and poor prognosis[10]

Table 1: Differentially methylated genes for triple negative breast cancer are linked with prognosis and survival

RESULTS

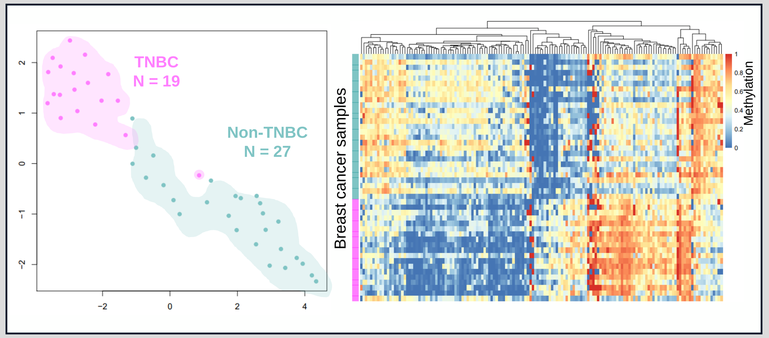

Figure 1. Biopsy samples shows clear separation by risk group. (Left) UMAP projection of 46 TNBC and non-TNBC tissue samples using the 150 differential regions, (Right) Heatmap showing per-region methylation levels of the 150 differentially methylated regions.

Classifier

We then built a feed-forward neural network classifier with classes TNBC and non-TNBC that used transfer-learning and the identified biomarkers to learn methylation signatures in the biopsy samples and map them to cell-free DNA. To emulate clinical workflow, where subtyping follows cancer diagnosis and tissue of origin identification, we evaluated cfDNA samples correctly identified as breast cancer using two independent machine learning models trained using 2,393 prospectively collected multi-indication cancer and non-cancer samples (NCT05435066).

RESULTS

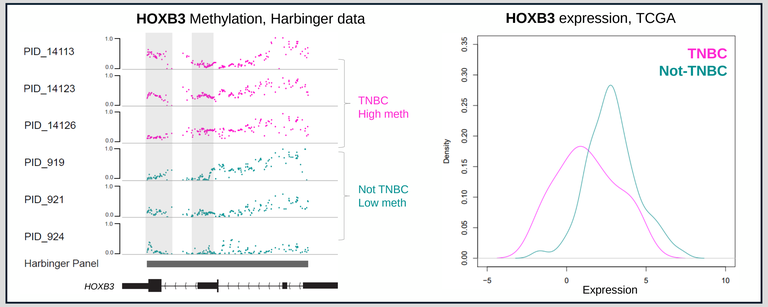

Figure 2: Differential methylation and expression of *HOXB3* in TNBC. (Left) CpG methylation levels within the HOXB3 gene, (Right) HOXB3 expression for TNBC and non-TNBC cases.

Performance

Subsequent TNBC subtyping of breast cancer-positive cases (N=56) accurately identified 77% (10/13) of TNBC samples and 91% (39/43) of non-TNBC cases, with overall accuracy of 84% (**Figure 3**). The estimated tumor content (TC) range for correctly predicted TNBC samples was 0.5-33% with the three mis-classified samples all showing very low TC (0.22%, 0.28%, 0.59%). Non-TNBC cases showed a TC range of 0.07-36% for correct and 2.3-14% for misclassified samples.

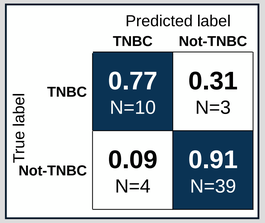

Figure 3: TNBC classification. Confusion matrix showing proportion correct (bold) and number of samples.

CONCLUSIONS

In conclusion, we identified novel methylation biomarkers that distinguish TNBC in the cell-free DNA of patients without needing invasive tissue biopsies. The high sensitivity of our assay at low tumor fractions has the potential to improve outcomes through earlier intervention and may enable earlier screening in high-risk groups, monitoring of minimal residual disease and longitudinal monitoring during treatment.

(1) Yen MC et al. *Oncol Rep*. (2017);37:3253–60. (2) Zhu L et al. *Cancer Manag Res* (2019) 11:1503–15. (3) Dai C et al. *Cancer Res* (2020) 80(19):4071–86. (4) Parris TZ et al. *Clin Cancer Res.* (2010);16:3860–3874. (5) Zhu L et al. *PeerJ*. (2020) Nov 20;8:e10421. (6) Zhang J et al. *Mol Genet Genomic Med*. (2021);9:e1598. (7) Sun C et al. *BMC Cancer.* (2019);19:1061. (8) Ren N et al. *Curr Issues Mol Biol*. (2021) Oct 26;43(3):1756-1777. (9) Ma Y et al. *Mol Cancer Res*. (2020) Dec;18(12):1825-1838. (10) Li J *et al. Cell Death Dis* 14, 569 (2023).