Iran. J. Biotechnol. April 2024;22(2): e3827 DOI: 10.30498/ijb.2024.422185.3827

Research Article

Investigation of Common Pathways and Putative Biomarker Candidates of Colorectal Cancer and Insomnia by Using Integrative *In-Silico* Approaches

Metehan Yaman[1], Dilek Pirim[1,2,3*]

[1]Institute of Natural and Applied Sciences, Department of Molecular Biology and Genetics, Bursa Uludag University, Bursa, Türkiye
[2]Institute of Health Sciences, Department of Translational Medicine, Bursa Uludag University, Bursa, Türkiye
[3]Department of Molecular Biology and Genetics, Bursa Uludag University, Bursa, Türkiye

[*]*Corresponding author*: Dilek Pirim, Institute of Health Sciences, Department of Translational Medicine, Bursa Uludag University, Bursa, Türkiye & Department of Molecular Biology and Genetics, Bursa Uludag University, Bursa, Türkiye. Tel/Fax: +90-2242942835, E-mail: dilekpirim@uludag.edu.tr

Received: 2023/11/04 ; **Accepted:** 2024/03/09

Background: Colorectal cancer (CRC) is one of the leading causes of cancer-related mortalities across the globe. Accumulating evidence shows that individuals having sleep disorders such as insomnia are at high risk of developing CRC, yet the association of sleep disorders with CRC risk is still unclear. Here, we investigated the potential molecular connections between CRC and insomnia using integrative *in silico* approaches.

Objective: This study aims to explore the potential molecular connections between CRC and insomnia utilizing integrative *in-silico* methodologies.

Methods and Methods: Gene expression microarray datasets for CRC and insomnia samples were retrieved from the NCBI-GEO database and analyzed using R. Functional enrichment analysis of common differentially expressed genes (DEGs) was performed by the g: Profiler tool. Cytoscape software was used to construct a protein-protein interaction network and hub gene identification. Expression profiles of hub genes in TCGA datasets were also determined, and predicted miRNAs targeting hub genes were analyzed by miRNA target prediction tools.

Results: Our results revealed a total of 113 shared DEGs between the CRC and insomnia datasets. Six genes (*HSP8A*, *GAPDH*, *HSP90AA1*, *EEF1G*, *RPS6*, and *RPLP0),* which were also differently expressed in TCGA datasets, were prioritized as hub genes and were found to be enriched in pathways related to protein synthesis. hsa-miR-324-3p, hsa-miR-769-3p, and hsa-miR-16-5p were identified as promising miRNA biomarkers for two diseases.

Conclusions: Our *in-silico* analysis provides promising evidence of the molecular link between CRC and insomnia and highlights multiple potential molecular biomarkers and pathways. Validation of the results by wet lab work can be utilized for novel translational and precision medicine applications to alleviate the public health burden of CRC.

Keywords: Colorectal cancer, Hub genes, In silico analysis, Insomnia, Pathway analysis

1. Background

Colorectal cancer (CRC) is the third most prevalent type of cancer globally that causes a significant global health burden (1). Environmental factors such as dietary habits, sedentary lifestyles, smoking, and alcohol consumption are well-established risk factors

for CRC (2). The interplay between epidemiological and genetic risk factors shapes tumor properties and tumor heterogeneity which causes a hurdle in developing accurate early diagnosis and treatment options (3-4). Thus, uncovering the contributions of the molecular pathways and biomolecules in CRC pathogenesis is of the utmost importance for developing appropriate diagnostic and treatment approaches for CRC management.

Previous studies suggested sleep disruption-induced molecular mechanisms may interfere with carcinogenesis and increase the risk of cancer development (5-9). Moreover, a growing body of evidence suggests sleep loss causes dysregulation of epigenetic mechanisms that result in alteration of cancer-related microRNA (miRNA) profiles and pathways in the circulation (8-11). Recently, epidemiological studies have provided intriguing evidence suggesting a link between CRC development and sleep disorders, thus sleep disorders are considered a risk factor for CRC (7, 12). Molecular changes resulting from sleep disruption may also involve molecular mechanisms underlying CRC and may promote the risk of CRC development (7, 13, 15). Recently, circadian clock genes including *Per1, Per2, Per3,* and *Cry 1* were found to be associated with tumorigenesis and cancer development (16-19). Additionally, intermittent hypoxia (IH) which leads to disturbed sleep, also contributes to the tumor progression and was reported to upregulate oncogenic miRNA expression in colorectal cancer cells (7).

The current knowledge highlights possible shared etiology for CRC and sleep disorders that need to be enlightened by further studies to advance therapeutic approaches and personalized medicine applications for CRC.

2. Objective

Existing literature suggests that there are shared molecular mechanisms between CRC and insomnia. Identifying hidden contributors to common molecular mechanisms of two diseases may provide insight into novel translational medicine approaches for CRC and insomnia management. Thus, this study aims to investigate the shared molecular pathways, potential biomarkers, and regulators involved in the pathogenesis of CRC and insomnia with integrated *in-silico* approaches.

3. Materials and Methods

3.1. Data retrieval and Differentially Expressed Gene (DEG) Analysis

Gene expression array datasets (GSE208668, GSE77953) used in this study were retrieved from the Gene Expression Omnibus (GEO) database. GSE208668 comprises 17 insomnia individuals and 25 controls whereas 17 individuals with CRC and 7 controls were included in the GSE77953 (**Supplementary Table 1**). Differentially expressed genes (DEGs) between cases and controls in both data sets were analyzed by using the 'limma' package in R. DEGs with a *P-adj*-value of <0.05 and log2FC> 1.5 or <-1.5 were considered statistically significant for both data sets. DEGs that were identified for insomnia and colorectal cancer were compared and overlapping DEGs (oDEGs) were analyzed in further downstream analyses **(Fig. 1).**

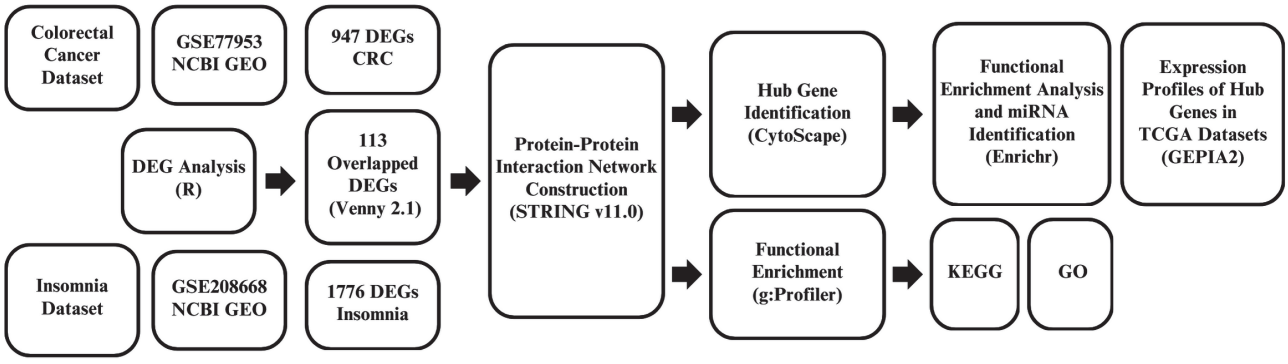

Figure 1. Methodological approach of the study. KEGG: Kyoto Encyclopedia of Genes and Genomes, GO: Gene Ontology

3.2. Network Analyses and Hub Gene Identification

Overlapped DEGs (oDEGs) were imported to the STRING database (v12.0) (https://string-db.org/) to analyze protein-protein interactions (PPI) and construct the network of oDEGs by selecting the combined score >0.4 in network construction settings (20). The network of the oDEGs was visualized and topological features of the network were analyzed by Cytohubba plug-in (21) in the Cytoscape software (Cytoscape v3.10) (https://cytoscape.org/) (22). Cytohubba calculates the interactions of the proteins and analyzed hub genes by employing 12 topological properties [Maximal Clique Centrality (MCC), Density of Maximum Neighborhood Component (DMNC), Maximum Neighborhood Component (MNC), Degree, Edge Percolated Component (EPC), Bottleneck (BN), EcCentricity, Closeness, Radiality, Betweenness, Stress and Clustering coefficient]. The hub genes defined in this study were identified by considering the top 10 oDEGs in the degree ranking based on the topological feature of the network.

3.3. Functional Analyses of the Overlapped Differentially Expressed Genes (oDEGs)

The g: Profiler (https://biit.cs.ut.ee/gprofiler) web tool was utilized to analyze the functions of the oDEGs in molecular pathways and biological processes. The determined oDEGs were enriched using the g: GOSt feature in a non-ordered query. Multiple testing correction was carried out by using the default settings of g: Profiler. Biological pathways were determined from the Kyoto Encyclopedia of Genes and Genomes (KEGG) and three Gene Ontology (GO) domains (Molecular Function, Biological Process, Cellular Component). KEGG and GO terms were calculated by the g: SCS algorithm (23), and $P<0.05$ was considered as a cut-off for statistically significant value for functional enrichment analysis.

3.4. Prediction of Common miRNA-Target Gene Interactions

MicroRNAs act as a key actor in gene regulation and common miRNAs targeting the hub genes may have possible roles in the etiology shared among two diseases. We used the Enrichr microRNA-target identification tool to analyze the miRNAs targeting the hub genes. We submitted the 10 hub genes as a group and the database retrieved the miRNAs targeting at least 3 hub genes by integrating the data with the miRTarBase 2017 algorithm (24). Retrieved miRNAs were ordered by *P-adj*-values using the Benjamini-Hochberg multiple hypothesis testing correction method.

3.5. Analysis of the Hub Genes in the Public Cancer Datasets

The Cancer Genome Atlas (TCGA) is a National Cancer Institute (NCI), National Human Genome Research Institute (NHGRI) supported project that collects and leads researchers to access clinical and transcriptomic data on many types of cancer. Gene Expression Profiling Interactive Analysis 2 (GEPIA2) is a publicly available online (http://gepia2.cancer-pku.cn) resource that incorporates omic and clinical data of samples from the Genotype-Tissue Expression Program and TCGA project (25). We analyzed the expression profiles of the identified 10 hub genes in the TCGA datasets [Colon adenocarcinoma (COAD) and rectum adenocarcinoma (READ)] by using the Expression DIY feature in the GEPIA2 web platform.

4. Results

4.1. DEG Analysis and Identification of Overlapped DEGs in Two Datasets

The analyses of the microarray data of the GSE208668 and GSE77953 revealed 947 and 1776 DEGs using logFC>1.5 logFC<-1.5 and *P-adj*<0.05 values for CRC and insomnia, respectively. A total of 113 protein-coding overlapping genes were successfully identified to be dysregulated in both datasets **(Supplementary Table 2) (Fig. 2)**. Of 113 oDEGs, 3 were upregulated in insomnia, in contrast, they were downregulated in CRC, and 8 oDEGs were upregulated in CRC whereas they were downregulated in insomnia **(Supplementary Table 3).**

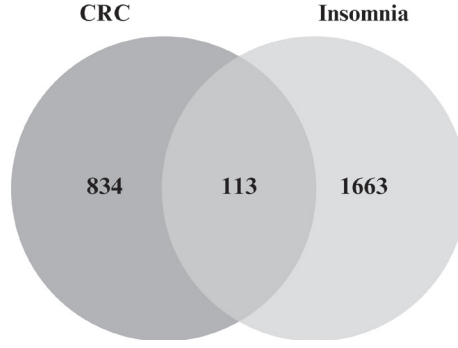

Figure 2. Venn diagram of the dysregulated genes in the GEO datasets.

Table 1. Topological features of the top 10 hub genes identified by Cytohubba

Gene ID	MCC	DMNC	MNC	EPC	Degree	Bottle Neckness	EcCentricity	Closeness	Radiality	Betweenness	Stress	Clustering Coefficient
EEF2	9.22E13	0.80	42.0	16.609	42.0	2.0	0.245	68.0	4.2	321.97	2920.0	0.5331
GAPDH	7.84E9	0.47	39.0	14.284	42.0	9.0	0.327	69.0	4.3	1670.77	11266.0	0.27526
HSPA8	4.58E13	0.58	42.0	15.882	42.0	11.0	0.327	70.0	4.3	899.37	6848.0	0.38792
RPSA	9.22E13	0.88	40.0	16.713	41.0	3.0	0.327	68.8	4.3	551.44	3672.0	0.56585
RPS20	9.22E13	0.89	40.0	15.98	40.0	2.0	0.245	67.5	4.2	230.98	2786.0	0.60128
EEF1G	9.22E13	0.82	40.0	16.517	40.0	4.0	0.245	67.8	4.2	359.12	3316.0	0.55513
HSP90AA1	1.83E8	0.47	39.0	13.991	39.0	9.0	0.327	68.3	4.3	874.18	6144.0	0.32389
RPLP0	9.22E13	0.97	36.0	16.066	36.0	3.0	0.245	64.8	4.1	131.72	1708.0	0.68095
RPS6	9.22E13	0.95	36.0	16.432	36.0	4.0	0.245	65.5	4.2	179.31	2096.0	0.66667
RPL13A	9.22E13	1.04	34.0	15.98	34.0	1.0	0.245	63.8	4.1	65.60	1120.0	0.74153

4.2. Protein-Protein Interaction (PPI) Network

We assessed the PPI of the 113 oDEGs in the STRING database and the network was constructed successfully with a PPI enrichment *P*-value of 1E-16. The network was imported to Cytoscape and visualization and topological features of the network were generated by Cytohubba**.** The top 10 hub genes that were suggested to be putative key genes in the shared pathways between CRC and insomnia were *EEF2*, *GAPDH*, *HSPA8*, *RPSA*, *RPS20*, *EEF1G*, *HSP90AA1*, *RPLP0*, *RPS6*, and *RPL13A* (**Table 1**). Notably, all hub genes were found to be upregulated in both datasets.

4.3. Enrichment of oDEGs in Molecular Pathways and Biological Functions

KEGG pathway and GO enrichment analyses were performed in the g: Profiler tool to determine the enrichments of the common dysregulated genes associated with CRC and insomnia in the molecular pathways and biological processes. The hub genes and oDEGs were analyzed separately for pathway enrichment analysis. Enrichments of oDEGs in KEGG terms revealed five significant pathways of which "Ribosome" was determined to be the top significant term (*P*=2.155E-15) (**Supplementary Table 4**). However, GO enrichment analysis revealed multiple significant GO terms that common DEGs were enriched "RNA Binding" (*P-adj*=6.214E-14), "structural constituent of ribosome" (*P-adj*=2.477E-9), "Nucleic Acid Binding" (*P-adj*=1.807E-7), "heterocyclic compound binding" (*P-adj*=6.237E-7) and "organic cyclic compound binding" (*P-adj*=1.028E-6) were found to be the most significant in molecular function (GO:MF) terms. The most significant 5 GO terms that related to biological processes were "Cytoplasmic Translation" (*P-adj*=5.705E-21), "Translation" (*P-adj*=4.917E-14), "Amide Biosynthetic Process" (*P-adj*=1.117E-13), "Peptide Biosynthetic Process" (*P-adj*=1.314E-13) and "Organonitrogen Compound Biosynthetic Process" (*P-adj*=7.964E-13) in GO: BP Domain.

GO Cellular Component Terms with most significant *P-adj* values were "Cytosolic Ribosome" (*P-adj*=2.385E-22), "ribosomal subunit" (*P-adj*=5.412E-17), "cytosolic large ribosomal subunit (*P-adj*=2.882E-16), "extracellular exosome" (*P-adj*=5.306E-15) and "extracellular vesicle" (*P-adj*=8.123E-15) (**Supplementary Table 5**).

4.4. Common miRNA Regulators of the Hub Genes

We used the Enrichr miRNA target prediction tool to identify the predicted miRNAs regulating the identified hub genes. Our analyses revealed 29 miRNAs targeting at least 2 hub genes with a *P-adj*<0.05. Seven hub genes (*EEF1G*, *HSPA8*, *HSP90AA1*, *RPLP0*, *RPS6*, *RPSA*, and *EEF2)* were found to be the target genes of hsa-miR-16-5p. However, five hub genes (*HSPA8*, *HSP90AA1*, *RPLP0*, *EEF2*, and *GAPDH*) were targeted by hsa-miR-324-3p, and hsa-let-7b-5p were

Table 2. Putative key miRNA regulators of the suggested hub genes associated with CRC and Insomnia

miRNAs	P-value	P_{adj}-Value	Odds Ratio	Combined Score	Hub Genes
hsa-miR-324-3p	3.146E-7	1.233E-4	59.030	883.794	*HSPA8*, *HSP90AA1 RPLP0 EEF2*, *GAPDH*
hsa-miR-769-3p	9.024E-7	1.769E-4	82.108	1142.793	*RPL13A*, *RPSA*, *EEF2*, *GAPDH*
hsa-miR-16-5p	1.649E-6	2.155E-4	27.798	370.133	*EEF1G*, *HSPA8*, *HSP90AA1 RPLP0,RPS6,RPSA,EEF2*
hsa-miR-25-3p	8.188E-5	0.008	25.311	238.184	*HSPA8*, *HSP90AA1*, *RPSA*, *GAPDH*
hsa-miR-320a	1.313E-4	0.010	22.310	199.405	*HSPA8*, *RPL13A*, *EEF2*, *GAPDH*
hsa-let-7b-5p	1.592E-4	0.010	15.534	135.850	*HSPA8*, *HSP90AA1*, *RPSA EEF2*, *GAPDH*
hsa-miR-484	6.594E-4	0.032	14.375	105.283	*HSP90AA1*, *RPLP0*, *EEF2*, *GAPDH*
hsa-miR-615-3p	6.622E-4	0.032	14.358	105.097	*HSPA8*, *RPSA*, *EEF2*, *GAPDH*
hsa-miR-149-5p	8.335E-4	0.034	21.370	151.515	*HSPA8*, *RPLP0*, *GAPDH*
hsa-miR-331-3p	8.897E-4	0.035	20.883	146.693	*HSPA8*, *RPLP0*, *GAPDH*

found to be a potential regulator of *HSPA8*, *HSP90AA1*, *RPSA*, *EEF2* and *GAPDH* genes. The results of miRNA Enrichment analysis and a list of the top 10 miRNAs targeting hub genes (hsa-miR-324-3p, hsa-miR-769-3p, hsa-miR-16-5p, hsa-miR-25-3p, hsa-miR-320a, hsa-let-7b-5p, hsa-miR-484 hsa-miR-615-3p, hsa-miR-149-5p, hsa-miR-331-3p) are shown in **Table 2.**

4.5. Expression Profiles of the Hub Genes in the Public Cancer Datasets

The GEPIA2 web tool was used to obtain the expression profiles of 10 top hub genes in the TCGA (COAD and READ samples) and GTEx (healthy samples) datasets. Six genes (*HSP8A*, *GAPDH*, *HSP90AA1*, *EEF1G*, *RPS6*, and *RPLP0*) were found to be significantly upregulated (*P-adj*<0.05) in COAD and READ datasets compared to GTEx datasets (**Fig. 3).** The results did not show a significant statistical difference (*P*>0.05) for the other four hub genes (*EEF2*, *RPSA*, *RPS20*, and *RPL13A*).

5. Discussion

Colorectal cancer remains a global public health concern with high incidence and morbidity rates despite the advances in genomic medicine and emerging knowledge related to its pathophysiology (1). Thus, there is an urgent need to identify novel biomarkers to utilize in its early diagnosis and develop cutting-edge therapeutic approaches. Insomnia is a prevalent sleep disorder that impairs the quality of life and can be seen as a primary condition, comorbidity, or symptom in many complex pathophysiologies, including mental disorders, cardiovascular diseases, and cancers (26-27). Recent studies suggest a potential link between insomnia and colon cancer, yet a lack of evidence exists to understand the common mechanism in both diseases (12,28). Therefore, determining the common molecular etiology of both two diseases could reveal promising biomarkers that could unravel the missing knowledge related to CRC pathogenesis.

In this study, we used GEO datasets and bioinformatics tools to investigate the potential common molecular mechanism underlying the pathophysiology of CRC and insomnia. We reanalyzed the GEO datasets related to CRC (GSE77953) and insomnia (GSE20866) and then defined differentially expressed genes in both datasets using R software. We identified 947 dysregulated genes in the CRC dataset, 1776 in the insomnia dataset, and

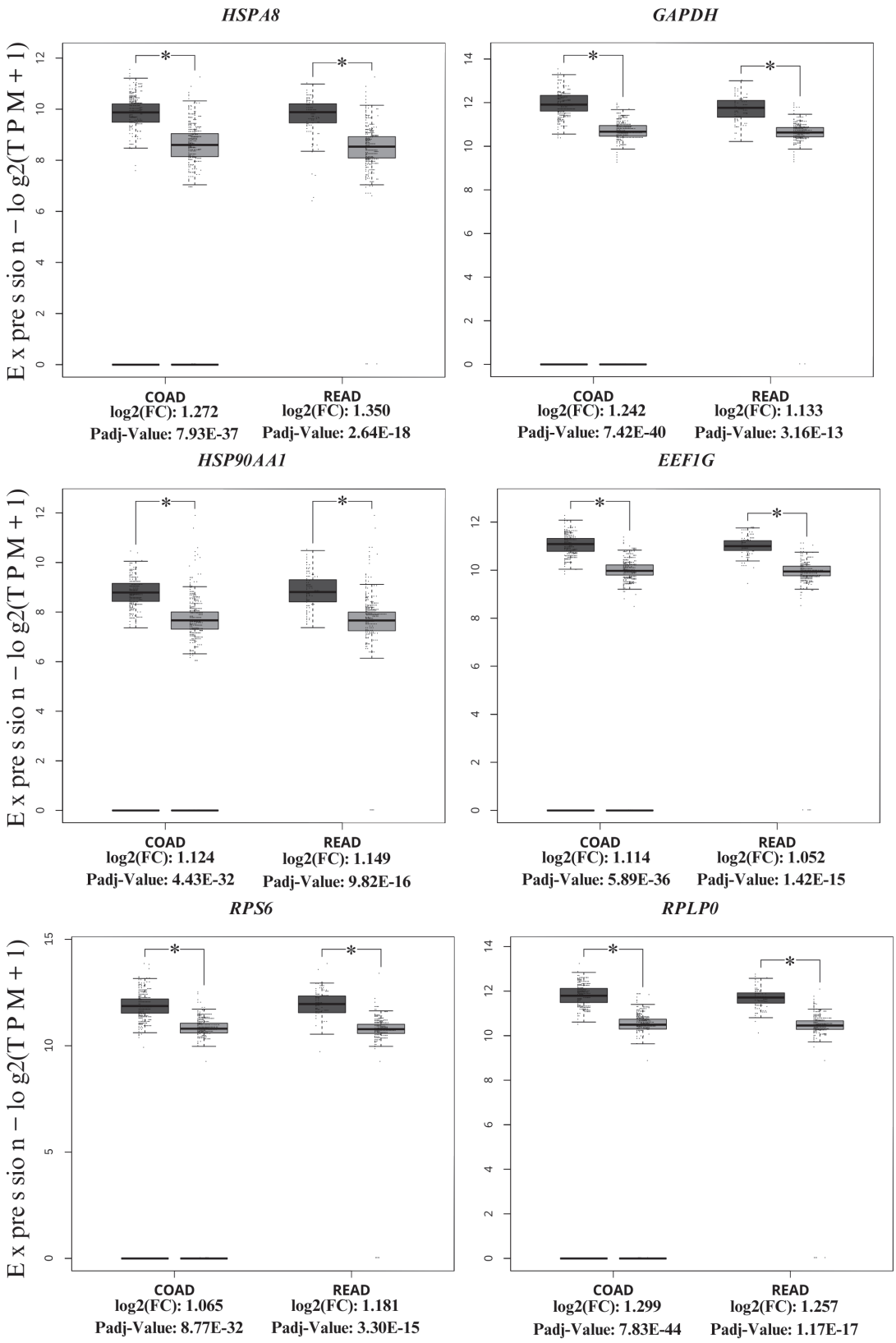

Figure 3. Expression profiles of hub genes in the TCGA datasets COAD and READ compared to the GTEx healthy samples. Black and grey represents TCGA tumor samples and GTEx healthy samples, respectively. COAD: Colon adenocarcinoma, READ: Rectal adenocarcinoma *Significantly dysregulated.

113 shared DEGs in both datasets. We further explored the enrichments of the commonly dysregulated 113 genes in molecular pathways and biological processes using *in silico* tools. Subsequently, we constructed the networks of the common DEGs and prioritized 10 top hub genes (*HSPA8*, *EEF2*, *GAPDH*, *RPSA*, *RPS20*, *EEF1G*, *HSP90AA1*, *RPLP0*, *RPS6*, and *RPL13A)* based on their topological properties in the network. Of note, the top 10 hub genes were observed to be upregulated in both datasets. Then, we investigated the expression levels of the hub genes in the colorectal cancer datasets of TCGA data. It reveals that six hub genes (*HSP8A*, *GAPDH*, *HSP90AA1*, *EEF1G*, *RPS6*, and *RPLP0*) were upregulated in COAD and READ samples compared to GTEx healthy samples.

Heat Shock Protein Family A (*Hsp70*) Member 8 (*HSPA8*) is a member of the *HSP70* gene family, which takes a role in autophagy and misfolded protein degradation. In a recent study, *HSP8A* and some other heat shock proteins including *HSP90* were upregulated in the hippocampus of sleep-deprived mice (29-30). Moreover, overexpression of *HSPA8* was found to be associated with different types of cancer, including breast cancer and endometrial carcinoma (31-32). Eukaryotic Translation Elongation Factor 1 Gamma (*EEF1G*) is responsible for the delivery of aminoacyl-tRNAs to the A site of the ribosome with other EEF1 complex subunits (33). *EEF1G* was found to be co-upregulated with TNF receptor-associated protein (*TRAP1*), which plays a role in mitochondrial integrity, programmed cell death, and oxidative stress in colorectal cancer patients (34). The potential impact of *EEF1G* has not been established in sleep disorders and further research is needed to determine its potential role in molecular mechanisms involved in sleep disruption. Glyceraldehyde 3-phosphate dehydrogenase (G*APDH)* plays a crucial role in energy metabolism, and elevated expression of G*APDH* is known to be associated with many types of cancer (35). Previous research showed that silencing *GAPDH* reduces glycolysis in colon cancer cell lines and suppresses epithelial-mesenchymal transition (36). Moreover, recently, a study showed that sleep deprivation causes decreased *GAPDH* levels in the prefrontal cortex of adult mice (37). Considering the crucial role of the *GAPDH* gene in energy metabolism, more clarification is needed on its relevance in the sleep mechanism. Heat Shock Protein 90 Alpha Family Class A Member 1 (*HSP90AA1)* is a molecular protein chaperone that is involved in multiple vital signaling pathways and other biological processes within cells (38-39). It has been shown that *HSP90* is overexpressed in colon cancer patients and linked to poor prognosis (40). Trials and emerging research in preclinical models indicate that HSP90 inhibitors increase the effectiveness of anti-neoplastic treatments when combined with therapeutic agents (41). However, increased levels of *HSP90* in the hippocampus of sleep-deprived rats were reported in a previous study, which suggested its potential effect on memory performance (30). Another hub gene, Ribosomal Protein L13a (*RPL13A*) existed in the exosomes derived from colorectal cancer cell lines implicating its association with metastasis (42-43). To date, a lack of evidence exists regarding the role of *HSP90* and *RPL13A* in sleep-related molecular pathways, which needs further examination. RPS6 phosphorylation and/or overexpression were found to be associated with many types of cancer and it was suggested as a potential therapeutic target for treatment interventions (44). The association between RPS6 and sleep disorders have not yet been fully understood. However, a study showed that the phosphorylation level of RPS6 was decreased in the brain of sleep-deprived mice, suggesting that RPS6 Kinase signaling activity may beneficially affect sleep mechanisms (45).

We also investigated the interactions of miRNAs with hub genes and found multiple miRNAs were the regulators of the hub gene network. miR-16-5p was found to be the common miRNA regulator of seven hub genes. Previous studies showed that hsa-miR-16-5p has a strong tumor suppressor effect in many cancers, including colorectal cancer (46). Strikingly, earlier research reported the down-regulation of hsa-miR-16-5p in prefrontal and somatosensory cortices of sleep-deprived rats (47). Furthermore, miR-324-3p was reported to have an association with many diseases and cancer types, including colorectal cancer (48). Notably, the miR-324-3p was shown to target multiple genes involving molecular clock mechanisms, and its possible role in sleep disorders requires further investigation (49). Additionally, a study reported decreased expressions of tumor suppressor miR-769-3p in colorectal cancer tissues and suggested miR-769-3p as a potential prognostic biomarker and a therapeutic target (50). However, the relevant contribution of miR-769-3p to sleep disturbance and related pathologies is mainly unknown.

Our analyses shed light on the putative roles of hub genes in protein synthesis. The excess protein requirement caused by neoplasia causes the translational machinery to work overtime. This may explain the overexpression of the genes involved in the protein synthesis and energy mechanism in CRC patients (51). Although the relationship between colorectal cancer and sleep disorder has not been fully defined and needs more clarification, the association may be direct or related to insomnia's metabolic outcomes, such as obesity, diabetes, and metabolic syndrome (52). Nevertheless, the understanding of the causal association between CRC and insomnia is still a challenge for researchers that need further investigations to improve public health strategies regarding both diseases.

This study has some limitations due to its *in-silico* design and requires further evaluation in independent data sets and wet lab work to understand better shared mechanisms related to both diseases. However, our findings are concordant with the current literature, which supports the strengths of our methodology and analyses.

Our results suggest that the pathogenesis of CRC and insomnia may share common pathways and molecular biomarkers that need to be considered as potential molecules while developing diagnostic, prognostic, and therapeutic applications for both diseases. The *in-silico* findings of the current study need to be also validated by further experimental research, which will ultimately pave the way for precision medicine interventions for both diseases.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author information Contributions

M.Y and D.P designed the methodology and the study concept. M.Y and D.P investigated the literature. M.Y curated the data. M.Y and D.P wrote the original draft. M.Y prepared the tables and the figures. M.Y and D.P wrote the manuscript. D.P critically reviewed and edited the manuscript. D.P supervised the study. Authors read and approved the manuscript.

Corresponding author

Correspondence to D.P

Ethics declarations

Ethics Approval and Consent to Participate

Not applicable.

Competing interests

Authors declare that they have no conflict of interest.

Data Availability

The public gene expression array datasets analyzed during the current study are available in the NCBI Gene Expression Omnibus (GEO) repository.

(GSE208668 https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=gse208668)

(GSE77953 https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=gse77953)

References

1. Morgan E, Arnold M, Gini A, Lorenzoni V, Cabasag CJ, Laversanne M, *et al*. Global burden of colorectal cancer in 2020 and 2040: incidence and mortality estimates from GLOBOCAN. *Gut*. 2023;**72**(2):338-344. doi:10.1136/gutjnl-2022-327736
2. Hossain MS, Karuniawati H, Jairoun AA, Urbi Z, Ooi DJ, John A, *et al*. Colorectal Cancer: A Review of Carcinogenesis, Global Epidemiology, Current Challenges, Risk Factors, Preventive and Treatment Strategies. *Cancers (Basel)*.2022;**29**;**14**(7):1732. doi:10.3390/cancers14071732
3. Sagaert X, Vanstapel A, Verbeek S. Tumor Heterogeneity in Colorectal Cancer: What Do We Know So Far? *Pathobiology*. 2018;**85**(1–2):72-84.doi:10.1159/000486721
4. Murphy N, Ward HA, Jenab M, Rothwell JA, Boutron-Ruault MC, Carbonnel F, *et al*. Heterogeneity of Colorectal Cancer Risk Factors by Anatomical Subsite in 10 European Countries: A Multinational Cohort Study. *Clin Gastroenterol Hepatol*. 2019;**17**(7):1323-1331.e6. doi:10.1016/j.cgh.2018.07.030
5. Berisha A, Shutkind K, Borniger JC. Sleep Disruption and Cancer: Chicken or the Egg? *Frontiers in Neuroscience* 2022;**16**:856235. doi:10.3389/fnins.2022.856235
6. Zhou L, Zhang Z, Nice E, Huang C, Zhang W, Tang Y. Circadian rhythms and cancers: the intrinsic links and therapeutic potentials. *J Hematol Oncol*. 2022;**4**;**15**(1):21. doi:10.1186/s13045-022-01238-y
7. Moriondo G, Soccio P, Minoves M, Scioscia G, Tondo P, Foschino Barbaro MP, *et al*. Intermittent Hypoxia Mediates Cancer Development and Progression Through HIF-1 and miRNA Regulation. *Archivos de Bronconeumología*. 2023;**59** (10):629-637. doi:10.1016/j.arbres.2023.07.001
8. Freitas LS, Silveira AC, Martins FC, Costa-Hong V, Lebkuchen A, Cardozo KHM, *et al*. Severe obstructive sleep apnea is associated with circulating microRNAs related to heart failure, myocardial ischemia, and cancer proliferation. *Sleep Breath*. 2020;**24**(4):1463-1472. doi:10.1007/s11325-019-02003-1
9. Assefa E, Tatin X, Lorenzetti M, Fins A, Tartar A, Tartar J. 0023 SLEEP DEPRIVATION RESULTS IN INCREASED EXPRESSION OF CANCER-RELATED MIRNAS IN HUMANS. *Sleep*. 2017;**40**(suppl_1):A9. doi:10.1093/sleepj/zsx050.022

10. Baek S-J, Ban H-J, Park S-M, Lee B, Choi Y, Baek Y, *et al*. Circulating microRNAs as Potential Diagnostic Biomarkers for Poor Sleep Quality. *Nat Sci Sleep*. 2021;**13**:1001-1012. doi:10.2147/NSS.S311541
11. awai S, Wong P-F, Ramasamy TS. Hypoxia-regulated microRNAs: the molecular drivers of tumor progression. *Crit Rev Biochem Mol Biol*. 2022;**57**(4):351-376. doi:10.1080/10409238.2022.2088684
12. Papantoniou K, Castaño-Vinyals G, Espinosa A, Turner MC, Martín-Sánchez V, Casabonne D, *et al*. Sleep duration and napping in relation to colorectal and gastric cancer in the MCC-Spain study. *Sci Rep*. 2021;**3**;**11**(1):11822. doi:10.1038/s41598-021-91275-3
13. Thompson CL, Larkin EK, Patel S, Berger NA, Redline S, Li L. Short duration of sleep increases risk of colorectal adenoma. *Cancer*. 2011;(4):841-847. doi:10.1002/cncr.25507
14. Jiao L, Duan Z, Sangi-Haghpeykar H, Hale L, White DL, El-Serag HB. Sleep duration and incidence of colorectal cancer in postmenopausal women. *Br J Cancer*. 2013;**15**;**108**(1):213-221. doi:10.1038/bjc.2012.561
15. Zhang X, Giovannucci EL, Wu K, Gao X, Hu F, Ogino S, *et al*. Associations of self-reported sleep duration and snoring with colorectal cancer risk in men and women. *Sleep*. 2013;**1**;**36**(5):681-688. doi:10.5665/sleep.2626
16. Fu L, Pelicano H, Liu J, Huang P, Lee C. The circadian gene Period2 plays an important role in tumor suppression and DNA damage response *in vivo*. *Cell*. 2002;**4**;**111**(1):41-50. doi:10.1016/s0092-8674(02)00961-3
17. Oshima T, Takenoshita S, Akaike M, Kunisaki C, Fujii S, Nozaki A, *et al*. Expression of circadian genes correlates with liver metastasis and outcomes in colorectal cancer. *Oncol Rep*. 201;**25**(5):1439-1446. doi:10.3892/or.2011.1207
18. Yu H, Meng X, Wu J, Pan C, Ying X, Zhou Y, *et al*. Cryptochrome 1 Overexpression Correlates with Tumor Progression and Poor Prognosis in Patients with Colorectal Cancer. *PLoS One*. 2013;**8**(4):e61679. doi:10.1371/journal.pone.0061679
19. Orhan T, Nielsen PB, Hviid TVF, Rosen AW, Gögenür I. Expression of Circadian Clock Genes in Human Colorectal Cancer Tissues Using Droplet Digital PCR. *Cancer Invest*. 2019;**37**(2):90-98. doi:10.1080/07357907.2019.1571079
20. Szklarczyk D, Kirsch R, Koutrouli M, Nastou K, Mehryary F, Hachilif R, *et al*. The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Res*. 2023;**6**;**51**(D1): D638-646. doi:10.1093/nar/gkac1000
21. Chin CH, Chen SH, Wu HH, Ho CW, Ko MT, Lin CY. cytoHubba: identifying hub objects and sub-networks from complex interactome. *BMC Syst Biol*. 2014;**8**(Suppl 4):S11. doi:10.1186/1752-0509-8-S4-S11
22. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, *et al*. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res*. 2003;**13**(11):2498-2504. doi:10.1101/gr.1239303
23. Raudvere U, Kolberg L, Kuzmin I, Arak T, Adler P, Peterson H, *et al*. g: Profiler: a web server for functional enrichment analysis and conversions of gene lists (2019 update). *Nucleic Acids Res*. 2019;**2**;**47**(W1): W191-198. doi: 10.1093/nar/gkz369
24. Xie Z, Bailey A, Kuleshov MV, Clarke DJB, Evangelista JE, Jenkins SL, *et al*. Gene Set Knowledge Discovery with Enrichr. *Curr Protoc*. 2021;**1**(3):e90. doi:10.1002/cpz1.90
25. Tang Z, Kang B, Li C, Chen T, Zhang Z. GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. *Nucleic Acids Res*. 2019;**2**;**47**(W1):W556-560. doi:10.1093/nar/gkz430
26. von Ruesten A, Weikert C, Fietze I, Boeing H. Association of sleep duration with chronic diseases in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study. *PLoS One*. 2012;**7**(1):e30972. doi:10.1371/journal.pone.0030972
27. Morin CM, Drake CL, Harvey AG, Krystal AD, Manber R, Riemann D, *et al*. Insomnia disorder. *Nat Rev Dis Primers*. 2015;**3**;**1**:15026. doi:10.1038/nrdp.2015.26
28. Loosen S, Krieg S, Krieg A, Leyh C, Luedde T, Vetter C, *et al*. Are sleep disorders associated with the risk of gastrointestinal cancer? -A case-control study. *J Cancer Res Clin Oncol*. 2023;**149**(13):11369-11378. doi: 10.1007/s00432-023-05009-1
29. Vecsey CG, Peixoto L, Choi JHK, Wimmer M, Jaganath D, Hernandez PJ, *et al*. Genomic analysis of sleep deprivation reveals translational regulation in the hippocampus. *Physiol Genomics*. 2012;**17**;**44**(20):981-991. doi: 10.1152/physiolgenomics.00084.2012
30. Rahimpour P, Nasehi M, Zarrindast MR, Khalifeh S. Dose-dependent manner of luteolin in the modulation of spatial memory with respect to the hippocampal level of HSP70 and HSP90 in sleep-deprived rats. *Gene*. 2023;**5**(852):147046. doi:10.1016/j.gene.2022.147046
31. Shan N, Zhou W, Zhang S, Zhang Y. Identification of HSPA8 as a candidate biomarker for endometrial carcinoma by using iTRAQ-based proteomic analysis. *Onco Targets Ther*. 2016;**9**:2169-2179. doi: 10.2147/OTT.S97983
32. Ying B, Xu W, Nie Y, Li Y. HSPA8 Is a New Biomarker of Triple Negative Breast Cancer Related to Prognosis and Immune Infiltration. *Dis Markers*. 2022;**2022**:8446857. doi:10.1155/2022/8446857
33. Biterge-Sut B. Alterations in Eukaryotic Elongation Factor complex proteins (EEF1s) in cancer and their implications in epigenetic regulation. *Life Sci*. 2019;**1**;238:116977. doi:10.1016/j.dib.2020.105162
34. Matassa DS, Amoroso MR, Agliarulo I, Maddalena F, Sisinni L, Paladino S, *et al*. Translational control in the stress adaptive response of cancer cells: a novel role for the heat shock protein TRAP1. *Cell Death Dis*. 2013;**10**;**4**(10):e851. doi: 10.1038/cddis.2013.379
35. Wang J, Yu X, Cao X, Tan L, Jia B, Chen R, *et al*. GAPDH: A common housekeeping gene with an oncogenic role in pan-cancer. *Comput Struct Biotechnol J*. 2023;**21**:4056-4069. doi: 10.1016/j.csbj.2023.07.034
36. Liu K, Tang Z, Huang A, Chen P, Liu P, Yang J, *et al*. Glyceraldehyde-3-phosphate dehydrogenase promotes cancer growth and metastasis through upregulation of SNAIL expression. *Int J Oncol*. 2017;**50**(1):252-262. doi: 10.3892/ijo.2016.3774
37. Muheim CM, Ford K, Medina E, Singletary K, Peixoto L, Frank MG. Ontogenesis of the molecular response to sleep loss. *Neurobiol Sleep Circadian Rhythms*. 2023;**14**:100092. doi:10.1016/j.nbscr.2023.100092
38. Schopf FH, Biebl MM, Buchner J. The HSP90 chaperone machinery. *Nat Rev Mol Cell Biol*. 2017;**18**(6):345-360. doi: 10.1038/nrm.2017.20
39. Poggio P, Sorge M, Seclì L, Brancaccio M. Extracellular

HSP90 Machineries Build Tumor Microenvironment and Boost Cancer Progression. *Front Cell Dev Biol*. 2021;**9**:735529. doi: 10.3389/fcell.2021.735529

40. Zhang S, Guo S, Li Z, Li D, Zhan Q. High expression of HSP90 is associated with poor prognosis in patients with colorectal cancer. *Peer J*. 2019;**7**:e7946. doi: 10.7717/peerj.7946
41. Kryeziu K, Bruun J, Guren TK, Sveen A, Lothe RA. Combination therapies with HSP90 inhibitors against colorectal cancer. *Biochim Biophys Acta Rev Cancer*. 2019;**1871**(2):240-247. doi: 10.1016/j.bbcan.2019.01.002
42. Chiba M, Kimura M, Asari S. Exosomes secreted from human colorectal cancer cell lines contain mRNAs, microRNAs and natural antisense RNAs, that can transfer into the human hepatoma HepG2 and lung cancer A549 cell lines. *Oncol Rep*. 2012;**28**(5):1551-1558. doi: 10.3892/or.2012.1967
43. Liu X, Li N, Zhang C, Wu X, Zhang S, Dong G, *et al*. Identification of metastasis-associated exoDEPs in colorectal cancer using label-free proteomics. *Transl Oncol*. 2022;**19**: 101389. doi:10.1016/j.tranon.2022.101389
44. Yi YW, You KS, Park JS, Lee SG, Seong YS. Ribosomal Protein S6: A Potential Therapeutic Target against Cancer? *Int J Mol Sci*. 2021;**21**;**23**(1):48. doi: 10.3390/ijms23010048
45. Kam K, Kang M, Eren CY, Pettibone WD, Bowling H, Taveras S, *et al*. Interactions between sleep disruption, motor learning, and p70 S6 kinase 1 signaling. *Sleep*. 2020;**12**;**43**(3):zsz244. doi:10.1093/sleep/zsz244
46. Ghafouri-Fard S, Khoshbakht T, Hussen BM, Abdullah ST, Taheri M, Samadian M. A review on the role of mir-16-5p in the carcinogenesis. *Cancer Cell Int*. 2022;**8**;**22**(1):342. doi: 10.1186/s12935-022-02754-0
47. Davis CJ, Bohnet SG, Meyerson JM, Krueger JM. Sleep loss changes microRNA levels in the brain: A possible mechanism for state-dependent translational regulation. *Neurosci Lett*. 2007;**422**(1):68-73. doi: 10.1016/j.neulet.2007.06.005
48. Kadkhoda S, Hussen BM, Eslami S, Ghafouri-Fard S. A review on the role of miRNA-324 in various diseases. *Front Genet*. 2022;**13**:950162. doi:10.3389/fgene.2022.950162
49. Kim JY, Kim W, Lee K-H. The role of microRNAs in the molecular link between circadian rhythm and autism spectrum disorder. *Anim Cells Syst* (Seoul). 2023;**27**(1):38-52. doi: 10.1080/19768354.2023.2180535
50. Han C, Song Y, Lian C. MiR-769 Inhibits Colorectal Cancer Cell Proliferation and Invasion by Targeting HEY1. *Med Sci Monit*. 2018;**24**:9232-9239. doi:10.12659/MSM.911663
51. Leibovitch M, Topisirovic I. Dysregulation of mRNA translation and energy metabolism in cancer. *Adv Biol Regul*. 2018;**67**:30-39. doi:10.1016/j.jbior.2017.11.001
52. Xi B, He D, Zhang M, Xue J, Zhou D. Short sleep duration predicts risk of metabolic syndrome: a systematic review and meta-analysis. *Sleep Med Rev*. 2014;**18**(4):293-297.doi: 10. 1016/j.smrv.2013.06.001