Gene expression associated with lymphovascular invasion and genomic risk in early-stage breast cancer

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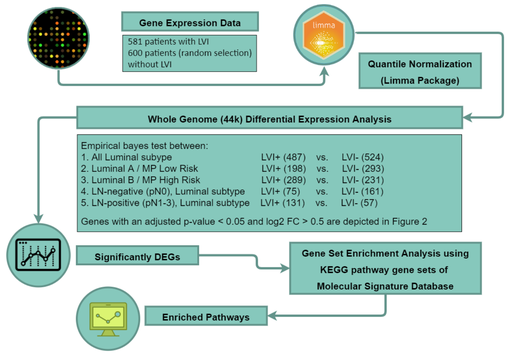
BACKGROUND

Lymphovascular invasion (LVI), the passage of carcinoma cells through lymphatic and blood vessels, is an important early step in metastasis; however, LVI is excluded from most breast cancer (BC) clinical risk assessments. Previous studies assessed the prognostic value of LVI to estimate clinical outcomes and have reported lower survival rates for patients with LVI (1-2). To gain understanding of the molecular basis of LVI, we evaluated differentially expressed genes (DEGs) between tumors with LVI *versus* those without LVI, stratified by the 70-gene signature (MammaPrint/MP) and 80-gene molecular subtyping signature (BluePrint/BP).

METHODS

Patients: The prospective, observational FLEX Study (NCT03053193) includes patients with stage I, II, and III invasive BC who receive MP/BP testing and consent to full transcriptome and clinical data collection. This sub-analysis included patients with LVI (n = 581) and without LVI (n = 600, randomly selected from all FLEX cases with LVI) enrolled from 2017 to present. Presence of LVI was determined by local pathology laboratories.

Analysis (Figure 1, below): Only Luminal subtype tumors with LVI (n = 487) and without LVI (n = 524) were included in the current analysis. Differential gene expression analysis of 44k Agilent microarray data was performed with limma R package. DEGs were compared within all BP Luminal subtype tumors, MP risk groups (Low Risk [LR]/Luminal A and High Risk [HR]/Luminal B), and by regional lymph node (LN) status. DEGs with a false discovery rate (FDR) < 0.05 were considered significant. Most patient clinical characteristics were compared using Chi square test or Fisher's Exact test; patient age distribution was compared using unpaired Student *t* test.



CLINICAL CHARACTERISTICS

Table 1. Clinical characteristics for patients with presence or absence of LVI for all Luminal-type tumors and for the subset negative regional lymph node involvement (pN0). There was no significant difference between LVI+ and LVI- groups in the distribution of patient race/ethnicity, body mass index (BMI) category, or diabetes status (p>0.05 for each comparison, not shown). **Here, regional lymph nodes were negative histologically (pN0), by IHC (pN0(-)), and/or by RT-PCR (pN0(mol-)). Tumors with regional lymph nodes with isolated tumor cells (pN0(+)) were excluded from this analysis to avoid confounding gene expression results.*

	All Luminal-type Tumors			pN0* Luminal-type Tumors		
Clinical Characteristics (*unknowns excluded*)	**LVI+ (n=487)**	**LVI- (n=524*)**	***p* value**	**LVI+ (n=75)**	**LVI- (n=161)**	***p* value**
Age, Median (years)	60	63		65	64	
Age, Mean (years)	59	61	**0.006**	63	61	0.323
Menopausal Status						
Pre or Peri	114 (26%)	99 (20%)	0.052	14 (20%)	29 (20%)	>0.999
Post	332 (74%)	391 (80%)		55 (80%)	118 (80%)	
Histologic Tumor Type						
IDC	383 (80%)	400 (78%)	0.340	61 (86%)	119 (80%)	0.590
ILC	55 (12%)	71 (14%)		4 (6%)	13 (9%)	
Mixed IDC/ILC	13 (3%)	8 (2%)		0	3 (2%)	
Other types	26 (5%)	34 (7%)		6 (8%)	13 (9%)	
Tumor Stage**						
cT1	154 (50%)	249 (72%)	**<0.0001**	45 (63%)	120 (79%)	**0.023**
cT2	120 (39%)	86 (25%)		26 (37%)	30 (20%)	
cT3/cT4	32 (11%)	12 (3%)		0	1 (1%)	
Nodal Stage						
cN0	164 (55%)	294 (89%)	**<0.0001**			
cN1	114 (39%)	37 (11%)				
cN2/cN3	17 (6%)	1 (<1%)				
Grade						
G1	67 (15%)	155 (31%)	**<0.0001**	10 (15%)	40 (28%)	**0.005**
G2	270 (58%)	280 (57%)		38 (57%)	85 (60%)	
G3	125 (27%)	59 (12%)		19 (28%)	17 (12%)	
Ki67% (IHC)						
Low (≤14%)	96 (30%)	161 (48%)	**<0.0001**	13 (26%)	42 (51%)	**0.006**
High (>14%)	223 (70%)	174 (52%)		37 (74%)	40 (49%)	
70-GS / MammaPrint						
Low Risk (Luminal A)	198 (41%)	293 (56%)	**<0.0001**	27 (38%)	95 (63%)	**0.001**
High Risk (Luminal B)	289 (59%)	231 (44%)		44 (62%)	56 (37%)	

***Clinical T staging results are reported for the group of Luminal subtype tumors; however, for the pN0 subset, pathological T (pT) staging results are reported.*

DEGs IN TUMORS WITH LVI

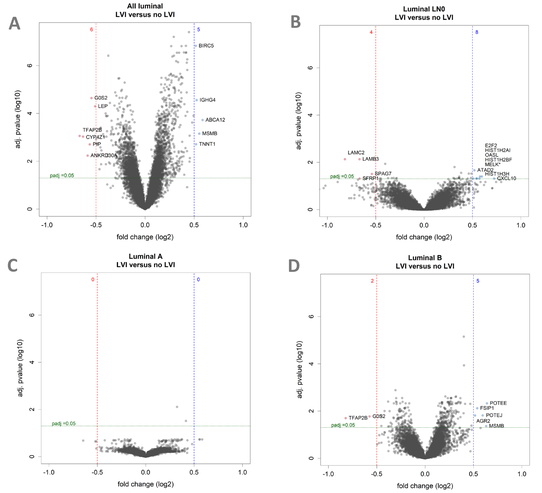


Figure 2: Volcano plots of DEGs between tumors with presence or absence of LVI in (**A**) all Luminal subtype, (**B**) lymph node-negative (LN0) Luminal, (**C**) Luminal A (MP Low Risk), and (**D**) Luminal B (MP High Risk) tumors. There were no significant DEGs between presence and absence of LVI in lymph node-positive (regardless of subtype) tumors (*not shown*). In each plot the number of DEGs upregulated in LVI is shown in red, and downregulated genes are shown in blue. **MELK is also found within MammaPrint signature*

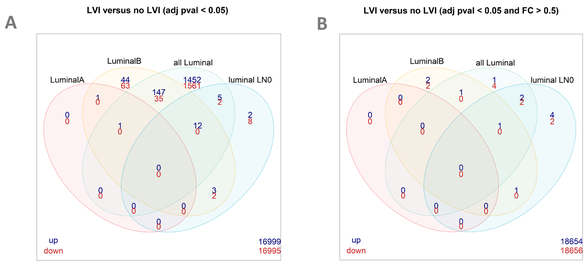


Figure 3: The numbers of (**A**) all significant (p < 0.05) DEGs, regardless of fold-change, and (**B**) the number of significant DEGs with FC > 0.5 in tumors with LVI compared with absence of LVI in each group comparison shown in Figure 2.

GENE SET ENRICHMENT

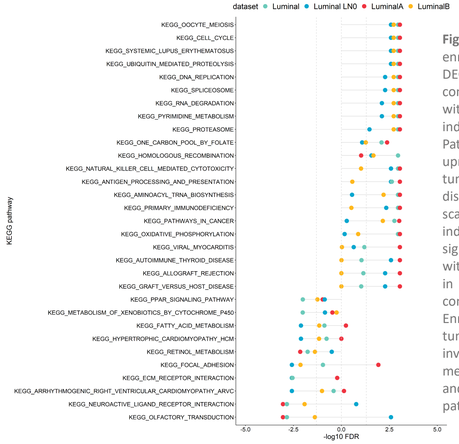


Figure 4: Gene set enrichment analysis of DEGs in tumors with LVI compared with those without LVI for each indicated data set. Pathways with upregulated genes in tumors with LVI are displayed on the positive scale. Dashed lines indicate threshold for significance. Gene sets with significant regulation in at least one comparison are shown. Enriched gene sets in tumors with LVI are involved in cell cycle, metabolism, immune, and extracellular matrix pathways.

CONCLUSIONS and FUTURE DIRECTIONS

- DEGs associated with LVI were primarily restricted to MP High Risk Luminal (Luminal B) and LN-negative tumors.
- Enrichment analysis suggested dysregulation of cell cycle, ECM organization, and cell adhesion pathways, consistent with previous reports (3-4), particularly in MP High Risk (Luminal B) and LN-negative tumors with LVI.
- Significant DEGs were associated with LVI presence *versus* absence in LN-negative tumors (Fig. 2B), but not in LN+ tumors (*data not shown*), suggesting that LVI assessment may provide less prognostic information in LN+ breast cancer.
- Although clinical outcomes are not yet available for these BC patients, the current analysis indicates few DEGs in LVI+ MP Low Risk (Luminal A) tumors; thus, the potential prognostic information gained from LVI-associated gene expression is likely already captured by the MP signature.
- Future studies will assess clinical outcomes associated with presence of LVI, as well as LVI-associated gene expression in BP Basal and HER2 subtype tumors.

References

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2. Song et al. *J Breast Cancer* 2011
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4. Kariri et al. *Pathobiology* 2020

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