Defining transcriptomic profiles of breast cancer with early lymph node metastases: a FLEX database sub-study

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INTRODUCTION

- Early lymph node (LN) metastasis often precedes systemic metastasis and corresponds with a 20% decrease in 10-year survival compared to patients without LN metastasis[1].
- Understanding biologic pathways involved in early LN metastasis could identify promising drug targets for early-stage breast cancer (EBC) treatment.
- While tumor size modestly correlates with the probability of clinical or occult axillary metastases, small T1 tumors are often found to have LN metastases, while many large T3 tumors are often LN negative.
- We compared large tumors without evidence of LN metastasis (pT2-3pN0) and small tumors with LN metastasis (pT1pN1+) by whole transcriptome analysis to elucidate molecular biological differences associated with the absence or presence of early LN metastasis at diagnosis.

METHODS

FLEX Study: The FLEX study (NCT03053193) enrolls patients with EBC who undergo standard of care MammaPrint (MP) and BluePrint (BP) testing, and consent to clinically annotated full transcriptome data collection.

Patient Cohort: MP classifies tumors as having High Risk (HR) or Low Risk (LR) of distant recurrence. MP combined with BP define tumor subtype as either Luminal A-Type (MP LR; 56.8%), Luminal B-Type (MP HR; 36.4%), HER2-Type (1.5%), or Basal-Type (5.3%). Our primary comparison was between pT1pN1+ and pT2-3pN0 tumors, with pT1pN0 and pT2-3pN1+ as controls (**Table 1**).

Gene Expression Analysis: R package 'limma' was used for quantile normalization and differential gene expression analyses. Differentially expressed genes (DEGs) with a false discovery rate <0.05 and $\log_2$ fold change >0.5 were considered significant for this exploratory analysis. Gene set enrichment analysis (GSEA) was performed using the Hallmark gene sets (MSigDB).

RESULTS

Table 1. Patient numbers by MammaPrint and BluePrint

MammaPrint Result	BluePrint Subtype	pT1pN0	pT1pN1+	pT2-3pN0	pT2-3pN1+
Low Risk (n=1334)	Luminal A (n=1334)	824	197	111	202
High Risk (n=1015)	Luminal B (n=855)	452	141	98	164
	HER2 (n=35)	24	7	1	3
	Basal (n=125)	84	10	25	6
Total: 2349	**2349**	**1384**	**355**	**235**	**375**

Figure 1. Principal component analysis (PCA) by pathological staging (A) and MP/BP (B).

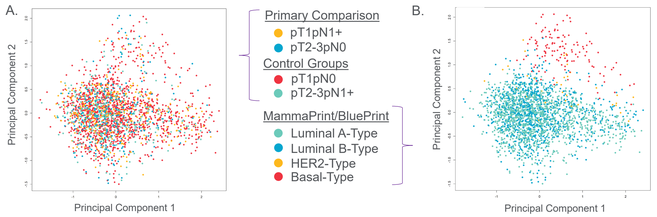

Figure 2. DEG analysis comparing pT2-3pN0 vs. pT1pN1+ tumors (A). As controls, we compared pN0 vs. pN1+ within (B) pT2-3 tumors and (C) pT1 tumors, and pT2-3 vs. pT1 tumors within (D) pN0 and (E) pN1+ tumors. Genes upregulated (blue) or downregulated (red) with fold change >1.4 and p value <0.05 are indicated.

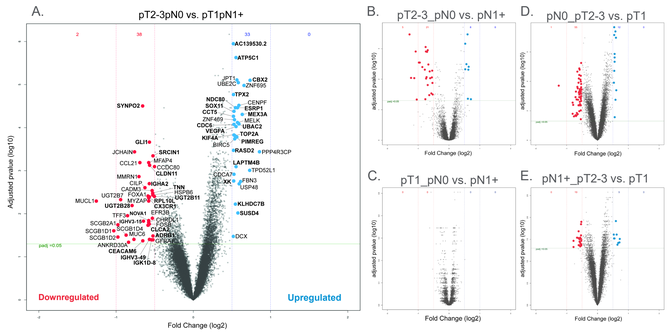

Figure 3. GSEA within MP High Risk tumors.

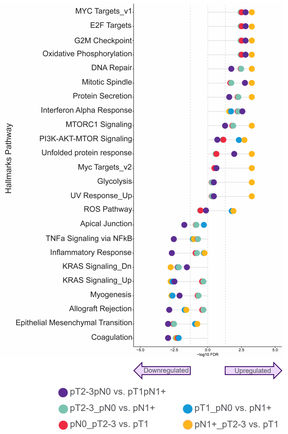

Figure 4. Number of unique genes per group.

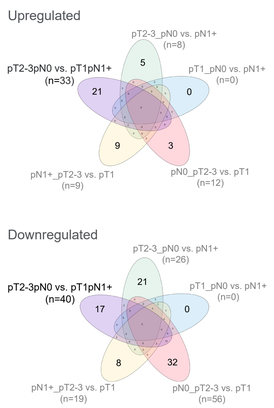

Table 2. Upregulated and downregulated genes within MP/BP HR tumors that were unique when comparing pT2-3pN0 with pT1pN1+ (not identified in control comparisons).

	Genes	Gene Functions
Upregulated	AC139530.2, ATP5C1, CBX2, CCT5, CDC6, DCX, ESRP1, KIF4A, KLHDC7B, LAPTM4B, MEX3A, NDC80, PIMREG, RASD2, SOX11, SUSD4, TOP2A, TPX2, UBAC2, VEGFA, XK	PI3K/AKT signaling, Protein folding, Growth and proliferation, Immune T cell infiltration
Downregulated	ADRB1, CEACAM6, CLCA2, CLDN11, CX3CR1, GLI1, IGHA2, IGHV3-15, IGHV3-49, IGKV1D-8, NOVA1, RPL10L, SRCIN1, SYNPO2, TNN, UGT2B11, UGT2B28	Epithelial to Mesenchymal Transition, Immune signaling, Regulation of reactive oxygen species, Negative regulation of proliferation, Negative regulation of PI3K-Akt-mTOR signaling, metabolism

- PCA revealed more gene profile differences between MP/BP (**Figure 1B**) than by pathological stage (**Figure 1A**).
- Differential gene expression analysis comparing pT2-3pN0 to pT1pN1+ identified 30 DEGs (data not shown).
  - Within MP LR tumors, no DEGs were observed.
  - Within MP HR tumors, 73 DEGs were identified (**Figure 2**) and were associated with increased proliferation and decreased immunity (**Figure 3**).
  - By subtype, no DEGs were found in Luminal A-Type tumors, 34 DEGs were identified within Luminal B-Type tumors, Basal-Type and HER2-Type tumors were not analyzed due to small numbers (data not shown).
- Within HR tumors, 38 genes were uniquely differentially expressed in pT2-3pN0 vs. pT1pN1+ that were not detected in any control comparison (**Figure 4, Table 2**).
  - 21 upregulated genes were associated with proliferation and T cell immunity.
  - 17 downregulated genes were involved in EMT, negative regulation of proliferation, and immune signaling.

CONCLUSIONS

- This study provides a foundation for understanding the mechanisms that promote LN metastasis. Previous studies have correlated LN metastasis with EMT and immune function[2-4].
- Overall, we found most biological differences within MP HR tumors.
  - Proliferation-related pathways were upregulated and EMT and immune-related pathways were downregulated in pT2-3pN0 tumors compared with pT1pN1+ tumors.
  - Our data suggests these pathways may be involved in early LN metastasis. Dysregulated immune pathways could activate immune evasion/suppression mechanisms to escape immune surveillance and ultimately metastasize.
- Future studies will investigate antineoplastic therapies for EBC that modulate these dysregulated pathways to reduce early LN metastasis and subsequent systemic metastasis.

The authors wish to thank our patients who inspire us everyday. Navigating a cancer diagnosis is never easy, doing so during a pandemic requires heroic personal strength.

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