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Unraveling the Anticancer Mechanisms of Cannabidiol in Colorectal Cancer via Network Pharmacology and Molecular Docking

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ABSTRACT

Colorectal cancer (CRC) is one of the most common malignancies worldwide, and currently available treatments are often associated with notable adverse effects. Cannabidiol (CBD), a bioactive constituent of *Cannabis sativa*, has exhibited promising antitumor potential. However, its precise molecular mechanisms remain unclear. Therefore, this study aims to investigate the mechanism of action of cannabidiol in colorectal cancer using a network pharmacology approach supported by molecular docking analysis. This study employed an integrative framework combining network pharmacology with molecular docking. Putative targets of CBD and CRC-related genes were retrieved using Swiss Target Prediction, MalaCards, and DisGeNET databases. Protein–protein interaction (PPI) networks were constructed and examined through STRING and Cytoscape. Functional enrichment analysis was performed using ShinyGO, while gene expression patterns and immune infiltration were analyzed via UALCAN and TISIDB. A total of 95 overlapping genes between CBD-related targets and CRC-associated genes were identified. Six hub genes (ANXA5, IGF1R, JAK2, MAPK8, MDM2, and PARP1) emerged as particularly significant due to their strong network centrality and involvement in key biological processes. Molecular docking results indicated that CBD binds effectively with these targets, potentially influencing major signaling pathways such as RAS/MAPK and PI3K-AKT/FoxO, which are essential for regulating cell growth, apoptosis, and the cell cycle. Among these genes, ANXA5 and JAK2 showed strong associations with immune cell infiltration, suggesting their involvement in modulating the tumor immune microenvironment. CBD may exert multi-target regulatory effects in CRC by modulating key genes and signaling pathways, supporting its potential as an adjunct therapeutic to enhance efficacy and reduce the toxicity of conventional treatments.

Keywords: Network pharmacology, Molecular docking, Cannabidio, Colorectal cancer, Immunity

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Introduction

Colorectal cancer (CRC) is defined as the malignant, uncontrolled proliferation of epithelial cells in the colon and rectum, which are the terminal portions of the digestive system [1]. It is among the top five most frequently diagnosed cancers globally. In 2022, approximately 1.9 million new CRC cases were recorded worldwide, along with about 900,000 deaths [2]. In Mexico, CRC mortality showed an upward trend between 1998 and 2018, increasing annually by 1.3% in women and 2.7% in men [3].

The conventional management of CRC involves surgical resection—either open or laparoscopic—often accompanied by excision of regional lymph nodes. Radiotherapy is sometimes used as an alternative or adjunct approach. Chemotherapy remains a cornerstone of treatment, employing agents such as 5-fluorouracil combined

with folinic acid, irinotecan, and oxaliplatin, as well as targeted biologics including bevacizumab, cetuximab, and panitumumab. However, these therapeutic strategies, particularly chemotherapy and radiotherapy, are frequently linked to considerable toxicity and side effects [4]. As a result, there is growing interest in developing alternative or complementary approaches that can reduce adverse effects while maintaining or improving treatment outcomes. Emerging alternatives include nanomedicine approaches utilizing materials such as selenium and silver nanoparticles [5, 6]. In addition, natural products and plant-derived compounds—including extracts from *Curcuma longa*, *Panax quinquefolius*, *Emilia sonchifolia*, and *Cannabis sativa*—as well as their bioactive constituents, such as carotenoids and flavonoids, have been explored for CRC therapy [7, 8]. Among these, cannabidiol (CBD) from *Cannabis sativa* has attracted attention due to evidence suggesting its ability to regulate apoptosis, cell proliferation, autophagy, and tumor progression in CRC models [9-11]. Furthermore, CBD has been reported to influence immune cell functions, including natural killer cell activity, within colorectal cancer environments [12]. Despite these findings, the detailed molecular pathways underlying CBD's effects remain incompletely understood, necessitating further research.

Network pharmacology represents a shift from the traditional "one disease–one target–one drug" paradigm toward a systems-level perspective in drug discovery. It enables the exploration of interactions between bioactive plant compounds and complex biological networks, supporting the development of more holistic and less toxic therapeutic strategies. By targeting interconnected signaling modules rather than single molecular targets, this approach facilitates the design of multi-target interventions with synergistic effects. It relies on multiple bioinformatics resources and databases, such as InterPro, KEGG, and PubChem, which are essential for mapping protein–protein interactions and biological pathways [13, 14].

Molecular docking serves as a complementary computational method that supports network pharmacology analyses. It models the interaction between small molecules and biological macromolecules, allowing prediction of binding conformations and interaction energies. It can also be extended to analyze macromolecular interactions such as protein–protein or protein–DNA binding. This technique is widely used in drug discovery and nutraceutical research due to its efficiency and low cost, although it is less precise than experimental approaches. Its predictive accuracy continues to improve with advancements in machine learning techniques [15, 16].

Therefore, this study aims to investigate the mechanism of action of cannabidiol in colorectal cancer using a network pharmacology approach supported by molecular docking analysis.

Materials and Methods

Data acquisition

The molecular structure and SMILES representation of cannabidiol (CBD) were retrieved from the PubChem database [17]. Potential biological targets of CBD were subsequently predicted using Swiss Target Prediction [18] and the PharmMapper Server [19]. In Swiss Target Prediction, only targets with a probability ≥ 0.1 were retained, whereas in PharmMapper, a normalized fit score ≥ 0.5 was used as the cutoff. In both datasets, only targets corresponding to *Homo sapiens* were included.

CRC-related genes were collected from three independent resources: Malacards [20], DisGeNET [21], and the Comparative Toxicogenomics Database (CTD) [22]. For DisGeNET, entries with Score_gda ≥ 0.1 were selected, and only human genes (*Homo sapiens*) were considered across all databases.

After standardization and duplicate removal, shared targets between CBD-associated proteins and CRC-related genes were identified using Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/, accessed on 5 July 2024) by performing an intersection analysis of the datasets.

Enrichment of gene ontology and metabolic pathways

Functional enrichment of the 95 intersecting genes was carried out using the ShinyGO 0.81 platform [23]. The analysis included Gene Ontology classifications—biological process (BP), molecular function (MF), and cellular component (CC)—as well as Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment.

Settings were constrained to a minimum of 2 genes per term and a maximum of 5000 pathways, with *Homo sapiens* defined as the organism. Statistical significance was adjusted using the False Discovery Rate (FDR) based on the Benjamini–Hochberg correction method. Visualization of enriched terms was performed using SRplot (https://www.bioinformatics.com.cn/en, accessed on 5 November 2024) [24].

Construction of protein–protein interaction network

A protein–protein interaction (PPI) network was generated for the identified targets using the STRING database [25], limited to *Homo sapiens*. Only interactions with a confidence score greater than 0.7 were retained to ensure high reliability. The resulting network was imported into Cytoscape v3.10.3 for visualization and downstream analysis [26]. Key hub regions and highly connected nodes were identified using the cytoHubba plugin [27].

Molecular docking analysis between cannabidiol and hub genes

To evaluate the interaction potential between CBD and CRC-related hub genes, molecular docking analyses were performed using two independent structural sources and two docking approaches.

In the first pipeline, three-dimensional protein structures were retrieved from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) [28]. The selected entries included 6k22 (ANXA5), 3nw5 (IGF1R), 6vgl (JAK2), 3pze (MAPK8), 4hbm (MDM2), and 7kk2 (PARP1). Protein preparation involved removing water molecules and co-crystallized ligands, followed by the addition of polar hydrogen atoms using BIOVIA Discovery Studio. The CBD ligand structure was downloaded from PubChem in SDF format, converted using Open Babel within PyRx, and subjected to energy minimization with charge assignment using the universal force field. Docking simulations were performed with AutoDock Vina through PyRx 0.8 [29]. Eight docking runs were executed, grid parameters were optimized, and the most stable binding conformations were selected for analysis.

In the second pipeline, protein structures were obtained from the AlphaFold Protein Structure Database [30], restricted to reviewed Homo sapiens models. Blind docking was conducted using the CB-Dock2 platform [31], and the best binding poses were selected based on minimum binding energy values. Binding affinities were reported in kcal/mol. Visualization of docking complexes and generation of 2D interaction maps were performed using BIOVIA Discovery Studio Viewer (v21.1.0.20298).

Analysis of expression and infiltration of immune cells

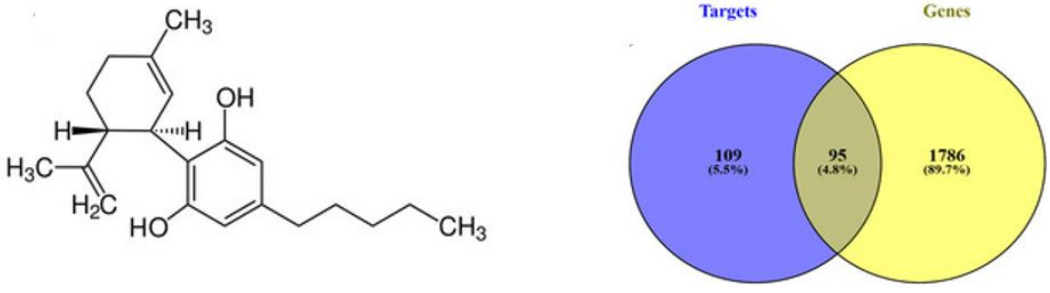
Expression differences of hub genes between CRC tissues and adjacent normal tissues were analyzed using the UALCAN database [32]. In addition, the TISIDB platform was used to investigate associations between hub gene expression and immune cell infiltration as well as immune-related molecular markers [33], focusing on colon adenocarcinoma (COAD) and rectum adenocarcinoma (READ).

Comparisons between tumor and normal tissues were conducted using Student's t-test. Correlation analyses were performed using Spearman's rank correlation test, with statistical significance defined as $P < 0.05$ for both analyses.

Results and Discussion

Analysis of cannabidiol targets and CRC-associated genes

The molecular structure of CBD was obtained from PubChem **(Figure 1a).** A total of 204 putative targets of CBD were identified through Swiss Target Prediction and PharmMapper Server. Meanwhile, 1881 CRC-related genes were retrieved from Malacards, DisGeNET, and CTD databases. Intersection analysis between both datasets revealed 95 overlapping genes **(Figure 1b).**



a) b)

Figure 1. (a) Chemical structure of cannabidiol, and (b) Shared targets between cannabidiol-related proteins and colorectal cancer-associated genes.

Gene ontology enrichment analysis and KEGG pathways

Gene Ontology analysis was performed using the 95 intersecting genes. The results indicated that the most significantly represented biological processes were primarily linked to programmed cell death (apoptosis) and cellular responses to diverse external stimuli, including chemical agents, organic compounds, and cyclic organic molecules.

For cellular component enrichment, these genes were predominantly localized to membrane-associated regions, especially lipid rafts, membrane microdomains, and vesicle-related structures. Regarding molecular function, enrichment signals were mainly observed in kinase-related activities and nucleotide-binding functions **(Figure 2).**

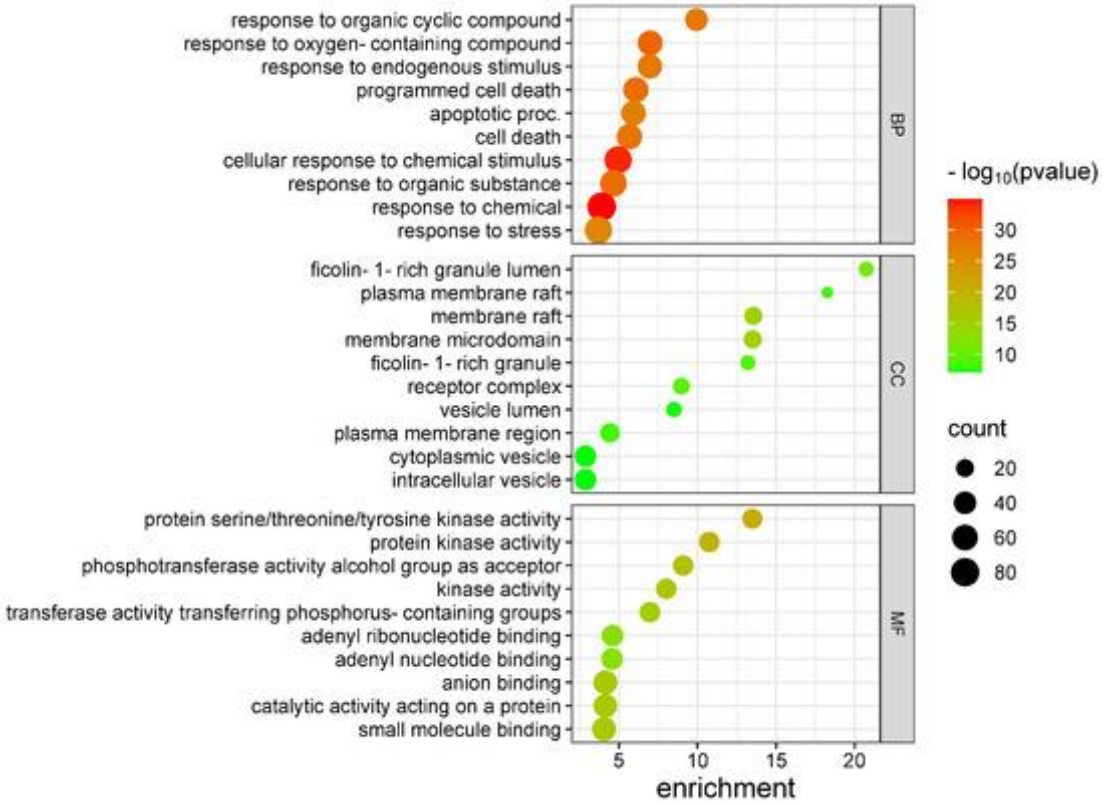


Figure 2. Gene ontology enrichment analysis. Abbreviations: BP = biological process, CC = cellular component, and MF = molecular function.

KEGG pathway enrichment revealed involvement in a broad range of signaling networks. The most prominent pathways included FoxO, RAS, MAPK, and PI3K-AKT signaling cascades. These were additionally linked to immune and endocrine regulation through pathways such as IL-17 signaling, lectin receptor pathways, relaxin signaling, and hormone-associated pathways, including estrogen and progestin signaling.

Moreover, disease-related pathways were also enriched, including Helicobacter pylori infection, atherosclerosis development, and multiple cancer-associated signaling routes **(Figure 3)**.

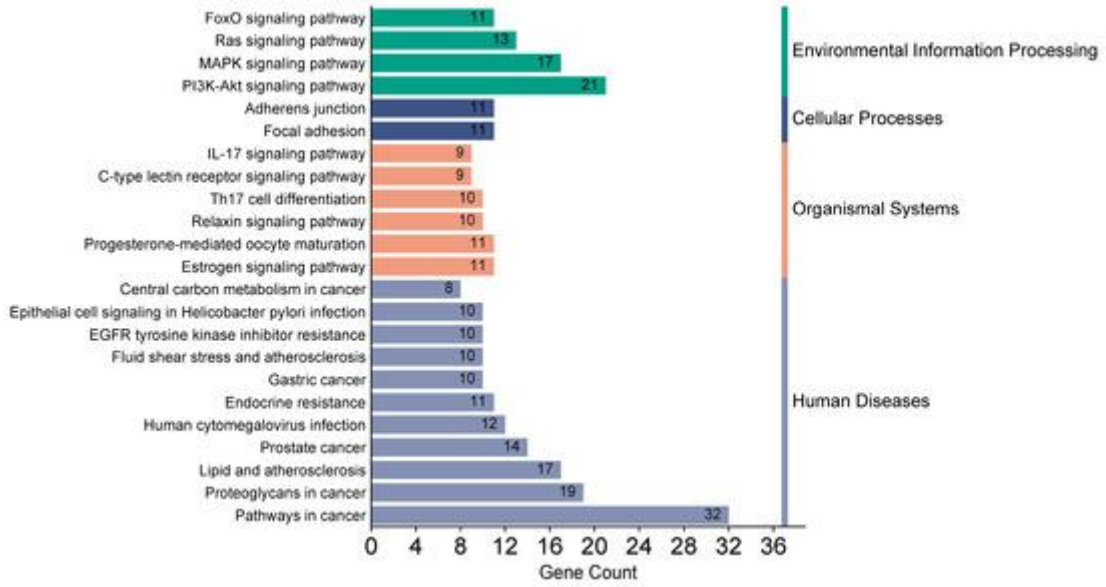


Figure 3. KEGG pathway enrichment analysis.

Construction of the Protein–Protein Interaction Network and Prediction of Hub Genes

The protein–protein interaction (PPI) network was generated and analyzed in Cytoscape. In this network, nodes represent proteins, and edges represent their interactions. The resulting network comprised 1015 interaction pairs, and **Figure 4** shows the 95 proteins ranked by connectivity, from highest to lowest.

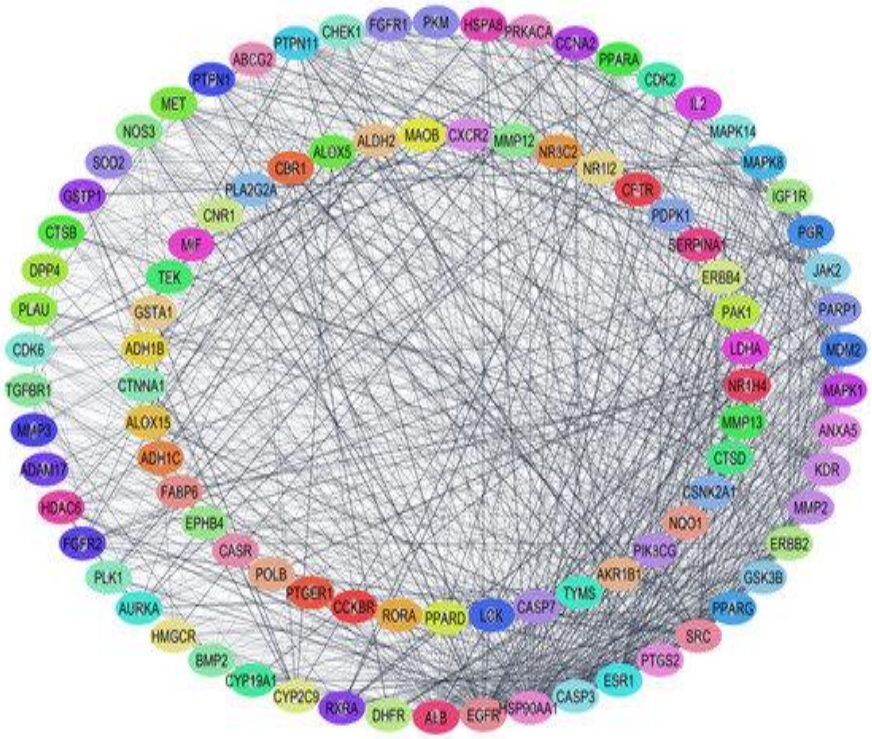


Figure 4. Protein–protein interaction network constructed from shared genes.

To determine potential key therapeutic targets of CBD in CRC, several network topology algorithms were applied, including degree centrality, maximum neighborhood component (MNC), density of maximum neighborhood component (DMNC), and maximal clique centrality (MCC). These metrics are widely used for identifying essential nodes in biological networks.

The degree score reflects the total number of direct connections associated with a node. MNC evaluates node importance based on the largest connected subnetwork within its neighborhood. DMNC further refines this by

incorporating both neighborhood size and internal density. MCC identifies nodes participating in fully connected substructures (cliques), where adding any additional node would disrupt complete connectivity.

Each algorithm produced a top 20-ranked gene list. A Venn diagram was then used to identify overlapping candidates, resulting in six common hub genes: annexin A5 (ANXA5), insulin-like growth factor 1 receptor (IGF1R), Janus kinase 2 (JAK2), mitogen-activated protein kinase 8 (MAPK8), MDM2 proto-oncogene, E3 ubiquitin-protein ligase (MDM2), and poly (ADP-ribose) polymerase 1 (PARP1). The identification process is summarized in **Figure 5.**

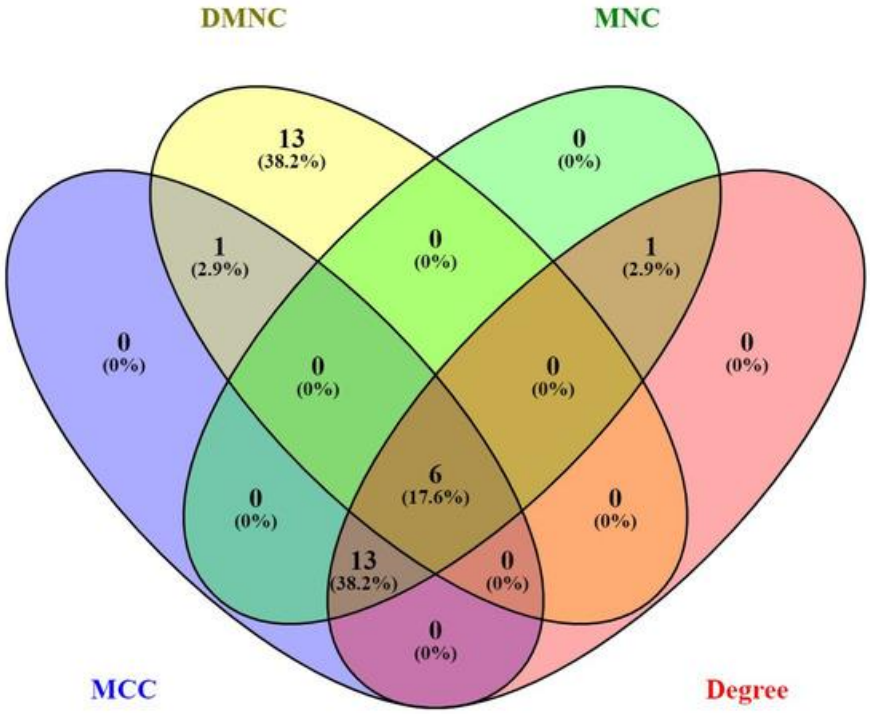


Figure 5. Venn diagram illustrating hub gene selection.

Molecular Docking Between Cannabidiol and Hub Genes

To evaluate the binding potential of CBD with CRC-related hub proteins (ANXA5, IGF1R, JAK2, MAPK8, MDM2, and PARP1), molecular docking analyses were performed. **Table 1** summarizes binding affinity values (kcal/mol) obtained using two docking platforms (AutoDock Vina and CB-Dock2) and two protein structure sources (experimental structures from RCSB PDB and predicted models from AlphaFold).

In this context, more negative binding energy values reflect stronger ligand–receptor affinity, with values below −5 typically indicating stable interactions. Among all tested combinations, the strongest binding was observed for MDM2 when CB-Dock2 was paired with RCSB structures, yielding a value of −8.2 kcal/mol.

Overall, differences among docking combinations for the remaining proteins were relatively minor, typically within 0.5–1 kcal/mol. Collectively, the results suggest that all six hub proteins exhibit stable interaction potential with CBD.

Two-dimensional interaction diagrams were also generated to visualize ligand–protein binding patterns (**Figure 6).**

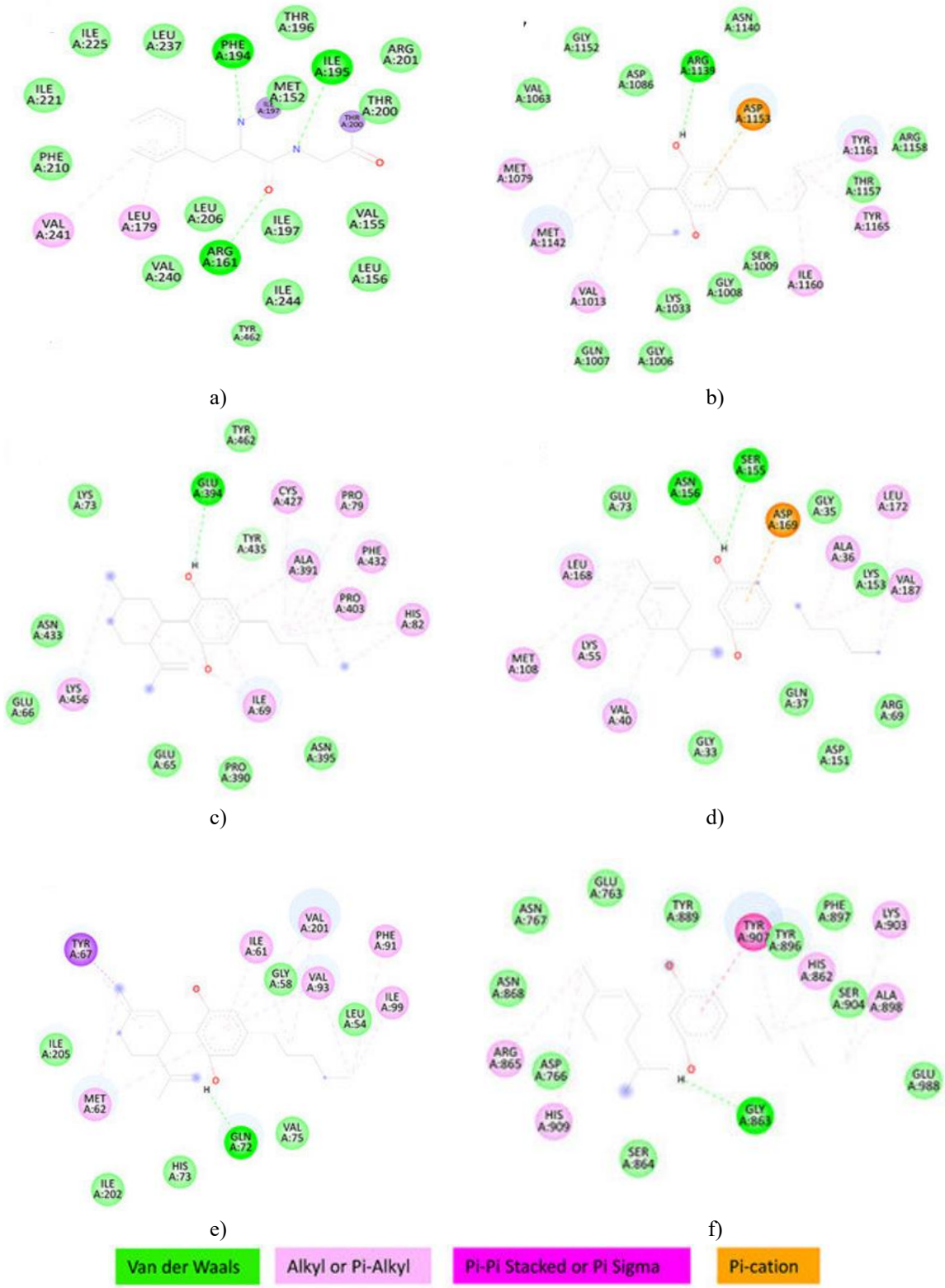


Figure 6. 2D interaction maps of cannabidiol with hub genes: (a) ANXA5, ΔG = −7.1; (b) IGF1R, ΔG = −6.8; (c) JAK2, ΔG = −7.8; (d) MAPK8, ΔG = −7.6; (e) MDM2, ΔG = −6.3; and (F) PARP1, ΔG = −8.1.

Table 1. Binding energies (kcal/mol) of cannabidiol with target proteins.

Protein	AutodockVina RCSB	AutodockVina AlphaFold	CB-Dock2 AlphaFold	CB-Dock2 RSCB
ANXA5	−6.6	−7.3	−7.1	−7.0
IGF1R	−7.3	−6.4	−6.8	−7.6

JAK2	−7.3	−6.9	−7.8	−7.6
MAPK8	−6.7	−7.7	−7.6	−7.2
MDM2	−7.2	−6.7	−6.3	−8.2
PARP1	−8.0	−7.2	−8.1	−8.1

Analysis of hub gene expression in CRC

Hub gene expression levels were investigated using the UALCAN platform, which analyzes transcriptomic data derived from The Cancer Genome Atlas (TCGA). For analytical purposes, colorectal cancer cases were separated into two anatomical subgroups: colon adenocarcinoma (COAD) and rectum adenocarcinoma (READ) **(Figures 7 and 8).**

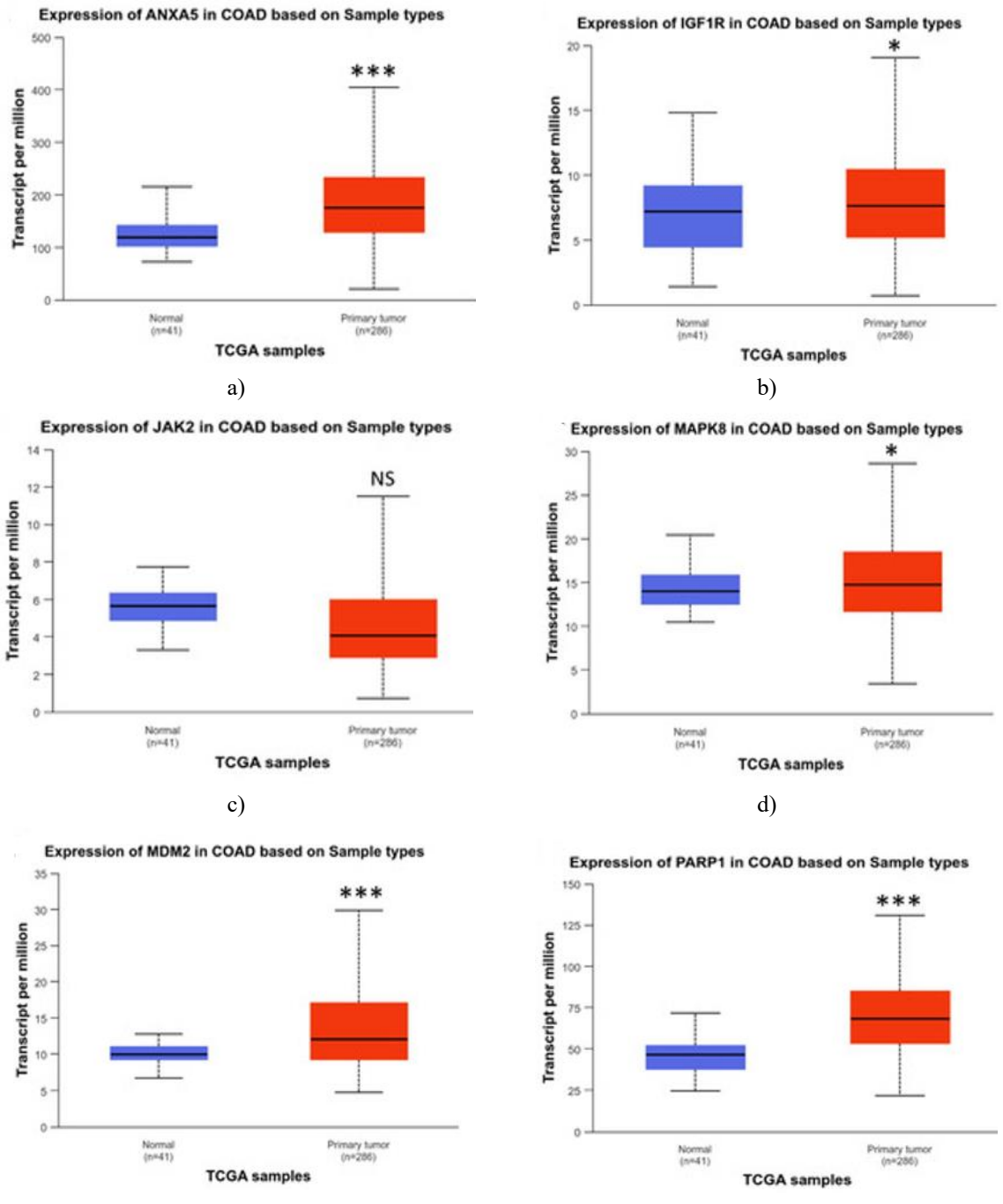


Figure 7. Expression profiling of hub genes in colon adenocarcinoma: (a) ANXA5, (b) IGF1R, (c) JAK2, (d) MAPK8, (e) MDM2, and (f) PARP1. * P < 0.05; *** P < 0.001. Abbreviation: NS = Not significant.

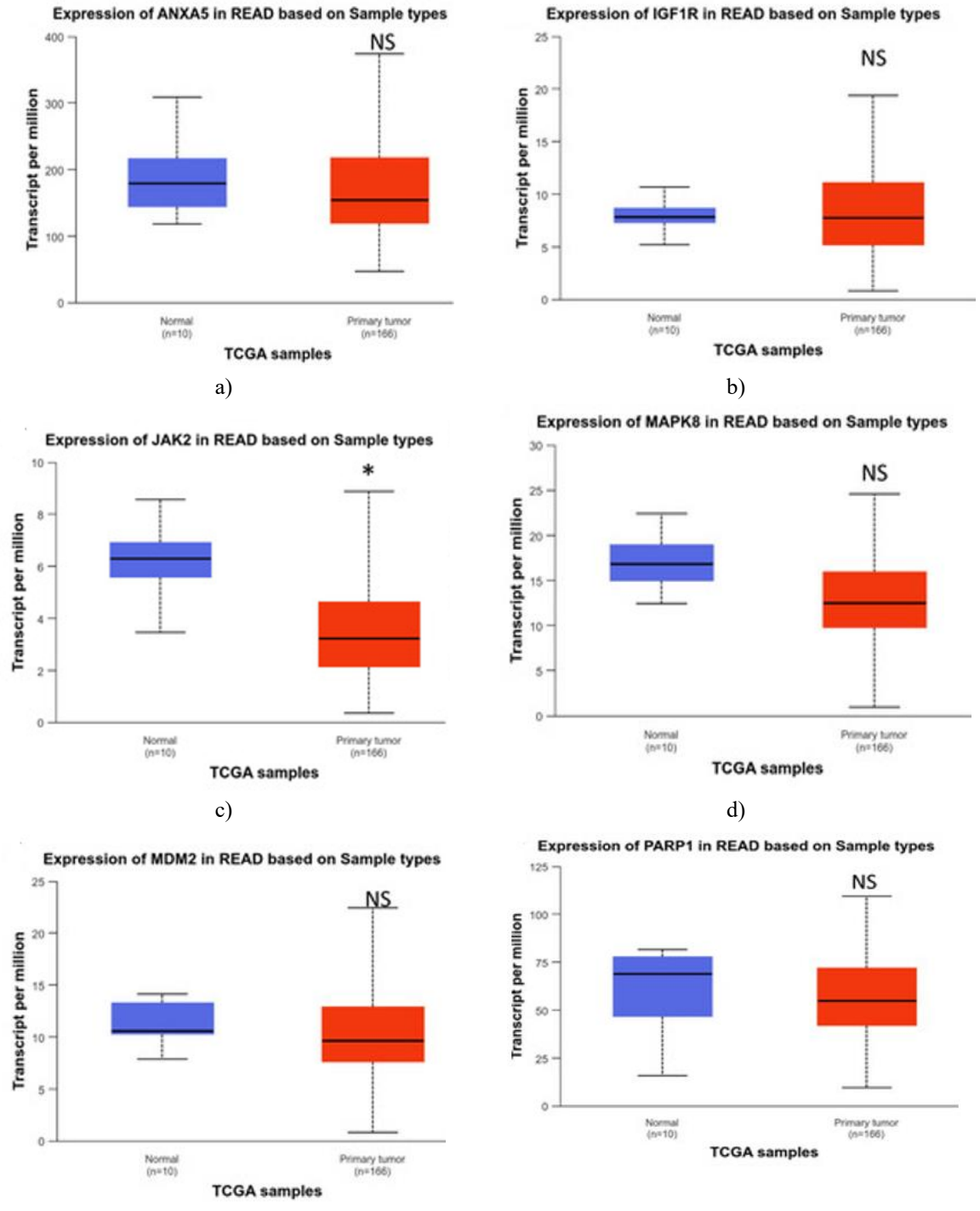


Figure 8. Expression profiling of hub genes in rectum adenocarcinoma: (a) ANXA5, (b) IGF1R, (c) JAK2, (d) MAPK8, (E) MDM2, and (f) PARP1. * P < 0.05. Abbreviation: NS = Not significant.

As shown in **Figure 7**, most hub genes (5 of 6) were significantly overexpressed in colon tumor tissues, with JAK2 being the only gene that did not show a statistically significant difference. Conversely, in rectal tumor samples, the expression pattern differed considerably, where only JAK2 exhibited significant differential expression. Taken together, these observations suggest that all six hub genes are dysregulated in CRC, though their expression profiles are subtype-dependent.

Immune cell infiltration analysis

To explore potential immunological associations, correlation analyses were conducted between hub gene expression and various immune cell populations, including CD8+ T lymphocytes, Th1, Th2, and Th17 subsets, and macrophages. In parallel, relationships with immune-related mediators, including IL10, TGF-β1, IL6, and CXCL8, were also evaluated. These analyses were performed for both the COAD and READ cohorts using data from the TISIDB database **(Figures 9 and 10).**

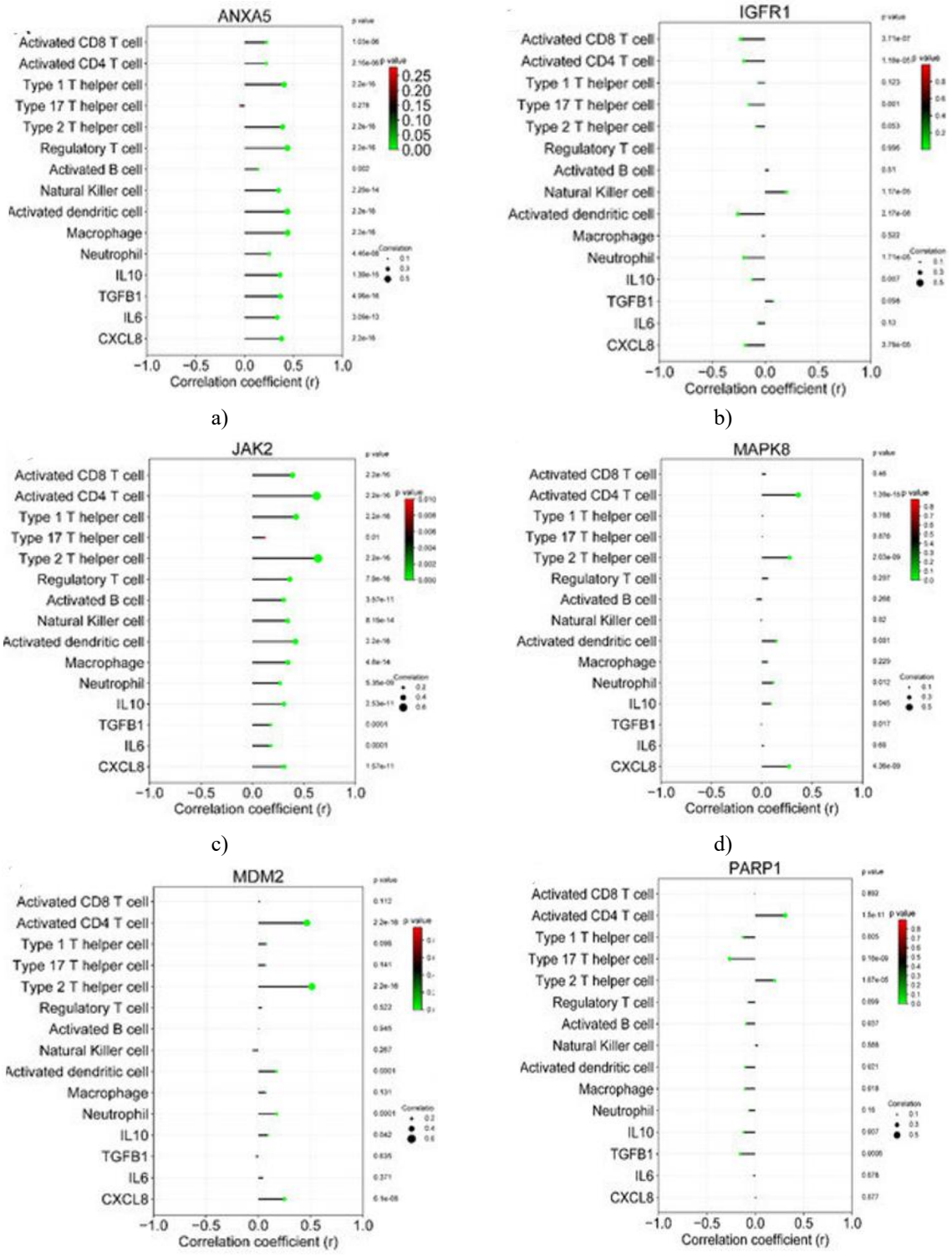


Figure 9. Correlation between hub genes and immune infiltration in colon adenocarcinoma: (a) ANXA5, (b) IGF1R, (c) JAK2, (d) MAPK8, (e) MDM2, and (f) PARP1.

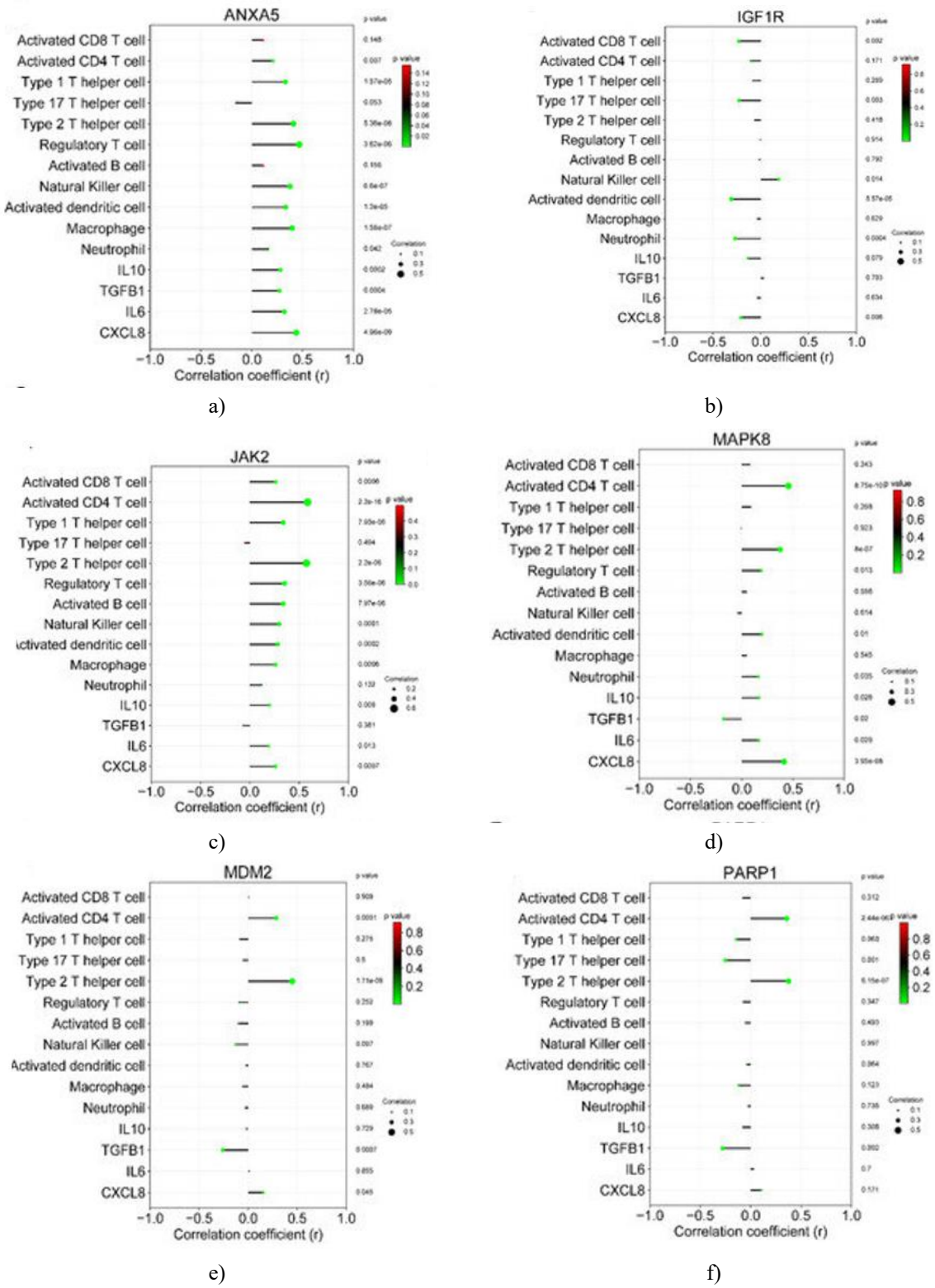


Figure 10. Correlation between hub genes and immune infiltration in rectum adenocarcinoma: (a) ANXA5, (b) IGF1R, (c) JAK2, (d) MAPK8, (e) MDM2, and (F) PARP1.

In colon adenocarcinoma **(Figure 9),** ANXA5, together with JAK2, showed the most pronounced and statistically significant associations with both immune cell infiltration and immune signaling molecules. A similar trend was observed in rectal adenocarcinoma **(Figure 10),** where ANXA5 and JAK2 again showed the strongest correlations.

Overall, these results indicate that cannabidiol may exert immunomodulatory effects in colorectal cancer, potentially through regulatory interactions involving ANXA5 and JAK2 within the tumor immune microenvironment.

The present study aimed to elucidate the mechanism through which cannabidiol exerts effects in colorectal cancer. Our findings indicate that CBD is associated with 95 distinct molecular targets in CRC, modulating biological processes including apoptosis, cellular responses to chemical stimuli, and kinase-related activity. These targets are mainly distributed within membrane-associated regions.

Moreover, the affected genes participate in major signaling cascades, including RAS, MAPK, FoxO, and PI3K-AKT pathways, as well as immune-related signaling, particularly IL-17 signaling. Among the identified targets, six hub genes—ANXA5, IGF1R, JAK2, MAPK8, MDM2, and PARP1—showed strong binding affinity with CBD, displayed differential expression in CRC tumor tissues, and were significantly associated with immune cell infiltration patterns.

Several previous investigations have also applied network pharmacology frameworks to explore the biological effects of CBD. Earlier studies focusing on *Cannabis sativa*, especially cannabidiol, have suggested that CBD may exert therapeutic potential across multiple cancer types, including colorectal cancer [34, 35]. Additionally, computational (in silico) studies have shown that CBD can modulate inflammatory processes, which are strongly linked to tumor development, implying potential activity across different cancers, including CRC [36, 37]. Similar network pharmacology-based research in ovarian cancer has reported that CBD may exert regulatory effects through PARP1, which is also identified as a hub gene in our analysis [38]. Collectively, these findings suggest that CBD may influence CRC and other cancer-related biological processes, particularly inflammation, through computationally supported mechanisms.

Gene Ontology analysis of the 95 CBD-related targets revealed strong enrichment for apoptotic regulation. Previous experimental evidence has demonstrated that CBD can downregulate anti-apoptotic factors while upregulating pro-apoptotic molecules in CRC cell lines, potentially through mechanisms involving reactive oxygen species (ROS) [9, 10, 39]. In addition, CBD-induced apoptosis in CRC has been linked to modulation of several protein kinases, including cyclin-dependent kinases (CDK2, CDK4, CDK6), mitogen-activated protein kinases (MAPKs), and protein kinase R (PERK) [9, 10, 40]. Furthermore, enrichment results indicated that CBD-related targets are predominantly membrane-localized. This aligns with previous findings showing that CBD exerts biological effects through membrane-associated molecules in colorectal cancer cells [12].

Pathway enrichment analysis further highlighted the significant involvement of RAS and MAPK signaling pathways, both of which regulate critical cellular functions, including proliferation, differentiation, metabolism, senescence, and cell cycle progression. Prior integrated computational and experimental research has shown that CBD may inhibit MAPK signaling via RAS modulation and may also interact with EGFR [10, 41]. In addition, the PI3K-AKT pathway, together with FoxO transcriptional regulation, is central to controlling cell survival, growth, and proliferation. Evidence suggests that CBD exerts anticancer activity in colon cancer by suppressing Akt signaling, thereby influencing FoxO activity. These observations support our findings that CBD's antitumor effects are mediated through RAS/MAPK and PI3K-AKT/FoxO pathways, consistent with previously reported studies [42].

Regarding immune-associated pathways, enrichment analysis identified significant enrichment of the IL-17 signaling pathway. IL-17 is known to play a critical role in CRC progression, metastasis, and patient prognosis [43]. Experimental evidence indicates that CBD can attenuate IL-17A-induced mucosal injury, suggesting a protective role for endocannabinoid signaling in inflammatory conditions [44]. Therefore, it is reasonable to propose that CBD may protect against CRC by modulating IL-17-related immune responses.

Protein–protein interaction analysis identified six key hub genes. The first, ANXA5, has been associated with advanced tumor stages and poor prognosis in colorectal adenocarcinoma [45]. Although no direct evidence currently links CBD to ANXA5 regulation, our molecular docking results revealed strong binding affinity between them, suggesting a possible regulatory interaction in CRC. Another hub gene, IGF1R, has been recognized as a therapeutic target in colorectal cancer [46]. Experimental evidence indicates that combined treatment with CBD and IGF-1 may reduce CBD's anti-proliferative effects, likely by interfering with the CBD–IGF1R interaction [47].

JAK2 is another central gene identified in our analysis, and inhibition of the IL-6/JAK2/STAT3 signaling axis has been proposed as a promising therapeutic strategy in CRC [48]. While direct regulation of JAK2 by CBD has not been reported, studies have shown that CBD can alleviate psoriasis-like inflammation in animal models by

modulating the JAK2-STAT3 pathway [49]. Based on these findings, it may be inferred that CBD could exert antitumor activity in CRC through interactions involving IGF1R and JAK2.

Multiple bioinformatics analyses have suggested that MAPK8, another key hub gene identified in this study, may serve as a promising therapeutic target in colorectal cancer [50, 51]. Furthermore, both computational (in silico) and experimental (in vitro) evidence indicate that MAPK8 expression or activity can be influenced by cannabidiol [10, 52]. In addition, the MDM2 rs2279744 polymorphism has been linked to an elevated susceptibility to CRC [53]. Although no direct association between CBD and cancer has been widely reported, studies using human gingival mesenchymal stem cells have shown that CBD can downregulate MDM2 expression [54].

Conversely, other findings suggest that CBD significantly increases reactive oxygen species (ROS) production primarily in cells harboring wild-type p53 (HCT116 and LS174T), a process associated with PARP1 accumulation [55]. Taken together, these observations support the idea that CBD may regulate CRC-related processes through MAPK8, MDM2, and PARP1, a notion further reinforced by our molecular docking results.

Subsequently, validation of hub gene expression differences between tumor and normal tissues was performed using the UALCAN database. Notably, JAK2 did not exhibit significant differential expression in colon adenocarcinoma; however, it was significantly dysregulated in rectal adenocarcinoma. Overall, these results support the conclusion that all six hub genes show differential expression patterns in CRC, although with tumor-site specificity.

The molecular docking findings further suggest that the origin of protein structures—experimental versus computational—can influence predicted ligand binding outcomes. In certain cases, such as IGF1R and MDM2, experimentally derived RCSB structures tend to yield stronger binding affinities, likely because they more accurately represent active binding sites. In contrast, AlphaFold-predicted structures yielded better docking scores for proteins such as ANXA5 and MAPK8, suggesting improved modeling of flexible regions or alternative conformational states of binding pockets.

Additionally, CB-Dock2, when combined with either RCSB or AlphaFold structures, occasionally yielded more negative binding energies (e.g., MDM2 and PARP1), suggesting that its scoring algorithm or docking search strategy may capture interaction modes that AutoDock Vina does not fully optimize. However, variations within a 0.5–1 kcal/mol range should be interpreted cautiously, as they fall within the expected error margin of docking methodologies.

Therefore, these results highlight an important methodological insight: neither docking software nor protein structure source (AlphaFold or RCSB) is universally superior. Instead, predictive accuracy depends on the specific protein target and the particular combination of tools applied.

Correlation analyses between hub genes and immune cell infiltration in COAD and READ revealed that ANXA5 and JAK2 consistently showed the strongest and most significant associations with immune-related parameters. ANXA5 is known to act as a functional link between innate and adaptive immunity, thereby contributing to an immune-activating tumor microenvironment. Although its role in CRC-specific antitumor immunity has not been extensively described, evidence from other malignancies, including hepatocellular carcinoma and head and neck squamous cell carcinoma, indicates that ANXA5 may play a key role in antitumor immune regulation [56, 57].

The strong association observed between JAK2 and immune components is consistent with its central role in cytokine-mediated signaling pathways, which are critically involved in cancer progression, including CRC [48]. Although CBD is widely recognized for its immunomodulatory properties [58, 59], the present findings are particularly notable because they suggest that this immune regulation may, at least in part, be mediated through ANXA5 and JAK2 in colorectal cancer.

Finally, based on the integrated analyses, a proposed molecular mechanism of CBD action in CRC was constructed, emphasizing the involvement of key hub genes and major signaling pathways. This proposed mechanism is illustrated in **Figure 11.**

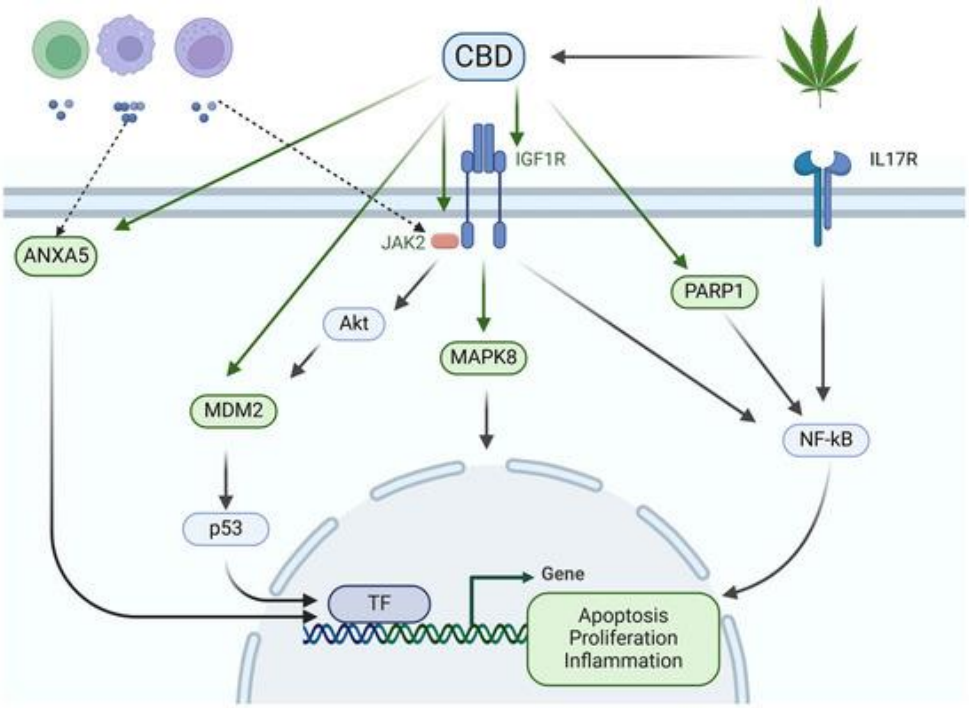


Figure 11. Proposed molecular mechanism of cannabidiol action in colorectal cancer. *Cannabis sativa*-derived cannabidiol (CBD) can interact with ANXA5 and MDM2, thereby modulating apoptosis in colorectal cancer (CRC) cells. In addition, CBD may bind to IGF1R, with JAK2 and Akt signaling involved, thereby regulating MAPK8 and MDM2. This cascade subsequently activates transcription factors (TFs) that control CRC cell proliferation and apoptosis. Moreover, CBD interaction with PARP1 may influence NF-κB signaling within the IL-17 pathway, thereby regulating inflammatory responses in CRC. Ultimately, ANXA5 and JAK2 are associated with immune cell infiltration and immune-related molecular activity within tumor tissue. Hub genes are highlighted in green.

Although this study provides new computational insights, it is important to recognize the limitations associated with in silico approaches. A major constraint is the reliance on computational analyses, which, while useful for predicting potential interactions between cannabidiol (CBD) and colorectal cancer–related genes, cannot replace the experimental validation required to confirm these observations.

Moreover, while protein–protein interaction mapping and molecular docking analyses provide meaningful predictions regarding binding affinity and pathway modulation—particularly for signaling routes such as RAS/MAPK and PI3K-AKT/FoxO—these computational outcomes still require confirmation through both in vitro and in vivo experiments to establish their biological and clinical significance.

In addition, current computational frameworks are not fully capable of reproducing the full complexity and heterogeneity of the tumor microenvironment in colorectal cancer, which limits the direct translation of these findings into real biological systems.

Further research is also needed to determine the most effective route of CBD administration and to clarify potential interactions with other drugs or dietary components. Previous studies have addressed some of these aspects. For example, intranasal and inhalation routes have been reported to enable rapid systemic and central nervous system delivery. In contrast, oral, transmucosal, and transdermal administration tend to result in slower absorption profiles [60]. In addition, oral CBD bioavailability may be enhanced by food intake, potentially due to lipid-mediated absorption mechanisms [61, 62].

It is also worth emphasizing that CBD and similar compounds should be considered as adjunctive agents rather than primary therapeutic options. Overall, the present findings add to the expanding body of literature aimed at clarifying the molecular mechanisms of cannabidiol in colorectal cancer.

Conclusion

This study demonstrated that cannabidiol interacts with 95 distinct molecular targets in colorectal cancer, modulating key biological processes including apoptosis, cellular response to chemical stimuli, and kinase activity. These targets are predominantly localized to membrane-associated regions.

Furthermore, they are involved in major signaling pathways, including RAS, MAPK, FoxO, and PI3K-AKT, as well as immune-related regulation through the IL-17 pathway. Cannabidiol was also shown to bind effectively to six central hub genes—ANXA5, IGF1R, JAK2, MAPK8, MDM2, and PARP1—which exhibit differential expression in colorectal cancer tissues. This differential expression pattern is associated with immune cell infiltration, particularly involving ANXA5 and JAK2.

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