Published Online: 2025 June 29 **Research Article**

A Prospective to Regulatory Role of miRNAs on Wnt/β-catenin Signaling and Its Crosstalk to the Other Cellular Pathways in Tumorigenesis of Glioblastoma by a Systems Biology Approach

Morteza Saeidi [1], Alireza Pasdar [2], Farzad Rahmani [3], Abozar Ghorbani [4], Negar Mottaghi [5], Forouzan Amerizadeh [1, 6, *]

[1] Department of Neurology, Mashhad University of Medical Sciences, Mashhad, Iran
[2] Department of Medical Genetics and Molecular Medicine, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[3] Metabolic Syndrome Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[4] Nuclear Agriculture Research School, Nuclear Science and Technology Research Institute (NSTRI), Karaj, Iran
[5] Department of Pharmacognosy and Pharmaceutical Biotechnology, School of Pharmacy, Iran University of Medical Sciences, Tehran, Iran
[6] Department of Internal Medicine, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

[*] ***Corresponding Author:*** Department of Internal Medicine, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran. Email: amerizadehf951@gmail.com

Received: 4 November, 2024; **Revised:** 8 April, 2025; **Accepted:** 19 May, 2025

Abstract

Background: The Wnt plays a crucial role in the initiation, progression, and spread of glioblastoma (GBM). Recently, microRNAs (miRNAs) have been demonstrated to be key players in controlling cell growth and tumor formation.

Objectives: The present study offers the latest insight into miRNAs that influence the Wnt pathway and their interaction with protein-coding genes.

Methods: Previous studies on the regulatory function of miRNAs targeting the Wnt/catenin pathway were reviewed, and all miRNA-targeted genes were found in the miRDB database. Protein-protein interactions (PPIs) of miRNA-targeted genes were investigated using String and Cytoscape software, and hub proteins were examined. Gene-subnetwork Gene Ontology (GO) analysis was performed.

Results: At first, 13 downregulated and 25 upregulated miRNAs targeting the Wnt pathway were obtained, each targeting 1,685 and 1,313 genes, respectively; 12 and 15 hub proteins were found in dysregulated miRNA-targeted genes, which interacted with most genes. The PPI network analysis and subnetwork GO analysis showed these proteins cross-talk with many other proteins that have key roles in the pathways that cause proliferation and malignancy in cells.

Conclusions: Hub proteins are oncogenic proteins that increase gene replication and suppress apoptotic pathways, or tumor suppressors that prevent cancer. By focusing on hub proteins alone or as part of a multi-target approach, it is possible to treat GBM tumors successfully.

Keywords: Glioblastoma, Wnt/β-catenin Signaling, Systems Biology, Protein-Protein Interaction, Subnetwork Analysis

1. Background

Glioblastoma (GBM) represents one of the most common cancers affecting the central nervous system. Despite recent improvements in therapeutic methods, there is an immediate need to discover new and efficient treatment options for managing GBM, since the average survival duration ranges from 12 to 15 months (1). The progression of GBM is a complex process marked by numerous genetic and epigenetic alterations, including deletions and/or amplifications of chromosomal regions, loss of heterozygosity (LOH), single-nucleotide polymorphisms (SNPs), and uncontrolled promoter methylation, resulting in the downregulation of tumor-inhibiting genes or activation of oncogenic genes (1).

How to Cite: Saeidi M, Pasdar A, Rahmani F, Ghorbani A, Mottaghi N, et al. A Prospective to Regulatory Role of miRNAs on Wnt/β-catenin Signaling and Its Crosstalk to the Other Cellular Pathways in Tumorigenesis of Glioblastoma by a Systems Biology Approach. Int J Cancer Manag. 2025; 18 (1): e156834. https://doi.org/10.5812/ijcm-156834.

Recent molecular research suggests that microRNAs (miRNAs) act as either oncogenes or tumor suppressors. They are capable of regulating various cellular processes, including growth, migration, angiogenesis, cell death (apoptosis), and metastasis by regulating the expression of their associated genes (2, 3). The miRNAs are naturally occurring small RNA molecules (18 - 23 nucleotides) that control the expression of specific mRNAs by directly binding to their target sequences and regulating their transcription and translation processes. The miRNAs are emerging as novel prognostic biomarkers for GBM, which are associated with drug resistance, tumor metastasis, and recurrence. The miRNAs may function as potential oncogenic or tumor-suppressive molecules by regulating multiple oncogenic cellular signaling routes, including the PI3K/AKT and Wnt/β-catenin signaling pathways, as supported by accumulating evidence. It has been shown that the upregulation of canonical Wnt signaling has an essential function in the proliferation and advancement of tumor cells across different human cancers, including GBM, breast, colorectal, and liver cancers (4, 5).

The miRNAs regulate Wnt/-catenin signaling by downregulating tumor suppressor proteins such as GSK-3 and APC or inhibiting Wnt signaling downstream targets such as cyclin D1 and -catenin proteins (Table 1). Some of them, like miRNAs such as miR-144-3p, miR-138-2-3p, miR-146b-5p, miR-370, miR-181c, and miR-150-5p, have all been shown to inhibit GBM initiation and invasion by targeting catenin proteins (6-11). Recent findings indicate that the regulatory mechanism of miRNAs on canonical Wnt signaling may occur through the regulation of specific transcription factors, such as T-cell factor (TCF) (12). Further studies demonstrated that miR-24, miR-27a, and miR-92b have inhibitory effects on GBM cell growth and metastasis by suppressing TFs like TCF4, SOX7, or FBXW7 (12-15). However, additional research is needed to explore the regulatory roles of miRNAs, transcription factors, and mRNAs in GBM tumorigenesis. Microarray analysis shows the changes in all genes that are expressed at a given time point, and the analysis of these data has important results about gene interactions.

2. Objectives

In this study, we identified Wnt-related differentially expressed genes (DEGs) controlled by miRNAs and transcription factors in GBM. This research helps in a more precise identification of gene interactions in GBM tumorigenesis, offering valuable information for future studies.

3. Methods

3.1. Literature and Database Mining

The initial selection of miRNAs associated with the Wnt/β-catenin signaling pathway was based on a previously published review article titled 'Regulatory role of miRNAs on Wnt/β-catenin signaling in tumorigenesis of glioblastoma' by Rahmani et al. (16). miRNAs were divided into 2 groups: Upregulated and downregulated miRNAs. The miRNA-targeted genes were identified using the miRDB database, an accessible resource containing annotated and published miRNA sequences, and miRNA-gene interactions were selected with a score of > 90.

3.2. Protein Network Analysis

In order to predict protein-protein interactions (PPIs), the STRING database version 11.5 was employed. This database compiles both direct and indirect interaction data. These interactions are sourced from computational methods, cross-species knowledge transfer, and curated information from primary literature. For further analysis and visualization of these complex networks, Cytoscape software (version 3.9.1), an open-source tool for visualizing biological networks, was utilized. Cytoscape provides a flexible framework for integrating various attribute data, making it a crucial tool for network analysis. To identify key hub proteins within the network, the Cytohubba plugin (version 0.1) was used, which includes multiple topological algorithms. These methods provide a comprehensive approach for identifying significant hub proteins in the network. The ranking of hub proteins, as shown in Tables 2 and 3, was determined based on their scores calculated using 3 topological algorithms: Maximal Clique Centrality (MCC), Degree, and Maximum Neighborhood Component (MNC) (within the CytoHubba plugin in Cytoscape. Higher-ranked proteins demonstrate greater centrality and potential regulatory significance within the PPI network.

3.3. Functional and Pathway Enrichment Analysis

Table 1. List of the Dysregulated MicroRNAs Inhibiting Glioblastoma Tumorigenesis, Their Molecular Alterations, and Targets in the Wnt/β-catenin Signaling Pathway

Molecular Alteration	Target
Upregulation	
miR-19	β-catenin/TCF4
miR-21	β-catenin and Sox2
miR-22-3-p	β-catenin
miR-22-5-p	
miR-24	β-catenin/TCF4
miR-27a	β-catenin/TCF4 and SFRP1
miR-92b	β-catenin/TCF4 and NLK
miR-106a-5p	APC
miR-135b	GSK-3 β
miR-296-3p	β-catenin
miR-603	β-catenin, WIF1, and CTNNBIP1
miR-1249	APC2
miR-4476	APC
Downregulation	
miR-34a	GSK-3β
miR-101	GSK-3β
miR-124a	IQGAP1 and β-catenin
miR-126-3p	β-catenin and Sox2
miR-137	EZH2 and β-catenin
miR-138	AKT and MMP2
miR-138-2-3p	β-catenin
miR-139-5p	Flt1 and β-catenin
miR-142-5p	Wnt3a and β-catenin
miR-144-3p	β-catenin
miR-146b-5p	β-catenin
miR-150-5p	β-catenin
miR-181c	β-catenin
miR-188	β-catenin
miR-206	Frizzled 7
miR-211	β-catenin
miR-320a	β-catenin, cyclin D1, and MMPs (2, 7)
miR-370-3p	β-catenin
miR-370	β-catenin
miR-449b-5p	Wnt2b
miR-505-3p	AKT
miR-577	LRP6 and β-catenin
miR-708	β-catenin
miR-769-3p	ZEB2
miR-1825	CDK-14

Abbreviation: miRNA, microRNA.

Table 2. Key Hub Proteins in Genes Modulated by Downregulated MicroRNAs

Hob Proteins	Method	Rank
FN1	MCC/MNC/Degree	2, 4, 4
JUN	MCC/MNC/Degree	3, 2, 1
RHOA	MCC/MNC/Degree	5, 3, 3
PTEN	MNC/Degree	1, 2
SIRT1	MNC/Degree	5, 5
CD44	MCC	1
IGF1	MCC	4
AGK	DMNC	1
BCLAF1	DMNC	2
TRAK1	DMNC	3
SGCE	DMNC	4
AEBP2	DMNC	4

To better understand the roles and interactions of the identified genes, enrichment analyses were performed. Gene Ontology (GO) analysis was carried out using STRING version 11.5. The GO provides a comprehensive framework for annotating genes or gene products by examining 3 main domains: Biological processes (BPs), molecular functions (MFs), and cellular components (CC), giving insights into their functional roles and cellular locations. The Pathway analysis was

Table 3. Key Hub Proteins in Genes Modulated by Upregulated MicroRNAs

Hob Proteins	Method	Rank
EGFR	MNC/Degree	1, 1
KRAS	MNC/Degree	2, 2
STAT3	MNC/Degree	3, 3
SIRT1	MNC/Degree	4, 5
GRIA2	MNC/Degree	5, 4
SNAP25	MCC	1
SYP	MCC	2
SLC17A7	MCC	3
CPLX2	MCC	4
SLC17A6	MCC	5
PANK1	DMNC	1
NCALD	DMNC	1
SV2B	DMNC	3
CCNJL	DMNC	4
GNS	DMNC	5

Abbreviations: MNC, maximum neighborhood component; EGFR, epidermal growth factor receptor; MCC, maximal clique centrality.

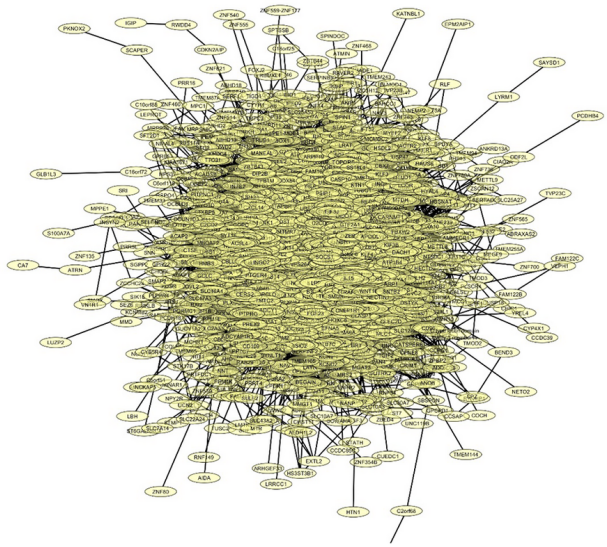

Figure 1. Network of proteins targeted by the downregulated microRNA (miRNA) presented by cytoscape software

conducted through KEGG enrichment using the STRING platform.

3.4. Analysis of the Network's Clusters

The network's nodes were grouped using CytoCluster (version 2.1.0) to facilitate the identification of significant clusters. For cluster analysis within the subnetwork, the identification of protein complexes was conducted using the integrated protein complex analysis (IPCA) technique. A threshold value of 2 was applied to define the clusters. STRING (version 11.5) was, then, employed to perform a detailed analysis of each cluster's genes, focusing on identifying the KEGG pathways associated with them (17, 18).

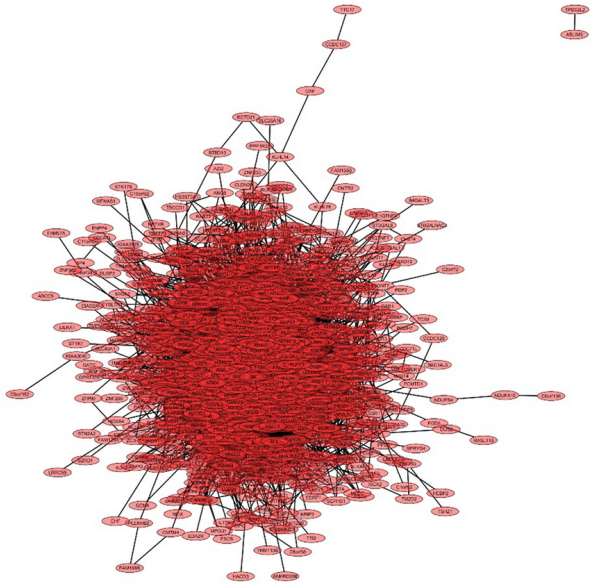

Figure 2. Network of proteins targeted by the upregulated microRNA (miRNA) presented by cytoscape software

3.5. Promoter Motif Analysis of Hub Genes

To examine the promoter regions of hub genes, upstream flanking regions (UFRs) covering 1 kilobase pair (1 kbp) were obtained from the Ensembl database. These sequences were analyzed for motif identification using MEME Suite (version 5.5.2). The default settings were used, with specific adjustments to the P and E values, which were set to 0.01 for enhanced accuracy (19). To remove redundant motifs and detect known cis-regulatory elements (CREs), Tomtom (version 5.5.2) was applied, utilizing the JASPAR CORE 2022 database for reference (20). Additionally, the GoMo tool was employed to predict the potential biological functions of the identified motifs. This analysis provided deeper insights into the regulatory elements within the promoter regions of the hub genes (21).

4. Results

4.1. Protein-Protein Interaction Networks and Hub Analysis of Dysregulated MicroRNA-Targeted Genes

In this study, we analyzed the role of dysregulated miRNAs in regulating the Wnt/β-catenin signaling pathway in GBM. A total of 1 685 and 1 313 target genes were identified for downregulated and upregulated miRNAs, respectively. The gene interaction networks are illustrated in Figures 1 and 2. A variety of miRNAs show altered expression patterns in GBM, playing significant roles in the proliferation and spread of cancer cells by directly influencing specific oncogenes or tumor-suppressing genes in glioma (22, 23). These miRNAs contribute to GBM development by modulating key oncogenes and tumor suppressors. To better understand their impact, we conducted a network analysis using proteins with interaction scores above 90. Hub proteins were identified using the CytoHubba plugin in Cytoscape (Tables 2 and 3).

For downregulated miRNAs, key hub proteins included FN1, JUN, RHOA, PTEN, and SIRT1, identified by multiple topological algorithms (MCC, MNC, degree). Additional hubs like CD44, IGF1, AGK, and TRAK1 were detected by single algorithms. In the upregulated group, hub proteins such as EGFR, KRAS, STAT3, SIRT1, and GRIA2 were prominent, with other hubs including SNAP25, SYP, and PANK1.

Overall, 12 unique hub proteins were consistently identified, several of which (e.g., SIRT1, STAT3) appeared

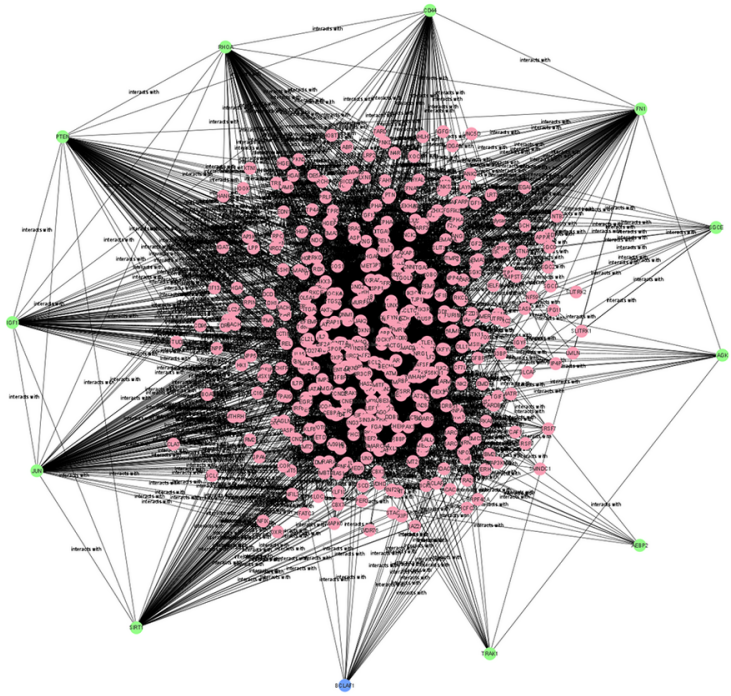

Figure 3. Subnetwork of hob proteins targeted by downregulated microRNA (miRNA) shown in Table 3.

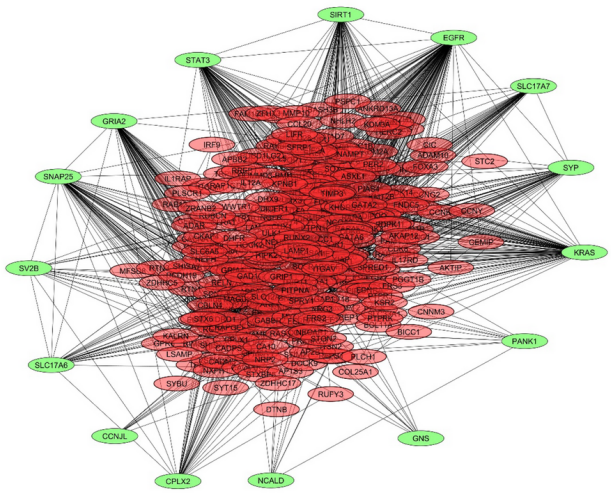

Figure 4. Subnetwork of hob proteins targeted by upregulated microRNAs (miRNAs) shown in Table 4.

in both regulatory groups, suggesting central roles in the Wnt/β-catenin network and its crosstalk with other

Table 4. Leading 5 Subnetwork Clusters from CytoCluster Analysis for Downregulated MicroRNA Targets

Clusters	Ranks	Nodes	Edges	Functions
1	1	28	249	
				Metabolism of inositol phosphate
				EGFR tyrosine kinase inhibitor resistance
				Endocrine resistance
				MAPK pathway
				ErbB pathway
2	2	26	216	
				Bacterial attack of epithelial cells
				Renal cell carcinoma
				EGFR tyrosine kinase inhibitor resistance
				Aldosterone-regulated sodium reabsorption
				T cell receptor pathway
3	3	26	199	
				EGFR tyrosine kinase inhibitor resistance
				Colorectal cancer
				FoxO pathway
				AGE-RAGE pathway in diabetic complications
				Apoptosis - multiple species
4	4	24	195	
				Adherens junction
				TGF-beta pathway
				AGE-RAGE pathway in diabetic complications
				Colorectal cancer
				Bacterial invasion of epithelial cells
5	5	24	204	
				EGFR tyrosine kinase inhibitor resistance
				Bacterial invasion of epithelial cells
				Renal cell carcinoma
				Aldosterone-regulated sodium reabsorption
				Central carbon metabolism in cancer

Abbreviation: EGFR, epidermal growth factor receptor

oncogenic pathways (Figures 3 and 4).

4.2. Functional and Pathway Enrichment Analysis

Subnetwork analysis is used to predict key pathways and significant processes within hub protein connections. In order to elucidate crucial pathways and processes in miRNA-targeted genes, hub protein interactions were subjected to subnetwork analysis. The GO database is one of the most comprehensive global resources for information on gene function, offering a basis for computational studies in large-scale molecular biology and genetic research (24). The GO analysis was conducted by examining BP, MF, and CC of the hub protein subnetwork (Figure 5). The Go analysis recognized 1,708 BPs, including positive regulation of BPs, negative regulation of cellular processes, regulation of developmental processes, positive cellular regulation, and regulation of multicellular organismal processes. In addition, 132 CCs were identified, including intracellular, nucleoplasm, nuclear lumen, intracellular organelle lumen, and protein-containing complex. Furthermore, 134 MFs were found, including protein binding, enzyme binding, MF regulator, and signaling receptor binding.

KEGG pathway analysis identified 162 pathways, encompassing pathways related to cancer, including PI3K-Akt signaling, FoxO signaling, axon guidance, and focal adhesion, enriched between subnetwork genes in interaction with the hub proteins targeted by downregulated miRNAs. As expected, cancer pathways

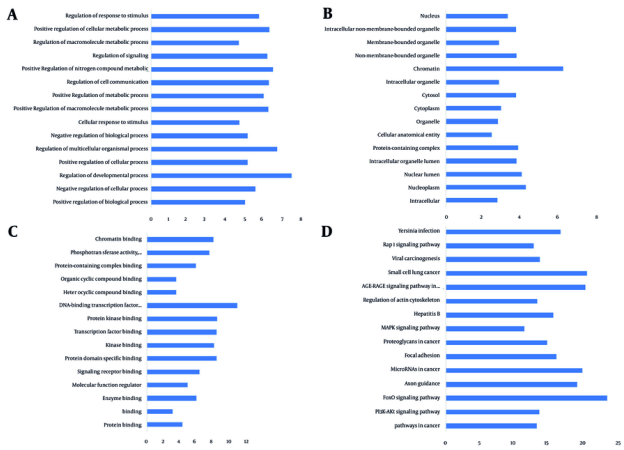

Figure 5. A, Biological process; B, Gene Ontology (GO): Cellular component (CC); C, GO: Molecular function (MF); D, KEGG pathway enrichment for subnetwork genes modulated by downregulated microRNAs (miRNAs).

are enriched in the group of downregulated miRNA-targeted genes, including important proliferation pathways such as PI3K and MAPK.

The GO analysis for the upregulated miRNAs revealed 1 075 BPs, such as localization control, positive regulation of biological activities, cell-to-cell communication, signaling pathways, and cellular process regulation. A total of 166 CCs were recognized, including cell junctions, synaptic regions (presynaptic and postsynaptic), and the plasma membrane. Furthermore, 109 MFs were identified, such as protein binding, enzyme interaction, MF regulation, and protein kinase association. Also, KEGG pathway analysis revealed that miRNAs are involved in targeting several pathways, such as cancer, PI3K-Akt, focal adhesion, and neurotrophin signaling, in genes influenced by upregulated miRNAs (Figures 5 and 6).

4.3. Analysis of the Network by Clusters

The organization of biological networks can be uncovered through cluster analysis, which is a crucial technique for detecting practical modules, forecasting protein complexes, and categorizing biomarkers within networks. In this study, subnetwork cluster analysis identified 817 clusters for downregulated miRNA-targeted genes and 706 clusters for upregulated miRNA-targeted genes, from which clusters ranked 1 to 5 were selected. As can be seen in Tables 4 and 5, the proteins that are often targeted by miRNAs with low expression are the proteins and pathways responsible for cell proliferation, angiogenesis, and carbon metabolism, such as HIF and TGF-beta. The reduction of miRNAs targeting these proteins causes an increase in these proteins, which increases the proliferation and growth of cells. On the contrary, the proteins that are targeted by miRNAs with higher expression target most of the pathways of cell connections and communication, and this disruption and reduction of cell communication can reduce the communication between cells, and the messages that prevent cell proliferation between cells are not transferred.

4.4. Promoter Motif Analysis of Hub Genes

Promoter motif analysis of the hub genes targeted by dysregulated miRNAs revealed several conserved CREs. For downregulated miRNAs (Figure 7), the identified motifs were predominantly associated with functions such as negative regulation of signal transduction, transcription corepressor activity, ion transport, and neuron fate commitment. These motifs may contribute to the suppression of tumor-inhibitory pathways when their targeting miRNAs are downregulated. In contrast, motifs identified in the upregulated miRNA-targeted hub genes (Figure 8) were enriched in regulatory functions such as transcription inhibition from RNA polymerase II promoters, signal transduction

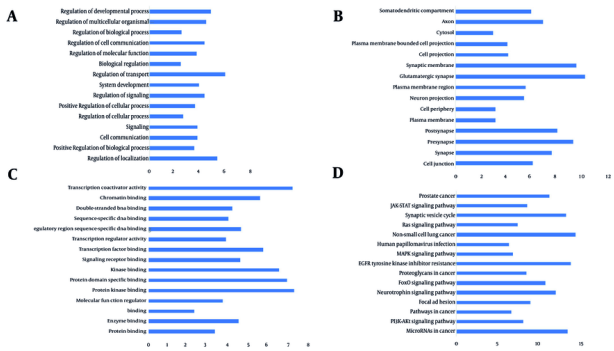

Figure 6. A, Biological process; B, Gene Ontology (GO): Cellular component (CC); C, GO: Molecular function (MF); D, KEGG pathway enrichment for subnetwork genes modulated by upregulated microRNAs (miRNAs).

Table 5. Leading 5 Subnetwork Clusters from CytoCluster Analysis for Upregulated MicroRNA Targets

Clusters	Ranks	Nodes	Edges	KEGG Pathway Enrichment of Nodes
1	1	20	125	
				Nicotine addiction
				Synaptic vesicle cycle
				Glutamatergic synapse
				Retrograde endocannabinoid signaling
				GABAergic synapse
2	2	20	124	
				Nicotine addiction
				Synaptic vesicle cycle
				Glutamatergic synapse
				Retrograde endocannabinoid signaling
				GABAergic synapse
3	3	18	102	
				Nicotine addiction
				Synaptic vesicle cycle
				Glutamatergic synapse
				Retrograde endocannabinoid signaling
4	4	18	114	
				Nicotine addiction
				Synaptic vesicle cycle
				GABAergic synapse
				Retrograde endocannabinoid signaling
5	5	18	110	
				Nicotine addiction
				Synaptic vesicle cycle
				Glutamatergic synapse
				Retrograde endocannabinoid signaling

modulation, and inner ear morphogenesis. Notably, common motifs such as SP1, SP2, and ZN467 were shared across both groups, indicating potential shared regulatory mechanisms. Overall, these findings suggest that dysregulated miRNAs modulate the transcriptional landscape of GBM by targeting key regulatory motifs in promoter regions of critical genes involved in cell signaling, apoptosis, and differentiation (Figures 7 and 8).

5. Discussion

In this study, we explored the regulatory role of dysregulated miRNAs on the Wnt/β-catenin signaling pathway in GBM using a systems biology approach. Our analysis revealed that both upregulated and downregulated miRNAs target a wide array of genes within the Wnt signaling network, many of which are critically involved in tumorigenesis, cellular proliferation, and therapy resistance in GBM. Among the downregulated miRNAs, we identified key targets such as PTEN, RHOA, and SIRT1, which are known tumor suppressors. The reduced expression of these miRNAs may lead to overactivation of oncogenic pathways, thereby promoting glioma cell proliferation and

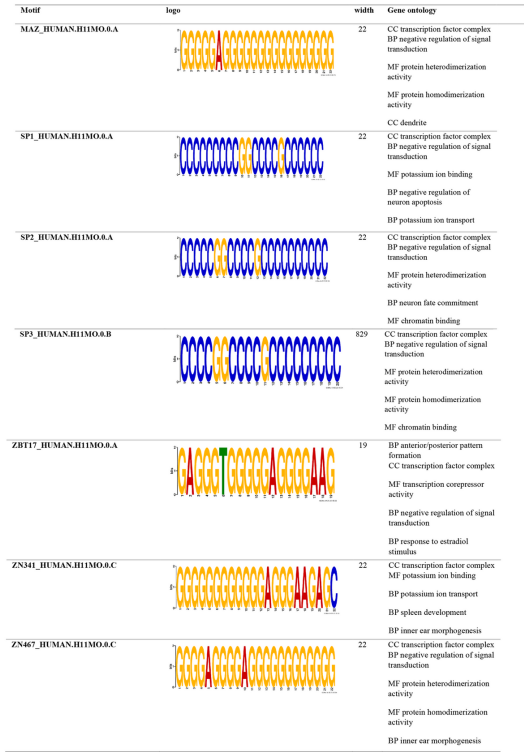

Figure 7. Promoter analysis of downregulated microRNA (miRNA)-targeted hub proteins

invasion. In contrast, upregulated miRNAs were found to target genes like epidermal growth factor receptor (EGFR), KRAS, and STAT3, which are pivotal drivers in GBM progression. These findings highlight the dual and context-dependent roles of miRNAs as either oncogenes or tumor suppressors, depending on their expression levels and target genes.

The identification of hub proteins through PPI network analysis further emphasized the centrality of certain genes in the regulation of tumor-promoting signaling pathways. Proteins such as FN1, JUN, and STAT3 emerged as major nodes in the network, indicating that they may serve as effective therapeutic targets or biomarkers for GBM. In a recent study, Song et al. demonstrated that the upregulation of FN1 reduced the levels of protein tyrosine phosphatase receptor type M (PTPRM) through enhanced methylation, which subsequently led to increased STAT3 phosphorylation and the stimulation of GBM cell proliferation (25). Thompson illustrated how the transcription factor JUN collaborates with YAP-TEAD to promote tumor growth in GBM and also works alongside MRTF-SRF to intensify the activation of cancer-associated fibroblasts, matrix stiffening, and metastasis (26). Cui et al. explored the proliferation of glioma cells, finding that RhoA and COX-2 levels were elevated in brain glioma tissues (27). An animal study performed by Li et al. showed that RhoA protein has a tumor suppressor role in glioma cancer. They demonstrated that Pard3 controls the levels, localization within the cell, and transcriptional activity of RhoA. Experiments using mouse models demonstrated that elevated RhoA expression suppresses glioma cell proliferation in living organisms (28).

PTEN, a well-known tumor suppressor present in nearly all body tissues, has been shown to carry mutations in multiple cancer types, such as glioma, breast, and colorectal cancers. In addition to the role of this protein in causing or promoting the onset of cancers, Ma et al. showed that phosphorylation of PTEN at Y240, facilitated by FGFR, is key to radiation resistance

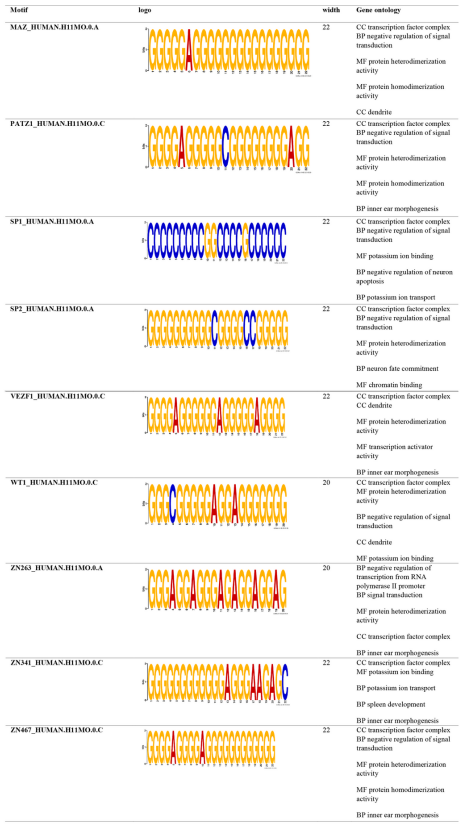

Figure 8. Promoter analysis of upregulated microRNA (miRNA)-targeted hub proteins

and may serve as a promising target to improve radiotherapy outcomes (29).

In glioma tissues and cell lines, SIRT1 expression was significantly reduced, with elevated levels being linked to better prognosis in glioma patients. Therefore, this protein can be considered a tumor suppressor (30). Increased mRNA levels of EGFR, a type of receptor tyrosine kinase, have been detected in various cancer types and are thought to stimulate the growth of solid tumors (31).

KRAS, a key hub protein, functions as a central node for cellular signaling pathways that drive cell growth and proliferation. Mutations in this protein have been found in various cancer types, including colorectal, breast, prostate, and lung cancers (32). Around 90% of GBM tissues and cell lines showed STAT3 phosphorylation at Tyr-705 and Ser-727, which was positively associated with higher histopathological grades and decreased patient survival (33, 34).

Cluster analysis revealed distinct functional patterns for genes targeted by upregulated and downregulated miRNAs. In the case of downregulated miRNAs, enriched clusters were mainly associated with cancer-related pathways such as MAPK signaling, TGF-β signaling, and central carbon metabolism, suggesting that reduced miRNA expression may lead to the activation of oncogenic processes and enhanced cell proliferation.

In contrast, clusters of upregulated miRNA targets were enriched in synaptic signaling and neuronal communication pathways like glutamatergic synapse, GABAergic synapse, and endocannabinoid signaling. These findings imply that upregulated miRNAs may suppress genes involved in neural-like signaling, potentially affecting tumor–neuron interactions and microenvironmental dynamics.

Overall, the distinct clustering patterns highlight the dual role of miRNAs in GBM, influencing both intrinsic tumor behavior and its interaction with the neural microenvironment.

Promoter analysis demonstrated that certain regulatory elements are commonly found in both downregulated and upregulated miRNA-targeted hub gene groups (Figures 7 and 8). In downregulated miRNA targets, regulatory motifs were associated with anterior/posterior pattern formation, transcription corepression, and estradiol response. Conversely, motifs in upregulated miRNA targets were linked to inhibition of RNA polymerase II-driven transcription and signal transduction. More clearly, the reduction of miRNAs targeting corepressors reduces transcription inhibition and ultimately facilitates transcription and protein synthesis. Anterior-posterior patterning involves the regionalization process that forms distinct regions of cell differentiation along the anterior-posterior axis, leading to cellular polarity. The loss of cellular polarity has been documented in multiple types of cancer (35). Inhibiting transcription and signal transduction pathways can lead to enhanced protein synthesis and increased cell growth (36).

5.1. Conclusions

This study provides a systems-level understanding of how dysregulated miRNAs influence the Wnt/β-catenin signaling pathway in GBM. By integrating bioinformatics tools, we identified key hub genes such as PTEN, STAT3, KRAS, SIRT1, and FN1, which play central roles in tumor progression and resistance mechanisms. Our cluster and promoter motif analyses revealed distinct regulatory patterns for upregulated and downregulated miRNAs, linking them to critical pathways including MAPK, TGF-β, and synaptic signaling. These findings suggest that specific miRNAs and their target genes may serve as potential diagnostic biomarkers or therapeutic targets in GBM. The results of this study pave the way for future experimental validation and the development of miRNA-based precision therapies for GBM.

5.2. Study Limitations

One of the primary limitations of this study is the lack of laboratory validation of the findings. While our research provides valuable insights into the interactions between miRNAs and the Wnt/β-catenin signaling pathway, the conclusions drawn are largely based on computational analyses and existing literature. This approach has several implications; the findings are reliant on previously published data, which may have inherent biases or limitations. Without direct experimental validation, the accuracy and applicability of these results to clinical settings remain uncertain. Biological systems are complex and can exhibit variability that is not captured in computational models. Laboratory experiments can account for this variability and provide a more nuanced understanding of the biological mechanisms involved. To address this limitation, we recommend that future studies include laboratory experiments that validate the computational findings. This could involve in vitro and in vivo studies to confirm the roles of specific miRNAs and their target genes in the Wnt pathway.

Acknowledgements

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.

Footnotes

Authors' Contribution: Study concept and design: F. A. and A. P.; Acquisition of data: M. S. and F. R.; Analysis and interpretation of data: F. A., A. G., and N. M.; Drafting the manuscript: M. S.; Study supervision: F. A.

Conflict of Interests Statement: The authors declare no conflict of interest.

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

Ethical Approval: IR.MUMS.MEDICAL.REC.1402.372

Funding/Support: This work was supported by a grant from Mashhad University of Medical Sciences, under project code 4021147.

References

1. Hanif F, Muzaffar K, Perveen K, Malhi SM, Simjee Sh U. Glioblastoma Multiforme: A Review of its Epidemiology and Pathogenesis through Clinical Presentation and Treatment. *Asian Pac J Cancer Prev*. 2017;**18**(1):3-9. [PubMed ID: 28239999]. [PubMed Central ID: PMC5563115]. https://doi.org/10.22034/APJCP.2017.18.1.3.
2. Soleimani A, Rahmani F, Saeedi N, Ghaffarian R, Khazaei M, Ferns GA, et al. The potential role of regulatory microRNAs of RAS/MAPK signaling pathway in the pathogenesis of colorectal cancer. *J Cell Biochem*. 2019;**120**(12):19245-53. [PubMed ID: 31512778]. https://doi.org/10.1002/jcb.29268.
3. Rahmani F, Ziaeemehr A, Shahidsales S, Gharib M, Khazaei M, Ferns GA, et al. Role of regulatory miRNAs of the PI3K/AKT/mTOR signaling in the pathogenesis of hepatocellular carcinoma. *J Cell Physiol*. 2020;**235**(5):4146-52. [PubMed ID: 31663122]. https://doi.org/10.1002/jcp.29333.
4. Ji J, Yamashita T, Wang XW. Wnt/beta-catenin signaling activates microRNA-181 expression in hepatocellular carcinoma. *Cell Biosci*. 2011;**1**(1):4. [PubMed ID: 21711587]. [PubMed Central ID: PMC3116242]. https://doi.org/10.1186/2045-3701-1-4.
5. Rahmani F, Tadayyon Tabrizi A, Hashemian P, Alijannejad S, Rahdar HA, Ferns GA, et al. Role of regulatory miRNAs of the Wnt/ beta-catenin signaling pathway in tumorigenesis of breast cancer. *Gene*. 2020;**754**:144892. [PubMed ID: 32534060]. https://doi.org/10.1016/j.gene.2020.144892.
6. Huang L, Li X, Ye H, Liu Y, Liang X, Yang C, et al. Long non-coding RNA NCK1-AS1 promotes the tumorigenesis of glioma through sponging microRNA-138-2-3p and activating the TRIM24/Wnt/beta-catenin axis. *J Exp Clin Cancer Res*. 2020;**39**(1):63. [PubMed ID: 32293515]. [PubMed Central ID: PMC7158134]. https://doi.org/10.1186/s13046-020-01567-1.
7. Yang W, Yu H, Shen Y, Liu Y, Yang Z, Sun T. MiR-146b-5p overexpression attenuates stemness and radioresistance of glioma stem cells by targeting HuR/lincRNA-p21/beta-catenin pathway. *Oncotarget*. 2016;**7**(27):41505-26. [PubMed ID: 27166258]. [PubMed Central ID: PMC5173075]. https://doi.org/10.18632/oncotarget.9214.
8. Lu M, Wang Y, Zhou S, Xu J, Li J, Tao R, et al. MicroRNA-370 suppresses the progression and proliferation of human astrocytoma and glioblastoma by negatively regulating β-catenin and causing activation of FOXO3a. *Experimental and Therapeutic Medicine*. 2017. https://doi.org/10.3892/etm.2017.5494.
9. Tian W, Zhu W, Jiang J. miR-150-5p suppresses the stem cell-like characteristics of glioma cells by targeting the Wnt/beta-catenin signaling pathway. *Cell Biol Int*. 2020;**44**(5):1156-67. [PubMed ID: 32009256]. https://doi.org/10.1002/cbin.11314.
10. Sun J, Ma Q, Li B, Wang C, Mo L, Zhang X, et al. RPN2 is targeted by miR-181c and mediates glioma progression and temozolomide sensitivity via the wnt/beta-catenin signaling pathway. *Cell Death Dis*. 2020;**11**(10):890. [PubMed ID: 33087705]. [PubMed Central ID: PMC7578010]. https://doi.org/10.1038/s41419-020-03113-5.
11. Gai SY, Yuan ZH. Long non-coding RNA SOX21-AS1 promotes cell proliferation and invasion through upregulating PAK7 expression by sponging miR-144-3p in glioma cells. *Neoplasma*. 2020;**67**(2):333-43. [PubMed ID: 31973536]. https://doi.org/10.4149/neo_2020_190509N412.
12. Arnott M, Sampilo NF, Song JL. Transcription of microRNAs is regulated by developmental signaling pathways and transcription factors. *Front Cell Dev Biol*. 2024;**12**:1356589. [PubMed ID: 38721525]. [PubMed Central ID: PMC11076791]. https://doi.org/10.3389/fcell.2024.1356589.
13. Grafals-Ruiz N, Sanchez-Alvarez AO, Santana-Rivera Y, Lozada-Delgado EL, Rabelo-Fernandez RJ, Rios-Vicil CI, et al. MicroRNA-92b targets tumor suppressor gene FBXW7 in glioblastoma. *Front Oncol*. 2023;**13**:1249649. [PubMed ID: 37752997]. [PubMed Central ID: PMC10518455]. https://doi.org/10.3389/fonc.2023.1249649.
14. Rivera-Diaz M, Miranda-Roman MA, Soto D, Quintero-Aguilo M, Ortiz-Zuazaga H, Marcos-Martinez MJ, et al. MicroRNA-27a distinguishes glioblastoma multiforme from diffuse and anaplastic astrocytomas and has prognostic value. *Am J Cancer Res*. 2015;**5**(1):201-18. [PubMed ID: 25628931]. [PubMed Central ID: PMC4300691].
15. Wang S, Liu N, Tang Q, Sheng H, Long S, Wu W. MicroRNA-24 in Cancer: A Double Side Medal With Opposite Properties. *Front Oncol*. 2020;**10**:553714. [PubMed ID: 33123467]. [PubMed Central ID: PMC7566899]. https://doi.org/10.3389/fonc.2020.553714.
16. Rahmani F, Hashemian P, Tabrizi AT, Ghorbani Z, Ziaeemehr A, Alijannejad S, et al. Regulatory role of miRNAs on Wnt/beta-catenin signaling in tumorigenesis of glioblastoma. *Indian J Cancer*. 2023;**60**(3):295-302. [PubMed ID: 37787188]. https://doi.org/10.4103/ijc.IJC_251_21.
17. Li M, Li D, Tang Y, Wu F, Wang J. CytoCluster: A Cytoscape Plugin for Cluster Analysis and Visualization of Biological Networks. *Int J Mol Sci*. 2017;**18**(9). [PubMed ID: 28858211]. [PubMed Central ID: PMC5618529]. https://doi.org/10.3390/ijms18091880.
18. Mai TL, Hu GM, Chen CM. Visualizing and Clustering Protein Similarity Networks: Sequences, Structures, and Functions. *J Proteome Res*. 2016;**15**(7):2123-31. [PubMed ID: 27267620]. https://doi.org/10.1021/acs.jproteome.5b01031.
19. Bailey TL, Boden M, Buske FA, Frith M, Grant CE, Clementi L, et al. MEME SUITE: tools for motif discovery and searching. *Nucleic Acids Res*. 2009;**37**(Web Server issue):W202-8. [PubMed ID: 19458158]. [PubMed Central ID: PMC2703892]. https://doi.org/10.1093/nar/gkp335.
20. Gupta S, Stamatoyannopoulos JA, Bailey TL, Noble WS. Quantifying similarity between motifs. *Genome Biol*. 2007;**8**(2):R24. [PubMed ID: 17324271]. [PubMed Central ID: PMC1852410]. https://doi.org/10.1186/gb-2007-8-2-r24.
21. Buske FA, Boden M, Bauer DC, Bailey TL. Assigning roles to DNA regulatory motifs using comparative genomics. *Bioinformatics*. 2010;**26**(7):860-6. [PubMed ID: 20147307]. [PubMed Central ID: PMC2844991]. https://doi.org/10.1093/bioinformatics/btq049.
22. Soleimani A, Rahmani F, Ferns GA, Ryzhikov M, Avan A, Hassanian SM. Role of Regulatory Oncogenic or Tumor Suppressor miRNAs of PI3K/AKT Signaling Axis in the Pathogenesis of Colorectal Cancer. *Curr Pharm Des*. 2018;**24**(39):4605-10. [PubMed ID: 30636581]. https://doi.org/10.2174/1381612825666190110151957.
23. Rahmani F, Ferns GA, Talebian S, Nourbakhsh M, Avan A, Shahidsales S. Role of regulatory miRNAs of the PI3K/AKT signaling pathway in the pathogenesis of breast cancer. *Gene*. 2020;**737**:144459. [PubMed ID: 32045660]. https://doi.org/10.1016/j.gene.2020.144459.
24. Yadav DK, Sharma A, Dube P, Shaikh S, Vaghasia H, Rawal RM. Identification of crucial hub genes and potential molecular mechanisms in breast cancer by integrated bioinformatics analysis and experimental validation. *Comput Biol Med*. 2022;**149**:106036.

[PubMed ID: 36096037]. https://doi.org/10.1016/j.compbiomed.2022.106036.

25. Song J, Zhao D, Sun G, Yang J, Lv Z, Jiao B. PTPRM methylation induced by FN1 promotes the development of glioblastoma by activating STAT3 signalling. *Pharm Biol*. 2021;**59**(1):904-11. [PubMed ID: 34225581]. [PubMed Central ID: PMC8259858]. https://doi.org/10.1080/13880209.2021.1944220.
26. Thompson BJ. YAP/TAZ: Drivers of Tumor Growth, Metastasis, and Resistance to Therapy. *Bioessays*. 2020;**42**(5). e1900162. [PubMed ID: 32128850]. https://doi.org/10.1002/bies.201900162.
27. Cui X, Cui N, Pan J, Sun X. Expression of RhoA and COX-2 and their roles in the occurrence and progression of brain glioma. *Pakistan Journal of Pharmaceutical Sciences*. 2021;**34**.
28. Li J, Xu H, Wang Q, Fu P, Huang T, Anas O, et al. Pard3 suppresses glioma invasion by regulating RhoA through atypical protein kinase C/NF-kappaB signaling. *Cancer Med*. 2019;**8**(5):2288-302. [PubMed ID: 30848088]. [PubMed Central ID: PMC6536976]. https://doi.org/10.1002/cam4.2063.
29. Ma J, Benitez JA, Li J, Miki S, Ponte de Albuquerque C, Galatro T, et al. Inhibition of Nuclear PTEN Tyrosine Phosphorylation Enhances Glioma Radiation Sensitivity through Attenuated DNA Repair. *Cancer Cell*. 2019;**35**(3):504-518 e7. [PubMed ID: 30827889]. [PubMed Central ID: PMC6424615]. https://doi.org/10.1016/j.ccell.2019.01.020.
30. Fang DZ, Wang WJ, Li FY, Liu J, Hui XB, Liu D, et al. Circ_0005075 stimulates the proliferation and metastasis of glioma via downregulating SIRT1. *Eur Rev Med Pharmacol Sci*. 2020;**24**(1):258-66. [PubMed ID: 31957839]. https://doi.org/10.26355/eurrev_202001_19918.
31. Nicholson RI, Gee JM, Harper ME. EGFR and cancer prognosis. *Eur J Cancer*. 2001;**37 Suppl 4**:S9-15. [PubMed ID: 11597399]. https://doi.org/10.1016/s0959-8049(01)00231-3.
32. Mustachio LM, Chelariu-Raicu A, Szekvolgyi L, Roszik J. Targeting KRAS in Cancer: Promising Therapeutic Strategies. *Cancers (Basel)*. 2021;**13**(6). [PubMed ID: 33801965]. [PubMed Central ID: PMC7999304]. https://doi.org/10.3390/cancers13061204.
33. Brantley EC, Nabors LB, Gillespie GY, Choi YH, Palmer CA, Harrison K, et al. Loss of protein inhibitors of activated STAT-3 expression in glioblastoma multiforme tumors: implications for STAT-3 activation and gene expression. *Clin Cancer Res*. 2008;**14**(15):4694-704. [PubMed ID: 18676737]. [PubMed Central ID: PMC3886729]. https://doi.org/10.1158/1078-0432.CCR-08-0618.
34. Lin GS, Chen YP, Lin ZX, Wang XF, Zheng ZQ, Chen L. STAT3 serine 727 phosphorylation influences clinical outcome in glioblastoma. *Int J Clin Exp Pathol*. 2014;**7**(6):3141-9. [PubMed ID: 25031733]. [PubMed Central ID: PMC4097241].
35. Muthuswamy SK, Xue B. Cell polarity as a regulator of cancer cell behavior plasticity. *Annu Rev Cell Dev Biol*. 2012;**28**:599-625. [PubMed ID: 22881459]. [PubMed Central ID: PMC3997262]. https://doi.org/10.1146/annurev-cellbio-092910-154244.
36. Martin RD, Hebert TE, Tanny JC. Therapeutic Targeting of the General RNA Polymerase II Transcription Machinery. *Int J Mol Sci*. 2020;**21**(9). [PubMed ID: 32397434]. [PubMed Central ID: PMC7246882]. https://doi.org/10.3390/ijms21093354.