Molecular profiles of genomically High Risk ER+ HER2- breast cancer tumors classified as functionally Basal or Luminal B by the BluePrint

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BACKGROUND

- The 80-gene signature (BluePrint/BP) classifies early-stage breast cancers based on functional molecular signaling pathways as Luminal, HER2, or Basal-type[1].
- In the NBRST study, 13% of immunochemistry (IHC) defined ER+ HER2- cancers were reclassified as Basal-type by the BP assay (ER+ Basal). These tumors had worse prognosis but responded better to neoadjuvant chemotherapy compared to ER+ HER2- cancers classified as genomically Luminal-type[2].
- The 70-gene risk of recurrence signature (MammaPrint/MP) further stratifies Luminal-type cancers into Low Risk Luminal A or High Risk (HR) Luminal B[1].
- HR Luminal-type cancers can be further stratified into MP High 1 (H1) or MP High 2 (H2), and the I-SPY2 trial has shown higher pathologic complete response rates in ER+ cancers classified as H2.
- Here, we investigated the biological differences among ER+ Basal, ER- Basal, H1 Luminal B, and H2 Luminal B cancers by full transcriptome analysis.

METHODS

FLEX Study: The FLEX Study (NCT03053193) is an ongoing, prospective study of stage I-III breast cancer patients that receive the MammaPrint 70-gene signature test with or without the BluePrint 80-gene signature test and consent to clinically annotated transcriptome data collection.

Patient Cohort: 1501 breast cancer samples with known IHC ER status were classified into subtypes by the MP and BP tests: 103 ER+ Basal, 210 ER- Basal and 1188 ER+ Luminal B (H1 n=1034, H2 n=154).

Gene Expression Analysis: Differentially expressed genes (DEGs) were detected using R package 'limma' and pathway analyses were performed with gene set enrichment analysis (GSEA). DEGs with a fold change >2 and FDR < 0.05 were considered significant.

Statistical Analysis: Clinical factors were assessed by either the Chi-square or Fisher's exact tests; ANOVA or *t* test were used to analyze age.

Table 1: Patient-Tumor clinical characteristics.

	ER- Basal (n = 128)	ER+ Basal (n = 63)	ER+ Luminal B (n = 698)	Significance	ER+ Luminal B H1 (n = 615)	ER+ Luminal B H2 (n = 83)	Significance
Mean Age	56.81	54.19	59.97	p < 0.001	60.38	56.98	p = 0.02
Lymph node							
cN0	113 (73%)	54 (86%)	523 (80%)	p = 0.13	482 (78%)	55 (66%)	p = 0.014
≥cN1	41 (27%)	9 (14%)	141 (20%)		133 (22%)	28 (34%)	
Grade							
G1	4 (3%)	2 (3%)	97 (15%)	p < 0.001	95 (16%)	2 (2%)	p < 0.0001
G2	16 (14%)	10 (16%)	406 (61%)		380 (65%)	26 (32%)	
G3	98 (83%)	50 (81%)	163 (24%)		110 (19%)	53 (66%)	
T Stage							
cT1	56 (44%)	30 (48%)	394 (56%)	p = 0.08	361 (59%)	33 (40%)	p = 0.005
cT2	60 (47%)	29 (46%)	254 (38%)		212 (34%)	42 (51%)	
≥cT3	11 (9%)	4 (6%)	50 (6%)		42 (7%)	8 (9%)	
Ethnicity							
White	74 (58%)	37 (59%)	505 (72%)	p = 0.001	458 (74%)	47 (57%)	p = 0.001
AA	30 (23%)	12 (19%)	75 (11%)		57 (9%)	18 (22%)	
LA	14 (11%)	6 (10%)	48 (7%)		43 (8%)	5 (6%)	
other	10 (8%)	8 (12%)	70 (10%)		57 (9%)	13 (15%)	
*unknowns excluded							
AA = African American							
LA = Latin American							

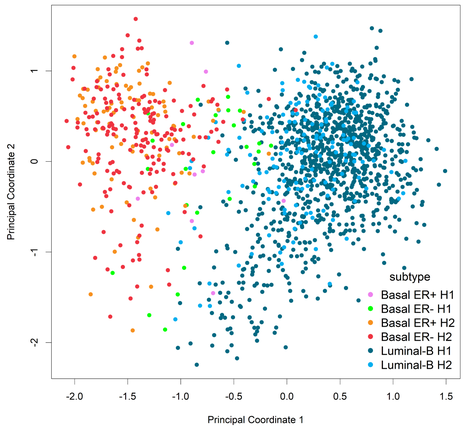


Figure 1: PCA analysis showing Luminal (blue) and Basal (ER+ = orange, ER- = red) samples explain the highest variance between the groups.

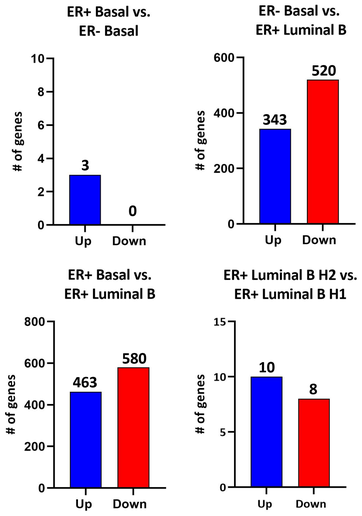
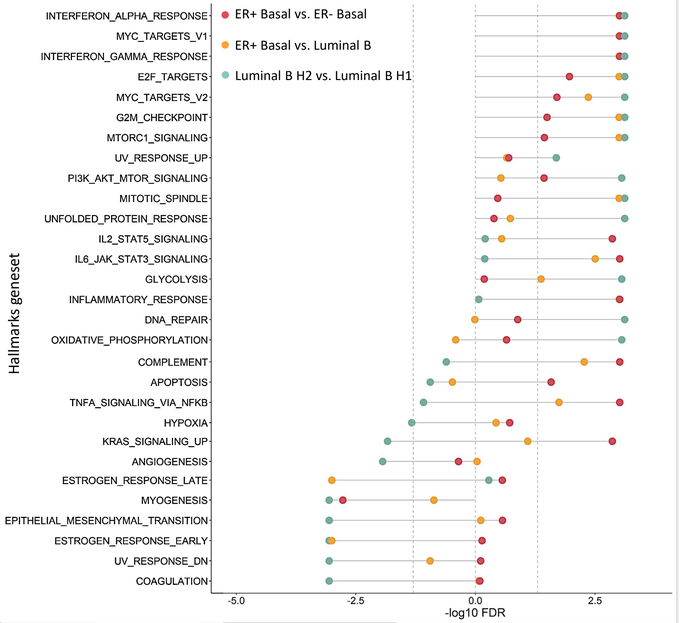


Figure 2: Number of differentially expressed genes between tumor subtypes.



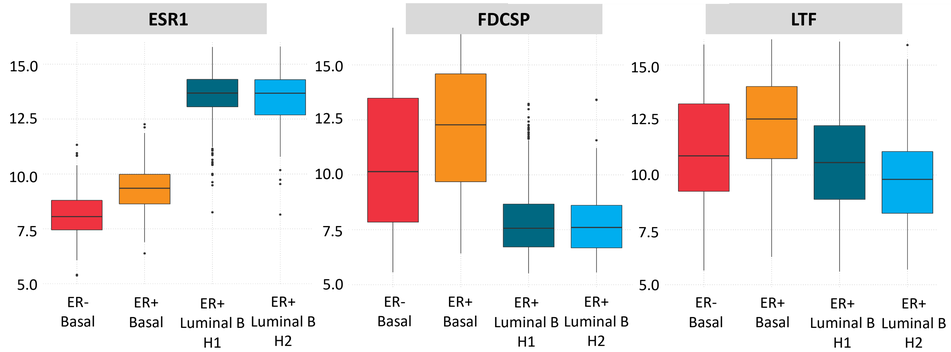


Figure 3: Genes differentially expressed among ER+ Basal, ER- Basal, and ER+ Luminal B tumor subtypes

Figure 4: Results from gene set enrichment analysis using Hallmark gene sets from Molecular signature database for ER+ Basal vs. ER- Basal (red), ER+ Basal vs. Luminal B (orange), and Luminal B H2 vs. Luminal B H1 (blue).

RESULTS

- Basal-type cancers (ER+/ER-) were larger and higher grade than Luminal B cancers (**Table 1**).
- Clustering analysis showed similar transcriptional profiles between ER+ Basal and ER- Basal cancers, distinct from Luminal B cancers **(Figure 1).**
- Only three upregulated genes were detected in ER+ Basal compared to ER- Basal cancers: *ESR1* and two immune-related genes (*FDCSP* and *LTF*) **(Figure 2 & 3).**
- In contrast, significantly more DEGs were found between Basal and Luminal B cancers, regardless of ER expression (**Figure 2**).
- Enrichment analysis of DEGs indicated increased immune activation and cell proliferation in ER+ Basal and ER- Basal cancers, and decreased estrogen response between ER+ Basal and Luminal B cancers (**Figure 4**).
- 18 DEGs were detected between Luminal B H1 and H2 cancers and enrichment analysis showed that H2 cancers had higher immune activation and cell proliferation and lower estrogen response (**Figures 2 & 4**).

CONCLUSIONS

- Reclassification by BluePrint of IHC defined ER+ HER2- cancers identified a subgroup of ER+ cancers that are biologically closer to ER- Basal than Luminal-type cancers.
- Significant differences in response to neoadjuvant chemotherapy that have been seen between ER+ Basal and Luminal B breast cancers lend support to the clinical importance of these findings.
- These data explain the poor prognosis observed in patients with ER+ Basal cancers and suggest that optimized chemotherapy, such as that for triple negative cancer, might be of benefit.
- BluePrint provides clinically actionable information beyond pathological subtyping, which may guide neoadjuvant treatment recommendations.

References

1. Krijgsman *et al.* 2012, Breast Cancer Res Treat
2. Groenendijk *et al.* 2019, npj Breast Cancer