Immunopathologia Persa
http immunopathol.com
DOI:10.34172/ipp.2025.43928

Network-based analysis of microRNAs targeting the RAS/MAPK signaling pathway in colorectal cancer

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Received 20 May 2025
Revised: 25 Aug. 2025
Accepted 31 Oct. 2025
ePublished 7 Dec. 2025

Keywords: Colorectal cancer, RAS/MAPK signaling pathway, MicroRNA, Drug repurposing, Systems biology

Abstract

Introduction: Colorectal cancer remains one of the leading causes of cancer-related morbidity and mortality worldwide. The rat sarcoma/mitogen-activated protein kinase (RAS/MAPK) signaling pathway plays a pivotal role in the regulation of cellular proliferation, differentiation, and survival, and its dysregulation is strongly associated with colorectal tumorigenesis. Recent studies suggest that microRNAs (miRNAs) critically influence cancer progression by targeting key components of this pathway.
Objectives: This study aimed to systematically investigate dysregulated miRNAs that target the RAS/MAPK signaling pathway in colorectal cancer and identify core regulatory genes, enriched biological functions, and potential therapeutic targets using a systems biology approach.
Materials and Methods: In this in silico study, differentially expressed miRNAs in colorectal cancer were identified from comprehensive literature and validated via the miRDB database (score >90). High-confidence target genes were analyzed using STRING to construct protein-protein interaction (PPI) networks. Hub genes were detected using CytoHubba in Cytoscape through multiple centrality algorithms (MNC, MCC, DMNC, Degree). Gene ontology (GO), KEGG, and transcription factor enrichment were performed using Enrichr. Promoter motif analysis was conducted via Tomtom and GOMO, and DrugBank was used to evaluate druggability of hub proteins.
Results: Several hub proteins, including STAT3, JUN, PTEN, ESR1, KRAS, and HIF1A, were identified as central components of the miRNA-target network. Gene ontology and KEGG enrichment highlighted their roles in transcriptional regulation, MAPK cascade, and cell proliferation. Promoter analysis revealed key regulatory motifs, while DrugBank analysis identified several approved and investigational compounds targeting these hub proteins.
Conclusion: The integration of miRNA –gene interaction analysis with network biology and drug discovery tools provided novel insights into the regulatory mechanisms underlying colorectal cancer. Hub proteins identified in this study show potential for targeted therapy and biomarker development. This study highlights critical miRNAs regulating the RAS/MAPK pathway in colorectal cancer and provides a framework for therapeutic target prioritization through integrative network analysis.

Citation: Ataei Azimi S, Farzadifar E, Pasdar A, Amerizadeh F. Network-based analysis of microRNAs targeting the RAS/MAPK signaling pathway in colorectal cancer. Immunopathol Persa. 2026;12(2):e43928. DOI:10.34172/ipp.2025.43928.

Introduction

Globally, colorectal cancer ranks as the third most frequently diagnosed cancer and represents the second primary cause of mortality among cancer patients, constituting a major public health burden (1). Despite advances in screening and therapeutic strategies, high rates of recurrence, metastasis, and drug resistance continue to hinder effective management. Therefore, identifying novel molecular markers and therapeutic targets is of paramount importance for improving diagnostic and clinical outcomes in colorectal cancer patients (2).

Cell signaling pathways play pivotal roles in tumor initiation and progression. Among them, the rat sarcoma/mitogen-activated protein kinase (RAS/MAPK) signaling pathway is one of the most critical cascades in colorectal tumorigenesis. This pathway orchestrates essential cellular functions, including proliferation, differentiation, apoptosis, migration, and survival (3). Dysregulation of the RAS/MAPK cascade often driven by activating mutations in genes such as KRAS, NRAS, and BRAF, or by aberrant regulation of upstream or downstream effectors is a hallmark of colorectal cancer and is frequently associated with poor prognosis and resistance to targeted therapies (4).

In recent years, microRNAs (short non-coding RNA molecules that post-transcriptionally regulate gene expression) have emerged as crucial players in cancer biology. By binding to the 3′ untranslated

Key point

- High-confidence target genes were used to build a protein-protein interaction (PPI) network and identify hub proteins (e.g., STAT3, KRAS, ESR1, HIF1A).
- Promoter motif and transcription factor analyses revealed shared regulatory signatures among hub genes.
- DrugBank analysis identified several FDA-approved and investigational drugs targeting key hub proteins.
- Findings support the potential of miRNA-guided, network-based approaches for precision therapy in colorectal cancer.

regions (3′UTRs) of their target mRNAs, miRNAs can repress translation or induce mRNA degradation (5). Dysregulated transcript levels of microRNAs has been widely reported in colorectal cancer and has been shown to affect numerous cancer hallmarks (5). Notably, miRNAs can function either as oncogenes (oncomiRs) or tumor suppressors , depending on the cellular context and the nature of their target genes (6).

Previous studies found that, miRNAs targeting components of the RAS/MAPK pathway can exert profound effects on cancer development by modulating the expression of key molecules such as KRAS, BRAF, MAP2K1/2, MAPK1 , and transcriptional effectors including FOS, JUN, and MYC, Therefore, comprehensive analysis of these miRNAs within molecular interaction networks can provide insights into the regulatory architecture underlying colorectal cancer progression and uncover potential biomarkers and therapeutic candidates (7-9).

Objectives

This study utilized an integrated systems biology strategy to investigate dysregulated miRNAs in colorectal cancer that selectively target the RAS/MAPK signaling pathway. Utilizing a combination of advanced bioinformatics tools (including miRDB, STRING, Cytoscape, Enrichr, Tomtom, GOMO, and DrugBank) we systematically identified key miRNAs, their high-confidence target genes, and the resulting protein-protein interaction (PPI) networks. We performed hub gene identification, functional enrichment analyses, promoter motif discovery, transcription factor mapping, and drug repurposing evaluations. This integrative analysis provides a comprehensive understanding of miRNA-driven regulatory networks in colorectal cancer and offers new avenues for therapeutic intervention.

Materials and Methods

Data collection

Data on differentially expressed miRNAs in colorectal cancer were gathered from the review article titled "The potential role of regulatory microRNAs of RAS/MAPK signaling pathway in the pathogenesis of colorectal cancer. "After identifying relevant miRNAs, the MiRDB database (version 6.0) was utilized to pinpoint their target proteins (10). Only target proteins with a score above 90 were selected to ensure the accuracy and reliability of subsequent analysis (11).

To identify hub proteins and perform subnetwork analysis

The PPI network was reconstructed by inputting target genes with a score ≥ 90 into the STRING database (version 11.5) (12). The network was imported into Cytoscape (version 3.10.1) for advanced analysis. Screening for hub proteins was conducted with the CytoHubba plugin (version 0.1) through the calculation of centrality indices. Finally, various algorithms, namely MNC, MCC, DMNC, and Degree, were employed to extract and analyze hub protein subnetworks, leading to the identification of critical interactions and functional clusters (13).

Gene ontology and KEGG pathway enrichment studies along with an assessment of transcription factors associated with the target genes

Gene ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed on the subnetworks using the Enrichr tool (version 2023) to explore associated biological processes, molecular functions, cellular component and pathways. To elucidate the transcriptional control mechanisms, we interrogated the ENCODE and ChEA Consensus transcription factors databases via the Enrichr platform. This analysis revealed pivotal transcription factors likely governing the expression of the target genes (14).

Identification of functional modules in the hub protein network

Protein complex detection was carried out using the CytoCluster plugin (v1.5) within the Cytoscape environment (v3.10.1), employing the ClusterONE algorithm with specific parameters (minimum cluster size: 3, automatic density threshold, combined score for edge weighting). This approach enabled the detection of highly interconnected protein modules within the PPI network, revealing functionally relevant molecular complexes (15).

Investigation of hub gene promoter regions

To elucidate the transcriptional regulation of hub genes, their promoter regions were analyzed for conserved motifs and transcription factor binding sites using the Tomtom (v5.4.1) and GOMO (v5.1.1) tools. This analysis yielded critical insights into the regulatory mechanisms and key transcription factors governing the expression of these pivotal genes (16).

Drug analysis

A screening of the DrugBank database (version 5.1.10) was conducted to identify FDA-approved and investigational compounds with potential activity against the critical hub proteins. This approach sought to discover drug repurposing opportunities for colorectal cancer therapy by leveraging existing pharmacological agents (17).

Statistical analysis

Protein-protein interaction networks were constructed using STRING (version 11.5) for target genes with a score above 90 and analyzed in Cytoscape (version 3.10.1) with the CytoHubba plugin. Hub genes were identified through four centrality algorithms (MCC, MNC, Degree, DMNC) (Table 1), with STAT3 and KRAS showing the highest connectivity scores ($P<0.01$, FDR-corrected). Functional enrichment via Enrichr revealed significant associations with MAPK signaling (KEGG $p=3.5\times10^{-7}$) and transcriptional regulation (GO: 0045944, $P=1.2\times10^{-10}$). Cluster analysis using ClusterONE identified two significant modules (7-node: $P=4.9\times10^{-4}$; 6-node: $P=0.037$) with full density (1.0). Drug-target interactions from DrugBank (version 5.1.10) identified 12 FDA-approved compounds (e.g., Celecoxib for STAT3, Sotorasib for KRAS) and 23 investigational drugs.

Table 1. Top-ranked hub proteins identified in the PPI network based on four centrality algorithms (MCC, MNC, DMNC, and Degree)

Rank	Hub proteins	Algorithm
1, 4, 3	STAT3	MCC, MNC, Degree
2, 2, 2	JUN	MCC, MNC, Degree
4, 1, 1	PTEN	MCC, MNC, Degree
3, 4	ESR1	MNC, Degree
5, 5	KRAS	MNC, Degree
3	HIF1A	MCC
5	SMAD2	MCC
1	ICE2	DMNC
1	TTF2	DMNC
3	SH3D19	DMNC
4	SS18L1	DMNC
5	ELL2	DMNC

MCC: Maximal clique centrality; MNC: Maximum neighborhood component; DMNC: Density of maximum neighborhood component.

Results

Discovery of key protein and subnetwork exploration

The gathered data revealed a detailed collection of miRNAs that are with significant expression changes in colorectal cancer. These miRNAs were associated with 2525 high score genes implicated in the progression of the disease. Figure 1A depicts the extensive interaction network derived among the identified target genes, highlighting a complex web of interactions that emphasize the intricate regulatory mechanisms underlying pathogenesis of colorectal cancer. The high-confidence scoring of the identified genes established a solid foundation for constructing a reliable PPI network, enabling robust computational investigations.

Gene ontology and WikiPathways exploration

Gene ontology pathway analysis identified significant biological processes and pathways linked to colorectal cancer. Key processes included the positive regulation of DNA-templated transcription and RNA polymerase II-mediated transcriptional regulation, highlighting their importance in governing gene expression and modulating cellular processes. Molecular functions highlighted sequence-specific double-stranded DNA binding. Cellular component analysis revealed critical elements such as the nucleus, membrane-bound intracellular organelles, neuron projection, and cell-substrate junction, suggesting their involvement in cell function and organization and cellular processes relevant to colorectal cancer. WikiPathways analysis uncovered pathways including VEGFA-VEGFR2 signaling, insulin signaling and cancer pathways, which are associated with cell growth, survival, angiogenesis, and

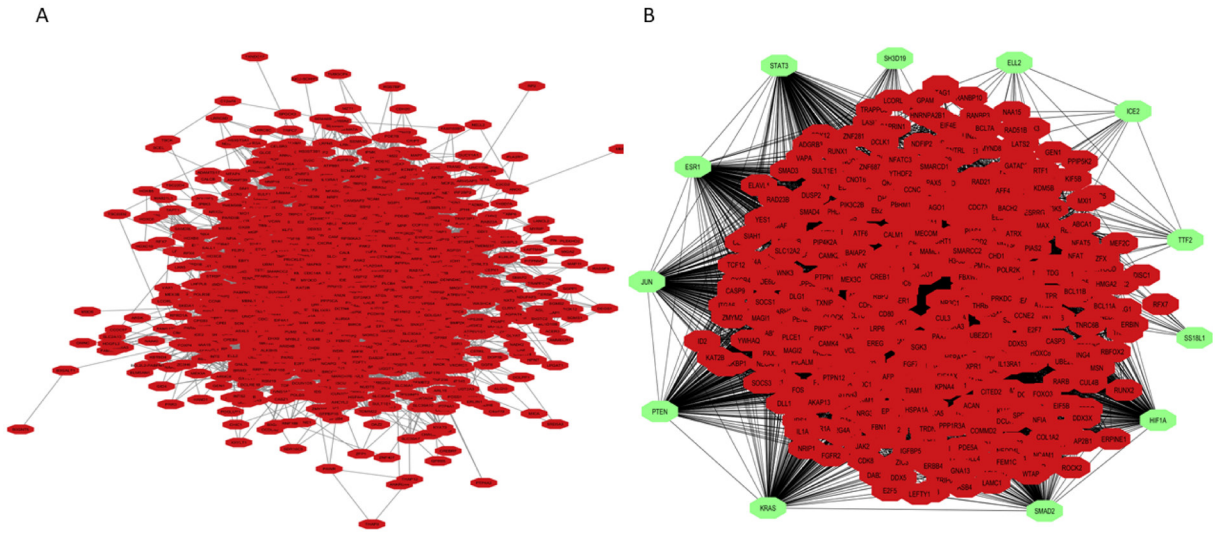


Figure 1. (A) Protein–protein interaction (PPI) network of target proteins constructed and visualized using Cytoscape (v3.10.1) based on STRING database input (confidence score > 0.7). (B) Highlighted hub proteins in the PPI network identified using the CytoHubba plugin in Cytoscape. Hub nodes are shown in green and ranked based on topological algorithms including MCC, MNC, DMNC, and Degree.

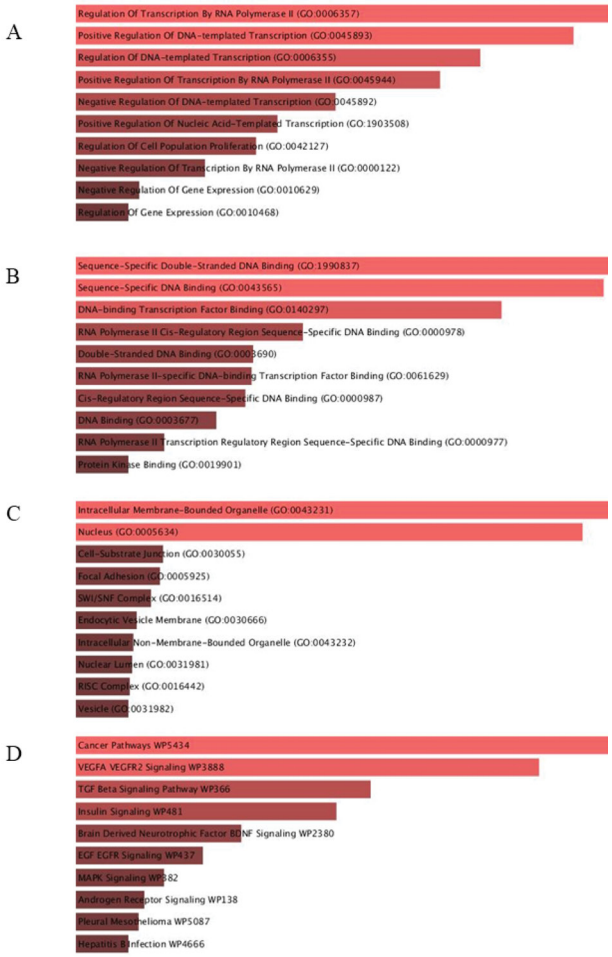


Figure 2. (A) Biological process. (B) Molecular function. (C) Cellular component of all target proteins. (D) Wiki pathway analysis

metabolism. These findings indicate potential overlaps in disease mechanisms that may drive colorectal cancer progression. Figure 2 presents these enriched pathways and gene ontology terms, highlighting the pathways and genes significance to elucidate the intricate biological mechanisms of colorectal cancer.

Identification of functional modules in the hub protein network

Identification of functional modules in the hub protein network was performed using the CytoCluster plugin in Cytoscape, utilizing the ClusterONE algorithm. This approach facilitated the identification and visualization of key protein clusters within the PPI network, specifically targeting clusters with a significance threshold of less than 0.05. These clusters offer valuable insights into essential protein interactions linked to colorectal cancer.

Several key clusters were identified, each exhibiting unique features: Cluster 1: Comprising 7 nodes, this cluster had a density of 1.000 and a quality of 0.636, with a P-value of 4.913E-4. The high density and high quality indicate a network of proteins that are involved in cancer-related signaling pathways and may influence disease progression and be involved in processes of metastasis, drug resistance, or cell proliferation. Proteins in this cluster can also be investigated as novel therapeutic targets for the development of anticancer drugs. Cluster 2: This cluster consisted of 6 nodes, with a density of 1.000 and a quality of 0.484, accompanied by a *P* value of 0.037. The high density indicates a tightly interconnected group of proteins, suggesting strong functional associations relevant to colorectal cancer. The robust connectivity within this cluster highlights the potential importance of these proteins in regulating pathways associated with the disease (Figure 3).

Analysis of promoters and transcription factors for hub proteins

To further investigate the transcriptional regulation of hub genes identified within the miRNAs–target interaction network, we performed promoter motif analysis using the MEME Suite (Tomtom and GOMO tools). This analysis aimed to uncover conserved DNA motifs within the upstream flanking regions of selected hub genes, providing insights into potential transcription factor binding sites and associated biological functions (16).

The analysis identified shared conserved motifs across the promoter regions of key genes including KRAS, PTEN, JUN, SMAD2, SH3D19, and SS18L1. Notably, a recurring motif signature was detected in multiple hub genes, suggesting the involvement of common regulatory programs. These motifs were highly associated with

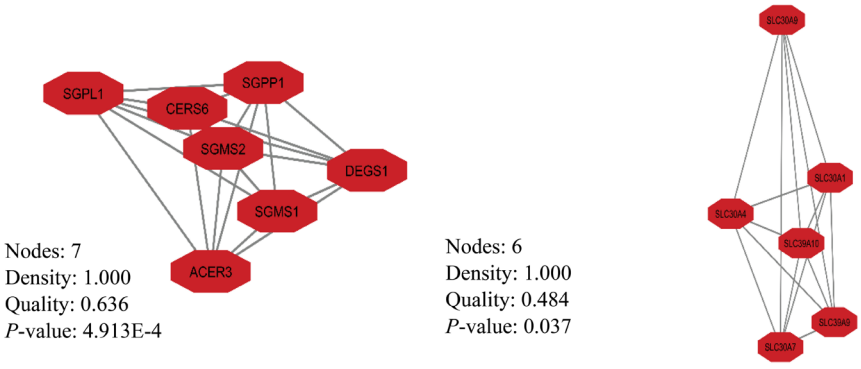
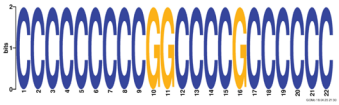
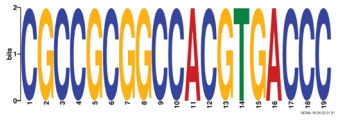
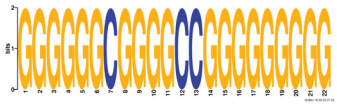
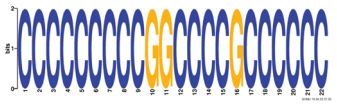
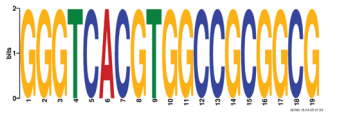
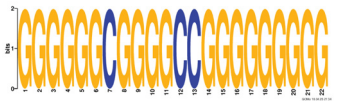


Figure 3. Significant protein clusters within the PPI network, focusing on clusters with a significance level below 0.05.

Table 2. Analysis of promoters and transcription factors for hub proteins

Motif	Logo	Length	Top 5 specific predictions
ENSG00000109686|SH3D19		22	CC transcription factor complex BP negative regulation of signal transduction MF protein heterodimerization activity BP neuron fate commitment MF chromatin binding
ENSG00000133703|KRAS		19	CC transcription factor complex BP negative regulation of signal transduction MF protein heterodimerization activity BP neuron fate commitment MF chromatin binding
ENSG00000171862|PTEN		22	CC transcription factor complex BP negative regulation of signal transduction MF potassium ion binding BP negative regulation of neuron apoptosis BP potassium ion transport
ENSG00000175387|SMAD2		22	CC transcription factor complex BP negative regulation of signal transduction MF potassium ion binding BP negative regulation of neuron apoptosis BP potassium ion transport
ENSG00000177606|JUN		22	CC transcription factor complex BP negative regulation of signal transduction MF protein heterodimerization activity BP neuron fate commitment MF chromatin binding
ENSG00000184402|SS18L1		19	CC transcription factor complex BP negative regulation of signal transduction MF protein heterodimerization activity BP neuron fate commitment MF chromatin binding
ENSG00000284792|PTEN		22	CC transcription factor complex BP negative regulation of signal transduction MF potassium ion binding BP negative regulation of neuron apoptosis BP potassium ion transport

transcription factor complexes (Cellular Component: CC), particularly those modulating signal transduction pathways.

Gene ontology enrichment of the motif-associated functions revealed several prominent terms: Biological Processes (BP): Suppressive role in cellular signaling cascades, negative regulation of neuronal apoptosis, and potassium ion transport. Molecular functions (MF): protein heterodimerization activity, chromatin binding, and potassium ion binding.

Interestingly, the presence of neuron-associated terms (such as neuron fate commitment and negative regulation of neuron apoptosis) in colorectal cancer-associated genes like PTEN and SMAD2 suggests a possible link between neurodevelopmental regulatory elements and colorectal cancer plasticity. Moreover, the enrichment of chromatin binding and transcription factor complex-related motifs points to a potential role for epigenetic and transcriptional remodeling mechanisms in governing the expression of these hub genes. Taken together, these findings highlight the presence of shared cis-regulatory elements across cancer-relevant hub genes, potentially modulated by dysregulated miRNAs. These motifs could serve as binding platforms for master transcription factors, contributing to the altered gene expression programs observed in colorectal cancer (Table 2).

To complement the motif-based promoter analysis, we performed transcription factor enrichment analysis using the ENCODE and ChEA consensus databases via the enrichr platform. This analysis aimed to identify key transcription factors that are potentially responsible for regulating the transcriptional activity of the hub genes identified in the miRNA–target network. This results revealed a set of significantly enriched TFs, with UBTF (ENCODE) and AR (CHEA) ranking as the top hits, followed by TCF3, NFE2L2, and SUZ12, suggesting that these factors may bind to shared cis-regulatory elements across multiple hub gene promoters.

Additionally, transcription factors with established oncogenic or tumor suppressive roles in colorectal cancer (such as SMAD4, STAT3, and CREB1) also emerged among the top regulators. The presence of STAT3 in both the miRNA–hub interaction network and the transcriptional regulator set indicates a possible autoregulation loop, where STAT3 may be both a target and a regulator within the colorectal cancer signaling landscape. The co-enrichment

of chromatin-associated factors such as SUZ12 and TAF1 further supports the role of epigenetic and transcriptional remodeling in controlling hub gene expression. The bar plot in Figure 4 visualizes the top-ranking transcription factors derived from consensus enrichment across both ENCODE and ChEA datasets, providing additional evidence of coordinated transcriptional regulation in colorectal cancer.

Therapeutic compound screening from DrugBank targeting key highly connected proteins

Screening of the DrugBank database aimed to pinpoint compounds that target particular key proteins. Bioactive compounds, including approved drugs and investigational agents, were identified for a subset of targets, whereas the remaining proteins lacked associated compounds in the screened databases. STAT3: This protein is one of the core proteins and several drugs such as Acitretin, Celecoxib, Quercetin and WP-1066 have been identified as its inhibitors. Most of these drugs are under investigation or experimental. JUN: Drugs such as Adapalene and T-5224 act as antagonists or inhibitors of this protein. Some of these drugs are approved (17). HIF1A: Drugs such as 2-methoxyestradiol and PT-2385 act as modulators or inhibitors of this protein. Most of these drugs are under investigation. PTEN: Phosphatidylethanolamine has been identified as a substrate for this protein. ESR1: Several drugs such as Tamoxifen, ethinylestradiol and testosterone cypionate act as agonists or antagonists for this protein. Many of these drugs are approved (17). KRAS: Drugs such as adagrasib and sotorasib act as inhibitors for this protein. These drugs are under investigation or approved (17). SMAD2: Dexfosfoserine has been identified as an experimental drug for this protein. Certain core proteins, such as STAT3 and ESR1, are associated with multiple drugs that could be explored as potential treatment options for colorectal cancer. Drugs like celecoxib and Tamoxifen, which are already approved, may be repurposed and investigated for their efficacy in treating colorectal cancer. For proteins like SMAD2, only a limited number of drugs are available, highlighting the need for additional research to discover new therapeutic options. This analysis indicates that while some key proteins involved in colorectal cancer have existing drugs that could be studied for treatment potential, other proteins lack effective drugs, underscoring the necessity for further research in this field (17) (Table 3).

Discussion

Colorectal cancer is a multifactorial malignancy driven by intricate molecular modifications in signaling networks that control cell growth, differentiation, programmed cell death, and immune evasion. Among these, the RAS/MAPK signaling pathway represents a canonical oncogenic cascade frequently dysregulated in colorectal cancer. This study provides a systems-level perspective on how dysregulated microRNAs contribute to aberrant

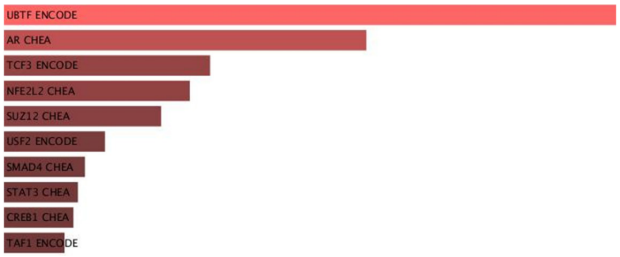


Figure 4. Transcription factor enrichment analysis of hub genes. Bar plot showing the top-ranked transcription factors enriched from ENCODE and ChEA consensus datasets that are potentially involved in regulating the expression of key hub genes identified in the miRNA–target interaction network.

RAS/MAPK signaling and colorectal cancer pathogenesis, highlighting both mechanistic insights and translational relevance.

At the core of the miRNA-regulated network identified in this study lies a panel of hub genes (STAT3, JUN, PTEN, ESR1, KRAS, HIF1A, SMAD2, ICE2, TTF2, SH3D19, SS18L1, and ELL2) each of which plays a pivotal role in colorectal cancer biology through distinct yet interconnected mechanisms. STAT3 and JUN are critical transcription factors activated downstream of MAPK signaling and are known to promote oncogenic transcriptional programs, immune evasion, and tumor cell survival (18,19). PTEN, a well-established tumor suppressor, resistance to therapy (20). ESR1, typically studied in hormone-sensitive cancers, is increasingly recognized for its role in colorectal epithelial cell regulation and has been linked to MAPK-mediated crosstalk (21). KRAS, a canonical proto-oncogene, is frequently mutated in colorectal cancer and drives constitutive activation of the MAPK cascade, fueling cell proliferation and therapeutic resistance (22). HIF1A mediates the cellular response to hypoxia, a common feature of the tumor microenvironment, by promoting angiogenesis and metabolic reprogramming (23). SMAD2 serves as a mediator of TGF-β signaling, often implicated in colorectal cancer metastasis and epithelial-to-mesenchymal transition (EMT) (24). Although less well characterized in colorectal cancer, ICE2, TTF2, SH3D19, SS18L1, and ELL2 emerged from our network analysis as structurally or functionally integrated elements, potentially contributing to transcriptional regulation, nuclear transport, chromatin modification, and RNA polymerase II elongation mechanisms increasingly recognized as drivers of cancer cell plasticity and adaptation. Together, these hub genes constitute a functionally diverse regulatory network that may serve as both a mechanistic foundation and a therapeutic opportunity in colorectal cancer (25,26).

Promoter motif analysis of selected hub genes provided critical insights into the upstream regulatory landscape governing gene expression in colorectal cancer. The detection of shared cis-regulatory motifs across promoters of genes such as KRAS, PTEN, JUN, SMAD2, SH3D19, and SS18L1 indicates the presence of common

Table 3. DrugBank compounds against key proteins

Drug	Drug group	Pharmacological action	Type	Actions
Acitretin	Approved	Unknown	STAT3	Inhibitor
Atiprimod	Investigational	Unknown	STAT3	Inhibitor
Celecoxib	Approved, investigational	Unknown	STAT3	Inhibitor
Diosmetin	Experimental	Unknown	STAT3	Inhibitor
ENMD-1198	Investigational	Unknown	STAT3	
Epigallocatechin gallate	Investigational	Unknown	STAT3	Inhibitor
Golotimod	Investigational	Unknown	STAT3	Inhibitor
Luteolin	Experimental	Unknown	STAT3	Inhibitor
MOL-4239	Investigational	Unknown	STAT3	Inhibitor
Napabucasin	Investigational	Unknown	STAT3	Inhibitor
NT-219	Investigational	Unknown	STAT3	Inhibitor
OPB-111077	Investigational	Unknown	STAT3	Inhibitor
Quercetin	Experimental, investigational	Unknown	STAT3	Inhibitor
TTI-101	Investigational	Unknown	STAT3	Inhibitor
WP-1066	Investigational	Unknown	STAT3	Inhibitor
Adapalene	Approved	Yes	JUN	Antagonist
Arsenic trioxide	Approved, investigational	Yes	JUN	Inducer
Irbesartan	Approved, investigational	Unknown	JUN	Other/unknown
LGD-1550	Investigational	Unknown	JUN	
T-5224	Investigational	Unknown	JUN	Inhibitor
Vinblastine	Approved	No	JUN	Other/unknown
2-Methoxyestradiol	Investigational	Yes	HIF1A	Modulator
Carvedilol	Approved, investigational	Unknown	HIF1A	Modulator
ENMD-1198	Investigational	Unknown	HIF1A	Inhibitor
FG-2216	Investigational	Unknown	HIF1A	
Hydralazine	Approved	Unknown	HIF1A	Inducer
PT-2385	Investigational	Unknown	HIF1A	Inhibitor
PX-478	Investigational	Yes	HIF1A	Inhibitor
Vadadustat	Approved, investigational	Unknown	HIF1A	Stabilization
Phosphatidylethanolamine	Experimental	Unknown	PTEN	Substrate
Conjugated estrogens	Approved	Yes	ESR1	Agonist
Danazol	Approved	Yes	ESR1	Agonist
Desogestrel	Approved	Yes	ESR1	Agonist
Dobutamine	Approved	Unknown	ESR1	Agonist
Enzacamene	Approved	Unknown	ESR1	
Estrone	Approved	Yes	ESR1	Agonist
Estrone sulfate	Approved	Yes	ESR1	Agonist
Ethinylestradiol	Approved	Yes	ESR1	Agonist
Ethynodiol diacetate	Approved	Yes	ESR1	Agonist
Eugenol	Approved	Unknown	ESR1	Agonist
Fluoroestradiol F-18	Approved	Unknown	ESR1	Binder
Gestrinone	Approved	Yes	ESR1	Antagonistagonist
Mestranol	Approved	Yes	ESR1	Agonist
Methyltestosterone	Approved	Unknown	ESR1	Partial agonist
Mitotane	Approved	Yes	ESR1	Binder
Norethynodrel	Approved	Unknown	ESR1	
Polyestradiol phosphate	Approved	Yes	ESR1	Agonist
Quinestrol	Approved	Yes	ESR1	Agonistmodulator
Synthetic Conjugated Estrogens, A	Approved	Yes	ESR1	Ligand
Synthetic Conjugated Estrogens, B	Approved	Yes	ESR1	Ligand
Tamoxifen	Approved	Yes	ESR1	Antagonistagonist
Testosterone cypionate	Approved	Unknown	ESR1	Inhibitor
Testosterone enanthate	Approved	Unknown	ESR1	Inhibitor
Zinc sulfate, unspecified form	Approved, experimental	Unknown	ESR1	Binder
Fluoxymesterone	Approved, illicit	Yes	ESR1	Antagonist
Arzoxifene	Approved, investigational	Unknown	ESR1	Inhibitor
Bazedoxifene	Approved, investigational	Yes	ESR1	Antagonistagonist
Clomifene	Approved, investigational	Yes	ESR1	Antagonistagonist
Dienestrol	Approved, investigational	Yes	ESR1	Agonist
Elacestrant	Approved, investigational	Yes	ESR1	Antagonist
[(3,7,11-Trimethyl-dodeca-2,6,10-trienyloxycarbamoyl)-methyl]-phosphonic acid	Experimental	Unknown	KRAS	
Adagrasib	Approved, investigational	Yes	KRAS	Inhibitor
Farnesyl diphosphate	Experimental	Unknown	KRAS	
Sotorasib	Approved, investigational	Yes	KRAS	Inhibitor
Dexfosfoserine	Experimental	Unknown	SMAD2	Actions

transcriptional control elements (16). These motifs were highly enriched in terms associated with transcription factor complex binding, chromatin interaction, and the negative regulation of signal transduction. The consistent appearance of neuronal developmental terms (e.g., neuron fate commitment and regulation of neuron apoptosis) in a colorectal cancer context suggests a potential rewiring of developmental programs in tumor cells, a phenomenon previously reported in cancers with high cellular plasticity (27). Furthermore, the enrichment of terms related to chromatin binding and protein heterodimerization implies that alterations in chromatin state and cooperative transcription factor binding may contribute to dysregulation of gene expression in colorectal cancer (16).

The transcription factor enrichment analysis conducted using ENCODE and ChEA databases further substantiated these findings by identifying key regulatory proteins potentially driving the expression of hub genes. Notably, UBTF, AR, NFE2L2, and TCF3 emerged as top-ranked transcription factors, some of which have documented roles in chromatin remodeling, stress response, and hormone signaling mechanisms central to cancer progression (28,29). Of particular interest was the identification of STAT3 and SMAD4, both of which not only act as downstream effectors in the RAS/MAPK and TGF-β pathways respectively, but also appeared as enriched transcriptional regulators of other hub genes (30). This dual role suggests feedback and feed-forward loops that may reinforce oncogenic signaling in colorectal cancer. The concurrent presence of epigenetic regulators such as SUZ12 (a core component of the PRC2 complex) further highlights the epigenomic modulation of gene expression as a layer of regulation shaped by upstream transcription factor binding and miRNA interference (31). Collectively, these findings suggest a highly orchestrated regulatory network where miRNAs, promoter motifs, transcription factors, and chromatin modifiers converge to govern the expression of genes critical to colorectal cancer progression (Figure 3).

To assess the translational potential of the identified hub genes, we performed a drug–target interaction analysis using the DrugBank database. This investigation revealed a range of clinically approved and investigational compounds targeting several key proteins within the miRNA-regulated network. Notably, STAT3, a central oncogenic transcription factor, was associated with multiple compounds such as celecoxib, quercetin, WP-1066, and TTI-101, many of which are under investigation for their anti-inflammatory and anti-proliferative properties (32,33). KRAS, a frequently mutated driver in colorectal cancer, is now therapeutically actionable through adagrasib and sotorasib, both of which have received regulatory approval in other cancer types and hold promise for colorectal cancer subtypes harboring KRAS mutations (34). ESR1, traditionally targeted in hormone-sensitive cancers, also emerged as a druggable node with a wide array of ligands including tamoxifen, ethinylestradiol, and fluoxymesterone, reflecting its broader role in transcriptional control and possible relevance in colorectal cancer (35,36). Additionally, HIF1A, which mediates hypoxia-induced signaling, was linked to inhibitors such as PX-478 and PT-2385, offering potential strategies to modulate the tumor microenvironment (37). While drugs for some of the lesser-characterized proteins (e.g., SMAD2, PTEN) were limited or experimental, the findings highlight that a subset of hub genes is already pharmacologically targetable, opening avenues for drug repurposing and miRNA-guided therapeutic intervention in colorectal cancer. These insights reinforce the utility of integrative network analysis in bridging molecular discovery with clinically actionable strategies.

However, it is important to acknowledge certain limitations. Computational predictions require experimental validation to confirm direct miRNA-mRNA interactions and drug efficacy. Moreover, the heterogeneity of colorectal cancer including variations across anatomical site (colon vs. rectum), molecular subtype (e.g., CMS classification), and tumor microenvironment necessitates contextualized interpretation of the findings.

Conclusion

This study presents a comprehensive bioinformatics-driven framework to decode the modulatory impact of miRNAs targeting the RAS/MAPK signal transduction axis in colorectal cancer. By integrating multiple layers of analysis including miRNA target prediction, network topology, functional enrichment, promoter motif mapping, and pharmacological annotation we identified critical hub genes and regulatory modules that orchestrate colorectal cancer progression.

Key findings include the identification of multi-targeting miRNAs, notably from the miR-548 family, and central oncogenic proteins such as STAT3, KRAS, ESR1, and HIF1A, which serve as promising candidates for further functional and therapeutic investigation. The convergence of these miRNAs on critical components of the RAS/MAPK pathway reinforces their importance as both upstream modulators and downstream effectors in colorectal tumorigenesis. Moreover, the discovery of clinically actionable drug targets within this regulatory network provides a valuable roadmap for drug repurposing and the development of miRNA-guided precision medicine in colorectal cancer. Subsequent investigations should prioritize in vitro and in vivo validation of predicted interactions, exploration of miRNA-based therapeutics, and stratification of patients based on microRNA-hub gene expression profiles to facilitate personalized treatment strategies. Overall, this investigation augments the expanding repository of scientific understanding on miRNA-mediated regulation of oncogenic pathways and opens new avenues for targeted therapeutic intervention in colorectal cancer.

Limitations and future directions

This study provides a systems-level insight into miRNA-mediated regulation of the RAS/MAPK signaling pathway in colorectal cancer. However, several limitations should be acknowledged. First, the analyses were entirely computational and relied on publicly available databases, which may not fully capture the context-specific expression and functional activity of miRNAs and their targets in colorectal cancer tissues. Experimental validation through in vitro assays (e.g., luciferase reporter, qRT-PCR, and western blotting) and in vivo models is essential to confirm the biological relevance of the predicted miRNA-gene interactions and drug-target relationships.

Second, the heterogeneity of colorectal cancer, including differences in tumor location (colon vs. rectum), molecular subtypes (e.g., CMS classification), and patient-specific genetic backgrounds, was not accounted for in this analysis. Future studies should incorporate patient-derived datasets with clinical annotations to allow for stratified analysis and improve translational applicability.

Third, the study focused on the RAS/MAPK pathway in isolation. Given the complex cross-talk between signaling cascades in colorectal cancer (e.g., Wnt/β-catenin, PI3K/AKT, TGF-β), a more integrative approach including multiple pathways may better reflect the regulatory landscape and uncover synergistic targets.

For future directions, we recommend experimental validation of key hub genes and miRNAs identified in this network, particularly those with therapeutic potential such as STAT3, KRAS, ESR1, and HIF1A. Additionally, exploration of miRNA-based therapeutics, including antisense oligonucleotides or miRNA mimics, could provide novel avenues for intervention. Integrating transcriptomic, epigenomic, and proteomic data with clinical outcomes may facilitate the development of miRNA-based biomarkers and support precision medicine in colorectal cancer.

Acknowledgments

We wish to express our sincere thanks to Mashhad University of Medical Sciences, the Nuclear Science and Technology Research Institute, and Iran University of Medical Sciences for their valuable support. We also acknowledge the Clinical Research Development Unit of Ghaem Hospital, Mashhad University of Medical Sciences, Mashhad, Iran, for their assistance.

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Conflicts of interest

The authors declare that they have no competing interests.

Data availability statement

The data that support the conclusions of this study can be made available by the corresponding author upon a reasonable request.

Ethical issues

This in silico study did not involve human/animal subjects or primary data collection.

Funding/Support

This work was supported by a grant from Mashhad University of Medical Sciences, under project code (Grant #4040284).

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