

Prognostic and Immune Microenvironment Significance of Opioid Receptor- and Pain-Related Gene Signatures in Gastric and Colorectal Cancer

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Abstract

Background

This study evaluated the prognostic and immune microenvironment relevance of opioid receptor- and pain-related gene signatures in gastric and colorectal cancers.

Results

CNR1 in TCGA-STAD and TRPV1 in TCGA-COAD showed adverse prognostic associations. The TCGA_ALL_GI risk score was independently associated with overall survival (adjusted HR = 1.90, 95% CI = 1.18–3.05; P = 0.008) and was supported in GSE39582 (HR = 1.43; P = 0.019) and GSE84437 (HR = 1.59; P = 0.004). High-risk tumors showed immune checkpoint differences and enrichment of inflammatory, epithelial-mesenchymal transition, and stromal pathways.

Conclusion

These findings indicate that the gene signature captures prognostic risk and inflammatory immune microenvironment states in gastrointestinal cancers, although its clinical relevance requires prospective validation, real analgesic exposure data, and functional studies.

1. Background

Gastric and colorectal cancers are digestive tract malignancies that impose a substantial global disease burden^{1–4}. Molecular classification, immunotherapy, and integrated treatment strategies have improved clinical management in recent years^{3–13}. Nevertheless, patients with similar TNM stage, treatment regimens, and pathological features may experience markedly different survival outcomes, indicating that anatomic staging alone cannot fully explain the biological heterogeneity of gastrointestinal cancers^{5–9}. Therefore, interpretable prognostic molecular signatures derived from public transcriptomic data remain valuable for precision risk stratification.

Current bioinformatics studies of gastrointestinal cancers mainly focus on immune infiltration, cell death, epithelial-mesenchymal transition (EMT), metabolic reprogramming, tumor stemness, and DNA damage repair, and many prognostic models and immune-related signatures have been reported^{14–18}. TCGA, GEO, and other public resources provide standardized transcriptomic profiles, clinical follow-up information, and external validation datasets, while single-cell sequencing data can help localize candidate gene expression across cellular subsets^{5–9,19–20}. Integrated public-database analysis is therefore an important strategy for identifying candidate mechanisms, screening prognostic biomarkers, and generating hypotheses for subsequent experimental validation^{21–29}.

Pain is a central clinical issue for patients with gastric and colorectal cancers during perioperative care, adjuvant therapy, recurrence, metastasis, and palliative treatment^{30–32}. Opioid analgesics exert their analgesic effects through opioid receptors, but opioid receptor-related signaling is not restricted to the nervous system^{30–34}. OPRM1, OPRK1, OPRD1, OPRL1, and OGFR represent classical or non-classical opioid receptor axes, whereas TLR4, PTGS2, TRPV1, P2RX7, CNR1, and CNR2 connect nociception, inflammatory responses, purinergic signaling, and the endocannabinoid system^{30,35–41}. These molecules may influence the tumor microenvironment through inflammatory mediator release, immune-cell activation, angiogenesis, stromal remodeling, and immune checkpoint regulation^{10,14–18}.

Perioperative pain after gastrointestinal surgery is dynamic rather than static. Recent trajectory-based studies have shown that patients undergoing gastrointestinal surgery can follow distinct acute postoperative pain courses and that early postsurgical pain trajectories are associated with later pain outcomes and recovery-related risk stratification^{42–43}. For gastric and colorectal cancer surgery, this concept is clinically important because perioperative nociceptive input, inflammatory activation, opioid requirement, and postoperative recovery occur within a biological window in which neuroimmune and tumor microenvironment signals may interact. Therefore, adopting a perioperative pain trajectory framework provides a clinical basis for studying opioid receptors and pain-related molecular mechanisms in gastrointestinal cancers.

Several gaps remain. First, studies of opioid receptor- and pain-related genes in gastrointestinal cancers are fragmented and often focus on a single gene or pathway rather than a predefined candidate-gene framework^{30–41}. Second, molecular signals relevant to pain medicine and anesthetic oncology have not been fully incorporated into conventional tumor prognostic models^{21–26,30–34}. Third, some public-database studies report positive findings in a single cohort without sufficient external validation, immune interpretation, or single-cell localization, limiting model generalizability and mechanistic interpretation^{5–9,19–20}. Finally, public databases usually lack analgesic regimens, opioid exposure doses, and pain scores; therefore, gene-signature association studies must be clearly distinguished from causal studies of drug exposure^{5–9,19–20}.

To address these gaps, this study focused on a predefined panel of opioid receptor- and pain-related genes and integrated TCGA gastric, colon, and rectal cancer cohorts with GEO external validation data and GSE146771 single-cell data. Candidate-gene differential expression, single-gene prognostic value, LASSO-Cox risk score, immune checkpoint associations, MSI/TMB proxy features, Hallmark pathway activity, single-cell expression localization, and clinical prediction performance were systematically assessed^{5–9,19–29}. The aim was to propose a testable molecular signature from the perspective of pain, neuroimmune regulation, and the tumor microenvironment, thereby providing candidate directions for future studies of perioperative analgesic exposure and functional mechanisms^{10,14–18,30–41}.

2. Materials and Methods

2.1 Data Sources and Study Population

The discovery cohorts included transcriptomic expression matrices and clinical follow-up data from TCGA-STAD, TCGA-COAD, and TCGA-READ⁵⁻⁶. External validation was performed using GEO sample-level validation data from GSE39582, GSE62254, and GSE84437^{7,9,19}. Single-cell localization was performed using GSE146771 colorectal cancer leukocyte data²⁰. All datasets were obtained from public databases or organized sample-level analysis tables, and no new patient recruitment was conducted.

2.2 Candidate Gene Definition and Expression Matrix Processing

Candidate genes were predefined according to their biological relevance to opioid receptor signaling, pain sensing, inflammatory responses, purinergic signaling, and the endocannabinoid system. The final panel included OPRM1, OPRK1, OPRD1, OPRL1, OGFR, TLR4, PTGS2, TRPV1, CNR1, CNR2, and P2RX7³⁰⁻⁴¹. TCGA expression matrices were organized by gene symbol and merged with clinical follow-up information⁵⁻⁶. GEO validation data retained the GEO accession, sample identifier, risk score, risk group, overall survival time, and outcome status^{7,9,19}.

2.3 Differential Expression, Survival Analysis, and Risk Score Construction

Candidate-gene expression differences between tumor and normal tissues were tested using the Wilcoxon test with false discovery rate (FDR) correction. Single-gene prognostic value was evaluated using Cox regression, Kaplan-Meier curves, and log-rank tests. In the combined TCGA_ALL_GI cohort, LASSO-Cox regression was used to select genes and construct the risk score; patients were classified into high- and low-risk groups according to the median risk score²¹⁻²³. Model performance was evaluated using Kaplan-Meier curves, time-dependent receiver operating characteristic analysis, univariate Cox analysis, and multivariable Cox analysis²⁴.

2.4 External Validation

The TCGA_ALL_GI risk score was applied to the external validation datasets, and each cohort was stratified into high- and low-risk groups according to the cohort-specific median risk score^{7,9,19}. For each GEO cohort, the Cox hazard ratio (HR), 95% confidence interval (CI), Cox P value, and log-rank P value for high-risk versus low-risk patients were calculated to evaluate cross-platform stability in independent cohorts.

2.5 Immune, Pathway, and Clinical Prediction Analyses

Immune microenvironment analysis included marker-based immune proxy scores and immune checkpoint gene expression comparisons^{10,18}. MSI status and a TMB proxy were used to explore the relationship between the risk score and immunotherapy-related molecular background¹¹⁻¹³. Pathway

analysis was performed using MSigDB Hallmark gene sets, including preranked GSEA and GSVA-based ssGSEA single-sample pathway scoring^{27–29}. The clinical prediction model included the risk score, age, pathological stage, and tumor cohort; nomogram, calibration curve, and decision curve analyses were then performed^{25–26}.

3. Results

The study workflow is presented in Fig. 1, and the classification and biological rationale of the candidate genes are summarized in Supplementary Table S1.

3.1 Candidate-Gene Expression Remodeling and Single-Gene Prognostic Signals

Candidate-gene expression remodeling and single-gene prognostic results in gastric and colorectal cancers are shown in Fig. 2 and Supplementary Table S2. Candidate genes showed cohort-specific expression patterns across gastric and colorectal cancers. In TCGA-COAD, CNR1, CNR2, OPRM1, OPRL1, and P2RX7 were generally lower in tumor tissues, whereas OGFR was higher. Similar decreasing trends for CNR1, CNR2, OPRM1, OPRL1, and P2RX7 were observed in TCGA-READ, and in TCGA-STAD, CNR1 was lower and OGFR was higher. Single-gene Cox analysis showed that higher CNR1 expression in TCGA-STAD was associated with poorer overall survival (HR = 1.09, 95% CI 1.03–1.16, P = 0.004, FDR = 0.045), while higher TRPV1 expression in TCGA-COAD was associated with increased mortality risk (HR = 1.42, 95% CI 1.02–1.97, P = 0.036).

3.2 LASSO-Cox Risk Score and Internal Validation

Kaplan-Meier curves, time-dependent receiver operating characteristic analysis, and Cox regression results for the TCGA_ALL_GI risk model are shown in Fig. 3 and Supplementary Table S3. In the TCGA_ALL_GI cohort, the LASSO-Cox model retained CNR1, OPRM1, P2RX7, OPRK1, TLR4, OPRL1, and PTGS2. The high-risk group had significantly shorter overall survival than the low-risk group, and the continuous risk score was associated with increased mortality risk in univariate Cox analysis (HR = 3.49, 95% CI 2.47–4.94, P < 0.001). After adjustment for age, stage, and tumor cohort, the risk score remained an independent prognostic factor in multivariable Cox analysis (HR = 1.90, 95% CI 1.18–3.05, P = 0.008).

3.3 External Validation

Kaplan-Meier curves and cohort-level external validation results are shown in Fig. 4 and Supplementary Table S4. The risk score was externally supported in two of the three independent cohorts. GSE39582 included 573 samples and 176 events; the HR for high-risk versus low-risk patients was 1.43 (95% CI 1.06–1.93, Cox P = 0.019, log-rank P = 0.018). GSE84437 included 431 samples and 163 events, with an HR of 1.59 (95% CI 1.16–2.17, Cox P = 0.004, log-rank P = 0.003). GSE62254 showed the same direction of effect but did not reach statistical significance (HR = 1.25, 95% CI 0.80–1.93, Cox P = 0.325),

suggesting that cohort source, cancer-type composition, platform differences, and the number of follow-up events may influence model generalizability.

3.4 Immune Microenvironment and Immune Checkpoint Associations

Differences in immune marker scores and immune checkpoint expression between high- and low-risk groups are shown in Fig. 5 and Supplementary Table S5. The high-risk group showed a more active inflammatory and immunoregulatory profile. Marker-based immune proxy analysis showed that several immune-cell-related scores were higher in the high-risk group, and immune checkpoint genes such as PDCD1LG2, HAVCR2, TIGIT, LAG3, PDCD1, CTLA4, and CD274 also differed between groups. These findings indicate that the risk score may capture not only survival risk but also inflammatory and immunoregulatory tumor microenvironment features.

3.5 Pathway Enrichment, MSI/TMB Proxy, and Single-Cell Localization

Hallmark GSEA and GSVA/ssGSEA results are shown in Fig. 6. MSI/TMB proxy analyses and single-cell expression localization are presented in Supplementary Figures S1 and S2, respectively. Hallmark GSEA and GSVA/ssGSEA indicated that the high-risk group was enriched for EMT, inflammatory response, complement, angiogenesis, TGF-beta, TNF-alpha/NF-kappaB, IL6/JAK/STAT3, and interferon-response pathways at both group and single-sample levels. MSI and TMB proxy analyses suggested an association between the risk score and immunotherapy-related molecular background. GSE146771 single-cell analysis showed cell-type-related expression differences of candidate genes in colorectal cancer leukocyte subsets, providing cell-level support for the link between the risk score and immune microenvironment features.

3.6 Clinical Prediction Model

Nomogram, calibration curve, and decision curve analysis results are presented in Supplementary Figure S3 and Supplementary Table S6. A multivariable Cox model including the risk score, age, stage, and tumor cohort was used to construct a 3-year overall survival nomogram. The calibration curve showed that predicted probabilities were generally close to observed outcomes, and decision curve analysis suggested that the combined model provided a higher net benefit than the treat-all and treat-none strategies across part of the threshold probability range.

4. Discussion

This study systematically evaluated the expression remodeling, prognostic value, immune microenvironment associations, and external validation performance of opioid receptor- and pain-related genes in gastric and colorectal cancers. Overall, the results suggest that these genes are not only related

to analgesia or neural regulation but also associated with inflammatory responses, stromal remodeling, and immunoregulatory states in gastrointestinal cancers^{10,14–18,30–34}. The LASSO-Cox risk score constructed from candidate genes showed independent prognostic value in the TCGA_ALL_GI cohort and was externally supported in GSE39582 and GSE84437. Although GSE62254 did not reach statistical significance, it showed a concordant direction, suggesting partial cross-cohort stability of the model.

At the expression level, candidate genes showed cohort-specific remodeling in gastric and colorectal cancers. Some opioid receptor- and pain-related molecules were downregulated in tumor tissues, whereas genes such as OGFR were increased in certain cohorts, indicating that these molecules do not exert a single uniform tumor-biological effect. In single-gene analysis, CNR1 in TCGA-STAD and TRPV1 in TCGA-COAD were associated with overall survival, but single-gene effects were limited and may be influenced by cancer type, platform, and sample composition. Therefore, this study used a LASSO-Cox model to integrate multigene information and capture the combined effects of multiple pain-, inflammation-, and opioid receptor-related signals^{21–23}.

At the model level, the risk score showed strong prognostic value in the internal cohort and remained independent after adjustment for age, stage, and tumor cohort, suggesting that this signature provides complementary information beyond traditional clinical variables. In external validation, GSE39582 and GSE84437 showed poorer outcomes for high-risk patients, whereas GSE62254 showed only a concordant trend. These results indicate that the model has promising but not uniform generalizability across GEO platforms and disease compositions. Presenting both successful and non-significant validation results provides a more balanced assessment than reporting internal positive findings alone.

At the immune and pathway levels, the high-risk group was enriched for Hallmark pathways including EMT, inflammatory response, complement, angiogenesis, TGF-beta, TNF-alpha/NF-kappaB, IL6/JAK/STAT3, and interferon response, accompanied by differences in immune markers and immune checkpoint expression^{10,14–18,27–29}. These findings suggest that the risk score may reflect a tumor microenvironment characterized by active inflammation, stromal remodeling, and increased immunoregulatory signaling. Associations between the risk score and MSI/TMB proxy features suggest a possible link with immunotherapy-related molecular background, but the current evidence is insufficient to equate this signature with MSI-H status, high tumor mutational burden, or immunotherapy benefit^{11–13}.

Previous TCGA and GEO studies have shown that gastric and colorectal cancers are molecularly heterogeneous, with subtypes differing in immune activation, stromal components, inflammatory responses, and clinical prognosis^{5–9}. In this study, the association of the risk score with survival outcomes, inflammatory pathways, and immune checkpoint expression is broadly consistent with current understanding of gastrointestinal cancer heterogeneity. In particular, enrichment of EMT, TGF-beta, inflammatory response, and complement pathways in the high-risk group supports the concept that inflammation, stroma, and immune regulation jointly promote tumor progression^{14–18}.

Evidence from pain and opioid receptor biology also supports the interpretation of these findings. Previous studies suggest that opioid receptor signaling, TLR4, COX-2/PTGS2, TRPV1, P2RX7, and the endocannabinoid system can participate in inflammatory responses, nociception, immune-cell activation, and tumor biology^{30–41}. This study integrated these dispersed signals into a unified candidate-gene framework and examined them across bulk transcriptomes, external validation datasets, and single-cell leukocyte data. The findings are consistent with prior knowledge of cancer-related inflammation, immune checkpoints, and the tumor immune microenvironment^{10,14–20}.

Compared with previous studies, the incremental value of this work is that it incorporates candidate genes of interest to pain medicine and anesthetic oncology into prognostic modeling for gastrointestinal cancers and simultaneously evaluates immune checkpoints, MSI/TMB proxy features, Hallmark pathways, and single-cell localization. This design goes beyond risk-score construction by exploring the immune microenvironment meaning of the score. However, the current results remain associative and cannot demonstrate that opioid drug exposure itself alters tumor progression or patient survival.

This study has several strengths. First, candidate genes were predefined according to opioid receptor signaling, pain sensing, inflammatory responses, purinergic signaling, and the endocannabinoid system, reducing the randomness associated with purely data-driven screening. Second, TCGA discovery cohorts, external validation data, and GSE146771 single-cell data were incorporated, providing traceability and cross-level interpretability for model assessment^{5–9,19–20}. Third, the analysis was not limited to survival modeling but also included immune markers, immune checkpoints, MSI/TMB proxy features, GSEA, GSVA/ssGSEA, nomogram, calibration curve, and decision curve analyses, allowing the results to be interpreted from prognostic, mechanistic, and clinical-prediction perspectives^{10–13,18,21–29}.

This study also has several limitations. First, all data were obtained from public databases or organized sample-level data; therefore, the analysis was retrospective and may be affected by sample selection, platform batch effects, endpoint definitions, and missing treatment information. Second, public databases lack key variables such as perioperative analgesic regimen, opioid exposure dose, pain score, anesthesia method, treatment line, and recurrence treatment, so the causal relationship between analgesic exposure and prognosis could not be evaluated. Third, immune analysis was mainly based on marker-derived immune proxies, and TMB was evaluated using a proxy measure; these approaches cannot fully replace standard immune-infiltration deconvolution or true TMB defined as mutations per megabase. Fourth, GSE146771 mainly covers colorectal cancer leukocyte populations and cannot fully represent tumor epithelial cells, fibroblasts, endothelial cells, or the gastric cancer microenvironment. Finally, independent prospective cohorts and functional experiments are lacking; therefore, the risk score should be regarded as a candidate molecular framework rather than a direct clinical decision-making tool.

5. Conclusion

Opioid receptor- and pain-related gene signatures have prognostic and immune microenvironment relevance in gastric and colorectal cancers. External validation supported the risk score in GSE39582 and GSE84437, while GSE62254 showed a concordant but non-significant trend. This signature may serve as a candidate molecular framework for research at the intersection of pain, neuroimmune regulation, and tumor progression. Future studies should integrate real perioperative analgesic exposure information, independent clinical cohorts, and functional experiments.

Abbreviations

CAF

Cancer-associated fibroblast

CI

Confidence interval

CNR1

Cannabinoid receptor 1

CNR2

Cannabinoid receptor 2

COAD

Colon adenocarcinoma

COX-2

Cyclooxygenase-2

EMT

Epithelial-mesenchymal transition

FDR

False discovery rate

GEO

Gene Expression Omnibus

GSEA

Gene set enrichment analysis

GSVA

Gene set variation analysis

HR

Hazard ratio

IL6

Interleukin 6

JAK

Janus kinase

LASSO

Least absolute shrinkage and selection operator

MSI

Microsatellite instability

MSigDB

Molecular Signatures Database

NF- κ B

Nuclear factor kappa-light-chain-enhancer of activated B cells

OGFR

Opioid growth factor receptor

OPRD1

Opioid receptor delta 1

OPRK1

Opioid receptor kappa 1

OPRL1

Opioid receptor-like 1

OPRM1

Opioid receptor mu 1

P2RX7

Purinergic receptor P2X 7

PTGS2

Prostaglandin-endoperoxide synthase 2

READ

Rectal adenocarcinoma

ROC

Receiver operating characteristic

ssGSEA

Single-sample gene set enrichment analysis

STAD

Stomach adenocarcinoma

STAT3

Signal transducer and activator of transcription 3

TCGA

The Cancer Genome Atlas

TGF- β

Transforming growth factor beta

TLR4

Toll-like receptor 4

TMB

Tumor mutational burden

TNF- α

Tumor necrosis factor alpha

TNM

Tumor, node, metastasis

TRPV1

Transient receptor potential cation channel subfamily V member 1

Declarations

Ethics Statement

This study was based on publicly available, de-identified datasets from TCGA and GEO. No new human participants were recruited, and no new biological specimens were collected. Therefore, additional institutional ethics approval and informed consent were not required(N/A).

Consent for Publication

Not applicable.

Availability of data and materials

The datasets analyzed in this study are publicly available from TCGA and GEO. The GEO datasets used in the study include GSE39582, GSE62254, GSE84437, and GSE146771. Additional processed data and analysis materials are available from the corresponding author on reasonable request

Competing interests

The authors declare that they have no competing interests

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Author Contributions

Zhaolong Zhao: Writing – original draft; Jing Liang: Writing – review & editing; Kun Wu: Software, Formal analysis; Jingge Cheng: Data curation; Danmei Li: Methodology; Hemei Wang: Conceptualization, Supervision. All authors read and approved the final manuscript.

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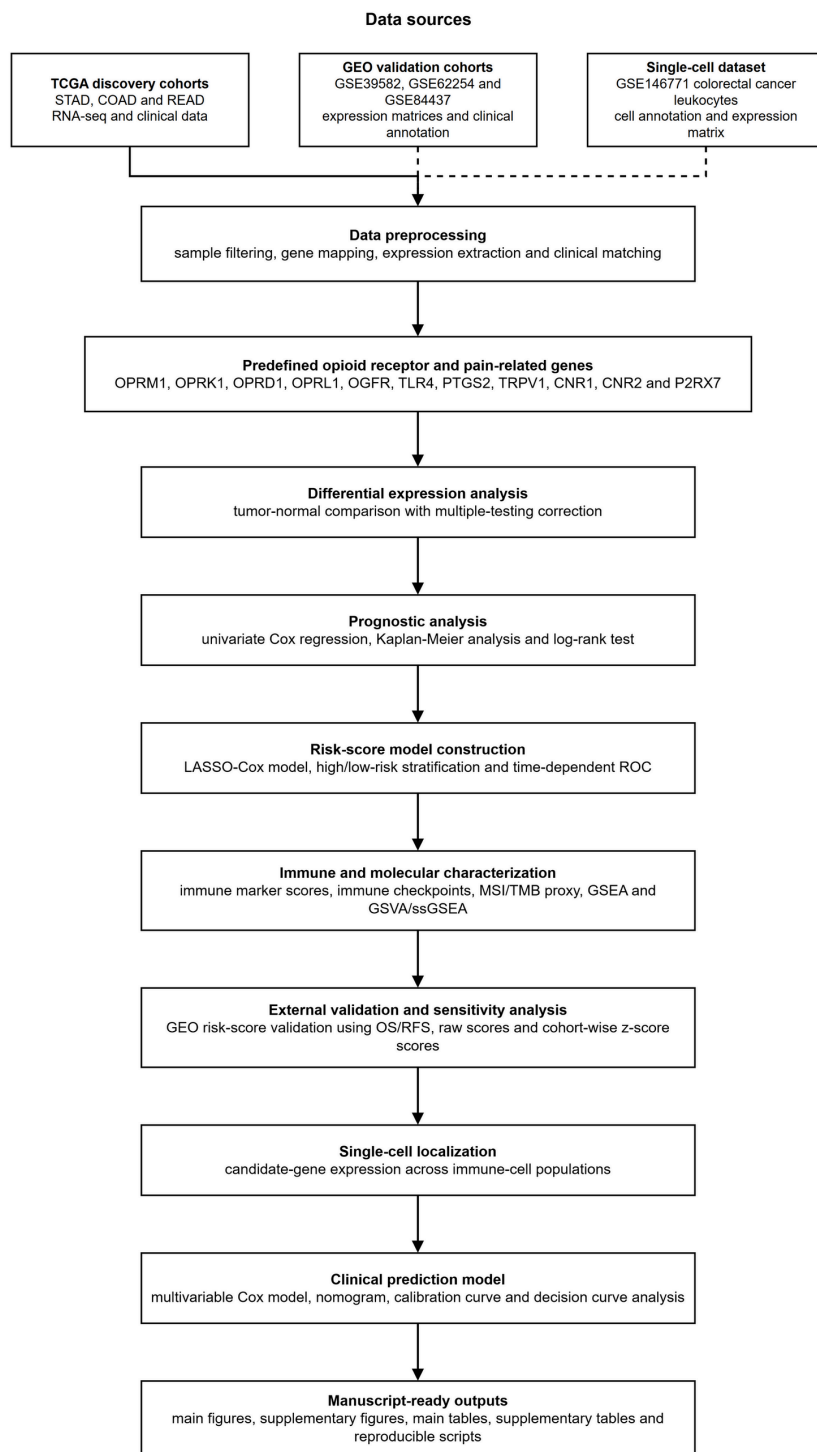
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Figures



All boxes are study steps; dashed arrows indicate supporting data sources entering preprocessing.

Figure 1

Study workflow.

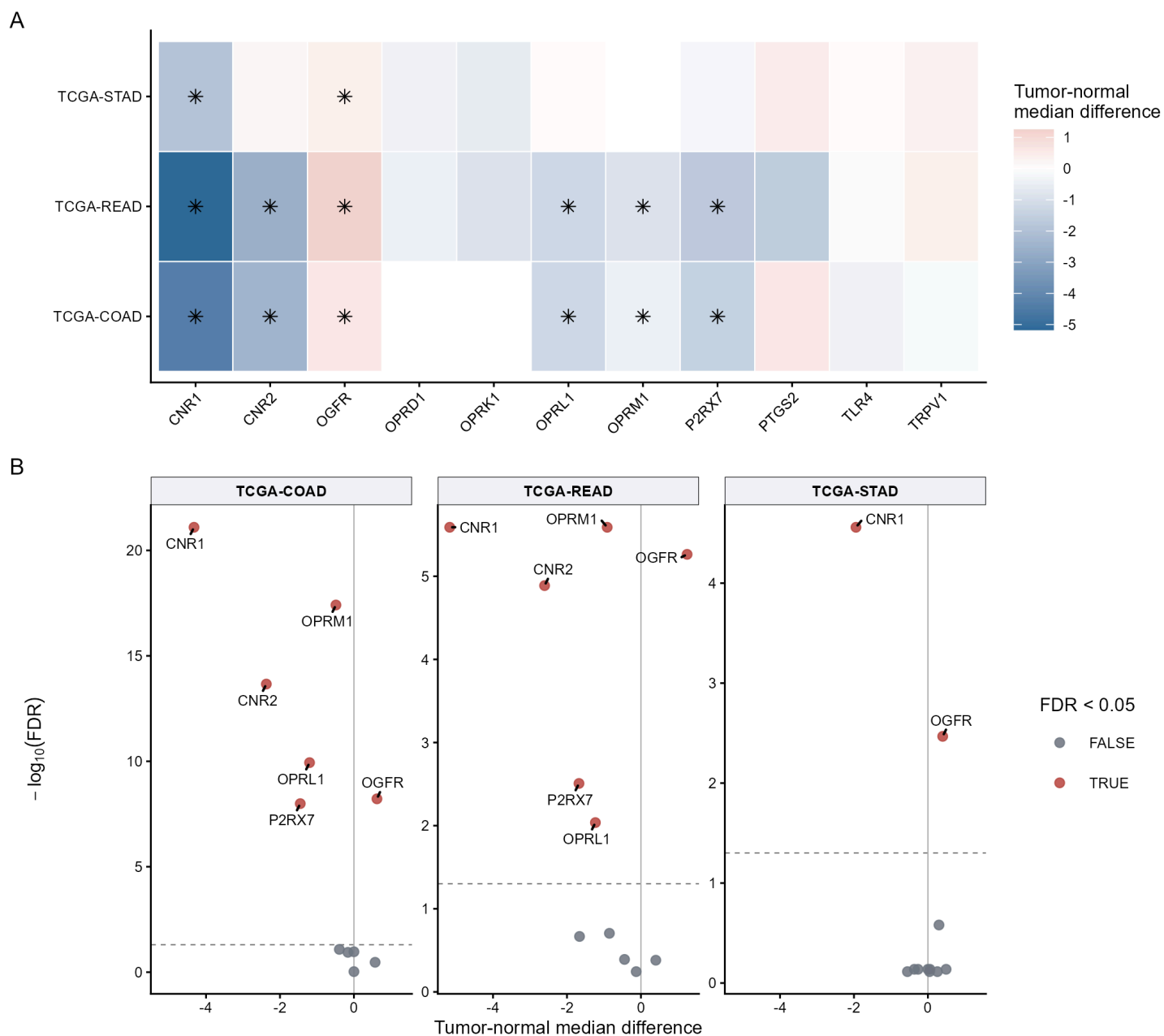


Figure 2

Differential expression of candidate genes in TCGA gastrointestinal cancer cohorts.

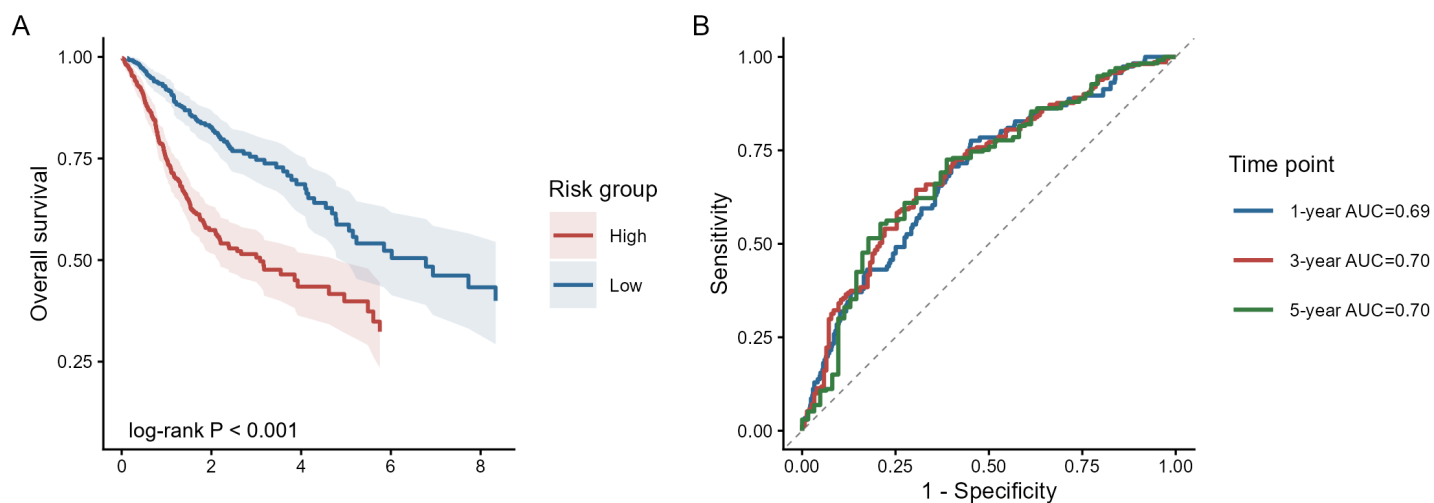


Figure 3

Internal validation of the TCGA_ALL_GI risk score.

GEO external validation of the risk score

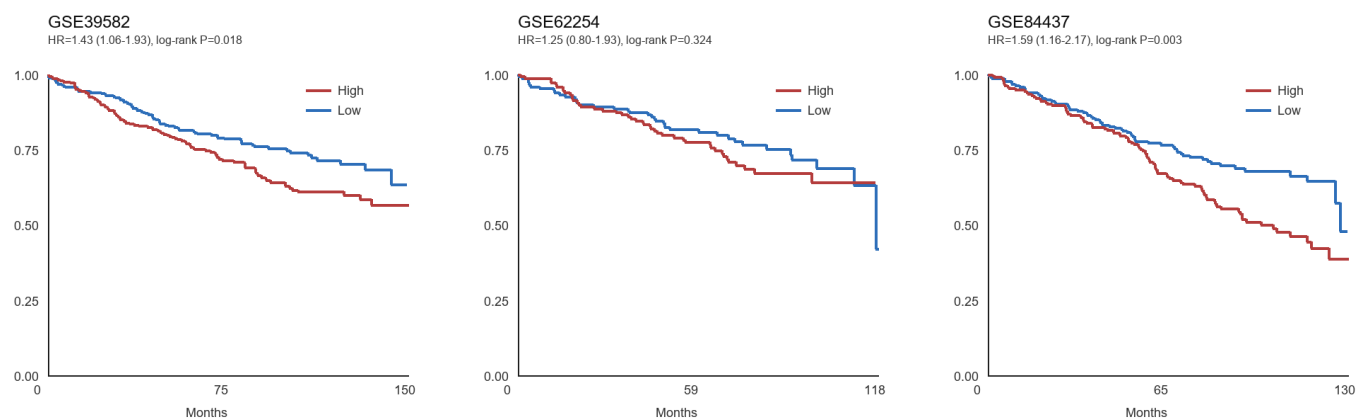


Figure 4

External validation Kaplan-Meier curves.

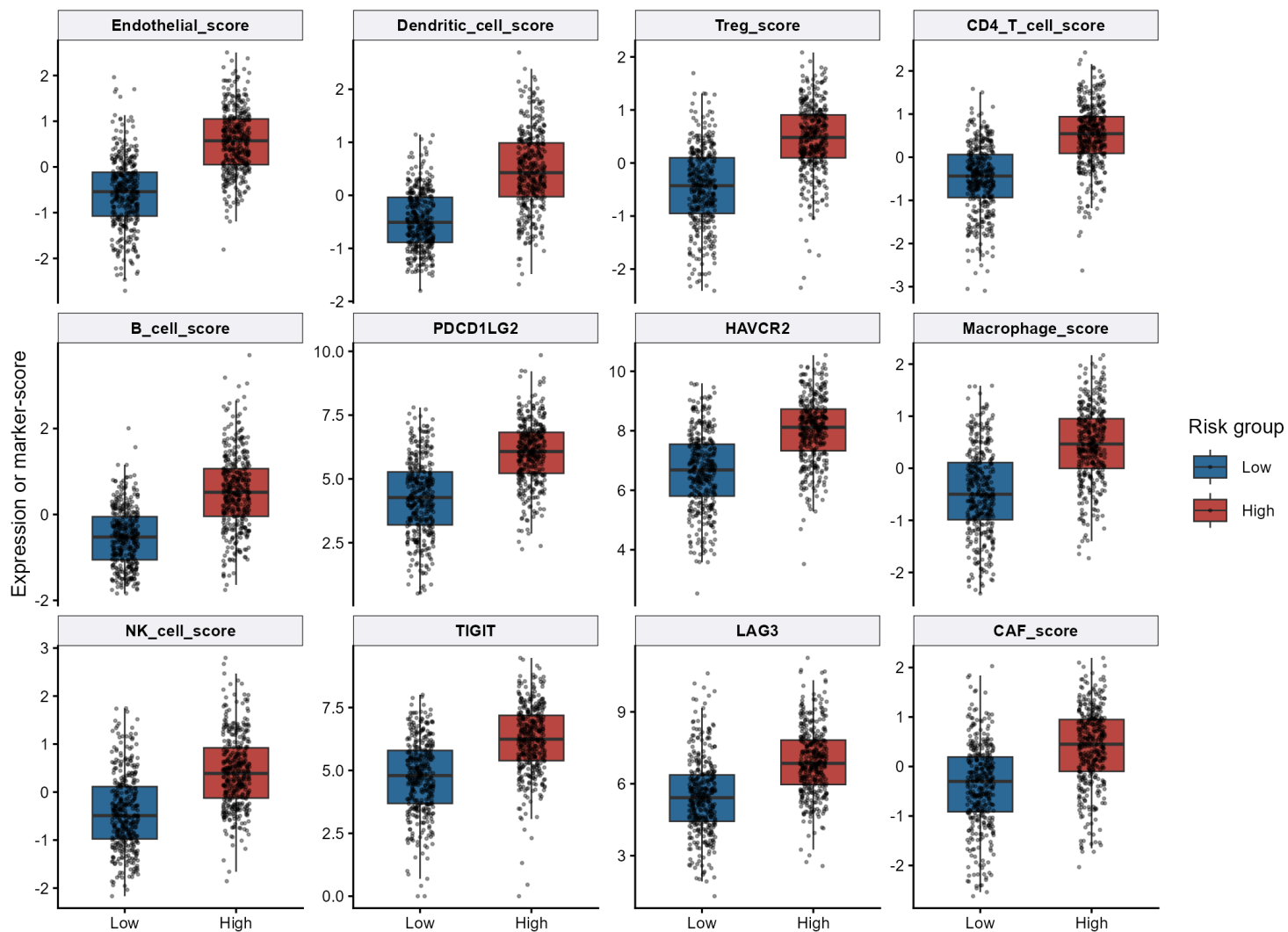


Figure 5

Immune marker and immune checkpoint differences between high- and low-risk groups.

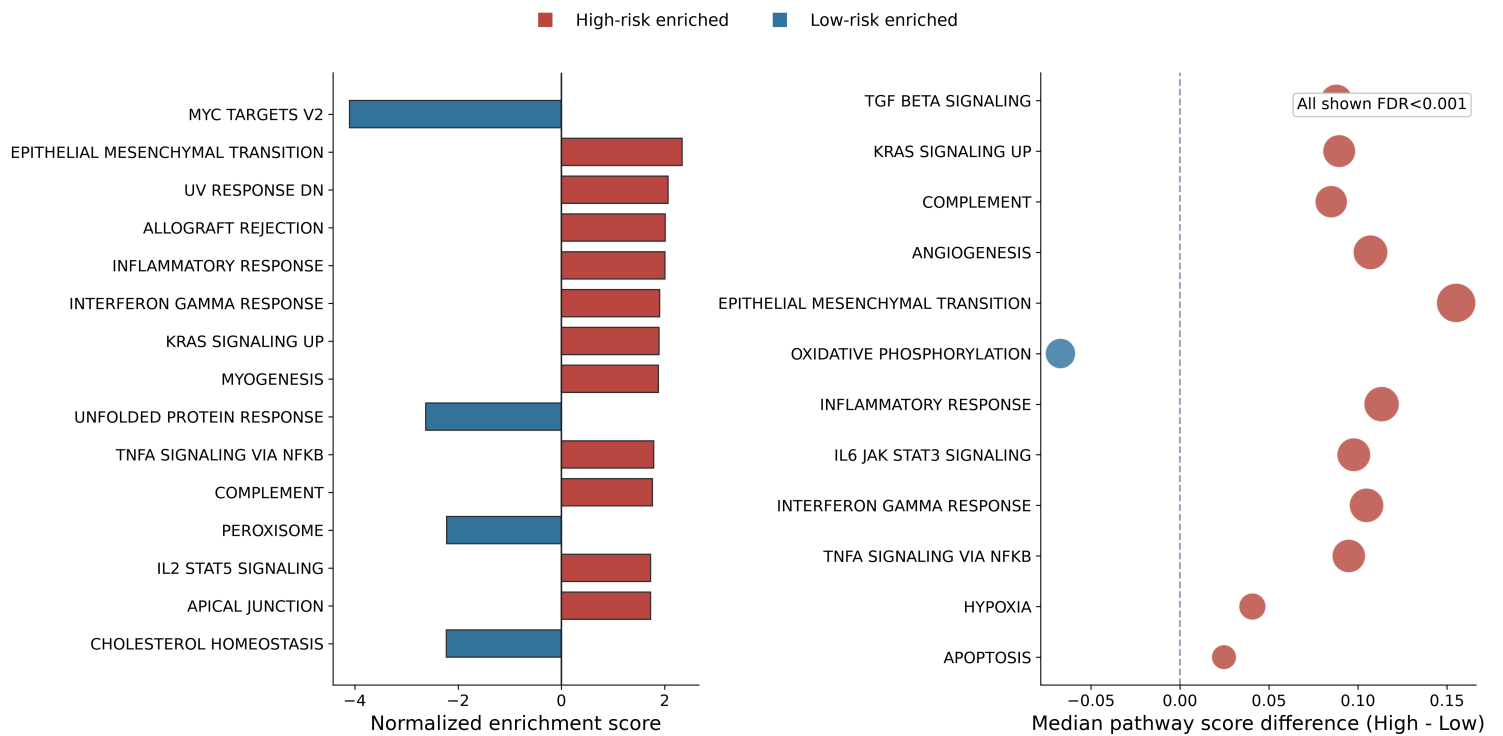


Figure 6

Hallmark GSEA and GSVA/ssGSEA pathway results associated with risk groups.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryMaterials.docx](#)