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Original Research

Upregulation of TPX2 and KIF20A in colorectal cancer

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ABSTRACT

Introduction: Colorectal cancer (CRC) is a leading cause of cancer-related deaths worldwide, with metastasis significantly reducing patient survival. Despite advances in treatment, the molecular mechanisms underlying the progression of colorectal cancer remain poorly understood. Recent studies highlight the role of TPX2 and KIF20A, two proteins involved in cell division, in the development of cancer. TPX2 plays a key role in the assembly of the mitotic spindle, while KIF20A is a member of the kinesin superfamily, which is important for intracellular transport and cytokinesis. Both genes are associated with various types of cancer, but their specific contribution to CRC remains unclear. The aim of this study is to investigate the expression and prognostic significance of TPX2 and KIF20A in CRC through bioinformatic analysis and experimental validation. **Methods**: To identify differentially expressed genes (DEGs) in CRC, five publicly available microarray datasets (GSE39582, GSE8671, GSE9348, GSE21510, and GSE44076) were analyzed using the Limma package in R. Functional enrichment analysis of DEGs was performed using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses. A protein-protein interaction (PPI) network was created using STRING and visualized using Cytoscape to identify hub genes. Survival analysis of hub genes was performed using the Gene Expression Profiling Interactive Analysis (GEPIA) tool with TCGA data. Receiver operating characteristic (ROC) curve analysis was used to evaluate the diagnostic potential of hub genes. Experimental validation was performed on 50 CRC samples using quantitative real-time PCR (qRT-PCR) to measure the expression of TPX2 and KIF20A. **Results**: The integrated analysis identified several DEGs significantly involved in CRC, with TPX2 and KIF20A emerging as key hub genes. GO and KEGG analyses showed that these genes are highly associated with cell cycle regulation and mitotic processes. Survival analysis showed that high TPX2 and KIF20A expression correlates with poorer prognosis in colorectal cancer patients. ROC curve analysis confirmed their potential as diagnostic biomarkers. Experimental validation showed significant upregulation of TPX2 and KIF20A in CRC tissues compared to normal controls, supporting the bioinformatic results. Further mechanistic evidence suggests that TPX2 and KIF20A contribute to colorectal cancer progression by promoting cell proliferation and tumor formation. Previous studies also suggest that KIF20A activates the JAK/STAT3 signaling pathway, thereby increasing the aggressiveness of colorectal cancer cells, while TPX2 is associated with chromosomal instability and tumorigenesis. These results suggest that targeting TPX2 and KIF20A may offer new therapeutic opportunities for the treatment of colorectal cancer. **Conclusion**: This study highlights the potential role of TPX2 and KIF20A as prognostic biomarkers and therapeutic targets in colorectal cancer. Their significant upregulation in tumor tissue and strong association with poor survival outcomes underscore their importance in colorectal cancer progression. Future research should focus on elucidating the molecular mechanisms underlying their oncogenic role and exploring targeted therapies aimed at modulating their activity to improve outcomes for colorectal cancer patients.

Key words: Colorectal cancer, TPX2, KIF20A

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INTRODUCTION

Colorectal cancer (CRC) remains a major global health burden despite advancements in treatment [1,2]. Approximately 50-60% of CRC patients develop metastases, significantly reducing their 5-year survival rate to around 14% [3,4]. The precise genetic mechanisms underlying CRC initiation and progression are still being investigated. Identifying novel gene targets and developing targeted therapies are crucial for improving patient outcomes. Current research focuses on understanding the molecular alterations in CRC and exploring precision medicine approaches to address the disease's heterogeneity and improve treatment effectiveness.

TPX2, a protein encoded on chromosome 20q11.1, is crucial for forming microtubules at kinetochores in mammalian cells [5]. It acts downstream of Ran-GTP and plays a central role in spindle assembly during cell division. The nominated function of TPX2 includes: in response to Ran-GTP in early mitosis, TPX2 is re-

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leased and interacts with Aurora A kinase. This interaction directs Aurora A to spindle microtubules, initiating their assembly[6,7]. TPX2 shields a critical site on Aurora A, preventing its inactivation and ensuring proper spindle formation. Cells lacking the TPX2/Aurora A complex have abnormal spindles and often fail to divide correctly[8,9]. TPX2 expression is tightly controlled throughout the cell cycle, suggesting its potential as a precise marker for tumor cell proliferation. Previous studies have shown that TPX2, a protein involved in cell division, is abnormally expressed in several cancers, including lung, prostate, liver, thyroid, and pancreatic cancer. However, the role of TPX2 in colon cancer has not been explored[10]. KIF20A is a newcomer to the kinesin superfamily-6[11,12]. This kinesin family is known for a special motor domain that allows them to ferry cargo around the cell. They play a vital role in various cellular activities like transporting molecules within the cell, separating chromosomes during cell division, and cell movement, all achieved by interacting with microtubules. Previous research has shown that KIF20A is located near the Golgi apparatus[13,14]. KIF20A acts like a molecular truck, using energy (in the form of GTP-bound RAB6A/B) to haul Golgi membranes back from the cell's periphery. Additionally, KIF20A relies on microtubules and its own directional movement to be involved in critical processes like chromosome separation and spindle formation during cell division[15,16]. Over the past decade, scientists have found KIF20A expressed in many organs throughout the body. Interestingly, cancer researchers have observed that KIF20A levels are elevated in various cancers, including breast, pancreatic, lung, and bladder cancers. Even more intriguing, evidence suggests KIF20A can promote aggressive behavior in pancreatic and breast cancers[17,18]. However, our understanding of KIF20A's role and how it functions in colorectal cancer (CRC) remains limited.

To investigate this, we analyzed publicly accessible gene expression data from the Gene Expression Omnibus (GEO) and the Cancer Genome Atlas (TCGA). Our bioinformatics analysis revealed that TPX2 and KIF20A play a critical role in managing cell cycle progression. These genes are essential for CRC cell proliferation and their ability to form 3D structures that mimic tumors. To validate these results, we investigated the expression of TPX2 and KIF20A in CRC specimens.

METHODS

Data Obtained and DEGs Acquired

The GEO is an online repository that allows researchers to store, share, and access functional genomics data, including gene expression data obtained through microarray, next-generation sequencing, and other high-throughput technologies. This platform enables users to search, review, and download data and gene expression profiles. For this study, microarray datasets containing gene expression profiles related to CRC were sourced from the GEO database. A total of five datasets were selected, specifically GSE39582, GSE8671, GSE9348, GSE21510, and GSE44076. These datasets included a total of 748 normal tissue samples and 790 tumor tissue samples.

Integrated Differential Gene Expression Analysis

To analyze gene expression data, we first normalized the raw data using the RMA method and then converted the values to a log2 scale. Next, we used the Limma package in R to identify genes that were expressed at significantly different levels between groups. We considered genes with a log2 fold change greater than 1 and an adjusted P-value less than 0.05 to be differentially expressed. Finally, we combined the lists of differentially expressed genes from all datasets to identify genes that were consistently changed across all experiments.

Functional Enrichment of Selected DEGs

To better understand what the identified genes might do and what biological processes they're involved in, we ran two analyses. Gene Ontology (GO) analysis helped us categorize the genes by their function (what they do), the molecules they interact with (how they do it), and the broader biological processes they contribute to (their role in the cell). KEGG pathway analysis further explored the specific pathways these genes might be involved in, providing a more detailed picture of their biological significance. We used a software package called ClusterProfiler in R along with a visualization tool called shinyGO to perform these analyses. Only pathways and functions with a very high likelihood (p-value less than 0.05) were considered significant.

Constructing PPI Network and Identification of Hub Genes

To explore how the DEGs might work together, we built a network of interacting proteins. We used a website called STRING, which is like a map of protein

connections across different organisms. This map includes both known and predicted interactions, showing how proteins might physically touch or work together in certain functions. We focused on interactions with high confidence scores (above 0.9) and excluded genes that didn't connect to others in the network. Next, we used software called Cytoscape to visualize this network of interacting proteins. To find key players within the network, we ran an analysis tool called MCODE. This identified clusters of highly connected genes, which are likely to be working together in important biological processes. Finally, another Cytoscape tool called CytoHubba helped us pinpoint the most central genes in the network, based on their connections to other genes. These central genes, called 'hub genes,' could be particularly important for understanding colorectal cancer.

Survival Analysis of Patients Using Hub Genes

To understand how our identified hub genes affect patient outcomes, we utilized an online tool called GEPIA. This tool lets us analyze data from The Cancer Genome Atlas (TCGA) project and create survival charts (Kaplan-Meier plots) for various genes across different cancers. We focused on colorectal cancer, specifically looking at both colon adenocarcinoma (COAD) and rectal adenocarcinoma (READ) patients. We divided the patients into four groups based on their expression levels of the hub genes and compared their overall survival rates. Genes with a statistically significant difference in survival between groups (p-value < 0.05) were considered to have prognostic significance and were chosen for further analysis.

Assessment of Survival-Related Hub Genes Expression Profile by TCGA Data

To further examine the expression patterns of these hub genes, we used the GEPIA online tool to compare their levels in tumor and normal tissues from the TCGA and GTEx datasets. We focused on COAD and READ samples, comparing them to matched normal tissues. Using a strict statistical cutoff, we identified significant differences in the expression of these genes between tumor and normal tissues, confirming their potential role in colon cancer.

ROC Curve Analysis of Hub Genes

To evaluate the diagnostic and prognostic value of the identified hub genes in CRC, we created receiver operating characteristic (ROC) curves using the GSE39582 dataset. ROC curves help us understand how well a test can distinguish between patients with and without CRC. The area under the curve (AUC) is a measure of this ability. By calculating the AUC for each hub gene, we assessed its potential as a biomarker for CRC diagnosis and prognosis.

Sample Collection, RNA Isolation, cDNA Synthesis, Real-Time Quantitative PCR

In short, after the approval of the ethics committee and the complete explanation of the project process to the participants, 50 samples (25 cases and 25 tumor margins as controls) were collected. Then RNA was extracted using a manual protocol and Trizol, and after quantitative and qualitative control of the extracted RNA, cDNA synthesis and RT-qPCR were performed according to previous studies [19,20].

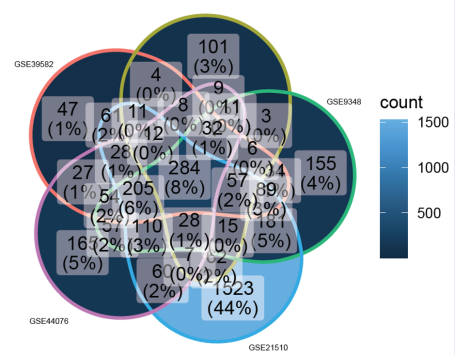
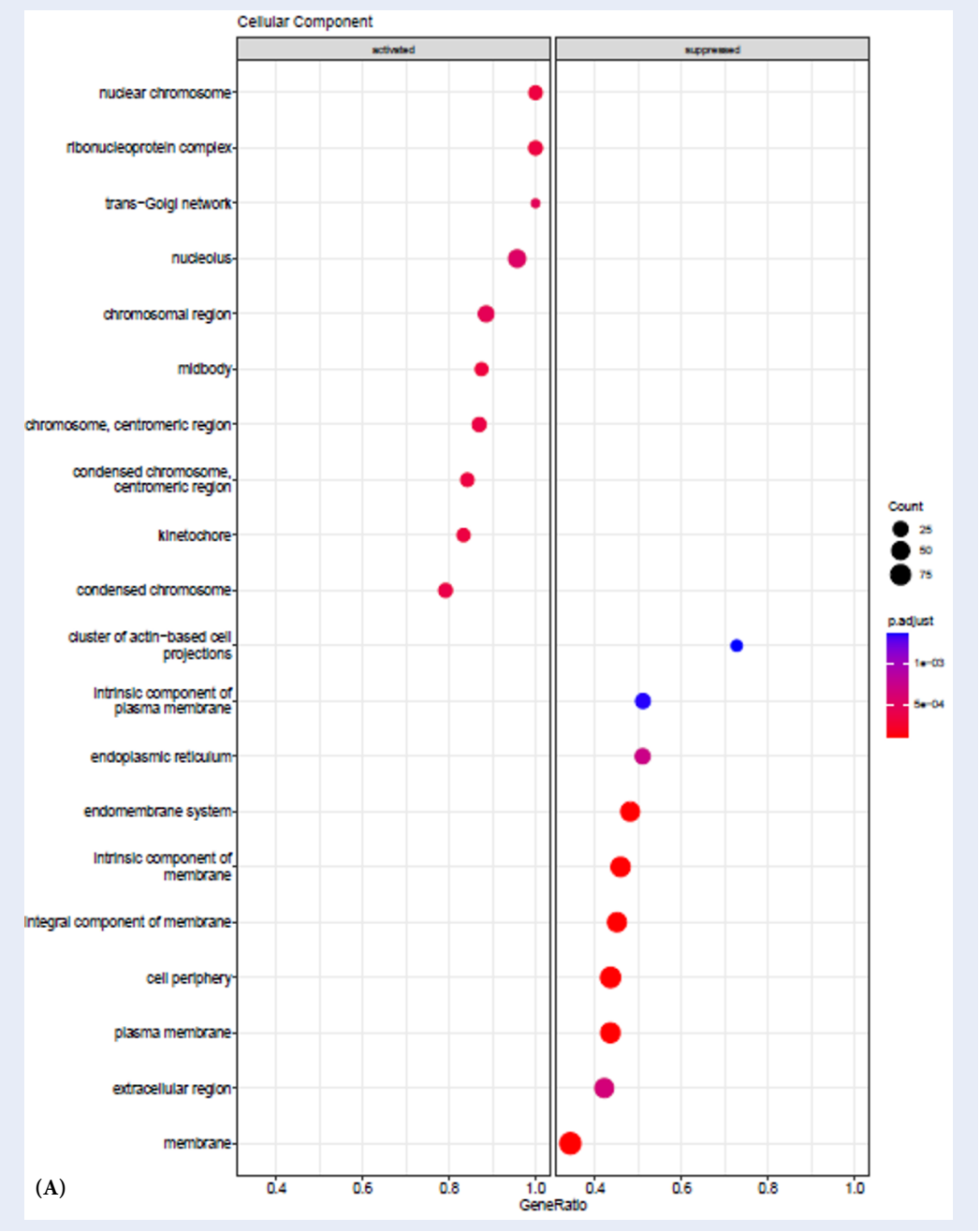


Figure 1: **This Venn diagram illustrates the overlap of upregulated and downregulated genes across the datasets labeled GSE39582, GSE9348, GSE44076, and GSE21510**. Each dataset is represented by a uniquely colored circle, and their intersections show the number of shared genes. The numbers within the overlapping and non-overlapping regions indicate the count of genes in each category, with percentages showing their proportion relative to the total gene count in the datasets. A blue color gradient on the right signifies gene counts, with darker shades depicting a greater number of overlapping genes. Key observations include: 1,523 genes (44%) are exclusive to the GSE21510 dataset. Smaller intersections range from single digits to several hundred genes. The central region displays the most significant overlaps, representing shared genes across multiple datasets. Minimal overlaps between some datasets suggest dataset-specific gene expression changes. Overall, the figure visually represents gene expression patterns across different datasets, highlighting both shared and unique differentially expressed genes.

RESULTS


Cellular Component
activated
suppressed
nuclear chromosome
ribonucleoprotein complex
trans-Golgi network
nucleolus
chromosomal region
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chromosome, centromeric region
condensed chromosome, centromeric region
kinetochore
condensed chromosome
cluster of actin-based cell projections
intrinsic component of plasma membrane
endoplasmic reticulum
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Figure 2A

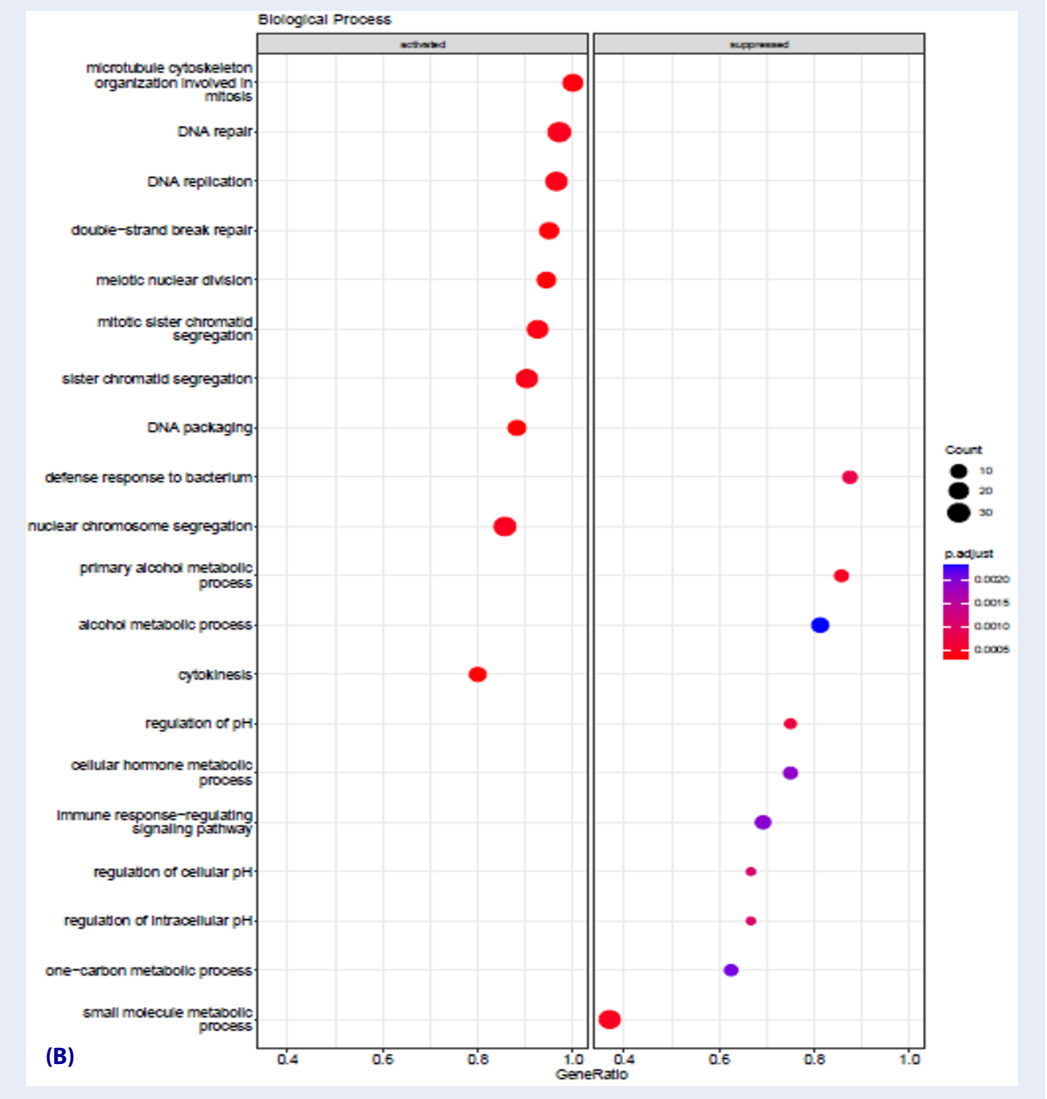

Figure 2B

Identification of Integrated DEGs

We analyzed five publicly available gene expression datasets to identify genes that are differentially expressed between colon cancer tissues and normal tissues. After data processing, we found a total of 2,084, 1,387, 2,296, 4,382, and 2,329 differentially expressed genes in the GSE39582, GSE8671, GSE9348, GSE21510, and GSE44076 datasets, respectively. Among these genes, more genes were downregulated than upregulated in most datasets (**Supplementary Tables 1-7**). A Venn diagram shows the genes that were consistently upregulated or downregulated across all five datasets (**Figure 1**).

GO and KEGG Pathway Enrichment Analysis of Common DEGs

We analyzed the common differentially expressed genes (DEGs) using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses. The results are shown in **Figure 2**. For cellular components (CC) (**Figure 2A**), the enriched genes were primarily related to the nucleus, chromosome, and Golgi apparatus. In terms of biological processes (BP) (**Figure 2B**), the DEGs were involved in DNA repair, replication, and packaging. For molecular functions (MF) (**Figure 2 C**), the enriched genes were mainly involved in helicase activity and RNA binding. Additionally, our KEGG pathway analysis (**Figure 3**) identified seven enriched

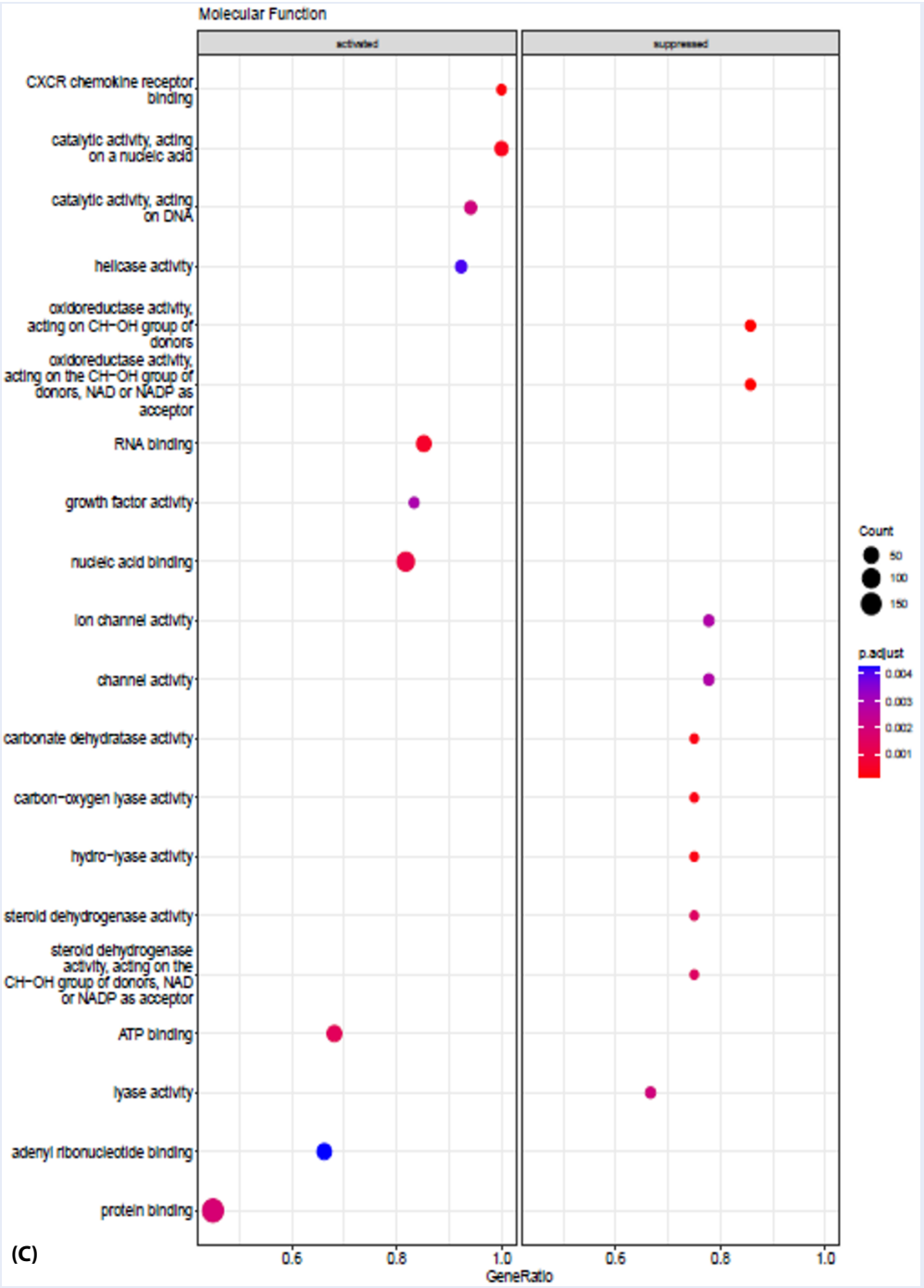

Figure 2: **Gene Ontology (GO) enrichment analyses for differentially expressed genes categorized into three functional aspects. A (Cellular Component)**: This plot highlights the cellular components where differentially expressed genes are significantly enriched. The left panel represents activated components, while the right panel represents suppressed components. Key activated components include the nuclear chromosome, ribonucleoprotein complex, nucleolus, chromosomal region, kinetochore, and plasma membrane. Each dot represents an enriched category, with size corresponding to the number of genes and color indicating adjusted p-values (statistical significance). **B (Biological Process):** This plot displays significantly enriched biological processes. The left panel shows activated processes, while the right panel shows suppressed processes. Activated processes include DNA repair, DNA replication, sister chromatid segregation, mitotic division, and cytokinesis. Suppressed processes involve immune response regulation, metabolic processes, and response to external stimuli. The dot size indicates the number of associated genes, and the color represents the significance level. **C (Molecular Function)**: This plot shows the molecular functions associated with differentially expressed genes. Activated functions include CXCR chemokine receptor binding, catalytic activity on DNA, helicase activity, and oxidoreductase activity. Suppressed functions involve ion channel activity, steroid dehydrogenase activity, and protein binding. Similar to the previous figures, dot size represents gene count, and color indicates statistical significance. These figures collectively provide insight into the functional implications of gene expression changes in the study, highlighting critical cellular structures, biological pathways, and molecular functions involved in the condition under investigation.

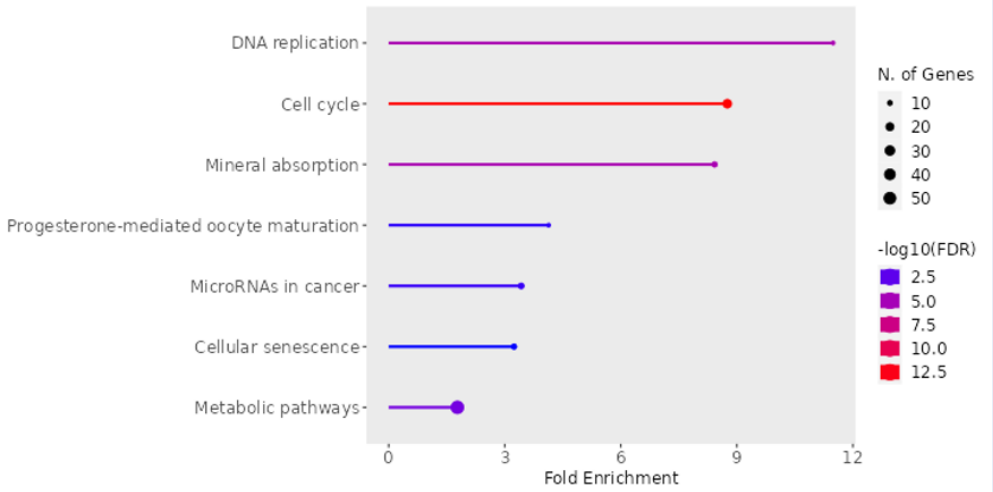

Figure 3: **This figure represents a pathway enrichment analysis, displaying the significantly enriched pathways based on gene expression data**. **Y-axis**: Lists the enriched biological pathways (*e.g.*, DNA replication, Cell cycle, MicroRNAs in cancer, Cellular senescence). **X-axis**: Represents the Fold Enrichment, indicating how much more frequently a given pathway is observed in the dataset compared to what is expected by chance. Dot Size: Indicates the number of genes associated with each pathway (larger dots represent more genes). **Color Gradient**: Represents the statistical significance of enrichment, measured as -log10(FDR) (False Discovery Rate). Red shades indicate highly significant pathways, while purple shades indicate lower significance. **Key Observations**: The "Cell cycle" pathway shows the highest enrichment and statistical significance (largest fold enrichment and red color). "DNA replication" and "Cellular senescence" are also significantly enriched. Other pathways, including "MicroRNAs in cancer" and "Mineral absorption," have lower enrichment scores and significance levels. This figure provides insight into the most affected biological pathways, aiding in the understanding of the functional impact of the differentially expressed genes in the dataset.

pathways, including DNA replication, cell cycle, mineral absorption, progesterone-mediated oocyte maturation, microRNAs in cancer, cellular senescence, and metabolic pathways.

Construction of PPI Network and Detection of Hub Genes

In the biological context, cells are integral components of a complex and elaborate network of interactions among biomolecules. Protein-protein interactions (PPIs) play a crucial role in these networks due to their diverse, specific, and adaptive nature. The PPI network derived from STRING, visualized through Cytoscape software, consists of 106 nodes and 1,446 edges, as depicted in **Figure 4**. Additionally, the most significant 15 hub genes within this network, recognized by the CytoHubba plugin, are detailed in **Figure 5**.

Prognostic Analysis of Hub Genes

Figure 6 and **Figure 7** show how the survival of patients with colon or rectal cancer is related to six key genes: *KIF20A, TPX2, DLGAP5, PBK, ARHGAP11B*, and *RNF146*. Our analysis found that changes in these genes can significantly affect how long patients live. For example, changes in DLGAP5 have a strong impact on survival, while changes in PBK have a smaller effect.

Assessment of Survival-Related Hub Genes Expression Profiles by TCGA Data

Our analysis of TCGA data revealed that all genes associated with survival were differentially expressed. Further analysis using GEPIA confirmed the expression patterns of KIF20A and TPX2, aligning with our initial findings. Based on these results, we selected these genes for further investigation (**Figure 8**).

ROC Curve Analysis

The genes we selected, which were confirmed using additional datasets, showed strong potential as diagnostic and prognostic markers for colorectal cancer. This is based on their high performance in predicting the disease, as measured by the area under the ROC curve (AUC) (**Figure 9**).

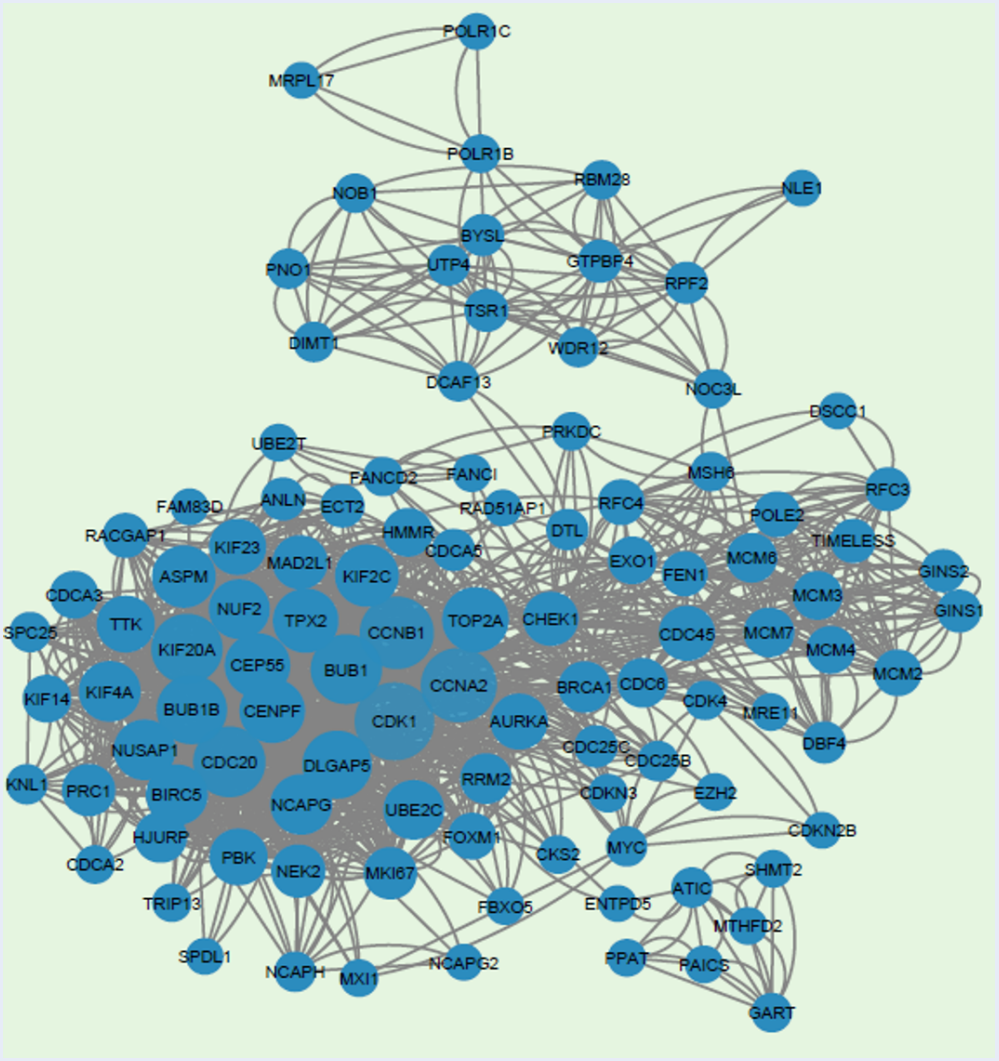

Figure 4: **This figure depicts a protein-protein interaction (PPI) network, where nodes (circles) correspond to proteins (labeled by gene names), and edges (lines) represent known or predicted interactions between them**. **Nodes (Blue Circles)**: Represent individual proteins with gene names labeled inside. **Edges (Gray Lines)**: Indicate interactions between the proteins. **Clusters**: The figure displays multiple clusters of proteins, suggesting functional modules or pathways. **Key Observations**: A large, densely connected cluster in the lower region suggests a highly interactive functional module, likely involved in essential biological processes such as the cell cycle, DNA replication, or mitosis. A smaller, less connected cluster at the top might represent a different biological process or a subset of proteins with fewer interactions. The presence of multiple well-connected hub proteins suggests that some proteins may play key regulatory roles. This network visualization aids in understanding the functional associations between proteins and identifying potential key regulators in a given biological context.

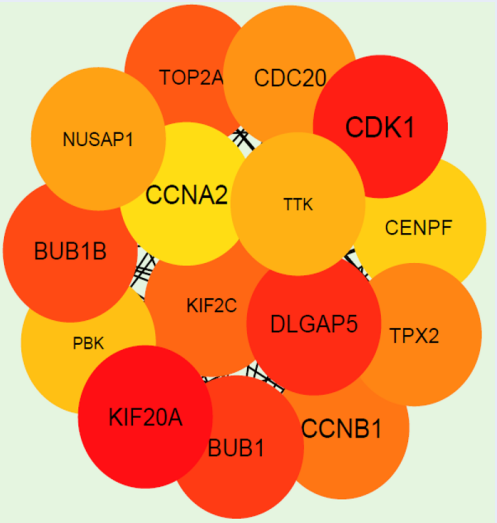

Figure 5: **This figure illustrates a hub-centered protein-protein interaction (PPI) network focused on key genes related to the cell cycle and mitosis**. **Nodes (Circles)**: Represent individual proteins, with gene labels inside. **Colors**: Indicate significance or importance within the network. Yellow represents central/hub genes, while red denotes highly connected genes. **Edges (Lines)**: Indicate interactions between proteins. **Key Observations**: CCNA2 (Cyclin A2): Identified as the central hub (yellow), indicating its crucial role in the regulatory network. **Other Important Genes**: Includes *CDK1*, *CCNB1*, *CDC20*, key regulators of mitotic progression. *KIF20A* and *KIF2C* are involved in mitotic spindle assembly, while *BUB1*, *BUB1B*, and *TTK* are critical for the spindle assembly checkpoint. **Color Gradient**: Ranging from yellow to red, may represent expression levels, connectivity, or pathway significance. This visualization underscores a core cell cycle/mitosis-associated gene module, beneficial for studying cancer, proliferation, or regulatory networks.

Expression Analysis of KIF20A and TPX2

As shown in **Figure 10**, after analyzing the results, it is clear that *TPX2* and *KIF20A* had a significant increase in expression in tumor tissue compared to normal tissue.

DISCUSSION

CRC continues to be a major health problem, even with improved diagnostic and treatment methods. KIF20A, a member of the kinesin protein family, has been implicated in various human cancers. Studies have shown that its overexpression is associated with tumorigenesis and cancer progression[21,22]. KIF20A plays a crucial role in cell division and organelle dynamics, and its aberrant expression is linked to several malignant tumors, but its role in CRC was previously unclear. This study reveals that KIF20A is overexpressed in CRC compared to normal tissue, at the mRNA levels. This overexpression was associated with advanced stages of the disease, including larger tumor size, lymph node involvement, and distant metastasis. Importantly, high KIF20A expression is associated with poor prognosis in CRC patients, suggesting its potential as a prognostic biomarker. Further investigations demonstrated that KIF20A promotes the aggressive behavior of CRC cells by enhancing their proliferation and ability to form colonies.

A study explored the relationship between KIF20A and the JAK/STAT3 signaling pathway, a known oncogenic pathway involved in cancer development. Results suggest that KIF20A may promote CRC cell growth and migration by activating this pathway. This finding highlights the potential of targeting KIF20A as a therapeutic strategy for CRC. Targeting KIF20A, particularly through the JAK/STAT3 pathway, may offer new therapeutic avenues for CRC treatment[23]. Further research is needed to fully elucidate its role in CRC and explore its potential as a therapeutic target.

A recent study by Wu *et al.* highlighted the importance of the KIF20A protein in colorectal cancer (CRC). They found that KIF20A levels were elevated in both animal models and cell cultures of CRC. When KIF20A was experimentally reduced (knocked down) in SW480 cells, these cells grew slower, migrated less, and were more likely to die. Conversely, increasing KIF20A levels (overexpression) in HT-29 cells had the opposite effect. Furthermore, the study showed that KIF20A affects cellular metabolism. Reducing KIF20A decreased the production of pyruvate, lactate, and ATP, while increasing KIF20A boosted

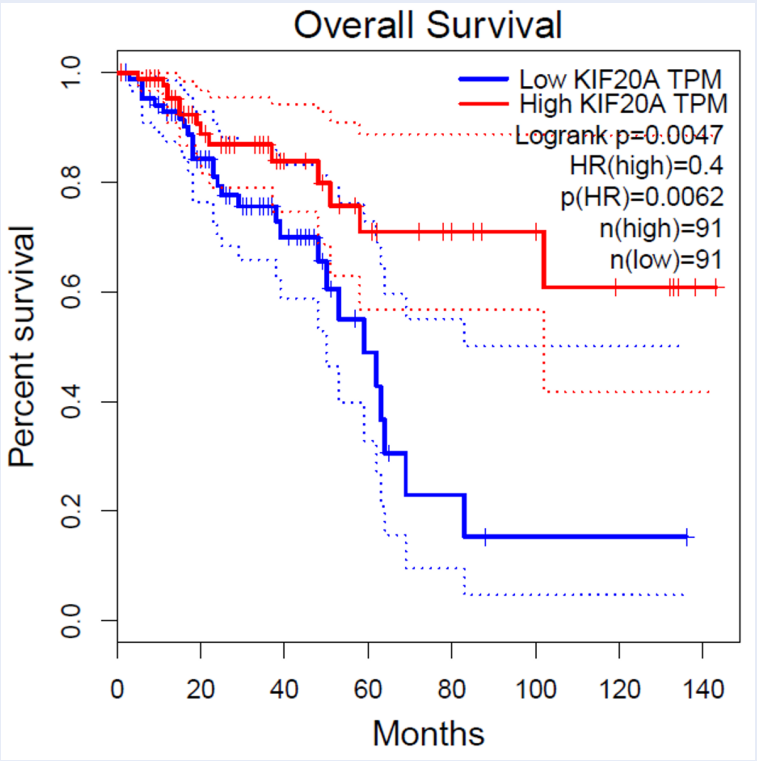

Figure 6: **This Kaplan-Meier survival curve depicts the overall survival (OS) rate stratified by KIF20A expression levels (TPM) in a given cohort**. **Key Observations**: **Blue Line (Low KIF20A TPM)**: Represents patients with low KIF20A expression. This group shows a lower survival rate over time, with a shorter median survival compared to the high-expression group. **Red Line (High KIF20A TPM)**: Represents patients with high KIF20A expression. This group demonstrates a higher survival probability, with the survival curve consistently above that of the low-expression group. **Statistical Significance**: Log-rank p-value = 0.0047: Indicates a statistically significant difference between the two groups. Hazard Ratio (HR) = 0.4: Suggests that patients with high KIF20A expression have a 60% lower risk of death compared to those with low expression. p(HR) = 0.0062: Indicates the hazard ratio is statistically significant. **Sample Size**: n (high) = 91, n (low) = 91: The groups are of equal size. In conclusion, higher KIF20A expression is associated with better overall survival, suggesting that KIF20A may have a protective role or be associated with a favorable prognosis in this context.

these metabolic markers, indicating a shift towards aerobic glycolysis (the Warburg effect). Western blot analysis revealed that KIF20A influences the levels of several proteins involved in cancer metabolism, including c-Myc, HIF-1α, PKM2, and LDHA. Rescue experiments further confirmed that KIF20A promotes the Warburg effect through its interaction with c-Myc and HIF-1α. These findings suggest that KIF20A plays a critical role in CRC progression by regulating both cell growth and metabolism. Targeting KIF20A could potentially be a promising strategy for treating CRC[24].

In a study by Li Cheng Zhang *et al*., researchers investigated the role of a circular RNA called Circ_0084188 in colorectal cancer (CRC). They found that Circ_0084188 was elevated in CRC cells, while a microRNA called miR-769-5p was reduced. When Circ_0084188 was experimentally decreased, CRC cells grew slower, migrated less, and were more likely to die. These effects were reversed when miR-769-5p was also reduced, suggesting that Circ_0084188 acts by regulating miR-769-5p. Further analysis revealed that KLF20A, a protein involved in cell growth and migration, is a direct target of miR-769-5p. By sponging to miR-769-5p, Circ_0084188 effectively increases the levels of KLF20A. This ultimately promotes the growth and spread of CRC cells. In animal models, reducing Circ_0084188 slowed the growth of CRC tumors. These findings suggest that targeting Circ_0084188 or its downstream target KLF20A might be potential strategies for treating CRC[25].

Yang and colleagues discovered that in colorectal cancer cells, the overproduction of KIF20A/NUAK1 can protect against the cell death caused by oxaliplatin.

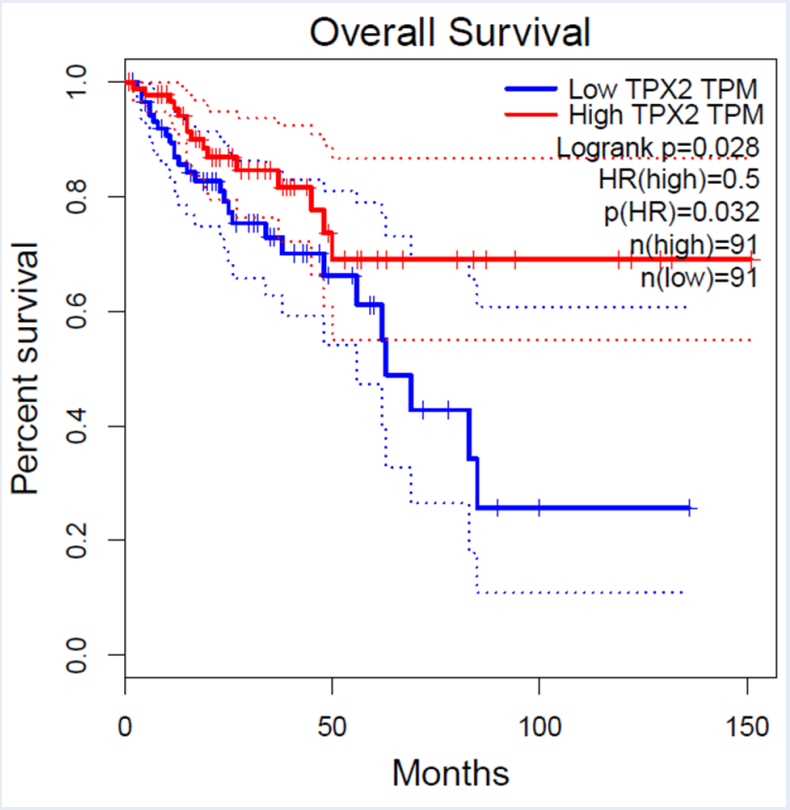

Figure 7: **This Kaplan-Meier survival curve illustrates the overall survival (OS) rates in relation to *TPX2* expression levels (TPM) within a specific cohort**. **Key Observations**: Blue Line (Low TPX2 TPM): Represents patients with low TPX2 expression, showing poorer survival outcomes as indicated by a steeper decline in survival probability. Red Line (High TPX2 TPM): Represents patients with high TPX2 expression, exhibiting a higher survival probability over time. **Statistical Significance**: Log-rank p-value = 0.028: Indicates a statistically significant difference in survival between the high and low expression groups. Hazard Ratio (HR) = 0.5: Suggests that patients with high TPX2 expression have a 50% lower risk of death compared to those with low expression. p(HR) = 0.032: Confirms the statistical significance of the HR. **Sample Size**: Both high and low TPX2 expression groups contain 91 patients each. The analysis indicates that higher TPX2 expression is associated with improved overall survival, suggesting TPX2's protective role or potential as a prognostic biomarker in this context. Patients with low TPX2 expression experience significantly worse survival outcomes.

This occurs by preventing oxidative stress and a process called ferroptosis. The researchers found that KIF20A/NUAK1 activates the GSK3β/Nrf2 pathway, which helps the cells resist chemotherapy. These findings suggest that targeting KIF20A/NUAK1 might be a promising strategy for overcoming chemotherapy resistance in colorectal cancer[26].

Tumorigenesis is a condition marked by cells dividing uncontrollably and forming tumors. This process is linked to changes in genes or proteins that normally keep cell growth, death, and DNA integrity in check. This often stems from changes in genes or proteins that regulate cell division, cell death, and maintaining the integrity of the genomic stability[27–29]. To develop effective treatments, identifying these genes and their protein products involved in the molecular steps leading to cancer is crucial. Our research focused on TPX2, a potential marker implicated in colon cancer development. We found that TPX2 levels were significantly elevated in CRC. This suggests that TPX2 might play a role in colon cancer progression and could be a valuable target for future therapeutic strategies.

A research team led by Ping Wei identified TPX2 protein overexpression in metastatic colon cancer lesions. Higher TPX2 levels correlated with worse patient outcomes, including metastasis and survival. In lab experiments, suppressing TPX2 expression reduced colon cancer cell growth, migration, and invasion. These findings suggest TPX2 as a potential biomarker for prognosis and a target for developing colon cancer therapies[30].

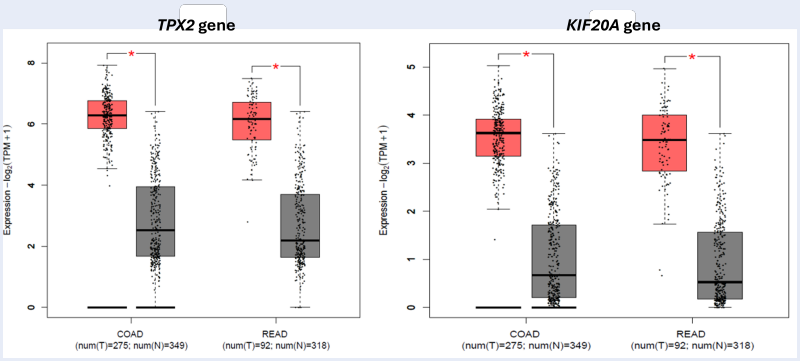

Figure 8: **The expression levels of *TPX2* gene and *KIF20A* gene in tumor (T) versus normal (N) tissues for two cancer types: COAD (Colon Adenocarcinoma) and READ (Rectum Adenocarcinoma)**. The y-axis displays the gene expression level in $\log_2(\text{TPM} + 1)$. The x-axis denotes the two cancer types (COAD and READ), with respective sample sizes provided below. Red box plots depict tumor tissues, while gray box plots represent normal tissues. Each dot corresponds to an individual sample. Asterisks (*) highlight statistical significance, indicating a significant upregulation of the gene in tumor tissues compared to normal tissues. The primary distinction between the two figures lies in the y-axis scale, affecting the numerical expression values while maintaining the overall trend.

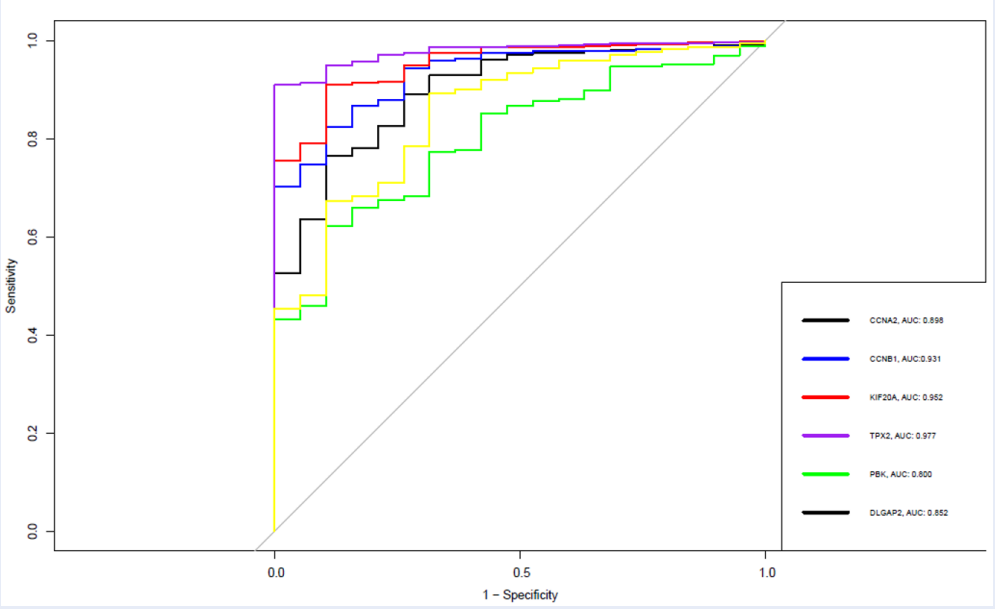

Figure 9: **This figure illustrates a Receiver Operating Characteristic (ROC) curve analysis used to assess the diagnostic performance of various genes in differentiating between two conditions (*e.g.*, cancer versus normal)**. **Key Features**: The x-axis represents (1 - Specificity), and the y-axis represents Sensitivity. Each colored line corresponds to a different gene, with their respective Area Under the Curve (AUC) values detailed in the legend. The diagonal gray line (y = x) denotes a random classifier with an AUC of 0.5, serving as a reference point. The closer the curve approaches the top-left corner, the more effective the gene is at classification. Among the genes analyzed, TPX2 (purple) exhibits the highest AUC of 0.977, indicating the strongest predictive power. KIF20A (red) and CCNB1 (blue) also demonstrate strong performance with AUC values of 0.952 and 0.931, respectively. On the other hand, PBK (yellow) shows the lowest AUC of 0.800, suggesting relatively weaker classification ability. Overall, this analysis highlights TPX2, KIF20A, and CCNB1 as promising biomarkers for disease classification.

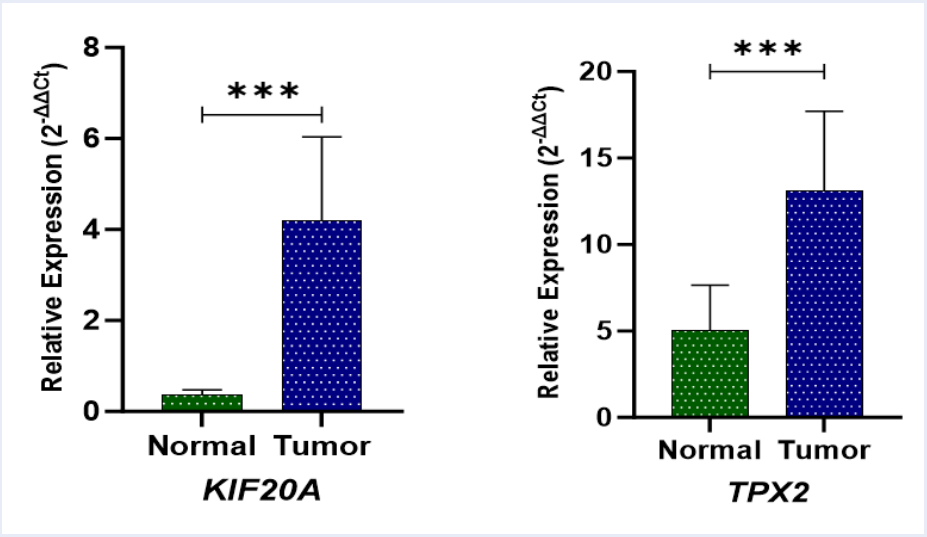

Figure 10: **These figures illustrate the differential expression of the KIF20A and TPX2 genes between normal and tumor samples using relative quantification ($2^{-\Delta\Delta Ct}$). KIF20A Expression**: The bar graph displays a significantly higher expression of KIF20A in tumor tissues compared to normal tissues. The y-axis represents relative expression levels, showing a marked increase in tumors. Statistical significance is indicated by "***", denoting a highly significant difference ($p < 0.001$). **TPX2 Expression**: Similarly, TPX2 expression is significantly elevated in tumor tissues compared to normal tissues. Bar heights reflect a substantial increase in TPX2 levels in tumors, with "***" signifying strong statistical significance ($p < 0.001$). These results suggest that KIF20A and TPX2 are upregulated in tumor samples, potentially indicating their role in tumorigenesis." Feel free to adjust further based on additional specifics or preferences.

A study evaluated TPX2 gene copy number, expression, and potential as a therapeutic target in pancreatic cancer. Findings show increased copy number and expression of TPX2 were observed in pancreatic cancer cell lines and tumor tissues compared to healthy controls. Silencing TPX2 using small interfering RNAs (siRNAs) in cancer cells resulted in reduced cell growth, apoptosis, and inhibited tumor growth in lab models and mice. TPX2 knockdown also increased the effectiveness of paclitaxel, a chemotherapy drug. In conclusion, TPX2 shows promise as a potential therapeutic target for pancreatic cancer[10].

A study by Y. Takahashi *et al.* utilized a combination of computer modeling (*in silico* analyses) and laboratory experiments (*in vitro* experiments) to identify two genes, *AURKA* and *TPX2*, as potential co-regulators of MYC in colorectal cancer cells. The research suggests that AURKA and TPX2 might collaborate with MYC, a well-known oncogene (cancer-promoting gene), to drive tumor development in colorectal cancers. Both AURKA and TPX2 reside on chromosome 20q, a region frequently amplified (increased copy number) across various cancers. The study revealed a high prevalence of co-amplification between the MYC locus (8q24) and chromosome 20q in diverse cancer types. While the exact mechanisms behind this co-amplification remain unclear, the research suggests that it might be driven by natural selection favoring the cooperative oncogenic activity of MYC with these two genes located on chromosome 20q. This work sheds light on the potential role of AURKA and TPX2 as co-regulators of MYC in colorectal cancer. Understanding the underlying mechanisms of their co-amplification and cooperative function with MYC could provide new targets for therapeutic strategies in MYC-driven cancers[31].

A study investigated the potential of targeting two genes, *TPX2* and *TTK*, for treating CRC. The study confirms, based on existing research, that *TPX2* and *TTK* are crucial genes for CRC development. Analysis of patient tumors revealed a complex network involving both genes, suggesting their coordinated role in CRC progression. When researchers inhibited *TPX2* and *TTK* function, it significantly reduced CRC cell proliferation, their ability to form colonies, and their growth in 3D models mimicking tumor environments. Further analysis revealed that specifically depleting *TPX2* and *TTK* impaired cell cycle progression in CRC cells, particularly under 3D culture conditions. This suggests that cell cycle regulation is a critical pathway affected by the loss of these

genes. The study found that elevated levels of *TPX2* and *TTK* correlated with a more aggressive tumor state in patient samples. This finding strengthens the evidence for the oncogenic role of the *TPX2/TTK* network in CRC development. Analyzing gene-drug interactions within the *TPX2/TTK* network identified several promising targets that could be potentially inhibited by existing drugs. Overall, this study provides compelling evidence for targeting the *TPX2/TTK* network as a promising therapeutic strategy for colorectal cancer. The identification of multiple actionable targets and potential drug interactions warrants further investigation to develop effective CRC treatments[32].

Another research study examined the expression levels of two molecules, miR-485-3p and TPX2, in CRC tissues. Their findings indicated a significant downregulation of miR-485-3p, while TPX2 exhibited upregulation in CRC tissues compared to healthy controls. Based on these observations, the study suggests that miR-485-3p might function as a tumor suppressor gene in CRC[33].

Despite the results, this study had significant shortcomings, such as a small sample size, no experiments to determine whether these genes actually affect cell proliferation, migration, invasion, or the ability of tumor cells to form 3D structures, the lack of the use of RNA sequencing data (RNA-seq), the lack of investigation of protein expression, and some data sets compare tumor tissue with normal tissue from healthy individuals, while others use adjacent non-tumor tissue from the same patients as controls.

CONCLUSION

TPX2 and KIF20A, previously linked to cancer cell growth and tumor formation, are also implicated in metastasis due to their tight regulation of the cell cycle. Invasion and metastasis are hallmarks of colon cancer and significantly impact patient outcomes. Identifying the molecular mechanisms underlying these processes is crucial for developing targeted therapies. Our bioinformatics analysis and RTqPCR results revealed upregulation of TPX2 and KIF20A in colon cancer cells. These findings suggest that TPX2 and KIF20A play critical roles in colon cancer invasion and metastasis, making them promising targets for new treatments. Our data also emphasize the importance of TPX2 and KIF20A in regulating cell cycle processes and driving colon cancer growth.

ABBREVIATIONS

AUC: Area Under the Curve, **AURKA**: Aurora Kinase A, **cDNA**: Complementary DNA, **COAD**: Colon Adenocarcinoma, **CRC**: Colorectal Cancer, **DEGs**: Differentially Expressed Genes, **GEO**: Gene Expression Omnibus, **GEPIA**: Gene Expression Profiling Interactive Analysis, **GO**: Gene Ontology, **GSK3β**: Glycogen Synthase Kinase 3 Beta, **GTEx**: Genotype-Tissue Expression, **HIF:1α**: Hypoxia-Inducible Factor 1-alpha, **JAK/STAT3**: Janus Kinase/Signal Transducer and Activator of Transcription 3, **KEGG**: Kyoto Encyclopedia of Genes and Genomes, **KIF20A**: Kinesin Family Member 20A, **KLF20A**: Krüppel:Like Factor 20A, **LDHA**: Lactate Dehydrogenase A, **MCODE**: Molecular Complex Detection, **miR-485-3p**: MicroRNA 485-3p, **Nrf2**: Nuclear Factor Erythroid 2–Related Factor 2, **NUAK1**: NUAK Family Kinase 1, **PKM2**: Pyruvate Kinase M2, **PPI**: Protein-Protein Interaction, **READ**: Rectal Adenocarcinoma, **RNA-seq**: RNA Sequencing, **ROC**: Receiver Operating Characteristic, **RMA**: Robust Multi:array Average, **RT-qPCR**: Reverse Transcription Quantitative Polymerase Chain Reaction, **STRING**: Search Tool for the Retrieval of Interacting Genes/Proteins, **TCGA**: The Cancer Genome Atlas, **TPX2**: Targeting Protein for Xklp2, **TTK**: Threonine Tyrosine Kinase.

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AUTHOR'S CONTRIBUTIONS

F.GH and M.T: performing the main steps of essay and writing the manuscript. H.M, E.B, S.K, A.J: Collecting the samples and helping to perform RNA extraction, qPCR, Analysis of results and doing statistical tests. M.D: Head of team and monitoring and fixing technical errors during all steps of the study. All authors read and approved the final manuscript.

FUNDING

None.

AVAILABILITY OF DATA AND MATERIALS

Data and materials used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study protocol was approved by the Institutional Ethics Committee of Shahid Sadoughi University of Medical Sciences of Yazd (approval number IR.SSU.MEDICINE.REC.1402.235). Prior to tissue sample collection, written informed consent was obtained from all participants.

CONSENT FOR PUBLICATION

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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