Distinct Transcriptional and Immunosuppressive Microenvironment Signatures in PIK3CA- and ESR1-Mutant Hormone Receptor Positive (HR+)/HER2- Metastatic Breast Cancer (MBC)

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INTRODUCTION

ESR1 and PIK3CA mutations drive endocrine resistance in HR+ MBC, with ESR1 mutations often acquired after therapy and PIK3CA mutations present early. The impact of these mutations and their co-occurrence on the tumor immune microenvironment and transcriptome remains unclear. We compared gene expression and immune cell infiltration in pts with PIK3CA and/or ESR1 mutations versus wild-type (WT; no PIK3CA or ESR1 mutation) HR+/HER2– MBC.

METHODS

We used Tempus Lens to identify pts with a primary diagnosis of MBC who had Tempus xT/xF and xR testing from the Tempus multi-modal database. Pts with tumor purity of at least 30% were assessed and all pathogenic or likely pathogenic somatic SNVs/indels for PIK3CA and ESR1 were extracted. Pts were stratified into groups based on the presence of PIK3CA-mut and/or ESR1-mut. RNA-seq data were normalized to correct for assay/batch effects, quantified as transcripts per million (TPM) for all protein-coding transcripts, and reported as log2(TPM + 1). Immune infiltration was quantified using quantiseq. Differential gene expression (DGE) analyses were performed using Wilcoxon rank-sum tests; p values were adjusted for multiple comparisons using the Benjamini-Hochberg false discovery rate method.

RESULTS

- The cohort included 4,583 patients with HR+/HER2– MBC: 48% WT, 36% PIK3CA-mut, 10% ESR1-mut, and 6% co-mut, with a median age of 57.
- ESR1-mutant and co-mutant tumors showed significantly increased expression of TFF1, FGA, FGB, ALB, and SCGB2A2 (all p<0.001), while SFRP2 was specifically downregulated in ESR1-mutant tumors (p<0.001).
- PIK3CA-mutant tumors were similar to WT except for elevated SCGB2A2 expression (p<0.001).
- Immune profiling revealed that WT tumors had the highest B-cell infiltration (median 3.92), while co-mutant tumors had the lowest (median 3.39, p<0.001).
- PIK3CA-mutant tumors demonstrated the greatest enrichment of M1/M2 macrophages and Tregs (p<0.001), whereas ESR1-mutant tumors had the lowest levels of these immunosuppressive cells (p<0.001).

Table 1. Differential Gene Expression by PIK3CA and ESR1 Mutation Status in HR+/HER2– Metastatic Breast Cancer

Gene Expression Median (Q1,Q3)	No-mut (N=2,203)	PIK3CA-only (N=1,668)	ESR1-only (N=436)	Co-mut (N=276)	p-value
TFF1	6.7 (3.2, 9.3)	7.9 (5.5, 9.7)	8.3 (6.0, 10.1)	9.3 (7.2, 10.8)	<0.001
FGA	0.5 (0.4, 2.3)	0.5 (0.4, 1.6)	7.2 (0.5, 10.9)	3.5 (0.5, 10.3)	<0.001
FGB	0.9 (0.5, 4.4)	0.9 (0.5, 3.3)	8.3 (1.0, 11.7)	5.1 (0.8, 11.0)	<0.001
ALB	2.9 (1.8, 6.4)	2.9 (1.8, 5.3)	10.9 (2.9, 14.5)	6.9 (2.3, 14.1)	<0.001
SCGB2A2	4.0 (0.7, 8.5)	7.7 (3.0, 10.4)	5.0 (0.9, 9.1)	8.2 (4.1, 10.8)	<0.001
SFRP2	6.1 (2.6, 8.2)	6.9 (3.7, 8.7)	2.3 (0.4, 5.7)	3.5 (0.8, 6.8)	<0.001

Table 1. Median (25th percentile, 75th percentile) are reported for all variables; all gene expression values are reported as log2(TPM + 1); p-values were calculated via Kruskal-Wallis tests.

Table 2. Immune Cell Infiltration by PIK3CA and ESR1 Mutation Status in HR+/HER2– Metastatic Breast Cancer

	Median % (Q1,Q3)	Stratified by PIK3CA/ESR1 SNV/indel Status				
	Overall	No PIK3CA nor ESR1 Alterations	Only a PIK3CA Alteration	Only an ESR1 Alteration	Both PIK3CA and ESR1 Alterations	p-value
Number of Observations	N=8,626	N=4,561	N=2,747	N=779	N=539	
Cell Type						
B Cells	3.83 (3.04, 4.80)	3.92 (3.09, 4.93)	3.82 (3.05, 4.77)	3.68 (2.92, 4.60)	3.39 (2.90, 4.15)	<0.001
Dendritic Cells	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	0.591
M1 Macrophages	3.22 (2.25, 4.47)	3.17 (2.12, 4.54)	3.40 (2.42, 4.57)	2.79 (2.05, 3.79)	3.26 (2.45, 4.40)	<0.001
M2 Macrophages	5.47 (4.12, 7.05)	5.33 (3.93, 6.94)	5.80 (4.53, 7.31)	5.09 (3.53, 6.53)	5.10 (3.99, 6.92)	<0.001
Monocytes	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	<0.001
NK Cells	2.32 (1.84, 2.93)	2.33 (1.84, 3.03)	2.32 (1.86, 2.88)	2.33 (1.82, 2.78)	2.17 (1.70, 2.78)	0.006
Neutrophils	5.99 (4.77, 7.44)	5.93 (4.65, 7.51)	6.08 (4.93, 7.51)	5.69 (4.53, 7.11)	6.13 (5.06, 7.30)	0.004
CD4 T Cells	0.00 (0.00, 0.76)	0.00 (0.00, 0.53)	0.00 (0.00, 1.21)	0.00 (0.00, 0.00)	0.00 (0.00, 0.79)	<0.001
CD8 T Cells	0.11 (0.00, 0.71)	0.12 (0.00, 0.70)	0.21 (0.00, 0.81)	0.00 (0.00, 0.37)	0.00 (0.00, 0.41)	<0.001
Tregs	2.64 (1.97, 3.68)	2.65 (1.99, 3.78)	2.80 (2.10, 3.83)	2.18 (1.57, 2.95)	2.32 (1.74, 3.08)	<0.001
Other Cells	74 (69, 77)	74 (69, 77)	73 (68, 77)	77 (73, 80)	76 (72, 79)	<0.001

Table 2..Quantification of immune cell populations (B cells, M1/M2 macrophages, regulatory T cells, CD4/CD8 T cells) stratified by wild-type, PIK3CA-mutant, ESR1-mutant, and co-mutant subgroups. Median and interquartile ranges are reported for each cell type.

Figure 1. Differential Gene Expression in ESR1-Mutant vs. WT HR+/HER2– Metastatic Breast Cancer

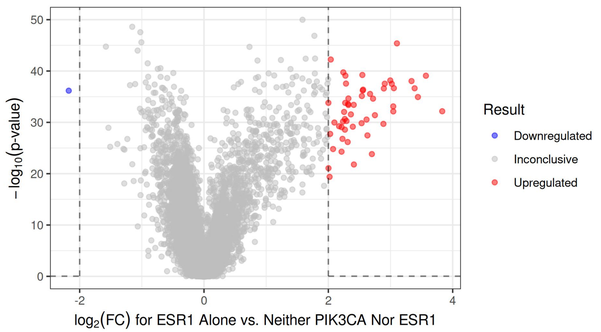

Figure 1. Volcano plot illustrating upregulated and downregulated genes in ESR1-mutant tumors compared to wild-type. Key genes with significant expression changes are highlighted.

Figure 3. Differential Gene Expression in PIK3CA-Mutant vs. WT HR+/HER2– Metastatic Breast Cancer

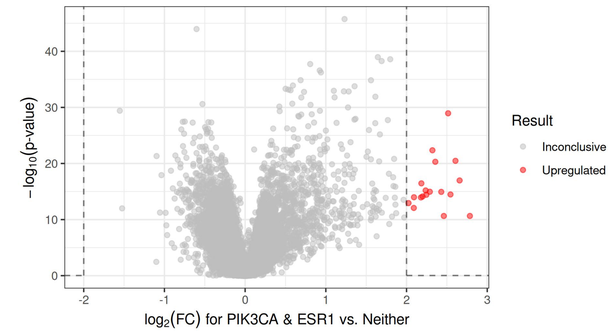

Figure 3. Volcano plot illustrating upregulated and downregulated genes in PIK3CA-mutant tumors compared to wild-type. Key genes with significant expression changes are highlighted.

Figure 2. Immune Cell Infiltration by PIK3CA and ESR1 Mutation Status in HR+/HER2– Metastatic Breast Cancer

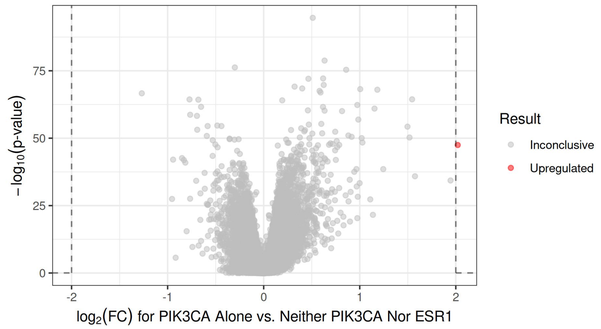

Figure 2. Volcano plot illustrating upregulated and downregulated genes in PIK3CA/ESR1 co-mutant tumors compared to wild-type. Key genes with significant expression changes are highlighted.

CONCLUSIONS

ESR1 and co-mutant tumors activate distinct estrogen-responsive gene signatures (TFF1, FGA, FGB, ALB, SCGB2A2) with downregulation of SFRP2, suggesting coordinated alterations in endocrine signaling and Wnt pathway regulation that may drive endocrine resistance.

Co-mutant tumors exhibit the most immunosuppressive phenotype, with lowest B-cell infiltration and altered macrophage/Treg balance, indicating immune evasion as a key mechanism of aggressive behavior. PIK3CA-mutant tumors paradoxically demonstrate highest macrophage and Treg enrichment despite WT-like transcriptomic profiles, suggesting PI3K activation independently shapes immune microenvironment.

These distinct transcriptomic and immune phenotypes provide therapeutic opportunities: ESR1-mutant tumors may benefit from estrogen pathway-directed therapy, while co-mutant and PIK3CA- mutant tumors warrant immunotherapy combinations to overcome suppressive immune landscapes.

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