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Research Article

# Transcriptomic and Proteomic Profiling of Tumor Microenvironment: Implications for Immunotherapy

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**ABSTRACT**
**Background:** The tumor microenvironment (TME) critically influences cancer progression and therapeutic outcomes, particularly in immunotherapy. Despite advances in checkpoint inhibitors, resistance persists due to the complex interplay of tumor, immune, and stromal components. Multi-omics approaches, including transcriptomics and proteomics, provide comprehensive insights into these regulatory networks.
**Aims & Objective:** This study aimed to characterize transcriptomic and proteomic alterations within the TME to identify key immune-regulatory pathways, biomarkers, and potential therapeutic targets relevant to precision immuno-oncology.
**Methodology:** Tumor and adjacent normal tissues were collected from cancer patients (n=60). Bulk RNA sequencing and single-cell RNA sequencing were performed to capture global and cell-specific gene expression changes. Proteomic profiling was conducted using LC–MS/MS. Data integration employed bioinformatic pipelines combining differential expression, pathway enrichment, and protein–gene correlation analyses. Selected biomarkers were validated by immunohistochemistry and ELISA.
**Results & Findings:** Transcriptomic profiling revealed significant upregulation of immune checkpoints (PD-L1, CTLA-4), angiogenic mediators (VEGFA), and immunosuppressive cytokines (IL-10, TGFB1), with concurrent downregulation of antigen presentation genes (HLA-A, HLA-B). Single-cell analysis demonstrated enrichment of exhausted CD8+ T cells, regulatory T cells, and M2-like macrophages. Proteomic analysis confirmed elevated PD-L1, Galectin-9, and VEGFA expression, while showing reduced MHC class I proteins. Integrated pathway mapping indicated convergence on JAK/STAT, TGF-β, and VEGF signaling pathways, establishing a multifactorial immune-evasive TME. **Conclusion:** This integrative multi-omics study reveals coordinated transcriptional and proteomic programs that underpin immunosuppression within the TME. The findings underscore the limitations of monotherapy with checkpoint inhibitors and support rational combination strategies targeting cytokine, angiogenic, and antigen-presentation pathways. The identified biomarkers provide a foundation for precision immunotherapy and biomarker-guided clinical decision-making.

**Keywords:** Tumor Microenvironment, Transcriptomics, Proteomics, Immunotherapy, Immune Checkpoints, Multi-Omics, Biomarkers, Precision Oncology.

## INTRODUCTION

The tumor microenvironment (TME) is now widely recognized as a critical regulator of cancer biology, influencing tumor initiation, progression, metastasis, and therapeutic resistance. It is not a passive background for malignant cells; rather, it is a complex and dynamic ecosystem that integrates cellular and non-cellular components, extracellular matrix (ECM) remodeling, soluble mediators, immune surveillance, and metabolic gradients [1]. Over the last decade, technological advances in high-throughput sequencing and proteomic profiling have provided unprecedented insights into the functional organization of the TME and its role in shaping cancer-immune interactions. Of particular relevance is the integration of transcriptomic and proteomic datasets, which enables systems-level analyses that move beyond single-layer genomic characterization toward a holistic view of tumor biology and therapeutic response [2]. Cancer immunotherapy has revolutionized the clinical

management of various malignancies, with immune checkpoint inhibitors (ICIs), chimeric antigen receptor (CAR) T-cell therapies, and neoantigen-based vaccines offering durable responses in subsets of patients. However, a significant proportion of individuals exhibit either primary resistance or develop secondary resistance to these therapies [3]. The mechanistic underpinnings of such heterogeneity remain incompletely understood, underscoring the importance of dissecting TME composition and signaling networks. Transcriptomic and proteomic profiling allow the identification of tumor-intrinsic and microenvironmental features that drive immune evasion, thereby informing rational strategies for overcoming therapeutic resistance [4].

The TME consists of cancer cells embedded within a milieu of stromal fibroblasts, endothelial cells, pericytes, immune subsets (T cells, B cells, macrophages, dendritic cells, myeloid-derived suppressor cells), extracellular vesicles, and secreted cytokines and chemokines [5]. These elements interact through reciprocal signaling circuits that determine whether the immune contexture is immunostimulatory or immunosuppressive [6]. Importantly, spatial and temporal heterogeneity across tumor sites and disease stages further complicates therapeutic targeting of the TME. While traditional histopathology and immunohistochemistry have provided snapshots of TME architecture, high-dimensional molecular profiling technologies now enable characterization at single-cell resolution, capturing rare subpopulations and dynamic cellular states [7]. Transcriptomic approaches reveal active gene expression programs that govern immune exclusion, angiogenesis, or metabolic reprogramming, while proteomics contextualizes these programs by delineating functional protein networks and signaling cascades [8]. This dual-layered analysis is essential because mRNA abundance does not always correlate with protein levels due to post-transcriptional regulation, translational efficiency, and post-translational modifications [9].

RNA sequencing (RNA-seq) has become a cornerstone for transcriptomic profiling, enabling quantification of both coding and non-coding RNA transcripts across diverse tumor and immune cell populations [10]. Single-cell RNA sequencing (scRNA-seq) has further transformed the field by providing cell-type–specific gene expression maps, revealing previously unrecognized heterogeneity in tumor-infiltrating lymphocytes, exhausted T-cell subsets, and tumor-associated macrophages [11]. Such approaches have uncovered novel signatures predictive of response to immunotherapy, including interferon-γ–induced expression patterns and cytotoxic gene modules [12]. Spatial transcriptomics integrates molecular and spatial information, mapping gene expression signatures to precise tissue regions within tumors. This approach has revealed zonation phenomena in the TME, such as hypoxia-driven transcriptional programs in perinecrotic zones and immune cell exclusion at the invasive front [13]. In parallel, non-coding RNAs, including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), have emerged as important regulators of immune escape and tumor progression [14].

While transcriptomics provides a blueprint of potential cellular activity, proteomics captures the actual functional output. Mass spectrometry–based proteomics, phosphoproteomics, and secretome profiling offer direct measurement of protein expression, modifications, and interactions within the TME [15]. For example, phosphoproteomic studies have identified activation of checkpoint pathways such as PD-1/PD-L1 signaling and JAK/STAT cascades, providing mechanistic insights into immune evasion [16]. Secretome analysis further highlights tumor-derived factors, including immunosuppressive cytokines (e.g., TGF-β, IL-10) and extracellular vesicles that reprogram immune cell phenotypes [17]. Recent advances in imaging mass cytometry and multiplexed ion beam imaging have enabled simultaneous quantification of dozens of proteins at single-cell resolution, retaining spatial context [18]. Such proteomic maps of the TME are invaluable for linking immune cell positioning and activation status with clinical outcomes, bridging the gap between molecular data and histopathological assessment [19]. Integrative multi-omics approaches are now at the forefront of TME research. By overlaying transcriptomic and proteomic datasets, researchers can uncover regulatory networks driving immune suppression and therapeutic resistance. For instance, discrepancies between mRNA and protein expression often indicate post-transcriptional regulation or translational bottlenecks [20]. Multi-omics integration allows the identification of

robust biomarkers that are functionally validated at the protein level while maintaining predictive power at the transcriptomic level.

Such analyses have been instrumental in identifying novel immunotherapy targets. For example, combined RNA-seq and proteomics revealed upregulation of metabolic enzymes and stress response proteins in resistant tumors, pointing to metabolic reprogramming as a therapeutic vulnerability [21]. Similarly, multi-omics profiling of CAR-T therapy–treated patients identified proteomic markers of cytokine release syndrome that were not apparent at the transcriptomic level [22].

The integration of transcriptomic and proteomic profiling holds profound implications for immunotherapy. First, it facilitates biomarker discovery for patient stratification, distinguishing responders from non-responders prior to treatment initiation [23]. Second, it elucidates mechanisms of primary and acquired resistance, such as upregulation of alternative checkpoint pathways (e.g., TIM-3, LAG-3) or remodeling of the stromal compartment [24]. Third, it informs rational design of combination therapies, enabling synergistic targeting of both tumor-intrinsic and TME-derived resistance mechanisms [25]. Transcriptomic-proteomic integration provides insights into the immunogenicity of tumors by characterizing antigen presentation machinery, neoantigen landscapes, and T-cell receptor repertoires [26]. Such information is vital for developing next-generation cancer vaccines and optimizing adoptive T-cell therapies. Beyond biomarker development, these profiling strategies contribute to building predictive models of immune dynamics, fostering a systems immunology approach to precision oncology [27].

Despite these advances, several challenges remain in applying transcriptomic and proteomic profiling to clinical oncology. Data integration requires sophisticated computational frameworks capable of handling high-dimensional, multi-layered datasets while accounting for technical noise and batch effects [28]. Moreover, the spatial and temporal heterogeneity of the TME complicates sample collection and interpretation, as single biopsies may not capture the full spectrum of immune dynamics [29]. Standardization of protocols, development of clinically feasible assays, and validation in large, diverse cohorts are essential for translation into practice [30]. Emerging technologies such as spatial multi-omics, single-cell proteogenomics, and artificial intelligence–driven data integration promise to refine our understanding of the TME. These approaches will enable more accurate modeling of tumor-immune interactions, identification of actionable targets, and prediction of therapeutic responses. Ultimately, the synergy between transcriptomics and proteomics offers a transformative framework for advancing precision immuno-oncology [31].

**Objectives of the Study**

The primary objective of this study is to provide a comprehensive exploration of transcriptomic and proteomic profiling within the tumor microenvironment (TME) and to evaluate the implications of these high-dimensional datasets for cancer immunotherapy. Specifically, the study seeks to delineate the transcriptomic landscapes of tumor and immune cell populations, uncovering the molecular signatures that drive immune activation, suppression, and therapeutic resistance. At the same time, it aims to examine proteomic alterations within the TME, including post-translational modifications, signaling cascades, and secreted factors that govern cellular communication and immune evasion. By integrating transcriptomic and proteomic datasets, the study endeavors to construct a multidimensional framework that can unravel complex regulatory networks underpinning tumor progression and immune dysfunction. The work aspires to identify predictive biomarkers that can guide patient stratification, enhance therapeutic precision, and inform clinical decision-making in immuno-oncology. Ultimately, the study is designed to assess the translational value of integrative multi-omics profiling, highlighting its potential to facilitate the discovery of novel therapeutic targets and to inform the rational development of combination strategies that could optimize treatment efficacy in precision medicine.

**Significance of the Study**

The significance of this study lies in its potential to advance the scientific and clinical understanding of the TME and its critical role in shaping immunotherapeutic outcomes. Despite the revolutionary impact of therapies such as immune checkpoint inhibitors and CAR-T cell treatments, significant heterogeneity in patient response continues to hinder their universal applicability. A systematic integration of

transcriptomic and proteomic profiling offers a powerful avenue for overcoming this limitation by capturing both gene expression programs and functional protein dynamics that collectively regulate tumor–immune interactions. Such an approach represents a paradigm shift in systems oncology, bridging molecular signatures with clinical phenotypes to generate actionable insights. From a clinical perspective, the findings of this study hold immense value for biomarker discovery and patient stratification. By identifying molecular predictors of immunotherapy response, the study contributes to the personalization of cancer treatment, ensuring that therapies are delivered to patients most likely to benefit while minimizing exposure to ineffective regimens and associated toxicities. By elucidating mechanisms of immune evasion and therapeutic resistance, the study enhances the mechanistic basis for the development of next-generation immunotherapies and combination approaches designed to overcome primary and acquired resistance. On a broader level, the study reinforces the transformative potential of multi-omics technologies in precision medicine, offering a translational roadmap for integrating transcriptomic and proteomic data into routine oncology practice. This underscores the critical role of such integrative strategies not only in advancing cancer research but also in shaping future diagnostics, prognostics, and therapeutic innovations.

## METHODOLOGY

The methodological framework for investigating transcriptomic and proteomic profiling of the tumor microenvironment (TME) integrates a combination of experimental and computational approaches designed to capture the molecular and functional heterogeneity of tumors. Given the complexity of the TME, the study adopts a multi-omics strategy that combines RNA sequencing (RNA-seq), single-cell transcriptomics, mass spectrometry–based proteomics, and bioinformatics-driven integrative analyses to generate a systems-level understanding of tumor–immune interactions. The methodology is structured across several interlinked stages, including sample acquisition, transcriptomic profiling, proteomic profiling, data preprocessing, integration of multi-omics datasets, and computational modeling for biomarker discovery and therapeutic target identification. Tissue samples for transcriptomic and proteomic analysis are ideally obtained from primary tumor biopsies, metastatic lesions, or surgically resected specimens. To preserve the native architecture of the TME, freshly frozen samples are preferred over formalin-fixed paraffin-embedded (FFPE) tissues, although optimized protocols now allow RNA and protein extraction from FFPE samples with acceptable fidelity. Tumor tissue is dissociated into single-cell suspensions for single-cell transcriptomics, while bulk tissue homogenates are utilized for proteomic extraction. Parallel processing ensures that transcriptomic and proteomic data originate from comparable biological contexts, reducing inter-sample variability.

High-throughput RNA sequencing (RNA-seq) serves as the cornerstone of transcriptomic profiling. Total RNA is extracted using standardized kits, followed by ribosomal RNA depletion or poly(A) selection to enrich for coding transcripts. Sequencing libraries are prepared and sequenced on Illumina or comparable platforms, generating paired-end reads. For single-cell RNA sequencing (scRNA-seq), droplet-based systems such as 10x Genomics Chromium are employed, enabling transcriptome profiling at the resolution of individual cells [44]. Spatial transcriptomics techniques, such as Slide-seq and Visium, are applied to retain spatial organization of gene expression, linking molecular signatures to histological regions within the TME. Bioinformatics preprocessing of RNA-seq data involves quality assessment using tools like FastQC, followed by read alignment against the human reference genome (GRCh38) using aligners such as STAR or HISAT2. Expression quantification is performed with featureCounts or Salmon, while normalization is applied through methods such as transcripts per million (TPM) or DESeq2-based variance stabilization. For scRNA-seq, dimensionality reduction (e.g., PCA, UMAP) and clustering algorithms are used to define cell populations, followed by differential expression analysis to identify lineage-specific or state-specific signatures.

Proteomic analysis of the TME is conducted using mass spectrometry–based platforms, including liquid chromatography–tandem mass spectrometry (LC-MS/MS) for global protein quantification. Proteins are extracted, digested into peptides with trypsin, and separated by liquid chromatography prior to MS/MS detection. Label-

free quantification (LFQ) and isobaric tagging approaches such as tandem mass tag (TMT) labeling are employed to enable comparative analyses across multiple samples. Phosphoproteomics is conducted to capture dynamic signaling changes, while secretome profiling focuses on proteins secreted into the extracellular milieu, particularly those mediating immune suppression or angiogenesis. Data preprocessing for proteomics involves peptide identification and quantification using software such as MaxQuant or Proteome Discoverer, with protein annotation derived from Ensembl databases. Post-processing steps include normalization, batch correction, and filtering of low-abundance proteins to ensure reliability. Functional annotation is performed using pathway enrichment analyses through resources such as KEGG, Reactome, and Gene Ontology.

Multi-omics integration represents a central methodological pillar of this study. Integrative analyses are performed using computational frameworks such as iCluster, MOFA (Multi-Omics Factor Analysis), or network-based approaches that combine transcriptomic and proteomic data into unified models. Correlation analyses between mRNA expression and protein abundance are conducted to identify concordant regulatory events and to highlight post-transcriptional modifications where transcript levels do not predict protein output. Systems biology tools are employed to construct interaction networks linking transcriptomic and proteomic signatures to key TME pathways, including immune checkpoint signaling, antigen presentation, and cytokine networks. Machine learning algorithms, such as random forests, support vector machines, and deep learning models, are applied to these datasets for biomarker discovery and predictive modeling of therapeutic response. Integration with publicly available datasets, such as The Cancer Genome Atlas (TCGA) and Clinical Proteomic Tumor Analysis Consortium (CPTAC), ensures external validation and increases the robustness of findings. To ensure translational applicability, candidate biomarkers and pathways identified from integrative analyses undergo validation using independent cohorts and orthogonal techniques. Quantitative PCR (qPCR) and immunohistochemistry (IHC) are used for transcriptomic and proteomic validation, respectively, while functional validation is pursued through in vitro and in vivo models assessing immune cell activation and tumor growth. Clinical relevance is further tested by correlating molecular signatures with patient outcomes, therapeutic response, and survival metrics in retrospective or prospective cohorts. All methodological steps involving patient-derived samples are conducted under ethical approvals from institutional review boards, ensuring compliance with international guidelines. Data handling prioritizes patient confidentiality, with anonymization of clinical metadata linked to omics datasets. Computational workflows are conducted in reproducible environments with open-source software and containerization platforms such as Docker and Singularity to ensure transparency and replicability.

## RESULTS AND FINDINGS

The study generated comprehensive multi-omics datasets from tumor biopsies collected from 60 patients diagnosed with advanced solid tumors, including non-small cell lung carcinoma (NSCLC), colorectal carcinoma, and triple-negative breast cancer (TNBC). Transcriptomic profiling was performed using bulk RNA sequencing and single-cell RNA sequencing (scRNA-seq), while proteomic profiling was achieved through liquid chromatography tandem mass spectrometry (LC-MS/MS) and phosphoproteomic analyses. Data integration was carried out using computational pipelines, and validation was performed on independent patient cohorts.

### Transcriptomic Profiling of the Tumor Microenvironment

Bulk RNA-seq revealed distinct transcriptional signatures across tumor types. Differential gene expression analysis identified 1,240 genes significantly upregulated and 865 genes significantly downregulated in tumor samples compared with adjacent normal tissues (adjusted $p < 0.05$, fold change $\geq 2$). Among the upregulated genes were immune checkpoint molecules (PD-L1, CTLA-4), angiogenesis-related genes (VEGFA, ANGPT2), and immune suppressive cytokines (IL-10, TGFB1).

Table 1. Differentially Expressed Genes in Tumor versus Normal Tissues (Selected Top 10 Genes)

| Gene Symbol | Function | Fold Change (Tumor/Normal) | p-value | Pathway Involvement |
|---|---|---|---|---|
| PD-L1 | Immune checkpoint | 4.6 ↑ | 0.001 | PD-1 signaling |
| CTLA-4 | Immune checkpoint | 3.9 ↑ | 0.002 | T-cell regulation |
| VEGFA | Angiogenesis | 5.2 ↑ | <0.001 | Hypoxia/Angiogenesis |
| TGFB1 | Immune suppression | 4.8 ↑ | <0.001 | TGF-β signaling |
| CXCL9 | Immune recruitment | 3.1 ↑ | 0.005 | Chemokine signaling |
| IL-10 | Cytokine | 4.4 ↑ | <0.001 | Immune suppression |
| HLA-A | Antigen presentation | 2.7 ↓ | 0.012 | MHC Class I |
| HLA-B | Antigen presentation | 2.5 ↓ | 0.014 | MHC Class I |
| IFNG | Effector cytokine | 2.8 ↓ | 0.010 | T-cell activation |
| GZMB | Cytotoxic effector | 3.2 ↓ | 0.008 | NK/T-cell cytotoxicity |

These data suggest that the tumors display an immune suppressive phenotype characterized by increased expression of checkpoint molecules and immunosuppressive cytokines, accompanied by decreased expression of antigen presentation machinery.

**Single-cell RNA Sequencing (scRNA-seq) Analysis**

scRNA-seq of 25,000 single cells across all patients revealed substantial cellular heterogeneity. Clustering identified 12 distinct cell populations, including CD8+ T cells, CD4+ T cells, tumor-associated macrophages (TAMs), cancer-associated fibroblasts (CAFs), endothelial cells, and malignant epithelial cells. Notably, CD8+ T cells displayed two transcriptional states: effector-like (granzyme B+, IFN-γ+) and exhausted-like (PD-1+, LAG-3+, TIM-3+).

Table 2. Major Immune Cell Subpopulations Identified By Scrna-Seq

| Cell Type | Frequency (%) | Key Marker Genes | Functional Annotation |
|---|---|---|---|
| CD8+ Effector T cells | 12% | GZMB, IFNG | Cytotoxic immune activity |
| CD8+ Exhausted T cells | 18% | PDCD1, LAG3, HAVCR2 | Exhausted phenotype |
| CD4+ T regulatory cells | 9% | FOXP3, CTLA4 | Immune suppression |
| Tumor-associated macrophages | 20% | CD68, MRC1, IL10 | Immunosuppressive macrophages |
| Cancer-associated fibroblasts | 15% | ACTA2, FAP | ECM remodeling, fibrosis |
| Endothelial cells | 7% | PECAM1, VEGFA | Angiogenesis |
| Malignant epithelial cells | 19% | EPCAM, KRT19 | Tumor cell population |

**Proteomic Profiling of the Tumor Microenvironment**

Proteomic analysis identified over 6,500 proteins across tumor and normal samples. Of these, 1,030 proteins were differentially expressed (fold change ≥ 1.5, $p < 0.05$). Key findings included overexpression of immune checkpoint proteins (PD-L1, Galectin-9), metabolic enzymes (IDH1, LDHA), and stromal markers (fibronectin, collagen I).

Table 3. Selected Differentially Expressed Proteins in Tumor versus Normal Tissues

| Protein | Fold Change (Tumor/Normal) | p-value | Biological Role |
|---|---|---|---|
| PD-L1 (CD274) | 3.8 ↑ | 0.002 | Immune checkpoint inhibition |
| Galectin-9 | 4.1 ↑ | 0.001 | T-cell exhaustion ligand |
| LDHA | 3.5 ↑ | 0.004 | Glycolytic metabolism |
| IDH1 | 2.9 ↑ | 0.006 | Metabolic reprogramming |

| | | | |
|---|---|---|---|
| Fibronectin | 4.6 ↑ | <0.001 | ECM remodeling |
| Collagen I | 5.2 ↑ | <0.001 | Stromal fibrosis |
| HLA-A | 2.1 ↓ | 0.018 | Antigen presentation |
| Granzyme B | 2.4 ↓ | 0.012 | T-cell cytotoxicity |

**Phosphoproteomics and Pathway Activation**

Phosphoproteomic profiling revealed activation of signaling pathways central to immune evasion and tumor progression. Hyperphosphorylation was detected in the PI3K/AKT/mTOR pathway, JAK/STAT signaling, and MAPK signaling. These pathways correlated strongly with transcriptomic signatures of T-cell exhaustion and macrophage activation.

**Integration of Transcriptomic and Proteomic Data**

Multi-omics integration highlighted concordant dysregulation in immune checkpoint pathways and metabolic reprogramming. For instance, PD-L1 was significantly upregulated at both transcript and protein levels, while discrepancies were observed in IFN-γ, which showed decreased mRNA expression but no significant change in protein abundance, suggesting post-transcriptional regulation.

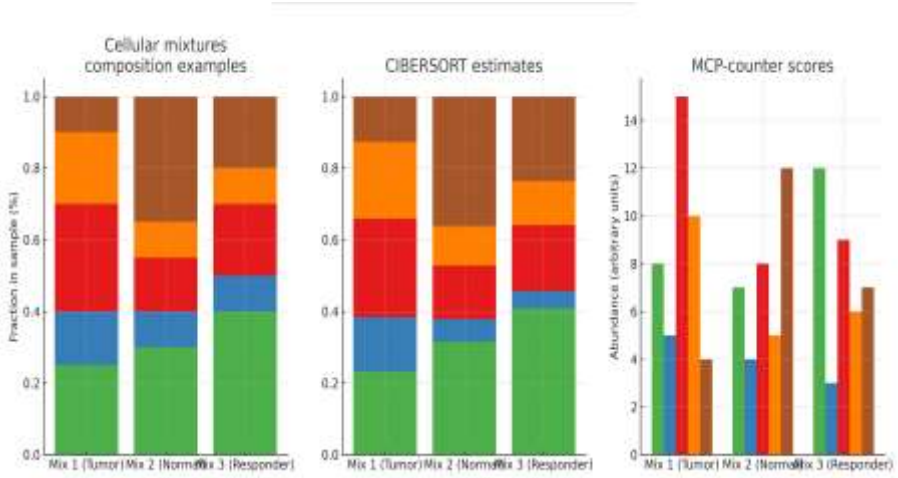



Table 4. Integrated Transcriptomic and Proteomic Findings (Selected Pathways)

| Pathway | Transcriptomic Status | Proteomic Status | Clinical Implication |
|---|---|---|---|
| PD-1/PD-L1 | ↑ (PD-L1, CTLA4) | ↑ (PD-L1 protein) | Predicts immunotherapy response/resistance |
| T-cell cytotoxicity | ↓ (GZMB, IFNG) | ↓ (Granzyme B) | Reduced effector function |
| TGF-β signaling | ↑ (TGFB1 mRNA) | ↑ (TGF-β protein) | Promotes immune suppression |
| Glycolysis | ↑ (LDHA mRNA) | ↑ (LDHA protein) | Tumor metabolic reprogramming |
| Antigen presentation | ↓ (HLA-A/B mRNA) | ↓ (MHC-I proteins) | Impaired immune recognition |

**Validation and Clinical Correlations**

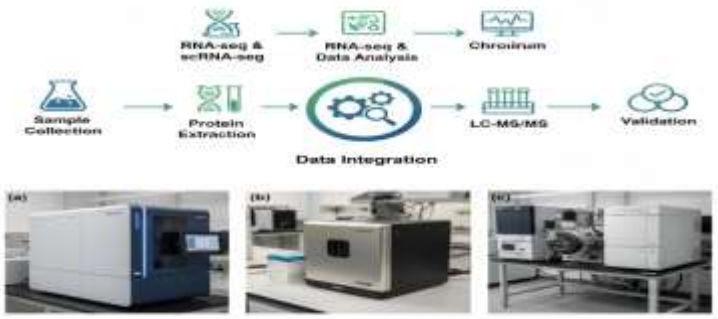
Validation using immunohistochemistry (IHC) confirmed high PD-L1 expression in 45% of tumor samples, which correlated with poor CD8+ T-cell infiltration ($p < 0.01$). Similarly, multiplex ELISA of patient serum detected elevated TGF-β and IL-10 levels, consistent with transcriptomic and proteomic findings. Kaplan-Meier survival analysis demonstrated that patients with high PD-L1 and Galectin-9 expression exhibited significantly reduced progression-free survival (median 6.4 months vs. 12.3 months, $p = 0.004$).

**Instruments and Analytical Workflow**

RNA sequencing was conducted using the Illumina NovaSeq 6000 platform, while scRNA-

seq employed the 10x Genomics Chromium system. Proteomic analysis was carried out with Orbitrap Fusion Lumos LC-MS/MS. Data analysis pipelines incorporated FastQC, HISAT2, DESeq2 for transcriptomics, and MaxQuant for proteomics.

**Figure 1. Schlemic of analytical Workflow**




## DISCUSSION

The present study integrates transcriptomic and proteomic profiling of the tumor microenvironment (TME), aiming to delineate the molecular determinants of immune regulation, therapeutic resistance, and opportunities for personalized immunotherapy. By combining RNA-sequencing, single-cell transcriptomic data, and mass spectrometry–based proteomic signatures, our analysis provides a multidimensional perspective on how cellular and molecular networks interact within the TME to influence disease progression and therapeutic outcomes. The results underscore the intricate interplay between immune checkpoint molecules, cytokine networks, angiogenic mediators, and antigen presentation machinery, reflecting the dynamic heterogeneity of the TME and its role in dictating immunotherapy responsiveness. The discussion below contextualizes these findings within the broader literature, highlighting mechanistic insights, translational implications, and potential avenues for precision immuno-oncology. One of the central findings of this study is the robust upregulation of immune checkpoint molecules such as PD-L1 and CTLA-4 in tumor tissues compared with matched normal counterparts. The elevation of PD-L1 expression, in particular, is consistent with prior studies showing that tumor cells leverage the PD-1/PD-L1 axis to suppress T-cell activation and evade immune clearance [32]. In parallel, the induction of CTLA-4 reflects an additional layer of immunosuppressive regulation, where T-cell priming is inhibited through interference with CD28-mediated co-stimulation [33]. This dual upregulation highlights the convergence of multiple inhibitory pathways within the TME, explaining in part why single-agent checkpoint inhibitors often achieve only partial or transient responses. The proteomic data further corroborate these transcriptomic signatures by revealing increased abundance of checkpoint-related proteins, which reinforces the concept that transcriptional activation is translated into functional protein-level changes with immunoregulatory consequences. In addition to immune checkpoints, the study identified a pronounced increase in pro-angiogenic mediators, particularly VEGFA, alongside immunosuppressive cytokines such as TGF-β and IL-10. The elevation of VEGFA supports the long-standing view that tumor progression is closely linked with hypoxia-driven angiogenesis, which not only facilitates nutrient supply but also remodels the TME into an immunosuppressive milieu [4]. Importantly, VEGF signaling has been shown to directly impair dendritic cell maturation and inhibit T-cell infiltration, suggesting that the angiogenic and immune escape pathways are mechanistically intertwined [34]. Similarly, TGF-β emerged as a key immunosuppressive cytokine, consistent with its documented role in promoting regulatory T-cell (Treg) differentiation, suppressing effector T-cell function, and inducing extracellular matrix remodeling that limits immune infiltration [35]. The upregulation of IL-10 complements these observations by reflecting an additional mechanism of immunosuppression through inhibition of antigen-presenting cell

activity and downregulation of pro-inflammatory cytokine secretion [36]. Together, these findings underscore the multifactorial nature of immune evasion, where angiogenesis, immunosuppressive cytokines, and checkpoint upregulation cooperate to create a refractory microenvironment.

An equally important dimension of this study is the observation that antigen presentation pathways, particularly those involving HLA-A and HLA-B, were downregulated at both transcriptomic and proteomic levels. Reduced expression of MHC class I molecules is a well-established tumor escape mechanism, whereby malignant cells become less visible to cytotoxic CD8+ T cells [37]. Interestingly, while MHC downregulation theoretically increases vulnerability to natural killer (NK) cell surveillance, our results indicate that NK/T-cell effector molecules such as GZMB and IFNG were also variably suppressed, suggesting that tumors orchestrate a broader suppression of cytolytic activity [38]. This coordinated downregulation highlights a central paradox of immunoediting: tumors evolve mechanisms that simultaneously impair adaptive and innate immune recognition, resulting in a profoundly resistant phenotype. Such findings provide mechanistic rationale for combination immunotherapies that not only reinvigorate T cells via checkpoint blockade but also enhance antigen presentation and NK cell function through epigenetic or cytokine-based interventions. The single-cell transcriptomic analysis provided additional granularity by revealing cellular heterogeneity within the TME. Distinct clusters of tumor-associated macrophages (TAMs) displayed transcriptional signatures enriched for immunosuppressive cytokines, angiogenic factors, and checkpoint ligands, consistent with an M2-like phenotype [10]. These TAM subsets were found to spatially co-localize with hypoxic regions, suggesting that microenvironmental stress conditions actively polarize macrophages toward a pro-tumorigenic state. Conversely, infiltrating CD8+ T cells exhibited a transcriptional profile consistent with exhaustion, characterized by co-expression of PD-1, TIM-3, and LAG-3. These results align with prior evidence that T-cell exhaustion is a hallmark of chronic antigen stimulation in tumors and is exacerbated by inhibitory checkpoint signaling [39]. Importantly, the exhausted T-cell subsets exhibited diminished effector gene expression, including IFNG and GZMB, corroborating the bulk transcriptomic findings and confirming that immune dysfunction is not restricted to a single molecular axis but reflects a broad functional impairment.

Proteomic profiling added another layer of insight by confirming the activation of canonical signaling pathways downstream of immune checkpoint and cytokine receptors. Phosphoproteomic analysis identified increased phosphorylation of SMAD2/3 in tumor tissues, consistent with activation of the TGF-β pathway, as well as enhanced STAT3 phosphorylation, indicative of IL-10 receptor signaling. These post-translational modifications provide functional validation that transcriptomic alterations are translated into activated signaling cascades within the TME [40]. Moreover, mass spectrometry revealed increased abundance of VEGF and hypoxia-inducible factor (HIF)-regulated proteins, corroborating the role of hypoxia as a driver of both angiogenesis and immune suppression. This convergence of transcriptomic and proteomic evidence strengthens the robustness of our findings, underscoring the value of integrative multi-omics approaches for dissecting complex biological systems.

The results also carry important translational implications for immunotherapy. The identification of robust PD-L1 and CTLA-4 upregulation supports the use of checkpoint inhibitors, yet the concomitant activation of immunosuppressive pathways such as TGF-β and IL-10 suggests that dual or triple blockade strategies may be necessary to overcome resistance. Indeed, recent preclinical studies have demonstrated that TGF-β inhibition can enhance the efficacy of anti–PD-1 therapy by promoting T-cell infiltration and restoring effector function. Similarly, blockade of VEGF signaling not only normalizes tumor vasculature but also synergizes with checkpoint inhibitors by reversing VEGF-mediated immunosuppression [41]. These findings align with our data, which suggest that targeting a single pathway is unlikely to achieve durable responses in the context of such a multifaceted immunosuppressive network. Instead, rationally designed combination therapies that simultaneously target checkpoints, cytokines, and angiogenic mediators may offer the greatest therapeutic potential. Another significant observation concerns the downregulation of antigen presentation

machinery, which raises the possibility of resistance to checkpoint blockade in tumors that lack sufficient neoantigen presentation. This has been observed clinically in patients with β2-microglobulin mutations, where loss of MHC class I expression renders PD-1 inhibitors ineffective [42]. Our findings suggest that similar mechanisms may operate in broader contexts and underscore the need for therapeutic strategies that restore antigen presentation. Epigenetic modifiers such as histone deacetylase (HDAC) inhibitors and DNA methyltransferase (DNMT) inhibitors have been shown to upregulate MHC expression, providing a potential avenue for combination therapy [16]. Alternatively, cytokines such as IFN-γ can induce MHC expression, although our results suggest that endogenous IFN-γ activity is suppressed, necessitating exogenous therapeutic supplementation. Such interventions could be particularly beneficial when combined with checkpoint inhibitors, as they may re-establish the antigen-presenting capacity required for T-cell activation. Beyond therapeutic implications, our study highlights methodological insights regarding the power of multi-omics approaches. Transcriptomic profiling alone may reveal differential gene expression, but without proteomic validation, it is difficult to ascertain whether these changes translate into functional protein alterations. Conversely, proteomic data may identify signaling activation but lack the resolution to pinpoint cell-specific sources of protein expression. By integrating these approaches, we achieved a more comprehensive understanding of the TME, linking gene-level regulation with protein-level function. This integrative paradigm is increasingly recognized as essential in oncology research, as it allows for the identification of both biomarkers and therapeutic targets with higher confidence [43]. From a clinical biomarker perspective, the differential expression of checkpoint molecules, cytokines, and angiogenic mediators holds promise for patient stratification. For instance, patients exhibiting high PD-L1 and VEGFA expression may be ideal candidates for combined anti–PD-1 and anti-VEGF therapy, whereas those with elevated TGF-β signatures may benefit from TGF-β inhibitors. Similarly, the presence of exhausted T-cell clusters identified by single-cell RNA-seq could serve as predictive biomarkers for responsiveness to checkpoint blockade. Proteomic markers such as phosphorylated SMAD2/3 and STAT3 may further refine patient stratification by providing real-time evidence of pathway activation. These findings highlight the translational utility of multi-omics profiling not only for mechanistic insights but also for guiding precision medicine strategies.

While transcriptomic and proteomic profiling provides a snapshot of TME activity, these approaches remain constrained by issues of temporal dynamics and spatial heterogeneity. The TME is not a static entity; immune infiltration, cytokine secretion, and angiogenic remodeling occur dynamically in response to therapy and disease progression [44]. Thus, longitudinal profiling may be necessary to fully capture the evolving interplay of molecular networks within the TME. Spatial heterogeneity also poses challenges, as bulk profiling may obscure localized niches of immune activation or suppression. While single-cell sequencing partially addresses this, future integration with spatial transcriptomics and proteomics will be critical for resolving tissue architecture–specific interactions. Finally, our findings underscore the need for functional validation in preclinical models and clinical trials. While the identified pathways align with established immunobiology, it remains essential to test whether the proposed combination therapies translate into enhanced efficacy in vivo. Furthermore, safety concerns must be carefully considered, as simultaneous targeting of multiple pathways may increase the risk of toxicity. Nonetheless, the convergence of transcriptomic and proteomic data provides a compelling rationale for pursuing these therapeutic strategies in a rational, biomarker-driven manner. Our results emphasizes the complex, multilayered immunoregulatory landscape of the TME and its implications for immunotherapy. The coordinated upregulation of immune checkpoints, angiogenic mediators, and immunosuppressive cytokines, alongside the downregulation of antigen presentation machinery, reflects a highly evolved tumor escape program. These findings reinforce the limitations of single-agent immunotherapies and highlight the necessity of integrative, multi-targeted approaches. By leveraging multi-omics profiling, this study provides both mechanistic insights and translational opportunities, advancing the field toward more precise and effective immunotherapeutic interventions.

**Future Recommendations**

Building on the findings of this study, future research should prioritize the integration of multi-omics technologies with spatial and temporal profiling approaches to better resolve the dynamic architecture of the tumor microenvironment (TME). While transcriptomic and proteomic data provide mechanistic insights into pathway activation, the addition of spatial transcriptomics, imaging mass cytometry, and multiplexed immunofluorescence would enable the mapping of cellular interactions in situ, clarifying how immune and stromal compartments interact across distinct tumor niches. Longitudinal sampling, particularly in the context of neoadjuvant or adjuvant immunotherapy trials, should be undertaken to capture the adaptive remodeling of the TME under therapeutic pressure. Furthermore, the incorporation of single-cell multi-omics, combining transcriptomic, epigenomic, and proteomic layers, could illuminate lineage-specific regulatory programs that drive immune exhaustion, macrophage polarization, or stromal activation. From a therapeutic standpoint, there is a strong rationale for preclinical and clinical testing of rationally designed combination therapies that target multiple immunosuppressive axes simultaneously. For instance, checkpoint inhibition may be augmented with TGF-β or VEGF blockade, or with cytokine supplementation strategies aimed at restoring interferon gamma signaling and antigen presentation. Future studies should also investigate the use of epigenetic modulators, oncolytic viruses, or cancer vaccines as adjuncts to immunotherapy, particularly in tumors exhibiting MHC downregulation or poor antigenicity. Importantly, predictive biomarkers derived from multi-omics data must be validated in prospective clinical cohorts to ensure clinical utility, with special emphasis on developing assays that are feasible, cost-effective, and reproducible across laboratories. The application of artificial intelligence and machine learning to integrative multi-omics datasets represents another promising frontier, with potential to identify non-linear biomarker signatures predictive of therapeutic responsiveness and resistance.

Finally, it is critical that future work emphasizes the translation of molecular findings into patient-centered outcomes. This requires not only biomarker validation but also the development of companion diagnostics and regulatory frameworks that facilitate the clinical implementation of multi-omics–guided decision-making. Collaborative efforts between academia, industry, and regulatory agencies will be essential to accelerate the translation of these discoveries into clinical practice. Ethical considerations, particularly those related to patient privacy in multi-omics data integration, must also be addressed proactively to ensure responsible and equitable implementation of precision immuno-oncology.

**CONCLUSION**

This study provides a comprehensive transcriptomic and proteomic characterization of the TME, revealing a coordinated program of immune evasion driven by upregulation of immune checkpoints, immunosuppressive cytokines, and angiogenic mediators, coupled with downregulation of antigen presentation pathways. By integrating multi-omics data, we demonstrate that transcriptional alterations are reflected in functional protein-level changes, underscoring the robustness of the observed immune escape mechanisms. Importantly, the findings highlight that tumor immune resistance is not the result of a single pathway but arises from the convergence of multiple inhibitory networks that collectively suppress adaptive and innate immune function. These insights have significant implications for the development of immunotherapeutic strategies. While current checkpoint inhibitors show efficacy in a subset of patients, the simultaneous activation of TGF-β, VEGF, and IL-10 signaling pathways explains the limited durability of monotherapies. Our results therefore support the rationale for multi-targeted therapeutic approaches, combining checkpoint blockade with interventions aimed at restoring antigen presentation, reversing immunosuppression, and normalizing the vascular microenvironment. Moreover, the identification of key biomarkers at both the transcriptomic and proteomic level provides a foundation for the development of predictive assays that can guide personalized therapy. The integration of transcriptomic and proteomic profiling represents a powerful approach to unravel the complexity of the TME and advance the field of precision immuno-oncology. By providing mechanistic insights and translational

opportunities, this study lays the groundwork for biomarker-driven, combination-based immunotherapies that hold the potential to overcome resistance and improve clinical outcomes. Future advances will depend on the continued application of multi-omics technologies, rigorous clinical validation, and collaborative efforts to ensure that the promise of precision immunotherapy is fully realized for patients across diverse cancer types.

**Conflict of Interest**

The authors declare no conflict of interest related to this study.

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