



Integrative Genomic and Transcriptomic Analysis of Metabolic and Inflammatory Signaling Pathways in Breast Cancer

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ABSTRACT

Objective: Breast cancer exhibits complex interactions between genomic alterations and transcriptional signaling pathways; however, the clinical significance of these distinct molecular architectures remains unclear.

Material and Methods: We performed an integrative analysis of genomic alterations, gene expression, and survival outcomes in The Cancer Genome Atlas breast cancer (n=1,084), and externally validated the findings in Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) (n=2,506), yielding a combined analysis of 3,590 tumors. Metabolic (including *PIK3CA*) and inflammatory (including *IL6*) pathways were evaluated. Survival was assessed using Kaplan-Meier and Cox regression models.

Results: Genomic analysis revealed marked heterogeneity, with frequent alterations in metabolic pathway genes, particularly *PIK3CA* (34%), but minimal genomic alterations in inflammatory genes (<1%). In contrast, inflammatory signaling demonstrated preserved, subtype-associated transcriptional activity, with relatively higher expression of *IL6* and *TNF* in basal-like tumors, a pattern reproducible in METABRIC. Co-occurrence analysis demonstrated a trend toward mutual exclusivity between *PIK3CA* alterations and *IL6* signaling ($\log_2$ odds ratio=-2.58; p=0.067), suggesting that these pathways may represent alternative biological states rather than co-operating drivers. *PIK3CA* alterations were associated with overall survival in univariable analyses [hazard ratio (HR): 1.22; p=0.024] and in subtype-adjusted multivariable analyses (HR: 1.32; p=0.006), whereas *IL6* expression was not associated with outcome. These survival associations were considered exploratory given the limited availability of detailed clinical and treatment-related variables. Molecular subtype remained the dominant determinant of survival.

Conclusion: Metabolic and inflammatory pathways demonstrate distinct genomic and transcriptional patterns across breast cancer cohorts. These exploratory findings provide a basis for further investigation into their biological and microenvironmental interactions.

Keywords: Breast neoplasms; gene expression profiling; molecular subtype; tumor heterogeneity

INTRODUCTION

Breast cancer remains the most frequently diagnosed malignancy among women worldwide, with an estimated 2.3 million new cases and approximately 670,000 deaths reported globally in 2022.¹ It is characterized by substantial molecular heterogeneity and variable clinical behavior, reflecting the complex interplay of genomic, transcriptomic,

and microenvironmental factors that drive tumor progression and treatment response.²

Beyond established genetic and hormonal determinants, breast cancer progression is shaped by complex interactions between genomic alterations and transcriptional signaling networks.³ Increasing evidence suggests that tumor behavior is not driven by single molecular events, but rather by

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coordinated pathway-level activity spanning metabolic, inflammatory, and endocrine signaling axes.⁴ These processes often operate through distinct mechanisms, including somatic mutations, copy-number alterations (CNAs), and context-dependent gene expression programs. However, the extent to which these diverse molecular layers converge or remain functionally independent within breast cancer remains incompletely understood.⁵

Key oncogenic pathways in breast cancer converge on the *PI3K-AKT-mTOR* axis, a central regulator of cellular growth, metabolism, and survival, and one of the most frequently altered signaling networks in this disease.⁶ In parallel, endocrine signaling pathways continue to play a critical role, particularly through estrogen receptor-mediated transcriptional programs that shape tumor proliferation and differentiation.⁷ Additionally, inflammatory signaling mediated by cytokines such as interleukin-6 and tumor necrosis factor- α (TNF- α) contributes to tumor progression through modulation of the microenvironment, immune response, and cellular plasticity.⁸ Together, these pathways represent distinct yet interconnected signaling axes that operate across genomic and transcriptional levels in breast cancer.

Although prior studies have characterized these pathways individually, most have focused on gene expression profiles, circulating biomarkers, or clinical associations rather than integrated genomic architectures.⁹ In contrast, while large-scale genomic studies have comprehensively characterized somatic mutations, CNAs, and functional dependencies in breast cancer, the integration of these alterations at the level of interacting signaling pathways has been less systematically examined across biological axes.¹⁰ Importantly, it remains unclear whether inflammatory signaling pathways contribute to tumor biology through direct genomic disruption or primarily through non-mutational, transcriptionally regulated mechanisms, and how these processes relate to canonical oncogenic drivers.¹¹

To address this gap, we performed an *in silico* analysis of genomic alterations and transcriptional activity across key signaling pathways in breast cancer using data from The Cancer Genome Atlas (TCGA). Alteration frequencies, subtype-specific distributions, and patterns of co-occurrence and mutual exclusivity were evaluated among genes involved in metabolic, endocrine, and inflammatory signaling. Expression patterns of inflammatory genes were further examined in the independent Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) dataset to assess the reproducibility.

The present study was designed as a descriptive and exploratory analysis of genomic and transcriptional patterns across obesity-associated metabolic, endocrine, and inflammatory pathways. The observed associations are intended to generate biological hypotheses and do not establish direct mechanistic causality.

This study adopts a pathway-level framework to distinguish genomic drivers from transcriptionally active signaling programs in breast cancer. Rather than focusing on individual genes, we examine how these pathways are organized across tumors, including the relationship between recurrent genomic alterations, such as *PIK3CA*, and transcriptionally regulated inflammatory signaling, such as *IL6*. By integrating data across 3,590 tumors, we identify patterns of mutual exclusivity and subtype-specific enrichment, providing a systems-level perspective on tumor heterogeneity and highlighting the distinction between molecular architecture and clinical behavior.

MATERIAL AND METHODS

1. Data Source and Cohort Characteristics

A retrospective *in silico* integrative analysis was conducted using data from TCGA PanCancer Atlas breast cancer cohort. Comprehensive multi-omic profiles, including somatic mutations, CNAs, and messenger RNA (mRNA) expression data, were obtained for 1,084 primary invasive breast carcinoma samples from cBioPortal for Cancer Genomics.

To assess the reproducibility of transcriptional findings, gene expression patterns were evaluated in an independent cohort from the METABRIC (n=2,506). The combined analysis of 3,590 primary tumors provided a large and diverse dataset for examining subtype-specific signaling patterns and pathway-level interactions.

2. Pathway Framework and Gene Selection

To minimize post-hoc selection bias and ensure biological consistency, a gene panel was predefined based on well-characterized roles in breast cancer signaling. Genes were organized into three functional axes representing key oncogenic processes: a metabolic axis comprising *PIK3CA*, *AKT1*, and *MTOR*, reflecting *PI3K-AKT-mTOR* signaling; an endocrine axis including *ESR1* and *CYP19A1*, representing estrogen receptor signaling and hormone biosynthesis; and an inflammatory axis consisting of *LEP*, *IL6*, and *TNF*, reflecting cytokine-mediated signaling. This pathway-based framework enabled a structured comparison of genomic alterations and transcriptional activity across distinct but interacting biological systems.

3. Genomic Alteration Profiling

Somatic mutation data (including non-synonymous variants) and putative CNAs, as defined by GISTIC 2.0 calls for high-level amplifications and deep deletions, were systematically analyzed. OncoPrint visualizations were generated to characterize the distribution and patterning of genomic alterations across the cohort. Subtype-specific alteration frequencies were further evaluated according to the *PAM50* molecular classification, including luminal A, luminal B, HER2-enriched, basal-like, and normal-like subtypes.

4. Transcriptomic and Expression Analysis

To evaluate activation of non-genomic pathways, mRNA expression of *IL6*, *LEP*, and *TNF* was analyzed using RNA-Seq V2 RSEM z-scores normalized to all samples in TCGA cohort, and microarray-derived z-scores from the METABRIC dataset. Differential expression across molecular subtypes was assessed using distributional analyses and boxplot visualizations to characterize subtype-specific transcriptional patterns in the absence of recurrent somatic alterations.

5. Ethical Considerations

This study used publicly available, de-identified data from TCGA and METABRIC. No new human participants were recruited, no interventions were performed, no biological samples were collected, and no identifiable personal data were used in this secondary *in silico* analysis. Therefore, additional institutional ethics committee approval and informed consent were not required. The original datasets were generated and made available in accordance with the ethical approvals and consent procedures of the respective source studies and consortia.

6. Pathway Interaction

Pairwise co-occurrence and mutual exclusivity of genomic alterations were assessed using Fisher's exact test. Associations were quantified using $\log_2$ odds ratios (OR), where positive values indicate co-occurrence and negative values indicate mutual exclusivity.

Statistical Analysis

To account for multiple comparisons, p-values were adjusted using the Benjamini-Hochberg method, and corresponding q-values were reported.

Differential gene expression across molecular subtypes was evaluated using distributional analyses and visualized with boxplots. Survival analyses were performed using Kaplan-Meier estimates and compared using the log-rank test. Cox proportional hazards regression models were used to estimate hazard ratios (HRs) for overall survival in univariable, multivariable, and interaction analyses, with adjustment for molecular subtype where appropriate.

All statistical tests were two-sided, with a significance threshold of 0.05. Visualizations and summary statistics were generated using the cBioPortal for Cancer Genomics and R software (version 4.5.3; R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

1. Cohort Characteristics

Genomic alteration analysis was performed using the TCGA Breast Invasive Carcinoma & PanCancer Atlas cohort comprising 1,084 primary tumor samples with available somatic mutation and CNA data. All cases underwent complete genomic profiling of the predefined gene panel. The distribution of genomic alterations across the analyzed genes is summarized in Figure 1.

OncoPrint visualization illustrates somatic mutations and CNAs across key signaling pathways, including metabolic (*PIK3CA*, *AKT1*, *MTOR*), endocrine (*ESR1*, *CYP19A1*), and inflammatory (*LEP*, *IL6*, *TNF*) axes in 1,084 TCGA breast tumors. Each column represents an individual tumor, and each row represents a gene. Colored annotations indicate alteration types, including missense mutations, truncating mutations, structural variants, amplifications, and deep deletions.

2. Alteration Landscape of Signaling Pathways

Substantial heterogeneity in genomic alteration frequency was observed across the predefined gene panel (Table 1). Within the metabolic axis, *PIK3CA* demonstrated the highest

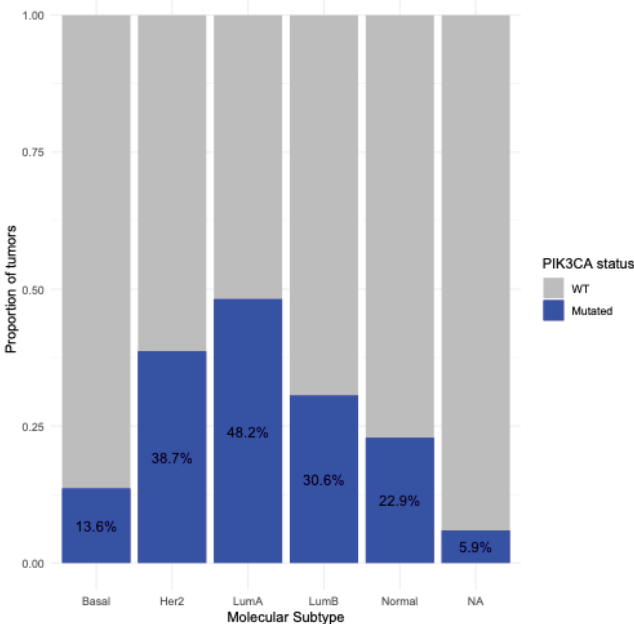


FIGURE 1: OncoPrint of signaling pathway alterations in TCGA breast cancer.

TCGA: The Cancer Genome Atlas; WT: Wild type

TABLE 1: Genomic alteration frequencies across signaling axes (n=1,084).

Functional axis	Gene symbol	Alteration frequency (%)	Primary alteration type
Metabolic axis	<i>PIK3CA</i>	34%	Missense mutation (Hotspot)
	<i>AKT1</i>	4.1%	Missense mutation/amplification
	<i>MTOR</i>	1.2%	Amplification/missense mutation
Endocrine axis	<i>ESR1</i>	2.5%	Amplification/missense mutation
	<i>CYP19A1</i>	0.8%	Amplification
Adipokine inflammatory axis	<i>LEP</i>	0.6%	Amplification/deep deletion
	<i>IL6</i>	0.5%	Deep deletion/amplification
	<i>TNF</i>	0.7%	Amplification

alteration frequency (34%), predominantly driven by hotspot missense mutations. In contrast, alterations in *AKT1* (4.1%) and *MTOR* (1.2%) were considerably less frequent.

Within the endocrine axis, *ESR1* showed a moderate alteration frequency (2.5%), primarily due to copy-number amplification, whereas alterations in *CYP19A1* were rare (0.8%).

Genes associated with inflammatory signaling, including *LEP*, *IL6*, and *TNF*, exhibited minimal frequencies of genomic alteration (<1%), with alterations largely limited to copy-number changes.

Overall, these findings indicate that genomic alterations are predominantly concentrated within the metabolic signaling axis, while inflammatory pathway genes show minimal direct genomic disruption.

Given the high prevalence of *PIK3CA* alterations, the mutation frequency of *PIK3CA* was evaluated across *PAM50* molecular subtypes in the TCGA cohort. Marked heterogeneity was observed, with the highest mutation frequency in the luminal A subtype (47.3%), followed by the HER2-enriched (32.5%) and the luminal B (29.7%) subtypes. In contrast, basal-like tumors demonstrated a substantially lower prevalence (7.0%) (Table 2).

These findings indicate a clear enrichment of *PIK3CA* alterations in hormone receptor-positive subtypes, consistent with the established association between *PI3K* pathway activation and luminal tumor biology (Figure 2).

Bar chart illustrating the proportion of tumors harboring *PIK3CA* somatic mutations across *PAM50* molecular subtypes in the TCGA breast cancer cohort (n=1,084). Mutation prevalence is highest in luminal A tumors (47.3%), followed by HER2-enriched tumors (32.5%) and luminal B tumors (29.7%), and is substantially lower in normal-like tumors (22.9%) and basal-like tumors (7.0%). These findings demonstrate marked subtype-specific enrichment of *PIK3CA* mutations within luminal breast cancers.

3. Expression Analysis of Inflammatory Genes

Despite low frequencies of somatic alterations, inflammatory pathway genes exhibited preserved transcriptional activity across molecular subtypes (Figure 3). mRNA expression analysis revealed detectable levels of *IL6*, *LEP*, and *TNF* across all *PAM50* subtypes, with substantial intra-subtype variability. Notably, higher expression levels of *IL6* and *TNF* were observed in basal-like tumors compared with other subtypes. These findings suggest that, unlike the enrichment of *PIK3CA* mutations in luminal tumors, inflammatory signaling is preferentially active in basal-like tumors through transcriptional rather than genomic mechanisms.

Box-and-whisker plots showing mRNA expression of *IL6* (A), *LEP* (B), and *TNF* (C) in the TCGA breast cancer cohort. Expression values represent log-transformed RNA-Seq V2 RSEM z-scores relative to all samples. All three genes demonstrated detectable transcriptional activity across

TABLE 2: Distribution of *PIK3CA* mutation frequency across *PAM50* molecular subtypes in the TCGA breast cancer cohort.

Subtype	Total samples (n)	Mutated samples (n)	% mutated
Luminal A	497	235	47.3%
HER2-enriched	77	25	32.5%
Luminal B	195	58	29.7%
Normal-like	35	8	22.9%
Basal-like	171	12	7.0%

TCGA: The Cancer Genome Atlas.

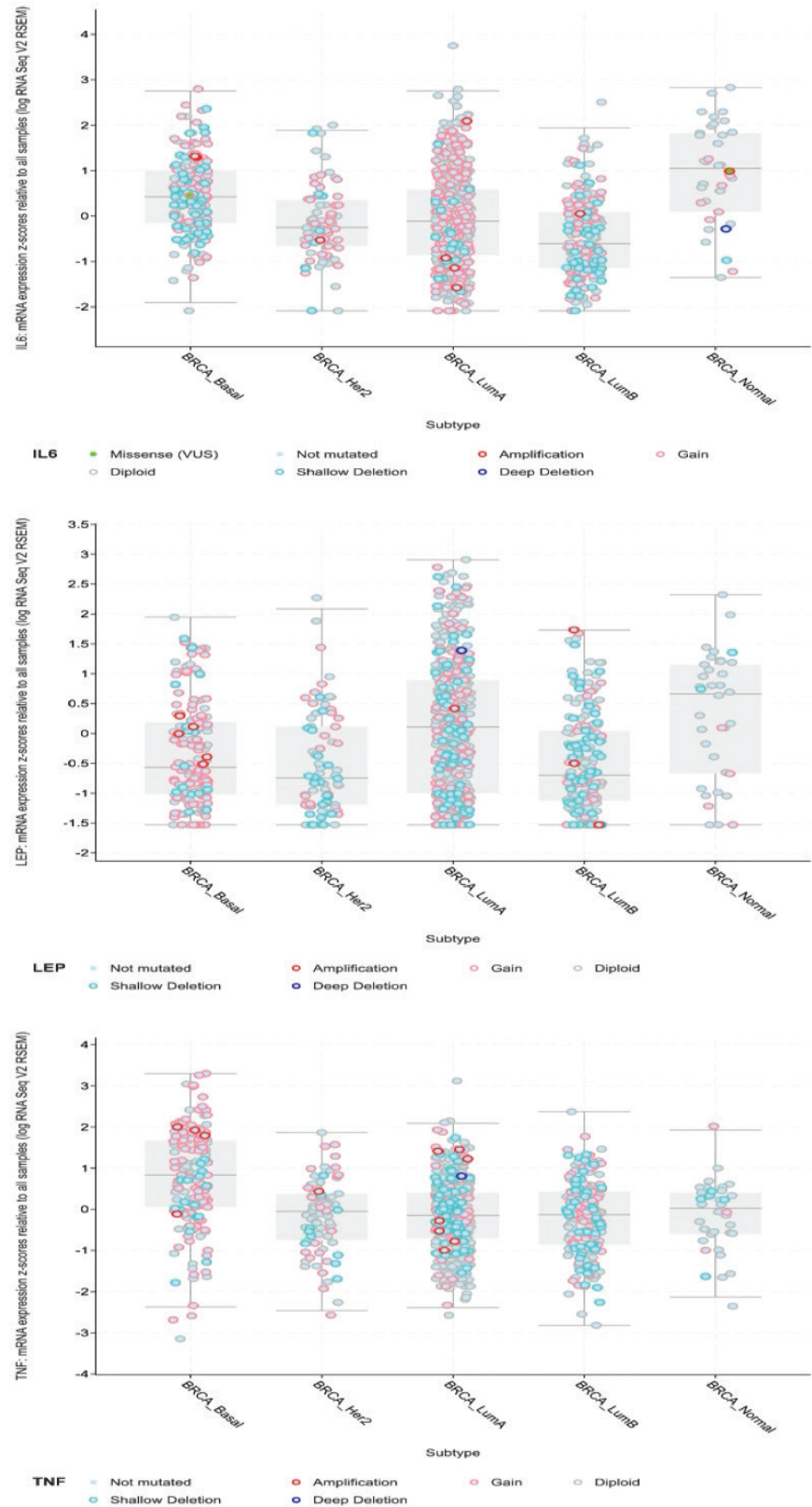


FIGURE 2: Subtype-specific distribution of *PIK3CA* mutations in breast cancer.

TNF: Tumor necrosis factor

molecular subtypes, with substantial intra-subtype variability. *IL6* and *TNF* expression showed a trend toward higher expression levels in basal-like tumors, although considerable overlap was observed across subtypes; *LEP* expression appeared relatively enriched in luminal subtypes.

4. Validation of Inflammatory Gene Expression in the METABRIC Cohort

To establish the reproducibility of the observed non-genomic activation patterns, we analyzed the expression of *IL6*, *LEP*, and *TNF* in the independent METABRIC cohort (n=2,506). Integrating this with the TCGA data creates a high-powered analysis of 3,590 primary tumors. Consistent with the low

frequency of genomic alterations observed in the primary cohort, all three inflammatory genes demonstrated robust transcriptional activity across molecular subtypes in the METABRIC dataset despite their minimal mutation frequencies. The validation confirms that the observed gene expression patterns are reproducible across independent cohorts and reflect consistent, subtype-associated transcriptional activity in breast cancer (Table 3, Figure 4).

These findings confirm that inflammatory gene expression patterns are reproducible across independent cohorts and are characterized by heterogeneous, subtype-dependent transcriptional activity rather than recurrent genomic alterations.

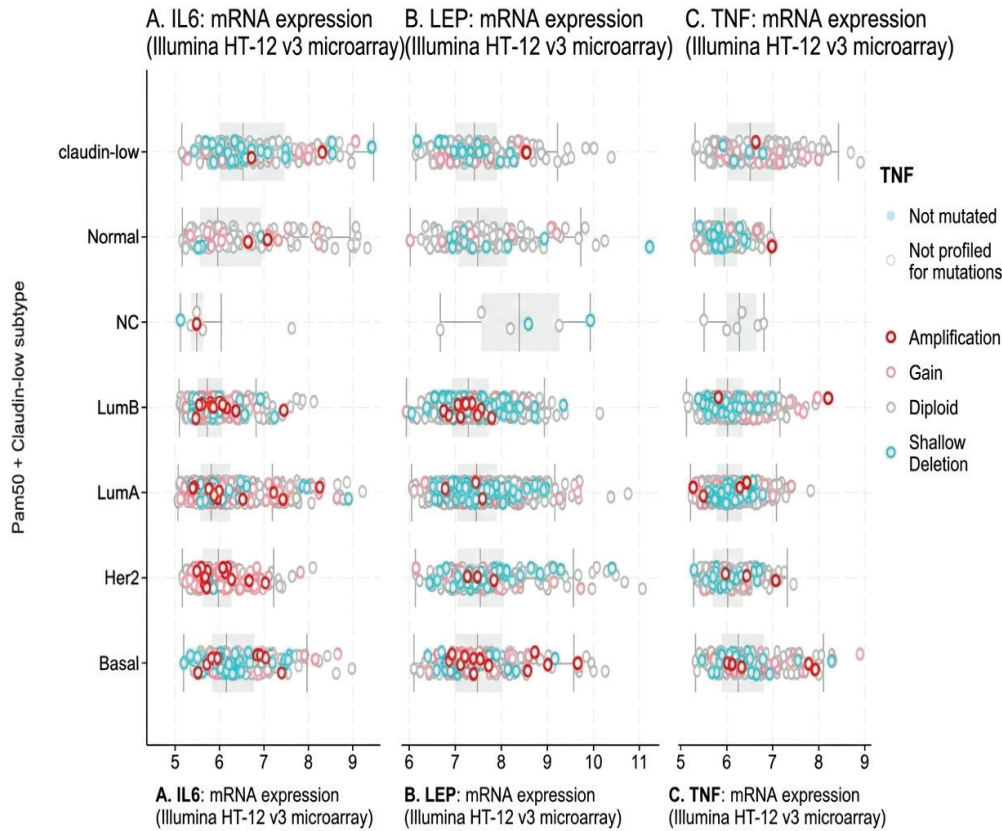


FIGURE 3: Expression of inflammatory pathway genes across PAM50 subtypes.

VUS: Variant of uncertain significance

TABLE 3: Summary of transcriptional patterns across TCGA and METABRIC cohorts

Gene	TCGA expression pattern	METABRIC expression pattern
<i>IL6</i>	Trend toward higher expression in basal-like tumors with substantial overlap across subtypes	Similar distribution with heterogeneous expression and no clear subtype separation
<i>LEP</i>	Relatively enriched in luminal subtypes	Consistent enrichment in luminal subtypes
<i>TNF</i>	Trend toward higher expression in basal-like tumors with marked variability	Similar heterogeneous pattern with broad overlap across subtypes

METABRIC: Molecular Taxonomy of Breast Cancer International Consortium; TCGA: The Cancer Genome Atlas.

Box-and-whisker plots illustrate the mRNA expression profiles of *IL6* (A), *LEP* (B), and *TNF* (C) across *PAM50* molecular subtypes in the METABRIC cohort. Expression values are represented as z-scores derived from microarray intensity data. Consistent with the TCGA findings, all three genes demonstrate preserved transcriptional activity across molecular subtypes despite low frequencies of genomic alterations. Expression patterns remain heterogeneous, with a trend toward higher *IL6* and *TNF* expression in basal-like and claudin-low subtypes, and a relative enrichment of *LEP* expression in luminal subtypes.

5. Patterns of Co-occurrence and Mutual Exclusivity

Pairwise analysis of genomic alterations revealed distinct interaction patterns across signaling pathways (Table 4).

Estrogen receptor-related genes tended to co-occur with inflammatory signaling components, including *ESR1-LEP* ($\log_2$ OR: 2.77, $p=0.05$) and *ESR1-TNF* ($\log_2$ OR: 2.30, $p=0.08$), suggesting potential coordination between endocrine and inflammatory axes.

In contrast, alterations in key metabolic drivers exhibited a trend toward mutual exclusivity with inflammatory pathway genes. *PIK3CA* and *IL6* alterations showed a trend toward mutual exclusivity ($\log_2$ OR: -2.58, $p=0.067$). The trend may indicate that tumors harboring *PI3K* pathway mutations may be less likely to exhibit inflammatory gene alterations. Similarly, *PIK3CA* and *AKT1* alterations demonstrated a tendency toward mutual exclusivity, consistent with their roles within the same signaling cascade. These findings suggest that breast tumors

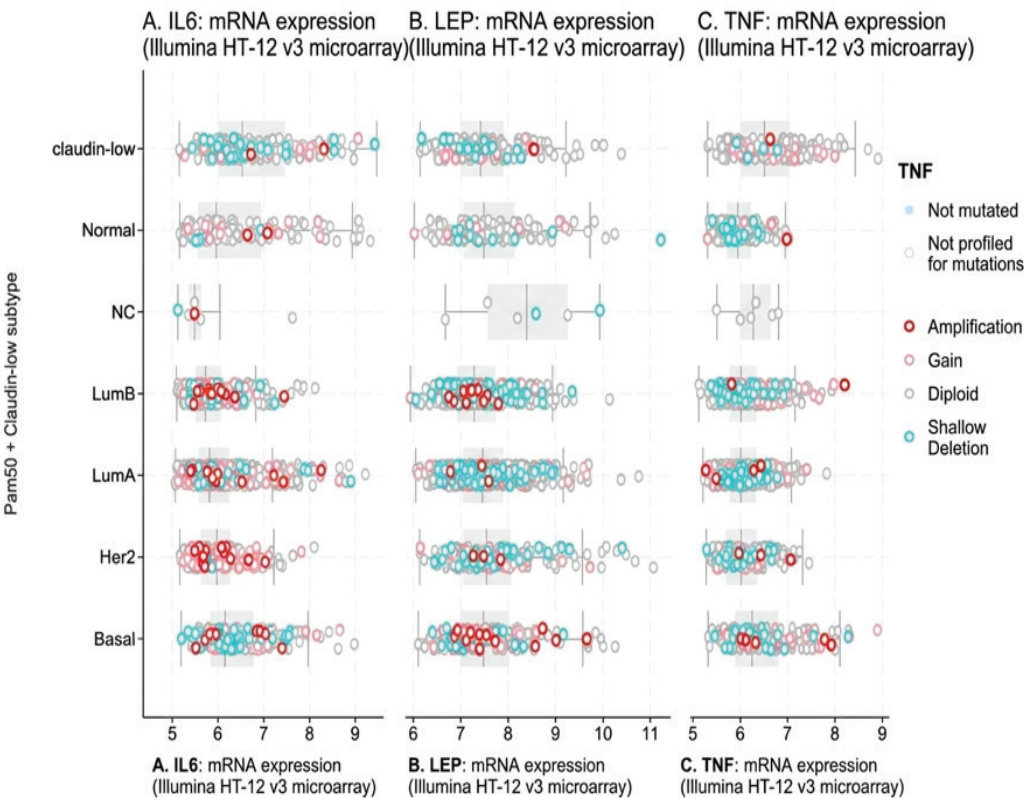


FIGURE 4: Independent validation of expression of inflammatory genes in the METABRIC cohort (n=2,506).

mRNA: Messenger RNA; METABRIC: Molecular Taxonomy of Breast Cancer International Consortium

TABLE 4: Pairwise co-occurrence and mutual exclusivity patterns across signaling pathways.

Gene pair (A vs. B)	Pathway interaction	$\log_2$ odds ratio	p-value	Association trend
<i>ESR1</i> vs. <i>LEP</i>	Endocrine-adipokine	2.77	0.050	Co-occurrence (borderline)
<i>ESR1</i> vs. <i>TNF</i>	Endocrine-inflammatory	2.30	0.081	Co-occurrence (trend)
<i>PIK3CA</i> vs. <i>IL6</i>	Metabolic-inflammatory	-2.58	0.067	Mutual exclusivity (trend)
<i>AKT1</i> vs. <i>MTOR</i>	Intra-metabolic	1.15	0.452	No significant association
<i>ESR1</i> vs. <i>PIK3CA</i>	Endocrine-metabolic	-0.42	0.320	No significant association

may preferentially engage either genomic *PI3K* pathway activation or transcriptionally-driven inflammatory signaling, rather than activating both concurrently.

Associations were assessed using Fisher's exact test. Positive $\log_2$ ORs indicate co-occurrence, whereas negative values indicate mutual exclusivity. These findings suggest that breast tumors may preferentially engage either genomic *PI3K* pathway activation or transcriptionally driven inflammatory signaling, rather than concurrently activating both pathways.

6. Survival Analysis

In univariable analysis, *PIK3CA* alteration was associated with overall survival [HR: 1.22; 95% confidence interval (CI): 1.03-1.46; $p=0.024$], whereas *IL6* expression was not associated with overall survival ($p=0.42$). In a multivariable Cox regression model adjusted for molecular subtype, *PIK3CA* alteration remained significantly associated with overall survival (HR: 1.32; 95% CI: 1.08-1.61; $p=0.006$), whereas *IL6* expression was not associated with outcome ($p=0.28$).

Among molecular subtypes, HER2-enriched tumors were associated with poorer survival compared with the reference group (HR: 2.16; $p=0.023$). Given the limited availability of detailed clinical and treatment-related variables in the publicly available dataset, these associations should be interpreted as exploratory and not considered evidence of an independent prognostic effect of *PIK3CA* alteration. Molecular subtype remained the dominant clinical correlate of survival in the available data.

7. Integrated Pathway-level Interpretation

When considered collectively, the observed alteration patterns highlight distinct signaling axes in breast cancer, with limited overlap between genomic and transcriptional processes. Metabolic pathway genes, particularly *PIK3CA*, exhibited frequent genomic alterations consistent with driver events, whereas inflammatory pathway genes demonstrated minimal genomic disruption but retained transcriptional activity. Estrogen receptor signaling exhibited partial overlap with inflammatory pathways, as reflected by trends toward co-occurrence, suggesting a potential, context-dependent integrative role across these signaling networks.

These findings support a model in which genomic activation of metabolic pathways and transcriptionally mediated inflammatory signaling represent distinguishable molecular patterns, while their biological interaction may be shaped by immune, stromal, endocrine, and cytokine-mediated processes within the tumor microenvironment.

DISCUSSION

Breast cancer is characterized by substantial molecular heterogeneity, yet the relative contribution of genomic alterations versus transcriptional regulation across key signaling pathways remains incompletely understood.^{11,12} By integrating data from the TCGA and METABRIC cohorts, this study delineates a distinct molecular architecture in which metabolic pathways are predominantly driven by somatic mutations, whereas inflammatory and related signaling pathways are largely maintained through transcriptional activity rather than recurrent genomic alterations.¹³⁻¹⁵

Among the analyzed pathways, the PI3K-AKT-mTOR pathway emerged as the most genomically altered network, driven predominantly by hotspot missense mutations in *PIK3CA*, which were observed in approximately one-third of tumors. This frequency is consistent with extensive evidence identifying the *PI3K-AKT-mTOR* axis as a central regulator of cellular growth, metabolism, and survival. Activation of this pathway integrates upstream growth factor signaling and promotes tumor proliferation, metabolic reprogramming, and resistance to apoptosis.^{16,17} Notably, in analyses stratified by subtype, *PIK3CA* mutations were enriched in luminal A tumors and comparatively rare in basal-like cancers, indicating preferential activation of *PI3K* pathway-driven oncogenic programs within hormone receptor-positive disease. Despite its high prevalence, *PIK3CA* alteration was statistically significantly associated with overall survival after adjustment for molecular subtype. However, this finding should be interpreted with caution because the available dataset did not provide comprehensive information on potentially important clinical and treatment-related determinants of outcomes. The observed association, therefore, should not be interpreted as establishing *PIK3CA* as an independent prognostic biomarker. Rather, it provides an exploratory observation that warrants validation in clinically annotated cohorts with detailed information on stage, treatment, age, comorbidities, and other established prognostic factors. Collectively, these findings support the concept that genomic activation of metabolic signaling pathways reflects intrinsic tumor biology, while its relationship with clinical outcome may depend on molecular subtype and other clinical factors.¹⁸⁻²⁰

A key finding of this study is the frequency of genomic alterations observed in inflammatory-pathway genes. Despite their established roles in tumor progression, *LEP*, *IL6*, and *TNF* demonstrated negligible mutation frequencies ($<1\%$).²¹ This observation is consistent with experimental and clinical evidence indicating that inflammatory signaling in breast cancer is largely mediated through transcriptional regulation, cytokine secretion, and tumor-microenvironment interactions

rather than recurrent somatic mutations.^{22,23} Cytokines such as *IL6* and *TNF- α* can promote tumor growth, angiogenesis, immune modulation, and stromal remodeling through paracrine and endocrine signaling mechanisms, largely independent of direct genomic alteration.^{24,25} Supporting this non-mutational model, our expression analyses demonstrated both preserved and heterogeneous transcriptional activity of *IL6*, *LEP*, and *TNF* across molecular subtypes, with relatively higher expression of *IL6* and *TNF* in basal-like tumors. These findings are consistent with a model in which inflammatory signaling in breast cancer is predominantly shaped by transcriptional and microenvironmental mechanisms rather than recurrent mutation-driven oncogenesis.²⁶

The relationship between inflammatory and metabolic signaling is likely influenced by the cellular and structural composition of the tumor microenvironment.²⁷ Although the present analysis identified distinct genomic and transcriptional patterns, expression of inflammatory mediators such as *IL6* and *TNF* cannot be attributed exclusively to malignant epithelial cells. These cytokines may be produced by infiltrating immune cells, cancer-associated fibroblasts, endothelial cells, and other stromal populations, and their activity may vary according to the composition and functional state of the tumor microenvironment.²⁸ Differences in immune cell infiltration, macrophage polarization, stromal remodeling, and extracellular matrix interactions may, therefore, contribute to the heterogeneous inflammatory transcriptional patterns observed across molecular subtypes. Cytokine signaling may also interact with metabolic and endocrine pathways through paracrine and autocrine mechanisms, potentially influencing *PI3K-AKT-mTOR* signaling, cellular proliferation, immune modulation, and therapeutic response. Thus, the distinction observed between mutation-associated metabolic signaling and transcriptionally active inflammatory signaling should not be interpreted as complete biological independence.²⁹ Rather, these pathways may remain interconnected through cellular and microenvironmental mechanisms that are not captured by genomic alteration analysis alone.

Estrogen receptor signaling represents a central biological axis in breast cancer, particularly within hormone receptor-positive subtypes. In our cohort, *ESR1* demonstrated moderate alteration frequency, predominantly through copy-number amplification, consistent with prior genomic analyses of luminal breast cancers.³⁰

Co-occurrence analysis revealed trends toward interactions between estrogen signaling and inflammatory pathways, including associations between *ESR1* and *LEP* and between *ESR1* and *TNF*. These patterns suggest that estrogen receptor

signaling may function as a context-dependent integrative node linking genomic and transcriptional signaling processes, although these associations did not reach statistical significance and should be interpreted with caution.

The observed trend toward mutual exclusivity between *PIK3CA* and *IL6* alterations raises the possibility of distinct biological states, although this finding requires further validation.³¹ Although *PIK3CA* alteration showed an exploratory association with overall survival, this finding should be interpreted cautiously given the limited clinical and treatment-related information available in the public datasets. Molecular subtype remained the dominant determinant of outcome in the available analysis.³²

Such divergent molecular strategies are consistent with established models of breast cancer heterogeneity, in which tumors exploit distinct biological programs to achieve similar malignant phenotypes.³³ These observations may have implications for therapeutic stratification, particularly in the context of pathway-directed and immune-targeted interventions.

Clinical and Translational Implications

Our findings provide a hypothesis-generating framework for understanding distinct genomic and transcriptional signaling breast cancer, and may inform future approaches to molecular stratification.

- **Genomic vs. Transcriptional Driver Identification:** The relative genomic quiescence of the inflammatory axis (*IL6*, *TNF*, *LEP*) suggests that standard DNA-based next-generation sequencing panels may fail to capture key drivers of tumor progression in a subset of patients. Integrated transcriptomic profiling or assessment of cytokine activity may therefore be required to fully characterize tumor biology, as these pathways appear to be driven by transcriptional regulation rather than recurrent somatic mutations.^{19,34-37}
- **Stratified Therapeutic Strategies:** The observed trend toward mutual exclusivity between *PIK3CA* and *IL6* alterations is hypothesis-generating and may warrant further investigation in relation to immune-cell composition, cytokine activity, and stromal features. Tumors with genomic activation of the *PI3K* pathway may benefit from established pathway-directed therapies, whereas inflammatory signaling may constitute a distinct therapeutic context that requires further evaluation of microenvironment-directed approaches. Integrated genomic, transcriptomic, immune, and microenvironmental studies are needed to determine whether these molecular patterns identify clinically meaningful therapeutic vulnerabilities.³⁸

• **Estrogen Receptor as a Regulatory Hub:** The observed co-occurrence between *ESR1* and inflammatory signaling components suggests that estrogen receptor activity may function as a context-dependent integrative node linking genomic and transcriptional signaling pathways. This raises the possibility that combining endocrine therapy with agents targeting inflammatory signaling could enhance therapeutic efficacy in selected molecular contexts, although this requires further validation.³⁹

• Our analysis may help explain the observed heterogeneity in responses to targeted therapies, informing clinical trial stratification. By distinguishing between mutation-driven metabolic signaling and non-genomic inflammatory pathway activation, future clinical trials may be better positioned to stratify patients based on underlying signaling dependencies, thereby improving therapeutic precision and clinical outcomes.⁴⁰

Importantly, this study is descriptive and exploratory. The identified patterns and associations between genomic alterations, gene expression, and molecular subtypes do not establish direct mechanistic or causal relationships. In particular, the observed differences between metabolic and inflammatory signaling should be considered hypothesis-generating and require validation through functional, cellular, and longitudinal studies.

Study Limitations

This study has several limitations. First, the analysis was based on publicly available genomic datasets with limited clinical annotation. Detailed information on age, disease stage, treatment exposure, treatment response, comorbidities, and other established prognostic factors was not consistently available for adjustment. Consequently, the observed associations between genomic alterations and survival should be considered exploratory and cannot establish independent prognostic effects. Second, bulk tumor transcriptomic data cannot distinguish tumor-cell-intrinsic expression from contributions of immune and stromal cells. Immune infiltration, cytokine activity, macrophage polarization, and stromal composition were not directly assessed and warrant evaluation in future studies using integrated microenvironmental and single-cell approaches. Finally, functional validation studies are required to confirm the biological mechanisms suggested by the genomic and transcriptional associations identified in this analysis.

CONCLUSION

Breast cancer signaling is not uniformly driven by recurrent genomic alterations. In this multi-cohort analysis, metabolic

pathways, particularly those involving *PIK3CA*, showed frequent genomic alterations, whereas inflammatory pathways demonstrated limited genomic alterations while preserving transcriptional activity. These findings suggest that metabolic and inflammatory pathways have distinct genomic and transcriptional patterns, while their biological interactions may involve additional endocrine, immune, and microenvironmental mechanisms. Although *PIK3CA* alterations showed an exploratory association with survival, molecular subtype remained the dominant determinant of clinical outcome. Overall, these descriptive findings support an integrative approach to understanding breast cancer biology and provide a hypothesis-generating framework for future studies investigating the relationships between genomic alterations, transcriptional regulation, and the tumor microenvironment.

Ethics

Ethics Committee Approval: This study used publicly available, de-identified data from TCGA and METABRIC. No new human participants were recruited, no interventions were performed, no biological samples were collected, and no identifiable personal data were used in this secondary *in silico* analysis.

Informed Consent: Therefore, additional institutional ethics committee approval and informed consent were not required. The original datasets were generated and made available in accordance with the ethical approvals and consent procedures of the respective source studies and consortia.

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Footnotes

Author Contributions

Concept: N.S., R.V., Design: N.S., Data Collection or Processing: N.S., B.S., Analysis or Interpretation: N.S., B.S., Literature Search: N.S., B.S., S.B., Writing: N.S., B.S., S.B., R.V.

Conflict of Interest: No conflict of interest was declared by the authors.

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AI Disclosure Statement: During the preparation of this work, the authors used generative AI tools to assist with language editing and to improve readability. The authors have reviewed and edited all generated content and take full responsibility for the content of the manuscript.

Data Availability: The genomic and transcriptomic datasets analyzed during the current study are available in the cBioPortal for Cancer Genomics (<https://www.cbioportal.org>), specifically the TCGA (PanCancer Atlas) and METABRIC cohorts.

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