Differential gene expression in Luminal-type invasive lobular carcinoma and invasive ductal carcinoma by MammaPrint risk stratification

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BACKGROUND

Invasive lobular carcinoma (ILC) comprises 10-15% of breast tumors and is the second most common histological type after invasive ductal carcinoma (IDC) (1). Patients with ILC are often diagnosed at an older age and more advanced stage than those with IDC (2). Despite the good prognostic phenotype of ILC, late recurrences and worse long-term survival (3) suggest the need for exploration of molecular pathways unique to ILC to optimize treatment strategies. Recent data from a MINDACT sub-analysis indicate that MammaPrint stratification of ILC predicts unfavorable survival outcomes for patients with tumors classified as High Risk (4). Although previous reports have described comprehensive transcriptomic profiling of ILC, these were limited by small sample sizes (5). Furthermore, global differential gene expression between ILC and IDC stratified by genomic risk has yet to be explored. Here we characterize differential gene expression between ILC and IDC in a large, age-matched patient subset categorized by 70-gene signature/MammaPrint (MP) risk and 80-gene signature/BluePrint (BP) subtype.

METHODS

The prospective FLEX Study (NCT03053193) includes stage I, II, and III primary invasive breast cancer patients who receive MP/BP testing and consent to full transcriptome and clinical data collection. This sub-analysis included 622 patients with ILC enrolled from 2017 to present. Compared with a random selection of patients with IDC (n=600, mean age, 60 years), patients with ILC were older (mean, 62 years, p<0.001). Thus, we selected an age-matched subset for differential gene expression analysis. There were few non-Luminal ILCs; thus, gene expression analyses were limited to BP Luminal tumors. A subset of age-matched (n=1136) patients with ILC and IDC were used for analysis. Gene expression data were quantile normalized using R limma package, and differentially expressed genes (DEGs) were compared between groups. DEGs with an adjusted p<0.05 and log2 fold change ≥ 1.0 were considered significant.

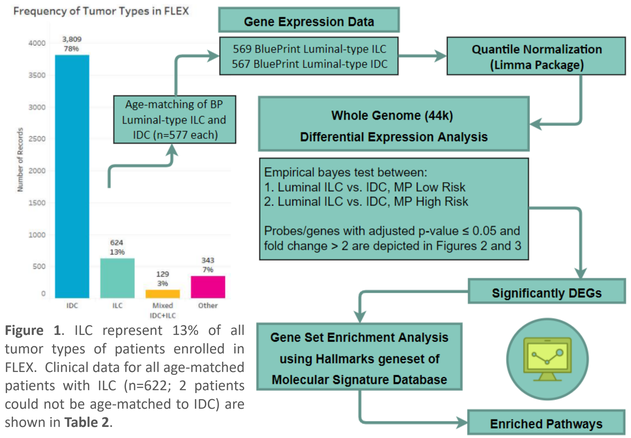

Figure 1. ILC represent 13% of all tumor types of patients enrolled in FLEX. Clinical data for all age-matched patients with ILC (n=622; 2 patients could not be age-matched to IDC) are shown in **Table 2**.

CLINICAL CHARACTERISTICS

Table 1	Patient Characteristics			
Patient Group	**Age, Mean**	**Age, Median**	**Pre/Peri-Menopausal**	**Post-Menopausal**
ILC (n=622)	62	63	94 (16%)	494 (84%)
IDC (n=600)	60	61	119 (21%)	443 (79%)
p-value	**<0.0001**		0.055	

Tables 1-2. Compared with randomly selected patients with IDC (n=600), patients with ILC were significantly older at diagnosis (p<0.0001, **Table 1**). An age-matched subset of patients with IDC was used for further comparisons. Ethnicity, nodal stage, distribution of BMI categories, and rate of type 2 diabetes were similar between patients with ILC and those with IDC (p>0.05). However, ILC were more frequently grade 1 or 2, T3, ER+ (when considering all subtypes), and more frequently MammaPrint Low Risk than IDC (**Table 2**).

Table 2	All Subtypes Age-Matched			BP Luminal only Age-Matched		
Patient Characteristics *(*unknowns excluded)*	**ILC (n=622)**	**IDC (n=622)**	***p*-value**	**ILC (n=577)**	**IDC (n=577)**	***p*-value**
Age, Mean	62	62	1.00	62	62	1.00
Age, Median	63	63		63	63	
Menopausal Status						
Pre/Peri	94 (16%)	84 (14%)	0.465	90 (16%)	82 (15%)	0.508
Post	494 (84%)	498 (86%)		456 (84%)	465 (85%)	
Grade						
G1	178 (29%)	151 (25%)	**<0.0001**	173 (30%)	199 (35%)	**<0.0001**
G2	375 (61%)	308 (50%)		340 (60%)	278 (49%)	
G3	30 (5%)	135 (22%)		27 (5%)	81 (14%)	
GX	29 (5%)	16 (3%)		27 (5%)	12 (2%)	
T stage						
T1	263 (60%)	229 (64%)	**0.003**	245 (60%)	271 (70%)	**<0.0001**
T2	128 (29%)	109 (31%)		120 (29%)	103 (27%)	
T3	49 (11%)	14 (4%)		41 (10%)	6 (2%)	
T4	2 (<1%)	3 (<1%)		2 (<1%)	5 (1%)	
N stage						
N0	337 (81%)	280 (84%)	0.232	307 (80%)	310 (85%)	0.457
N1	69 (17%)	51 (15%)		67 (18%)	52 (14%)	
N2/3	8 (2%)	3 (1%)		7 (2%)	4 (1%)	
ER status (IHC)						
Positive	583 (99%)	544 (91%)	**<0.0001**	545 (99%)	549 (99%)	0.547
Negative	7 (1%)	55 (9%)		4 (1%)	7 (1%)	
MammaPrint Result						
Low Risk	419 (67%)	289 (46%)	**<0.0001**	389 (67%)	317 (55%)	**<0.0001**
High Risk	203 (33%)	333 (54%)		188 (33%)	260 (45%)	
BluePrint Subtypes						
Luminal-type	577 (98%)	522 (87%)	**<0.0001**			
HER2-type	6 (1%)	20 (3%)				
Basal-type	3 (<1%)	56 (9%)				

GENE EXPRESSION DIFFERENCES BETWEEN AGE-MATCHED PATIENTS WITH ILC AND IDC

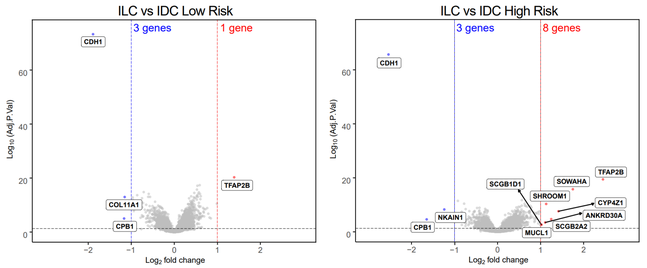

Figure 2. Volcano plots of DEGs for ILC compared with IDC, by MP risk group. MP Low Risk (n=310 ILC, n=385 IDC) comparison is shown in the left panel; MP High Risk (n=184 ILC, n=257 IDC) is shown in the right panel. In each plot the number of DEGs upregulated in ILC is shown in red, and downregulated genes/probes are shown in blue. Regardless of MP risk, expression of *CDH1* was downregulated in ILC compared with IDC, consistent with loss of E-cadherin that is considered a hallmark of ILC (1, 6-7). *TFAP2B*, a transcription factor, was upregulated in ILC regardless of MP risk, and has been shown to be associated with ILC proliferation and prognosis (8). *MUCL1*, which was upregulated in MP HR ILC, has also been shown to have a role in breast tumor cell proliferation (9), as well as associated with a variety of immune pathways.

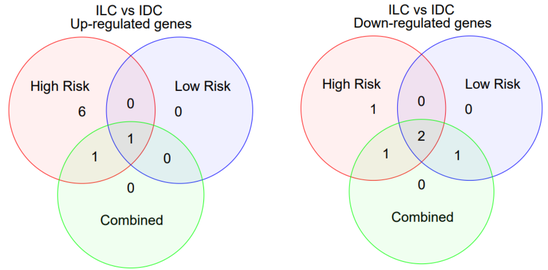

Figure 3. Venn diagrams showing up- and down-regulated genes in ILC relative to IDC. The number of DEGs in each comparison are given for MammaPrint Low Risk and High Risk groups, and all samples combined. As in other comparisons, sample were matched for patient age at diagnosis and for BluePrint subtype (all Luminal).

GENE SET ENRICHMENT IN ILC

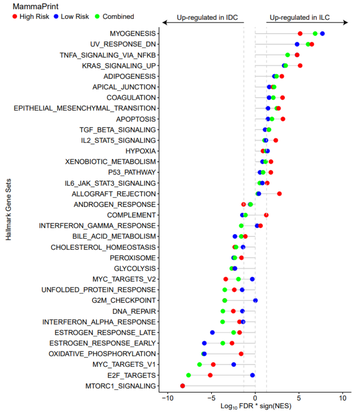

Figure 4. Gene set enrichment analysis results for ILC compared with IDC from age-matched patients. Pathways with upregulated genes in ILC are displayed on the positive scale. Dashed lines indicate threshold for significance. Compared with IDC, gene sets upregulated in ILC were enriched for immune pathways, including TNFα, KRAS, TGFβ, IL-2, IL-6 signaling, as well as adipogenesis, epithelial-mesenchymal transition, and apoptosis pathways.

CONCLUSIONS

- Approximately one-third of ILCs were MP HR, and recent MINDACT data demonstrate less favorable survival outcomes in patients with HR compared with LR ILC (4). Here we report a greater number and diversity of DEGs, as well as uniquely enriched gene sets, between HR ILC and HR IDC, suggesting greater heterogeneity in HR compared with LR tumors.
  - Further investigation of the associated pathways may elucidate molecular mechanisms associated with late recurrences and poorer clinical outcomes seen in patients with ILC and provide targets for treatment optimization.
- ILCs comprise 13% of the total cases in the FLEX trial, consistent with frequencies of ILC reported in the US breast cancer population (1), which highlights the capacity of FLEX to enroll a real-world breast cancer patient population.

FUTURE DIRECTIONS

- Significant differences between ILC and IDC in factors such as grade and T stage suggest that further stratification by clinical-pathological factors may be informative for characterization of biological pathways uniquely upregulated in ILC.
- Therapeutic strategies targeting upregulated pathways may be future avenues of exploration in ILC, although further studies are warranted to characterize underlying molecular mechanisms.

References

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