Cancer Research and Medicine
DOI: https://doi.org/10.63626/jttyf080
ISSN Online: 3104-4131
ISSN Print: XXXX-XXXX

Research Article

Expression of cyclin dependent kinase 1 in colorectal cancer: A cell cycle regulator with prognostic potential

Osheen Sajjad, Shamaila Habib
Department of Allied Health sciences, NUR International University, Lahore, Pakistan

Abstract
Background: Cell cycle regulation plays important role in maintaining cellular homeostasis. Dysregulation of the cell cycle is a hallmark of various diseases including cancer. In this study, the focus was to investigate expression profile of a major cell cycle related gene (CDK1) in colorectal cancer tissues and its active role in cell life.
Methods: Tissue samples were collected from the patients diagnosed with colorectal cancer. Total RNA was extracted and gene expression levels were quantified via quantitative real-time PCR. Relative expression levels were estimated using ΔΔCt Livak method. Curated interaction pathway of CDK1 was generated using the NDEx Integrated Query platform to highlight the associated signaling networks.
Results: CDK1 showed significant deregulation in the clinical samples compared to matched normal mucosa tissues. Overexpression of CDK1 was observed in the cases as compared to base level in control tissues. On average, a consistent induction was observed in stage I (2.6fold), stage II (4.7fold), stage III (3.5fold) and stage IV (3.99fold) cancerous tissues. Bioinformatic approach reflected the involvement of CDK1 in key cellular functions including G2 phase arrest, DNA replication, DNA repair, G1/S phase transition and mitotic activity, thereby indicating potential contribution to cancer progression and tumor development.
Conclusion: The findings reflect clear induction of CDK1 gene in colorectal cancer tissues and may serve as a potential biomarker for prognosis. However, validation in a larger cohort is needed to explore its utility in prognostic and therapeutic domains.
Key Words: Colorectal cancer, Cyclin dependent kinase 1, Expression profile, Biomarker

INTRODUCTION

Cyclin-dependent kinase 1 (CDK1) is a conserved serine/threonine kinase and works as major regulator of cell cycle progression [1]. Being a catalytic subunit of cyclin/CDK complexes, CDK1 manipulates temporal route of cell cycle transitions, particularly entry into mitosis [2]. CDK1 integrates the regulatory signals with downstream phosphorylation events to monitor and ensure accurate DNA replication and cell division [3]. CDK1 activity is controlled at multiple levels starting from cyclin binding to phosphorylation and subcellular localization. Association with the cyclins, primarily cyclin B, imposes a conformational change which in turn activates the kinase working [4].

Corresponding Author: Osheen Sajjad
Email: osheen.sajjad@niu.edu.pk
Received: 20.10.2025
Revised: 07.03.2026
Accepted: 09.03.2026
Published: 31.03.2026

Multilayered regulation of CDK1 ensures that the activation occurs after completion of DNA replication while following the response to specific checkpoint signaling [5]. Dysregulation of CDK1 has substantial pathological implications including oncogenesis [6]. Aberrant CDK1 activation and altered expression leads to uncontrolled proliferation, resistance to cell cycle checkpoints and chromosomal instability [7]. Consequently, it has earned considerable attention as potential therapeutic target in cancer biology leading to the efforts on developing selective inhibitors to exploit tumor-specific dependency on the cell cycle machinery [8]. Elucidating molecular mechanisms controlling CDK1 regulation and substrate selection provides deeper insight into the cell cycle control leading to the development of targeted therapeutic strategies. In the normal cells, CDK1 activity is highly controlled through association with the regulatory cyclins and reversible phosphorylation to ensure accurate DNA replication followed by the chromosome segregation. However, in cancer cells, regulatory framework is often disrupted causing aberrant CDK1

activation which contributes towards uncontrolled proliferation and genomic instability [9].

Cancer cells often exhibit higher CDK1 expression leading to hyperactivation and dysregulation of upstream regulators, functioning of cyclins and checkpoint proteins [10]. Overexpression of cyclin B or loss of inhibitory kinases can cause sustained CDK1 activity which enables the cells to bypass critical cell cycle checkpoints. Aberrant signaling promotes fast G2/M transition even in presence of damaged DNA or incomplete replication. This in turn facilitates mutation accumulation and chromosomal instability which are key hallmarks of tumor progression [11]. Importantly, cancer cells often display a delicate dependency on CDK1 activity as compared to normal tissues, offering a potential therapeutic vulnerability [12]. Inhibition of CDK1 has shown induction of mitotic catastrophe, senescence or apoptosis in various malignancies [13]. Consequently, CDK1 has emerged as promising drug target as a single agent or in combination with other agents [14]. Given its central role at the intersection of cell cycle regulation and genome maintenance, CDK1 represents critical check in cancer cell survival and progression. Further understanding of the CDK1 mediated signaling in the tumor contexts is essential for refining therapeutic approaches to aim targeted interventions.

Colorectal cancer is one of the leading causes of cancer-related deaths worldwide. This cancer is characterized by the progressive accumulation of genetic changes that drive uncontrolled proliferation, and metastatic dissemination [15]. Among molecular determinants, dysregulation of cell cycle machinery is fundamental hallmark of the colorectal tumorigenesis [16]. Among these, CDK1 plays a pivotal regulator of G2/M transition and mitotic progression. It has emerged as critical contributor towards colorectal cancer development and progression. Colorectal cancer cells may exhibit high dependency on the CDK1-mediated signaling, suggesting the potential therapeutic vulnerability [17]. Pharmacological targeting of CDK1 has shown induction of mitotic catastrophe and apoptotic events in the preclinical models. These findings make CDK1 a prognostic biomarker and a therapeutic target in colorectal cancer [18].

METHODS

Clinical Sampling and Expression Analysis

Cancerous tissues (35) along with normal mucosa (10) were collected from the colorecta cancer patients after informed consent and were stored immediately. Afterwards, total RNA was extracted from the frozen cancer tissue samples and normal mucosa specimens using a commercially available RNA extraction kit (RNeasy Mini Kit, Qiagen). RNA concentrations were measured with nanodrop technology. cDNA was synthesized by using commercially available kit with 200ng of RNA per reaction (RevertAid, Thermo Scientific). Prepared cDNA samples were checked and verified by using conventional PCR with amplification of a reference gene before further procedures.

Real Time PCR

Primers for CDK1 (*CATCTCAGTCCTTATGGCAGTTT, CAACATGGCAAGAAACTGATG*) were designed by using online tools and optimized by using gradient PCR method. Validated cDNA samples were utilized with gene-specific primers to evaluate the expression profile of CDK1 gene. Each sample was amplified in triplicate using SYBR Green Master Mix (Thermo Scientific) along with AriaMax Real-Time PCR system. Obtained Cq values were analyzed to determine relative fold changes using the Livak ($2^{-}\Delta\Delta Ct$) method. Data normalization was performed using Cq values of the housekeeping gene GAPDH, followed by comparative analysis between healthy control samples and the samples of four stages of colorectal cancer. Graphical analyses and visualizations were generated using GraphPad Prism version 10.5.

Bioinformatics Analysis

To investigate the interaction profile of CDK1 and its associated proteins, a protein–protein interaction network was constructed using the STRING database and visualized in Cytoscape software. For this, the target gene/protein was queried in the STRING software v.12 to identify known and predicted protein interactions. Sources included experimental evidence, database annotations co-expression were selected for *Homo sapiens*. A high confidence interaction score threshold (≥0.7) was applied to ensure reliability of the predicted associations. The generated protein–protein interaction network was exported from STRING into Cytoscpae software, v.3.10 for network visualization and topological analysis. The nodes represented proteins, while the edges represented functional or physical interactions between proteins. Network analysis was performed using the built-in Network Analyzer tool in Cytoscape to evaluate topological parameters, including node degree, clustering coefficient, while hub genes/proteins were identified based on high degree connectivity and centrality scores.

Table 1: Demographic details of the patient cohort

	Gender	Age	Numbers
Normal Tissues	Male/Females: 07/03	Average: 61.3Y	10
Cancer Tissues	Male/Females: 22/13	Average: 58.7Y	Stage I Patients: 07 Stage II Patients:12 Stage III Patients: 08 Stage IV Patients: 08

RESULTS

Cyclin-Dependent Kinase 1 High Expression in Colorectal Cancer

Quantitative real-time PCR analysis was conducted to assess the transcriptomic expression of CDK1 gene in healthy individuals and colorectal cancer patients across different disease stages. The study included 10 healthy controls and 35 colorectal cancer tissues, who were classified according to cancer stage (Stage I–IV). Relative CDK1 expression levels were compared among all groups. The analysis revealed substantial variation in CDK1 expression among the study participants. In healthy matched tissues, the mean expression level was 0.35-fold, reflecting basal gene expression. Control samples showed variable patterns, ranging from the maximum upregulation of 2.6-fold to maximum downregulation of -2.5-fold. In contrast, elevated CDK1 expression was noticed in colorectal cancer patients across all the disease stages. Stage I patients (n=7) demonstrated an average expression level of 2.6fold. Within group, highest upregulation was observed to be 5.2fold, while there was only one sample demonstrating the downregulation with value of -1.8fold. In stage II patients (n=12), a mean CDK1 expression of 4.7fold was noticed. The maximum induction was recorded in stage II group which was 11.4fold, while the lowest induction was 1.9fold. More importantly, all stage II samples showed upregulation of the CDK1 levels. Likewise, in stage III patients (n=8), consistent CDK1 overexpression with a mean expression level of 3.5fold was witnessed. Maximum upregulation detected in stage III group was 8.3fold, while downregulation was not observed in any of the stage III sample. This indicated a uniform gene induction pattern among these patients. In stage IV patients (n=8), mean expression level was found to be 3.99fold. Highest upregulation was 9.1fold, while minimum detected induction was 1.2fold. Like stage II and III, none of the stage IV samples demonstrated downregulation when compared to healthy controls. Overall, findings demonstrate a consistent trend of upregulation of CDK1 gene in the colorectal cancer tissues as compared to healthy matched samples. Elevated expressions were observed across all the cancer stages, which in turn suggests that CDK1 may play important role in colorectal cancer progression. It further highlights potential of CDK1 as a biomarker for disease development and progression (Figure 1).

Role of Cyclin-Dependent Kinase 1 in Central Cell Machinery

To further explore the molecular role of CDK1 in colorectal cancer, curated interaction pathway was generated by using NDEx Integrated Query platform while using CDK1 as a central gene. The analysis was performed to discover potential interacting partners; associated pathways and the functional relationships associated with CDK1 in colorectal cancer. Curated pathway revealed complex interaction network with multiple cell cycle regulatory partners, checkpoint components and molecules associated with tumor proliferation and progression. Functional analysis based on interactions indicated that CDK1 is involved in critical cellular processes, including G2 phase arrest regulation, DNA repair mechanisms, replication and G1/S phase transition. The interconnected pathways highlighted central role of the CDK1 in maintaining cell cycle progression and genomic stability. Observed molecular associations further supported involvement in pathogenesis and advancement of cancer while emphasizing its potential as a promising prognostic biomarker and therapeutic target (Figure 2).

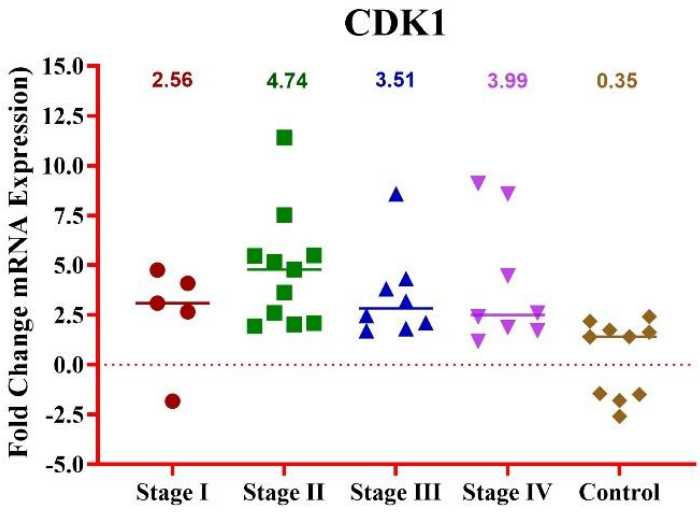

Figure 1: Expression levels of CDK1 gene in colorectal cancer tissues. The cancerous tissues and healthy mucosa were analyzed. Total RNA was extracted from the tissues and CDK1 gene expression was measured using real-time PCR method. Relative fold changes were calculated using Livak method and were compared among the groups.

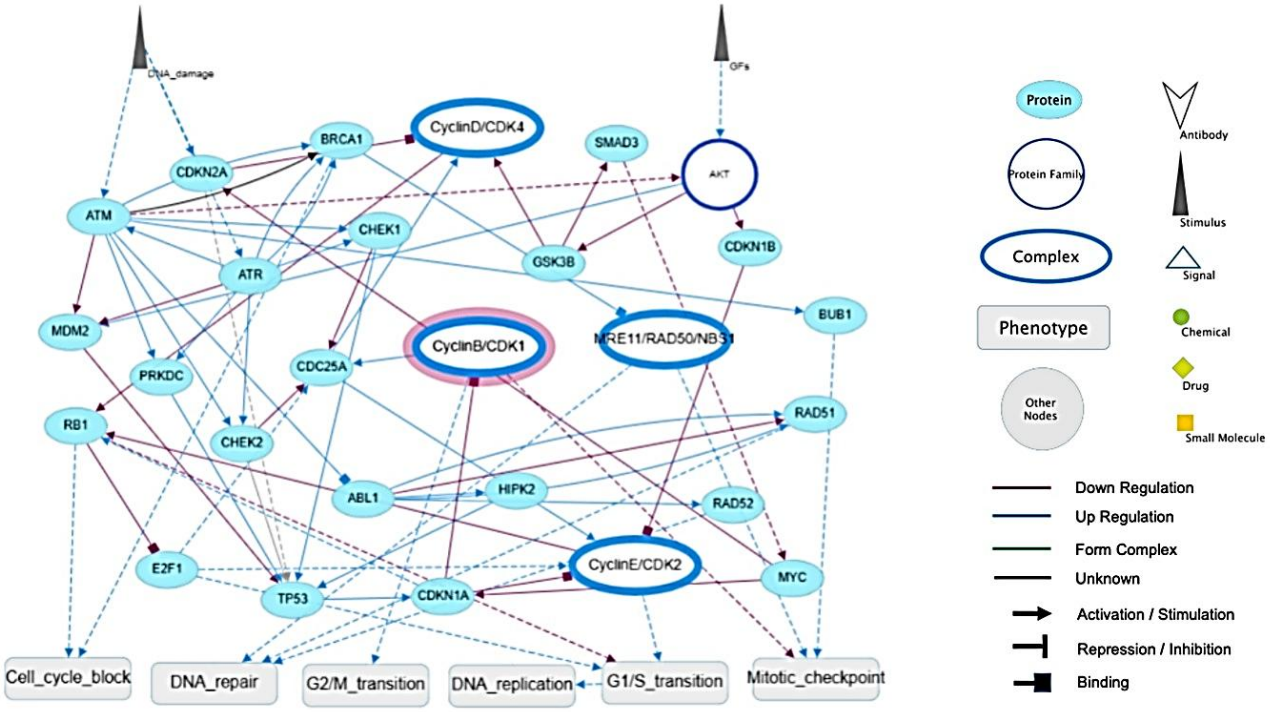

Figure 2: Curated interaction pathway of CDK1. The signaling network was generated using NDEx Integrated Query platform showing molecular interactions and the associated signaling networks. The network highlights involvement of CDK1 in critical processes including DNA repair, DNA replication, G2 phase arrest and G1/S phase transition. The analysis supporting potential role of CDK1 in cancer progression and tumor development.

DISCUSSION

Colorectal cancer is leading cause of cancer related deaths worldwide. Progression of colorectal cancer is strongly associated with the dysregulation of cell cycle machinery. Among the main players, CDK1 has gained attention as it plays central role in regulating the cell cycle including mitotic entry and genome stability [19]. The current study was designed to investigate the transcriptomic expression of CDK1 in colorectal cancer tissues of different stages and were compared with heathy mucosa. Furthermore, we explored the molecular interaction network by using bioinformatic pathway analysis. Findings demonstrated consistent upregulation of CDK1 gene in the colorectal cancer tissues as compared to healthy controls. This shows and supports the hypothesis that CDK1 contributes substantially towards colorectal tumorigenesis and progression.

The qRT-PCR analysis showed elevated expression of CDK1 in all stages of colorectal cancer including stage I, II, III, and IV patients. In contrast, healthy tissues exhibited relatively low basal expression with minor degree of individual variability. The observed high expression of CDK1 in cancer tissues is biologically plausible as CDK1 is a critical regulator gene for the G2/M transition and mitotic progression. Activation of CDK1, therefore contributes towards uncontrolled cellular proliferation, a central hallmark feature of the malignant transformation [20]. Findings of this study are also in agreement with the several previous investigations reporting increased CDK1 levels in lung, liver, and gastric cancers. Elevated CDK1 expression is associated with enhanced tumor aggressiveness, reduced overall survival and poor prognosis [21-24]. Present data demonstrated that cancerous tissues showed consistent higher mean expression values of CDK1. This finding indicates that CDK1 activation occurring during the tumor progression plays a significant role in promoting cellular proliferation in developmental and metastatic dissemination. Furthermore, absence of CDK1 downregulation in patients suggests persistent activation of this gene throughout the disease development. This continuous overexpression may contribute to sustained mitotic signaling, cellular division and resistance towards normal cell cycle checkpoints. On clinical grounds, consistent upregulation of CDK1 in this study hints the potential utility as a prognostic biomarker for colorectal cancer. Increased expression in all disease stages highlights that CDK1 serves as an early molecular indicator for tumor development. Moreover, as the CDK1 expression was elevated in advanced stages, it may also possess prognostic marker for monitoring the disease progression and therapeutic responses. In this context, several experimental studies have shown that CDK1 inhibitors can be instrumental anticancer agents.

Molecular pathway analysis strengthened the view about the biological significance of CDK1 in colorectal cancer. Curated interaction network generated by using NDEx Integrated Query platform showed that CDK1 interacts with the multiple partner proteins involved in cell cycle regulation, DNA replication, mitotic checkpoint control and genome maintenance. These findings support established role of the CDK1 as master regulator during the cell division [25].

Functional associations identified during the analysis highlighted that the involvement of CDK1 in critical cellular phases including DNA repair and replication, G1/S phase transition and mitotic activity [26]. Involvement of CDK1 in the DNA repair mechanisms is principally important in the context of cancer development. Cancer cells frequently accumulate genomic instability due to the defects in DNA repair machinery [27]. CDK1 has shown its ability to regulate proteins involved in homologous recombination and DNA damage responses, thereby influencing cell survival following genomic injury [28]. Dysregulation of the CDK1 may therefore contribute to both uncontrolled proliferation and faulty genome maintenance, which ultimately facilitate the tumor progression and mutation accumulation. At the same time, association of CDK1 with the G2 phase arrest and mitotic checkpoints suggests its role in controlling the transition phase including DNA synthesis to mitosis. Under normal physiological conditions, checkpoints of G2/M prevent the cells with damaged DNA from entering mitosis phase. However, activation of CDK1 may override these checkpoints, thus allowing genetically unstable cells to carry the proliferating state [29]. This explains the need for persistent overexpression of CDK1 observed during the advanced cancer stages in this study.

Pathway analysis also demonstrated that the involvement of CDK1 in DNA replication and G1/S transition processes. CDK1 is traditionally known for its role in the G2/M transition, however emerging evidence suggests that it can balance other cyclin dependent kinases and help in earlier phases of cell cycle [30]. High CDK1 activity may therefore increase overall cell cycle progression leading to rapid tumor cell proliferation. The findings collectively indicate the possibility that CDK1 acts as multidimensional regulator influencing several interconnected pathways in cancer. Protein–protein interaction analysis using STRING and Cytoscape software further showed extensive connectivity of CDK1 in cellular regulatory networks. High confidence interactions with the proteins responsible for mitotic spindle formation, chromosomal segregation and checkpoint signaling indicates that CDK1 carries a central hub position within tumor associated networks [31]. Hub genes are considered as critical determinants of the disease

progression as they are involved in regulating multiple downstream pathways at the same time. Thus, targeting CDK1 as a hub gene may deliver therapeutic benefits by targeting several oncogenic routes at once.

To conclude, present study showed significant upregulation of CDK1 in the colorectal cancer tissues of all disease stages. Furthermore, we identified the extensive involvement of CDK1 in key cellular pathways related to the tumor progression. Combined experimental and bioinformatic approach indicated the central role of CDK1 in tumor development, regulating cell cycle progression and mitotic activity in colorectal cancer. Despite these significant findings and interpretations, limitations of the study should also be considered. Most important one is the sample size which was relatively small and may influence generalizability of the observed results. Furthermore, focus was on transcriptomic expression while evaluating protein level expression of CDK1 is equally important to be explored. Future studies with larger patient cohorts, protein profiling, and necessary functional assays are required to provide a more comprehensive understanding about the biological importance of CDK1 in colorectal cancer. Lastly, considering clinical outcomes including patient survival and treatment responses will be helpful to establish prognostic significance of CDK1 in colorectal cancer.

REFERENCES

1. Massacci G, Perfetto L, Sacco F. The Cyclin-dependent kinase 1: more than a cell cycle regulator. British Journal of Cancer. 2023 Nov 23;129(11):1707-16.
2. Banyai G, Baïdi F, Coudreuse D, Szilagyi Z. Cdk1 activity acts as a quantitative platform for coordinating cell cycle progression with periodic transcription. Nature communications. 2016 Apr 5;7(1):11161.
3. Enserink JM, Kolodner RD. An overview of Cdk1-controlled targets and processes. Cell division. 2010 May 13;5(1):11.
4. Coudreuse D, Nurse P. Driving the cell cycle with a minimal CDK control network. Nature. 2010 Dec 23;468(7327):1074-9.
5. Lemmens B, Hegarat N, Akopyan K, Sala-Gaston J, Bartek J, Hochegger H, Lindqvist A. DNA replication determines timing of mitosis by restricting CDK1 and PLK1 activation. Molecular cell. 2018 Jul 5;71(1):117-28.
6. Bonelli P, Tuccillo FM, Borrelli A, Schiattarella A, Buonaguro FM. CDK/CCN and CDKI alterations for cancer prognosis and therapeutic predictivity. BioMed research international. 2014;2014(1):361020.
7. Anda S, Rothe C, Boye E, Grallert B. Consequences of abnormal CDK activity in S phase. Cell Cycle. 2016 Apr 2;15(7):963-73.
8. Wang Q, Bode AM, Zhang T. Targeting CDK1 in cancer: mechanisms and implications. NPJ precision oncology. 2023 Jun 13;7(1):58.
9. Malumbres M, Barbacid M. Cell cycle, CDKs and cancer: a changing paradigm. Nature reviews cancer. 2009 Mar;9(3):153-66.
10. Sweeney KJ, Swarbrick A, Sutherland RL, Musgrove EA. Lack of relationship between CDK activity and G1 cyclin expression in breast cancer cells. Oncogene. 1998 Jun;16(22):2865-78.
11. Santamaría D, Barrière C, Cerqueira A, Hunt S, Tardy C, Newton K, Cáceres JF, Dubus P, Malumbres M, Barbacid M. Cdk1 is sufficient to drive the mammalian cell cycle. Nature. 2007 Aug 16;448(7155):811-5.
12. Knudsen ES, Kumarasamy V, Nambiar R, Pearson JD, Vail P, Rosenheck H, Wang J, Eng K, Bremner R, Schramek D, Rubin SM. CDK/cyclin dependencies define extreme cancer cell-cycle heterogeneity and collateral vulnerabilities. Cell reports. 2022 Mar 1;38(9).
13. Castedo M, Perfettini JL, Roumier T, Kroemer G. Cyclin-dependent kinase-1: linking apoptosis to cell cycle and mitotic catastrophe. Cell Death & Differentiation. 2002 Dec;9(12):1287-93.
14. Zhang M, Zhang L, Hei R, Li X, Cai H, Wu X, Zheng Q, Cai C. CDK inhibitors in cancer therapy, an overview of recent development. American

journal of cancer research. 2021 May 15;11(5):1913.
15. Marcellinaro R, Spoletini D, Grieco M, Avella P, Cappuccio M, Troiano R, Lisi G, Garbarino GM, Carlini M. Colorectal cancer: current updates and future perspectives. Journal of Clinical Medicine. 2023 Dec 21;13(1):40.
16. Ahmad R, Singh JK, Wunnava A, Al-Obeed O, Abdulla M, Srivastava SK. Emerging trends in colorectal cancer: Dysregulated signaling pathways. International journal of molecular medicine. 2021 Mar;47(3):14.
17. Zhang P, Kawakami H, Liu W, Zeng X, Strebhardt K, Tao K, Huang S, Sinicrope FA. Targeting CDK1 and MEK/ERK overcomes apoptotic resistance in BRAF-mutant human colorectal cancer. Molecular Cancer Research. 2018 Mar 1;16(3):378-89.
18. Priyamvada P, Ramaiah S. Potential signature therapeutic biomarkers TOP2A, MAD2L1, and CDK1 in colorectal cancer: a systems biomedicine-based approach. Biochemical Genetics. 2024 Jun;62(3):2166-94.
19. Enserink JM, Kolodner RD. An overview of Cdk1-controlled targets and processes. Cell division. 2010 May 13;5(1):11.
20. Wang Z, Fan M, Candas D, Zhang TQ, Qin L, Eldridge A, Wachsmann-Hogiu S, Ahmed KM, Chromy BA, Nantajit D, Duru N. Cyclin B1/Cdk1 coordinates mitochondrial respiration for cell-cycle G2/M progression. Developmental cell. 2014 Apr 28;29(2):217-32.
21. Shen J, Gong X, Tan S, Zhang Y, Xia R, Xu S, Wang S, Zhou H, Jiang Y, Zhao T, Zhang Y. CDK1 acts as a prognostic biomarker associated with immune infiltration in pan-cancer, especially in gastrointestinal tumors. Current Medicinal Chemistry. 2025 Jul;32(23):4836-57.
22. Sung WW, Lin YM, Wu PR, Yen HH, Lai HW, Su TC, Huang RH, Wen CK, Chen CY, Chen CJ, Yeh KT. High nuclear/cytoplasmic ratio of Cdk1 expression predicts poor prognosis in colorectal cancer patients. BMC cancer. 2014 Dec 15;14(1):951.
23. Zhang J, Su G, Lin Y, Meng W, Lai JK, Qiao L, Li X, Xie X. Targeting cyclin-dependent kinases in gastrointestinal cancer therapy. Discovery Medicine. 2019 Jan 25;27(146):27-36.
24. Li M, He F, Zhang Z, Xiang Z, Hu D. CDK1 serves as a potential prognostic biomarker and target for lung cancer. Journal of International Medical Research. 2020 Feb;48(2):0300060519897508.
25. McCusker D, Denison C, Anderson S, Egelhofer TA, Yates 3rd JR, Gygi SP, Kellogg DR. Cdk1 coordinates cell-surface growth with the cell cycle. Nature cell biology. 2007 May;9(5):506-15.
26. Limas JC, Cook JG. Preparation for DNA replication: the key to a successful S phase. FEBS letters. 2019 Oct;593(20):2853-67.
27. Yao Y, Dai W. Genomic instability and cancer. Journal of carcinogenesis & mutagenesis. 2014 Feb 25;5:1000165.
28. Ira G, Pellicioli A, Balijja A, Wang X, Fiorani S, Carotenuto W, Liberi G, Bressan D, Wan L, Hollingsworth NM, Haber JE. DNA end resection, homologous recombination and DNA damage checkpoint activation require CDK1. Nature. 2004 Oct 21;431(7011):1011-7.
29. Fatma F, Kumar A. The cell cycle, cyclins, checkpoints and cancer. Asian Journal of Research in Pharmaceutical Science. 2021 Jun 1;11(2).
30. Hume S, Dianov GL, Ramadan K. A unified model for the G1/S cell cycle transition. Nucleic acids research. 2020 Dec 16;48(22):12483-501.
31. Khurshid S, Usmani S, Ali R, Hamid S, Masoodi T, Sadida HQ, Ahmed I, Khan MS, Abeer I, Albalawi IA, Bedaiwi RI. Integrating network analysis with differential expression to uncover therapeutic and prognostic biomarkers in esophageal squamous cell carcinoma. Frontiers in Molecular Biosciences. 2024 Aug 21;11:1425422.

Author Contributions: OS performed experiments and wrote the article. Shamaila Habib helped with data analysis.

Competing Interests: Authors declare no competing interests.

Ethical Statement: This study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by relevant Institutional Review Board.

Financial Support: No specific grant was availed for this research.

Data Availability: The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Open Access

This article is licensed under a Creative Commons Attribution 4.0 International License. The license permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source provides a link to the Creative Commons license, and indicates if changes were made. https://creativecommons.org/licenses/by/4.0/.