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Systematic Downregulation of T-Cell Pathways Distinguishes Recurrent from Non-Recurrent Colorectal Cancer

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Abstract: This study compared the transcriptomic profiles of recurrent and non-recurrent colorectal cancer (CRC) patients in the GSE39582 cohort and identified immune-related alterations—particularly those involving T-cell functional pathways—as the dominant molecular features associated with recurrence. Differential expression analysis revealed extensive downregulation of immune-related genes in recurrent patients, while heatmap clustering demonstrated a consistent reduction in transcripts involved in T-cell activation, proliferation, and effector functions. Gene Ontology (GO) enrichment showed that downregulated genes were predominantly enriched in key immune processes such as "T cell activation," "lymphocyte proliferation," and "leukocyte cell–cell adhesion." Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis further indicated suppression of multiple essential immune pathways, including Th1/Th2/Th17 differentiation, chemokine signaling, antigen processing and presentation, and B/T-cell receptor signaling. Immune checkpoint analysis showed reduced expression of LAG3 and CTLA4 in the recurrent group, whereas PDCD1, PDCD1LG2, and SIGLEC15 exhibited no significant differences. Collectively, these findings reveal a transcriptional signature characterized by T-cell immune suppression and an immune-cold phenotype in recurrent CRC, suggesting that impaired T-cell activation may be a key molecular driver of recurrence.
Keywords: Colorectal cancer; Recurrence; T-cell immunity; Transcriptomics; Immune-cold microenvironment

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1. Introduction

Colorectal cancer (CRC) remains one of the leading causes of cancer incidence and mortality worldwide[1]. Despite substantial progress in surgery, chemotherapy, targeted therapy, and immunotherapy, a considerable proportion of patients still experience disease recurrence following initial treatment[2]. Tumor recurrence is the primary determinant of long-term survival in CRC, underscoring the need to elucidate its underlying molecular mechanisms and to identify biomarkers capable of distinguishing recurrent from non-recurrent disease, thereby improving prognostic assessment and guiding personalized therapeutic strategies[3].

Tumor recurrence is recognized as a multifactorial biological process influenced not only by the intrinsic malignant properties of cancer cells but also by the complex regulatory dynamics of the tumor microenvironment (TME)[4]. Mounting evidence highlights the pivotal role of the immune system in CRC progression, with T-cell–mediated anti-tumor immunity serving as a critical barrier against tumor growth and relapse[5, 6]. High levels of tumor-infiltrating lymphocytes—

particularly $CD8^+$ T cells—have been strongly associated with improved disease-free and overall survival [7, 8]. In contrast, immune suppression, T-cell dysfunction, impaired antigen presentation, and disrupted cytokine signaling may facilitate immune evasion and contribute to recurrence[9,10]. Although the importance of anti-tumor immunity in CRC is widely acknowledged, whether recurrent patients exhibit reproducible, transcriptome-level immune alterations remains poorly characterized in large-scale cohorts[11].

Public databases such as TCGA and GEO provide extensive transcriptomic resources for exploring CRC biology. Among them, GSE39582 represents one of the largest CRC cohorts with detailed recurrence information and genome-wide expression data[12]. In this study, we systematically compared transcriptomic differences between recurrent and non-recurrent patients using this dataset, with a particular focus on immune-related biological processes.

Our findings revealed a prominent downregulation of immune-related genes in recurrent patients. Hierarchical clustering demonstrated that these downregulated genes were largely involved in key T-cell processes, including activation, proliferation, leukocyte adhesion, cytokine responsiveness, and effector function[13]. Consistent with these observations, Gene Ontology enrichment analyses showed significant suppression of terms such as “T cell activation,” “lymphocyte proliferation,” and “leukocyte cell–cell adhesion.” KEGG pathway analysis further revealed substantial inhibition of multiple essential immune pathways, including Th1/Th2/Th17 differentiation, chemokine signaling, antigen processing and presentation, cytokine–cytokine receptor interactions, and B/T-cell receptor signaling[14]. These results point toward a coherent biological pattern wherein recurrent CRC is characterized by broad and systemic attenuation of T-cell–mediated immunity[15].

To further assess immune regulatory status, we examined the expression of canonical immune checkpoint genes, including CTLA4, LAG3, PDCD1, PDCD1LG2, and SIGLEC15. Notably, LAG3 and CTLA4 were significantly downregulated in recurrent patients, whereas PDCD1, PDCD1LG2, and SIGLEC15 showed no significant differences. This pattern indicates that recurrent CRC does not exhibit typical features of immune activation or exhaustion; rather, it demonstrates an “immune-cold” or immune-excluded phenotype with insufficient T-cell infiltration and limited anti-tumor pressure. Together with the suppression of antigen presentation and cytokine signaling pathways, this immune-cold state may facilitate the survival of residual tumor cells and ultimately contribute to recurrence.

In summary, this study establishes a direct link between CRC recurrence and widespread suppression of immune pathways, particularly those governing T-cell function. These findings highlight the central role of impaired T-cell activation in the biology of recurrence and suggest that immune-related genes and pathways may serve as promising biomarkers for recurrence risk assessment. Moreover, they provide important insights for developing immunomodulatory strategies aimed at reducing recurrence and improving long-term outcomes in CRC patients.

2. Methods

2.1. Data Source and Preprocessing

Gene expression data were obtained from the Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/) database. The colorectal cancer cohort GSE39582, one of the largest publicly available datasets with detailed clinical follow-up, was selected for analysis. The dataset was generated on the Affymetrix platform and downloaded in MINiML format. Raw CEL files were processed using the R package *affy* with the Robust Multi-array Average (RMA) algorithm for background correction, normalization, and summarization. Probe identifiers were mapped to official gene symbols according to the platform annotation, and when multiple probes corresponded to the same gene, their average expression value was used. Clinical variables, including recurrence status and recurrence-free survival time, were extracted from the accompanying phenotype data. Based on recurrence information, patients were categorized into two groups: the recurrence group (G1; Recurrence = 1) and the non-recurrence group (G2; Recurrence = 0 with adequate follow-up). Only samples with complete clinical information and valid mRNA expression profiles were included in subsequent analyses.

2.2. Differential Expression Analysis

To compare gene expression differences between G1 and G2, differential expression analysis was performed using the *limma* package (version 3.40.2). A design matrix was constructed, and linear models were fitted, followed by empirical Bayes moderation. For each gene, log2 fold change (log2FC) and adjusted P values (Benjamini–Hochberg method) were calculated. Differentially expressed genes (DEGs) were defined by the thresholds $|log2FC| > 1–1.3$ (consistent with visualization criteria) and adjusted $P < 0.05$. Volcano plots were generated using the *EnhancedVolcano* package to illustrate the overall distribution of DEGs.

2.3. Clustering Analysis of Differentially Expressed Genes

To visualize the expression patterns of key DEGs across samples, the top 50 upregulated and top 50 downregulated genes were selected for hierarchical clustering. Heatmaps were generated using the *pheatmap* package, with sample groups annotated as G1 or G2. Expression values were scaled, and a blue-to-red color gradient was used to represent low to high expression levels. Clustering was performed using Euclidean distance and the complete linkage method.

2.4. GO and KEGG Functional Enrichment Analyses

To explore the biological functions and pathways associated with the identified DEGs, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were conducted using the *clusterProfiler* package (version 3.18.0). GO analysis included three major domains: Biological Process (BP), Molecular Function (MF), and Cellular Component (CC). KEGG pathway analysis was used to identify signaling pathways significantly associated with upregulated and downregulated genes. Enrichment significance was defined as $P < 0.05$ or false discovery rate (FDR) < 0.05. GO results were visualized using bar plots, and KEGG pathways were presented as bubble plots, where bubble size represented the number of enriched genes and color intensity reflected $-\log10$(P value).

2.5. Analysis of Immune Checkpoint Gene Expression

To evaluate the potential involvement of immune regulatory mechanisms in recurrence, the expression levels of representative immune checkpoint genes—CTLA4, PDCD1 (PD-1), PDCD1LG2 (PD-L2), LAG3, and SIGLEC15—were extracted from the normalized expression matrix. Differences between G1 and G2 were assessed using the Wilcoxon rank-sum test. Boxplots were generated using *ggplot2*, and significance levels were annotated as follows: $P < 0.05$; or for more significant differences; “ns” indicating non-significance.

3. Results

3.1. Differentially expressed genes distinguish recurrence from non-recurrence groups

Comparison of the recurrent (G1) and non-recurrent (G2) groups in the GSE39582 cohort revealed substantial differences in global gene expression profiles. The volcano plot illustrates the distribution of differentially expressed genes (DEGs) in the $\log_2$ fold-change and $-\log10$(P value) space, highlighting many significantly upregulated and downregulated genes associated with recurrence **(Figure 1)**. The wide separation of highly significant genes toward both extremes of the plot indicates that recurrence is accompanied by extensive transcriptional reprogramming, laying the foundation for downstream functional interpretation.

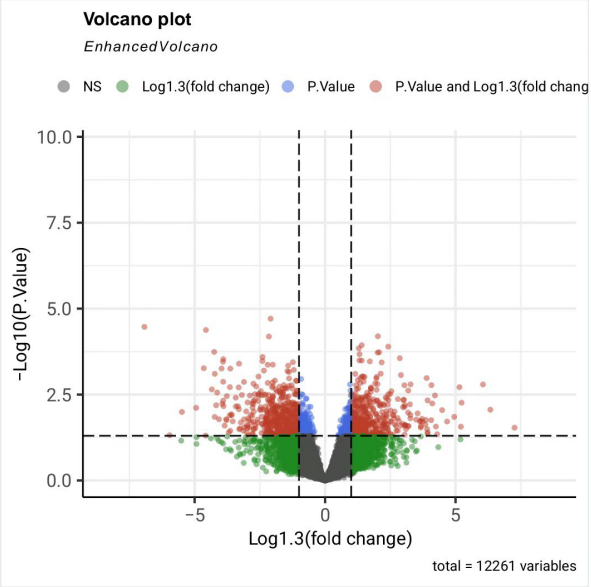

Figure 1. Volcano plot of differentially expressed genes between recurrent (G1) and non-recurrent (G2) CRC groups.

The volcano plot visualizes mRNA expression differences in the GSE39582 cohort. The x-axis shows $\log_2$ fold change, and the y-axis shows $-\log_{10}$(P value). Red dots indicate significantly up- or downregulated genes (large |logFC| and adjusted $P < 0.05$); green and blue dots mark genes meeting only one threshold; gray dots represent non-significant genes. This plot highlights candidate genes most altered between G1 and G2.

3.2. Top differentially expressed genes reveal a distinct transcriptomic signature in recurrence

The bidirectional hierarchical clustering of the top 50 upregulated and top 50 downregulated DEGs demonstrated a clear distinction between G1 and G2 **(Figure 2)** Samples segregated clearly according to G1 and G2, with recurrent patients exhibiting a consistent clustering pattern. Notably, the expression of downregulated genes in G1 showed a coordinated and marked reduction, forming an “immune hypoactivation” profile. This stable clustering pattern suggests that DEGs robustly distinguish recurrent from non-recurrent patients and that immune-related genes are collectively suppressed in the recurrent group.

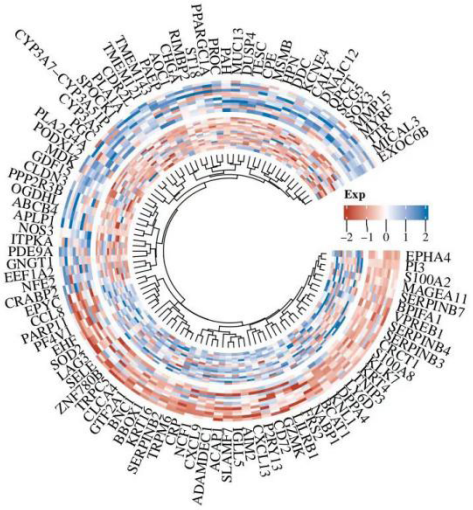

Figure 2. Circular heatmap of top 50 upregulated and 50 downregulated genes.

The circular clustered heatmap displays expression profiles of the 100 most significantly altered genes between G1 and G2. Each radial line represents a sample (outer ring) and each gene (inner ring), colored from blue to red for low to high normalized expression. Distinct clustering patterns clearly separate recurrent and non-recurrent samples.

3.3. GO and KEGG enrichment reveal broad suppression of T-cell–related immune pathways in recurrence

Functional enrichment analyses of upregulated and downregulated genes **(Figure 3)** revealed striking differences between groups. Upregulated genes were predominantly enriched in structural and signaling pathways involved in cytoskeletal organization, cellular morphology, and extracellular matrix remodeling. In contrast, downregulated genes showed strong enrichment for immune-related biological processes, including T cell activation, lymphocyte proliferation, leukocyte cell–cell adhesion, and immune effector functions. KEGG analysis further demonstrated significant suppression of multiple essential immune pathways in the recurrent group, such as Th1/Th2/Th17 differentiation, chemokine signaling, antigen processing and presentation, cytokine–cytokine receptor interactions, and B/T-cell receptor signaling. Together, these findings indicate a broad, systemic downregulation of T-cell–mediated immunity in recurrent CRC.

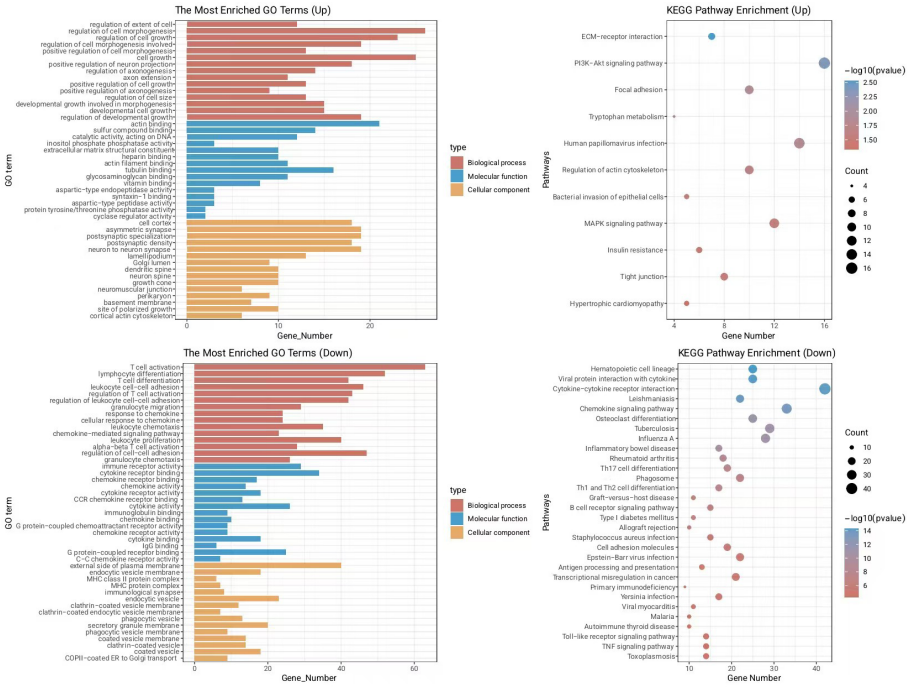

Figure 3. GO and KEGG enrichment analyses of up- and downregulated genes.

GO term barplots (left) and KEGG pathway bubble plots (right) show enriched biological processes for upregulated (top) and downregulated (bottom) genes in G1 vs. G2. Dot size indicates the number of enriched genes, and color depth corresponds to $-\log^{10}$ (P value). Downregulated genes are mainly involved in immune-related and inflammatory pathways such as T-cell differentiation, chemokine signaling, and antigen presentation.

3.4. Immune checkpoint analysis indicates reduced activation of LAG3- and CTLA4-mediated pathways

Analysis of immune checkpoint genes revealed significantly decreased expression of LAG3 and CTLA4 in recurrent patients, whereas PDCD1 (PD-1), PDCD1LG2 (PD-L2), and SIGLEC15 showed no significant differences between groups. This pattern suggests that recurrent CRC does not exhibit features of classical immune activation or T-cell exhaustion but instead reflects an immune-excluded or "immune-cold" phenotype **(Figure 4)**. This observation aligns with the widespread suppression of T-cell–related pathways observed in the enrichment analyses.

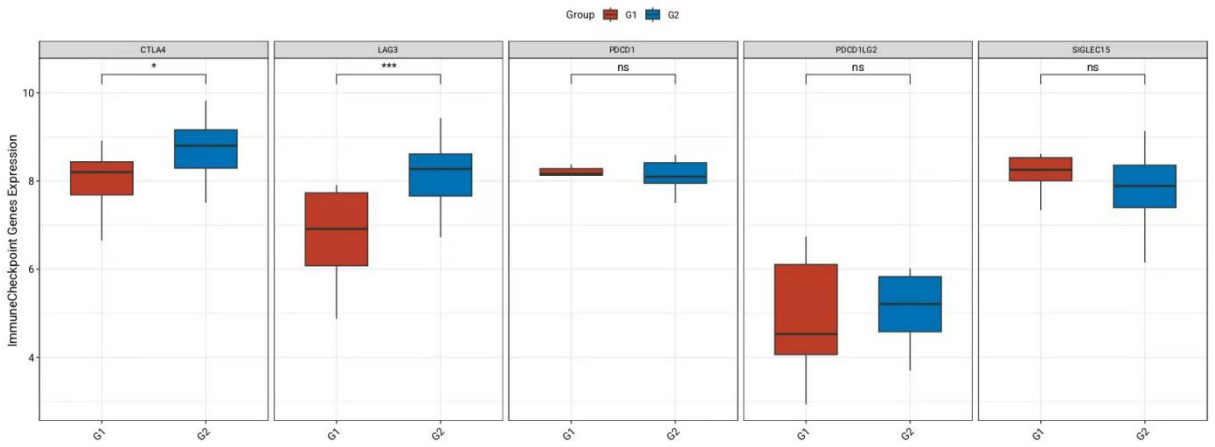

Figure 4. Expression of immune checkpoint–related genes between G1 and G2.

Boxplots compare normalized expression levels of CTLA4, LAG3, PDCD1, PDCD1LG2, and SIGLEC15 between recurrent and non-recurrent groups. Significance levels are shown (* $P < 0.05$; *** $P < 0.001$; ns, not significant). LAG3 and CTLA4 are significantly downregulated in G1, while others show no significant difference, indicating heterogeneous checkpoint activation across recurrence states.

4. Discussion

This study provides comprehensive transcriptomic evidence demonstrating that colorectal cancer recurrence is strongly associated with suppression of immune-related pathways, particularly those governing T-cell activation and effector function[2]. Through differential expression analysis of the GSE39582 cohort, we observed extensive downregulation of immune genes in recurrent patients, with functional enrichment analyses highlighting widespread inhibition of T-cell–mediated immune responses. These findings suggest that impaired anti-tumor immunity—rather than enhanced malignant potential alone—is a central biological mechanism driving CRC recurrence[16].

The most notable pattern identified in this study was the coordinated downregulation of pathways related to T-cell activation, lymphocyte proliferation, cytokine responsiveness, and leukocyte adhesion. These processes form the core machinery through which T cells recognize, migrate toward, and eliminate tumor cells. Their suppression implies a failure of immune surveillance, permitting residual tumor cells to escape detection following initial treatment [12]. This observation is consistent with previous immunological studies demonstrating that high densities of $CD8^+$ T cells and other tumor-infiltrating lymphocytes are strongly associated with improved survival and reduced recurrence risk [17]. The Immunocore, a clinically validated prognostic tool based on T-cell infiltration, further supports the critical role of T-cell immunity in CRC outcomes[18]. Our findings extend these observations by showing that recurrent CRC displays a transcriptome-level signature indicative of T-cell hypoactivity.

Furthermore, KEGG pathway analysis revealed that multiple essential immune signaling pathways including Th1/Th2/Th17 differentiation, chemokine signaling, antigen processing and presentation, and B/T-cell receptor signaling—were significantly inhibited in recurrent tumors. The Th1/Th2/Th17 axis is central to orchestrating adaptive immune responses, with Th1 responses particularly important in anti-tumor immunity through interferon-γ–mediated cytotoxic pathways[19]. Meanwhile, downregulation of chemokine signaling likely impedes immune cell trafficking into the tumor microenvironment, contributing to an immune-excluded phenotype[20]. Suppression of antigen presentation pathways further suggests that recurrent tumors may evade immune recognition by reducing MHC-I–mediated neoantigen display—an established mechanism of immune escape[21].Our immune checkpoint analysis reinforces this immune-cold phenotype. Recurrent patients showed reduced expression of LAG3 and CTLA4—markers that typically rise during chronic immune

activation or T-cell exhaustion[20]. Their reduction implies not exhaustion but insufficient T-cell activation from the outset. This aligns with emerging frameworks classifying tumors into "immune-hot," "immune-cold," and "immune-excluded" categories[22]. Recurrent CRC in our dataset exhibits features of immune-cold tumors, which are known to respond poorly to immune checkpoint blockade[23].

Clinically, the immune-related transcriptional patterns identified here may serve as valuable biomarkers for recurrence risk prediction[11]. Immune signatures often complement or outperform traditional TNM staging in prognostic accuracy. Although we did not construct a prognostic model in this study, the DEGs and pathways identified provide a strong foundation for developing immune-based predictors. Moreover, the recognition that recurrent tumors exhibit diminished T-cell activity underscores the potential value of immune-modulating interventions[24], such as dendritic cell activators, STING agonists, cytokine therapies, or agents that enhance chemokine gradients—to convert immune-cold tumors into immune-responsive ones.

This study has several limitations. As analyses were conducted on a single public dataset, validation in independent cohorts is needed. We inferred immune status based solely on gene expression without integrating immune cell infiltration data or histological confirmation. Additionally, mechanistic experiments are required to determine whether specific suppressed immune pathways directly promote recurrence[25]. In conclusion, we demonstrate that CRC recurrence is associated with extensive suppression of T-cell–mediated immunity and the emergence of an immune-cold tumor microenvironment[13]. These findings improve our understanding of recurrence biology and highlight the potential utility of immune-related biomarkers and therapeutic strategies aimed at restoring T-cell function to prevent disease relapse.

Disclosure statement

The author declares no conflict of interest.

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