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Exploring the Role of CDKN2A in Human Cancers Using an Integrative Pan-Cancer Approach

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Abstract: This study aims to investigate the expression variation, biological significance, and prognostic value of cyclin-dependent kinase inhibitor 2A (CDKN2A) as a common biomarker across 33 malignancies. Various bioinformatics tools, including UALCAN, GEPIA2, OncoDB, cBioPortal, TIMER2, STRING, DAVID, and the GSCA database, were employed for this pan-cancer analysis. The results revealed significant up-regulation of CDKN2A in 24 major human cancer subtypes ($P < 0.05$). This up-regulation was strongly associated with poor overall survival and tumor dissemination, particularly in uterine corpus endometrial carcinoma (UCEC), colon adenocarcinoma (COAD), and liver hepatocellular carcinoma (LIHC), highlighting its potential as a prognostic biomarker. Moreover, CDKN2A overexpression was linked to diverse clinicopathological characteristics of patients. Genetic alterations recorded via cBioPortal indicated minimal mutation rates in COAD, LIHC, and UCEC. Additionally, promoter methylation, drug sensitivity, and enrichment analyses were performed to explore associations with CDKN2A expression. Overall, the findings emphasize the potential of CDKN2A as a shared diagnostic and prognostic biomarker, as well as a therapeutic target in COAD, LIHC, and UCEC, particularly in patients with varied clinicopathological traits.
Keywords: CDKN2A; Cancer; Prognosis biomarker; Therapeutic target

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1. Introduction

Cancer, a complex and diverse group of diseases, remains a critical public health concern and the leading cause of mortality worldwide [1-3]. It is characterized by the uncontrolled proliferation and dissemination of abnormal cells, significant heterogeneity, intricate genomic alterations, and a wide array of molecular abnormalities that affect nearly every tissue and organ [4-6]. Global statistics indicate that the number of new cancer cases and fatalities rose to 20 million and 9.7 million, respectively, in 2022, compared to 18.1 million and 9.6 million in 2018 [7,8]. The multifactorial etiology of cancer involves the interaction of genetic variants, environmental exposures, and lifestyle factors [9-11]. Despite significant advancements in cancer diagnostics and treatments, including immunotherapy, targeted therapies, chemoradiotherapy, and surgery, the prognosis for cancer

patients remains poor, primarily due to tumor heterogeneity, distant metastases, acquired drug resistance, and recurrence [12-14]. As a result, the identification of novel diagnostic and prognostic biomarkers for various cancers is of paramount importance.

Cyclin-dependent kinase inhibitor 2A (CDKN2A), located on band p21.3 of human chromosome 9, is ubiquitously expressed in many tissues, including the adrenal glands, bladder, testis, stomach, spleen, and fat tissue [15,16]. CDKN2A functions as a tumor suppressor gene encoding two proteins, p14ARF and p16INK4a. These proteins play key roles in cell cycle regulation; p16INK4a inhibits CDK4, inducing G1 cell cycle arrest and preventing retinoblastoma phosphorylation, while p14ARF activates p53 [17-19]. Deletions, insertions, point mutations, and epigenetic changes are among the most common alterations of CDKN2A, with its deletion identified in 1.7% to 6.7% of cases, often associated with various cancers. Homozygous deletions of CDKN2A are linked to poor prognosis in IDH-mutant gliomas, supratentorial ependymomas, meningiomas, and malignant peripheral nerve sheath tumors (MPNST), and have been recognized as diagnostic criteria by the World Health Organization [20-23].

Single nucleotide polymorphisms (SNPs) in the p14ARF protein can also lead to different cancer types [24]. CDKN2A plays a significant role in inherited cancers, particularly in familial atypical multiple-mole melanoma (FAMMM), which increases the risk of melanoma and pancreatic cancer [25]. Alterations in CDKN2A, such as point mutations, translocations, homozygous and heterozygous losses, and abnormal promoter methylation, have been associated with melanoma, non-small cell lung cancer (NSCLC), head and neck cancers, prostate, esophageal, ovarian, kidney, colon, breast, and bladder cancers [26-28]. Moreover, aberrant CDKN2A expression correlates closely with immune infiltration and immune-regulatory gene levels. Several activated immune cells show a strong positive correlation with high CDKN2A expression, suggesting an intriguing role for CDKN2A in tumor immunity.

CDKN2A exhibits significant expression across various cancers, including breast cancer (BRCA), head and neck squamous cell carcinoma (HNSC), colon adenocarcinoma (COAD), kidney renal cell carcinoma (KIRC), stomach adenocarcinoma (STAD), lung adenocarcinoma (LUAD), liver hepatocellular carcinoma (LIHC), and uterine corpus endometrial carcinoma (UCEC) [29-31]. These findings indicate that CDKN2A may serve as a key target for cancer diagnosis and therapeutic intervention, given its critical involvement in cancer progression. However, the precise role of CDKN2A in pan-cancer contexts remains unclear.

This study aims to analyze CDKN2A expression across multiple human cancer subtypes and its association with various parameters, including promoter methylation levels, overall survival (OS), relapse-free survival (RFS), genetic mutations, copy number variations (CNVs), immune cell infiltration, gene enrichment, and gene-drug interaction networks, using several online databases and bioinformatics tools.

2. Materials and methods

2.1. Pan-cancer expression analysis of CDKN2A

UALCAN is an online database developed for the comprehensive examination of cancer-associated data across several cancer subtypes [32]. In this study, UALCAN's default settings were utilized to perform a pan-cancer analysis of CDKN2A expression in both tumor and normal samples, based on different parameters across 33 cancer types. A *P*-value of <0.05 was considered statistically significant.

2.2. Survival analysis

GEPIA2 is a robust bioinformatics tool designed to investigate variations in gene expression within different tissues and tumor types [33]. In the present study, the impact of CDKN2A expression on OS in various cancers was evaluated using the Survival Plot module of GEPIA2, with a *P*-value of < 0.05 set as statistically significant.

2.3. Promoter methylation analysis

OncoDB is an essential database functioning as a comprehensive platform for oncogenic mutations, with a specific emphasis on DNA methylation data [34]. In this study, OncoDB was employed to investigate the promoter methylation levels of CDKN2A across various cancers, enabling the identification of its potential as a biomarker.

2.4. Mutational analysis using cBioPortal

cBioPortal is a web-based application designed for the assessment of genetic alterations across numerous cancers [35]. In this study, cBioPortal's advanced features were used to conduct a thorough mutational characterization of the CDKN2A gene across diverse cancer subtypes.

2.5. Immunogenetics analysis

The relationship between immune cell infiltration and gene expression across distinct cancer types can be evaluated using the Tumor Immune Estimation Resource (TIMER2) database [36]. In this study, the associations between CDKN2A expression and the infiltration levels of various immune cell populations were analyzed using the TIMER2 algorithm.

2.6. Protein-protein interaction network and enrichment analysis

Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) is a significant bioinformatics resource that facilitates the visualization of protein-protein interaction networks [37]. In this study, the protein-protein interaction (PPI) network corresponding to CDKN2A and its interacting proteins was constructed using the STRING database. Database for Annotation, Visualization, and Integrated Discovery (DAVID) is another bioinformatics tool used to elucidate the functional significance of gene lists [38]. Gene enrichment analysis of CDKN2A was performed using the DAVID tool.

2.7. Drug sensitivity analysis

The GSCA database is a valuable resource for assessing pharmacological sensitivity [39]. In this study, the correlation between drug sensitivity and the mRNA expression of CDKN2A was explored using the GSCA database.

3. Results

3.1. Pan-cancer expression analysis of CDKN2A

The pan-cancer analysis of CDKN2A expression across 33 cancers was conducted using the TCGA and GTEx databases through UALCAN. The results demonstrated that CDKN2A was significantly ($P < 0.05$) overexpressed in 24 cancer subtypes, including squamous-cell carcinoma (SCC) of the lung, bladder urothelial carcinoma (BLCA), papillary renal cell carcinoma (PRCC), esophageal carcinoma (ESCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), stomach adenocarcinoma (STAD), BRCA, LIHC, COAD, HNSC, and UCEC (**Figure 1**).

3.2. Prognostic analysis of CDKN2A

GEPIA2 was utilized to further assess the role of CDKN2A overexpression on OS across various cancer types. The results indicated that upregulated CDKN2A expression was significantly ($P < 0.05$) correlated with worse OS and RFS in five cancer subtypes, including adenoid cystic carcinoma (ACC), COAD, LIHC, UCEC, and uveal melanoma (UVM) (**Figure 2**). This association of upregulated CDKN2A expression with poor OS in ACC, COAD, LIHC, UCEC, and UVM highlights the potential of CDKN2A as a prognostic biomarker.

3.3. CDKN2A expression in COAD, LIHC, and UCEC patients categorized by various features

The UALCAN database was employed to investigate CDKN2A expression in COAD, LIHC, and UCEC samples, stratified by attributes such as patient age, ethnicity, and clinical stages. Significant (*P*-value < 0.05) upregulation of CDKN2A expression was observed, and this expression demonstrated correlations with clinicopathological characteristics, including patient age, ethnicity, and cancer staging (**Figure 3**).

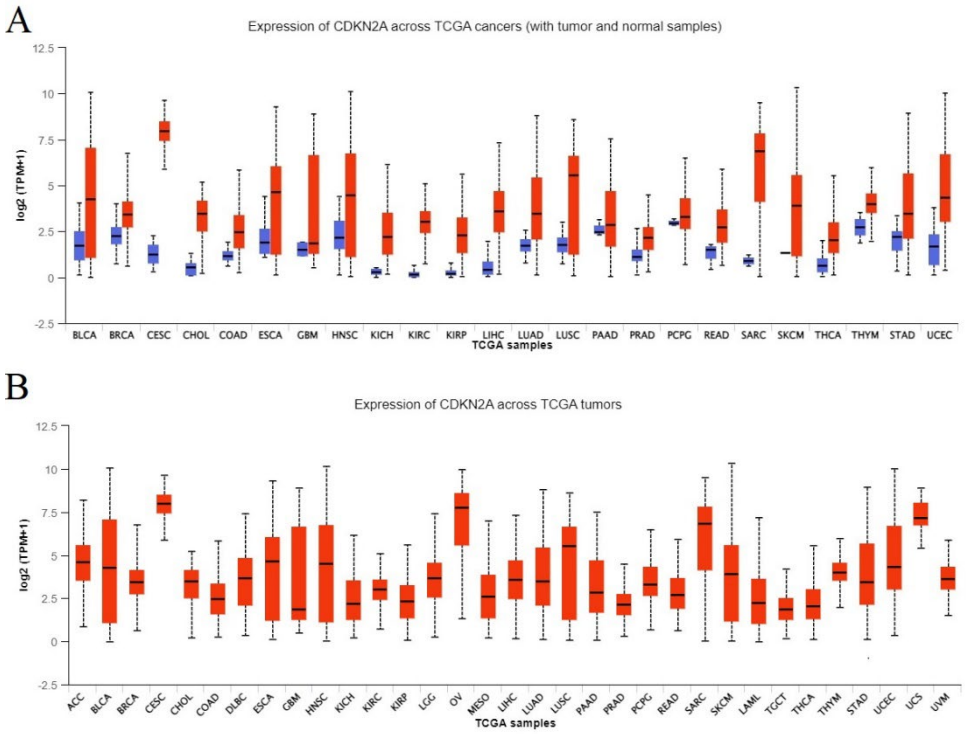

Figure 1. Differential transcription expression analysis of the CDKN2A gene via pan-cancer analysis using UALCAN from the TCGA database. (A) Analysis of CDKN2A between tumor and normal samples via UALCAN from the TCGA database. (B) Pan-cancer expression analysis results of CDKN2A in cancerous samples. **P* < 0.05 was considered significant

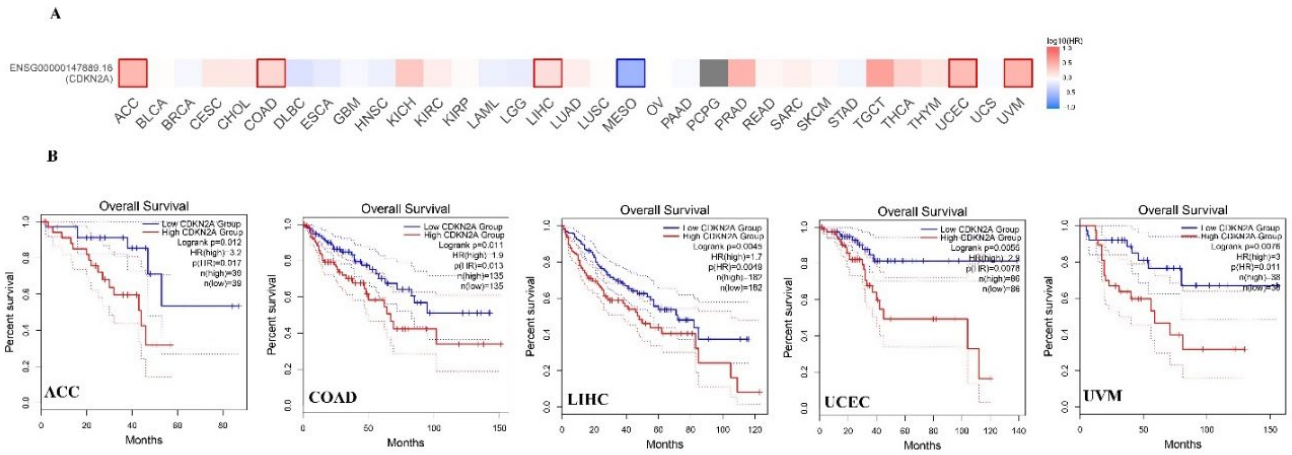

Figure 2. Pan-cancer prognosis analysis of CDKN2A expression. (A) The survival map based on CDKN2A expression across 33 cancer types. (B) The impact of CDKN2A expression on overall survival. **P*-value < 0.05

3.4. Analysis of CDKN2A promoter methylation levels

Dysregulated DNA methylation, including localized hypermethylation and genomic hypomethylation, is associated with various diseases, particularly cancer [40,41]. In this study, the UALCAN database was employed to examine CDKN2A promoter methylation levels in COAD, LIHC, and UCEC samples compared to normal tissues. The results indicated that CDKN2A was hypomethylated in COAD, LIHC, and UCEC samples, revealing a positive correlation between CDKN2A methylation and its expression levels (**Figure 4**).

3.5. Genetic alterations of CDKN2A

Genetic alterations are linked to cancer progression. In this study, cBioPortal was used to assess genetic modifications related to CDKN2A in COAD, LIHC, and UCEC samples. The results showed that CDKN2A mutations occurred in 1.8% of COAD, 8% of LIHC, and 0.8% of UCEC samples, with observed mutations including truncating, missense, amplification, and deep deletion (**Figure 5**). These findings provide insights into potential mutational pathways underlying cancer progression.

3.6. Analysis of immune cell infiltration

Immune cell infiltration significantly influences cancer initiation and progression [42]. TIMER2.0 was used to evaluate the relationship between CDKN2A expression and immune cell infiltration, including $CD8^+$ T cells, $CD4^+$ T cells, and B cells. In UCEC, an inverse relationship between CDKN2A expression and $CD8^+$ and $CD4^+$ T cells was observed, while weak correlations were found in LIHC and COAD (**Figure 6A–B**). Additionally, no correlation was detected between CDKN2A expression and B cell infiltration in UCEC, COAD, and LIHC (**Figure 6C**).

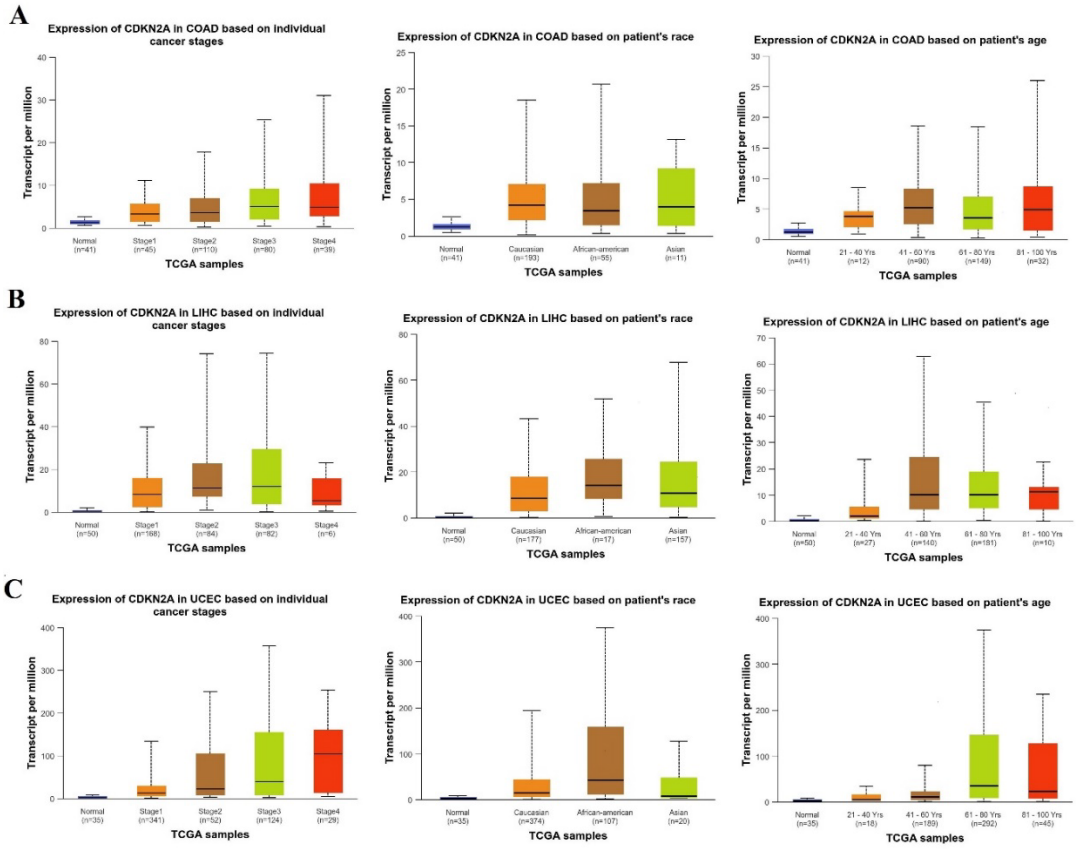

Figure 3. CDKN2A expression in COAD, LIHC, and UCEC samples based on various attributes, including patient age, race, and cancer stage. (A) CDKN2A expression in COAD samples. (B) CDKN2A expression in LIHC samples. (C) CDKN2A expression in UCEC samples

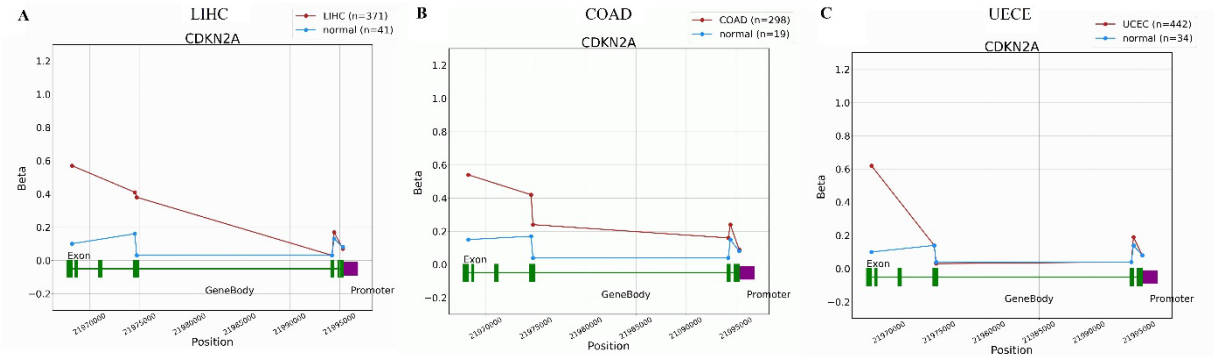

Figure 4. Correlation between CDKN2A mRNA expression and promoter methylation status in COAD, LIHC, and UCEC using the OncoDB database. Significance level = *P*-value < 0.05

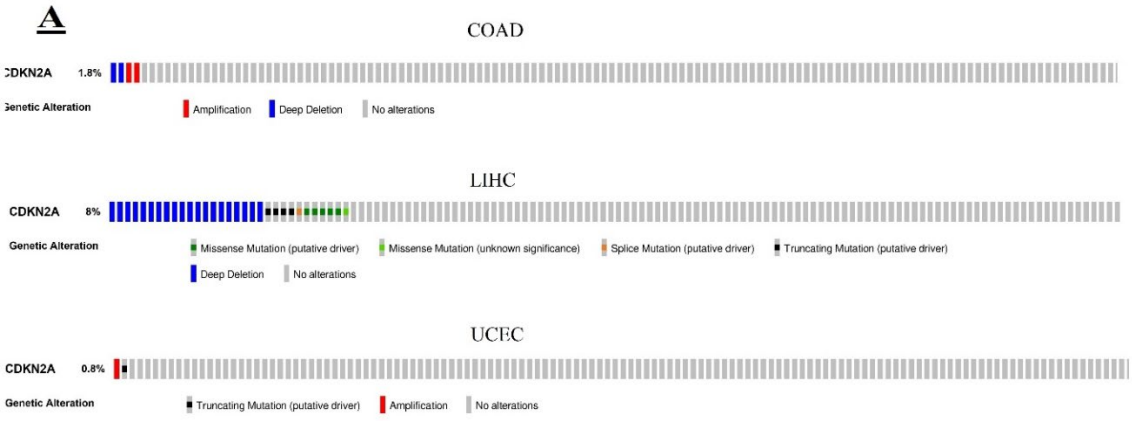

Figure 5. Insights into the mutational pathways underlying the progression of COAD, LIHC, and UCEC cancers

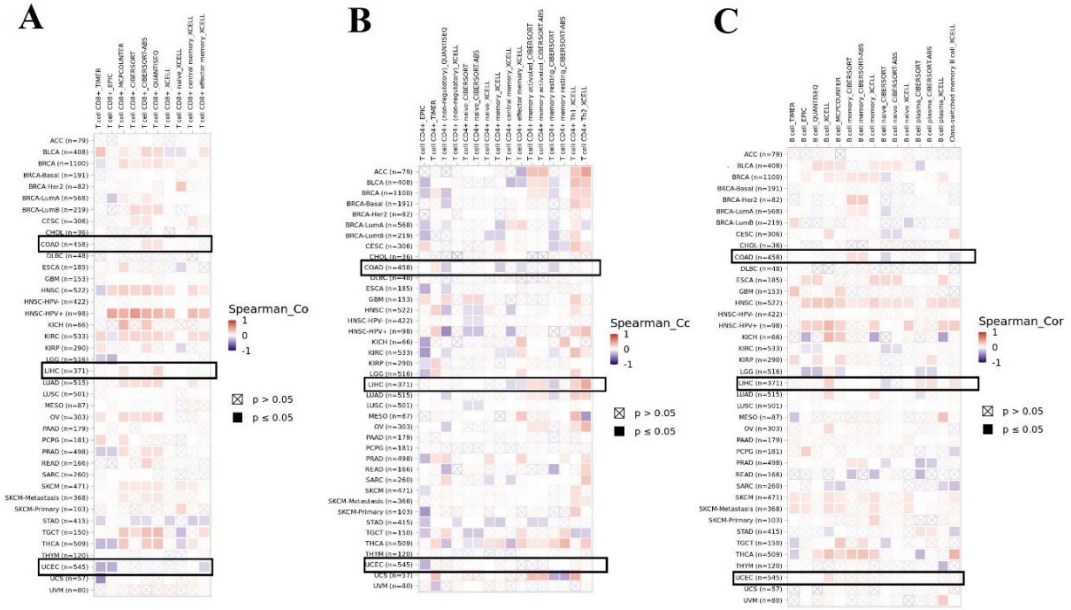

Figure 6. Correlation between CDKN2A expression and immune cell infiltration in COAD, LIHC, and UCEC samples. (A) Correlation with $CD8^+$ T cell infiltration. **(B)** Correlation with $CD4^+$ T cell infiltration. **(C)** Correlation with B cell infiltration. **P*-value < 0.05.

3.7. Protein-protein interaction (PPI) network and gene enrichment analysis

Further analysis was conducted to explore the molecular mechanisms associated with CDKN2A. The PPI network revealed ten CDKN2A-associated proteins: TP53, CCND1, CCND2, MDM2, CDK6, KRAS, CDK2, CDK4, MYC, and NPM1 (**Figure 7**). Gene Ontology (GO) and KEGG pathway enrichment analysis indicated that CDKN2A-linked genes were involved in processes such as the G1/S transition, G2/M transition, cellular senescence, Ras protein signaling, and cell division (**Table 1**).

Table 1. Enrichment analysis results for CDKN2A-associated genes

Gene term	Gene count	*P*-value	Genes
Biological processes			
GO:0000082~G1/S transition of mitotic cell cycle	6	6.050814181752417E-11	CDK6, CCND2, CCND1, CDK4, MYC, CDK2
GO:0010389~regulation of G2/M transition of mitotic cell cycle	4	1.871658073473485E-8	CDK6, CDKN2A, CDK4, CDK2
GO:0090398~cellular senescence	4	1.8957134610072728E-6	NPM1, CDKN2A, CDK2, TP53
GO:0007265~Ras protein signal transduction	4	7.304047046494659E-6	CDKN2A, CDK2, KRAS, TP53
GO:0051301~cell division	5	1.663144962206214E-5	CDK6, CCND2, CCND1, CDK4, CDK2
Cellular components			
GO:0000307~cyclin-dependent protein kinase holoenzyme complex	5	2.1409941400482225E-9	CDK6, CCND2, CCND1, CDK4, CDK2
GO:0005813~centrosome	6	3.815763739803654E-6	NPM1, CDK6, CCND2, CCND1, CDK2, TP53
GO:0005737~cytoplasm	10	9.096498278877434E-6	NPM1, CDK6, CCND2, CCND1, CDKN2A, CDK4, MYC, CDK2, KRAS, TP53
GO:0005654~nucleoplasm	9	1.4245291009844775E-5	NPM1, CDK6, CCND2, CCND1, CDKN2A, CDK4, MYC, CDK2, TP53
GO:0005730~nucleolus	6	1.3473568936354612E-4	NPM1, CCND2, CDKN2A, CDK4, MYC, TP53
Molecular function			
GO:0001046~core promoter sequence-specific DNA binding	3	2.3108479185177537E-5	NPM1, MYC, TP53
GO:0016538~cyclin-dependent protein serine/threonine kinase regulator activity	3	7.793826870580279E-5	CCND2, CCND1, CDK4
GO:0004693~cyclin-dependent protein serine/threonine kinase activity	3	7.793826870580279E-5	CDK6, CDK4, CDK2
GO:0030332~cyclin binding	3	1.1405424441090305E-4	CDK6, CDK4, CDK2
GO:0019901~protein kinase binding	4	0.0013063490610775586	NPM1, CCND2, CCND1, CDKN2A
KEGG pathway			
hsa04218:Cellular senescence	9	8.283955501554002E-15	CDK6, CCND2, CCND1, CDKN2A, CDK4, MYC, CDK2, KRAS, TP53
hsa04110:Cell cycle	8	4.022392188086772E-12	CDK6, CCND2, CCND1, CDKN2A, CDK4, MYC, CDK2, TP53
hsa04115:p53 signaling pathway	7	8.407192281962224E-12	CDK6, CCND2, CCND1, CDKN2A, CDK4, CDK2, TP53
hsa05220:Chronic myeloid leukemia	7	9.895313060617844E-12	CDK6, CCND1, CDKN2A, CDK4, MYC, KRAS, TP53
hsa05203:Viral carcinogenesis	8	2.5566041564744064E-11	CDK6, CCND2, CCND1, CDKN2A, CDK4, CDK2, KRAS, TP53

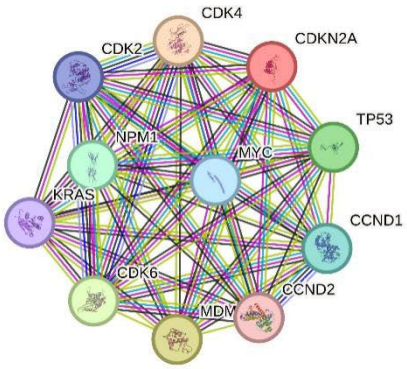

Figure 7. PPI network of CDKN2A using the STRING tool

3.8. Drug sensitivity analysis of CDKN2A

The correlation between CDKN2A mRNA expression and the efficacy of various therapeutic agents was investigated using the Gene Set Cancer Analysis (GSCA) database. The results revealed a strong positive correlation between CDKN2A expression and drug sensitivity to agents such as (5Z)-7-Oxozeaenol, Bleomycin, Cytarabine, Nutlin-3a (-), PD-0332991, and Midostaurin (**Figure 8**). CDKN2A emerged as a critical gene significantly associated with drug sensitivity, especially for drugs with an FDR of less than 0.05, making it a promising therapeutic target for treating COAD, LIHC, and UCEC.

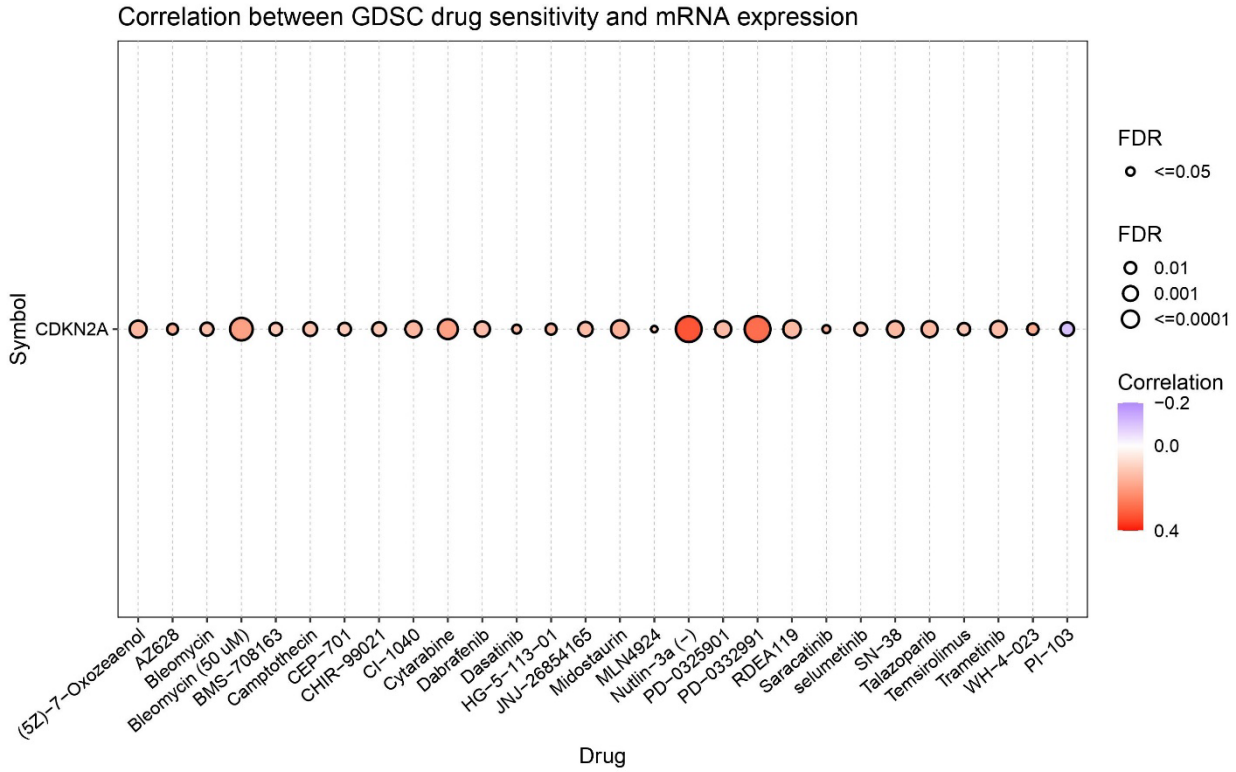

Figure 8. Drug sensitivity analysis using the GSCA database for CDKN2A. Blue indicates a negative correlation, while red indicates a positive correlation. **P*-value < 0.05.

4. Discussion

Cancer remains a major global cause of death, posing significant challenges to effective treatment and adversely impacting health [43]. The identification and validation of biomarkers associated with different cancer types are crucial for improving the detection and treatment of malignancies. CDKN2A, located on chromosome 9p21.3, encodes a cyclin-dependent kinase inhibitor and is categorized as a tumor suppressor gene, playing a pivotal role in cell cycle regulation. It encodes two critical proteins, p16INK4a and p14ARF, whose mutations can lead to uncontrolled cell division, contributing to tumor development [44,45]. Mutations in CDKN2A have been linked to various cancers, including melanoma, pancreatic cancer, glioblastoma, bladder cancer, and HNSC [46-49]. Understanding its role in cancer biology not only advances knowledge of carcinogenesis but also opens the door to research into its potential as a diagnostic and prognostic biomarker, as well as a therapeutic target.

The present study demonstrated that CDKN2A was significantly upregulated in the tissues of all 24 major cancer types ($P < 0.05$), including BLCA, ESCA, HNSC, COAD, LIHC, UCEC, and UVM compared to normal control samples. Additionally, the analysis revealed a strong correlation between upregulated CDKN2A expression and lower OS in UCEC, COAD, and LIHC. These findings suggest that CDKN2A plays a critical role in the initiation, development, and progression of UCEC, COAD, and LIHC. Therefore, these three cancer subtypes were the focus of further investigation in this study.

Subsequent analysis of CDKN2A expression across various clinicopathological features, including cancer stages, patient races, genders, and ages in UCEC, COAD, and LIHC, revealed notable overexpression in tumor samples compared to normal controls. Various factors, such as methylation profiles and genetic alterations, have been shown to significantly modulate gene expression [50,51]. Based on this, the present study investigated the genetic mutations and promoter methylation levels of CDKN2A in UCEC, COAD, and LIHC using the OncoDB and cBioPortal databases. The results revealed a positive correlation between CDKN2A expression and promoter methylation, emphasizing the complexity of gene regulation and the involvement of various factors. Furthermore, the analysis of CDKN2A mutations in COAD, LIHC, and UCEC revealed low mutation frequencies of 1.8%, 8%, and 0.8%, respectively. These findings suggest that while hypermethylation may have a significant impact on expression regulation, genetic mutations likely play a minor or negligible role in regulating CDKN2A expression in these cancers.

Immune cell infiltration plays a crucial role in tumor proliferation, metastasis, and invasiveness, influencing clinical outcomes and immunotherapy responses [52,53]. This study explored the relationship between CDKN2A expression and immune cell infiltration in UCEC, COAD, and LIHC using TIMER2.0. The results indicated a negative correlation between CDKN2A expression and the infiltration of $CD8^+$ and $CD4^+$ T cells in UCEC, while no correlation was observed between CDKN2A expression and the infiltration of $CD8^+$ T cells, $CD4^+$ T cells, or B cells in COAD and LIHC. These findings suggest that CDKN2A may have a specific role in immune modulation in UCEC, while its influence on immune cell infiltration in COAD and LIHC appears limited.

The PPI network analysis for CDKN2A identified direct interactions with ten genes and revealed significant enrichment of genes involved in the G1/S transition of the mitotic cell cycle, regulation of the G2/M transition, Ras protein signal transduction, and cell division ($P < 0.05$). The cyclin-dependent protein kinase holoenzyme complex and cyclin-dependent protein serine/threonine kinase regulator activity were among the enriched biological processes, molecular functions, and cellular components, along with KEGG terms related to the cell cycle, signaling pathways, chronic myeloid leukemia, and viral carcinogenesis. These results suggest that CDKN2A may be integrated into multiple pathways, modulating associated genes involved in tumorigenesis. Pathways such as the G1/S transition of the mitotic cell cycle and regulation of the G2/M transition are crucial for genomic replication, growth, and segregation [54-57]. Disruptions in these pathways have also been associated with adverse prognoses in cancer [55,58].

Moreover, analysis using the GSCA database revealed a strong correlation between CDKN2A mRNA expression and drug sensitivity, indicating a positive association with therapeutic agents such as (5Z)-7-Oxozeaenol, Bleomycin, Cytarabine, Nutin-3a (-), PD-0332991, and Midostaurin. These findings suggest that CDKN2A may serve as a predictive biomarker for favorable responses to specific therapies in UCEC, COAD, and LIHC, highlighting its potential as a therapeutic target.

5. Conclusion

This study demonstrated significant upregulation of CDKN2A in UCEC, COAD, and LIHC samples, which was associated with poorer prognoses in these cancers. The correlation between CDKN2A upregulation and the development and progression of UCEC, COAD, and LIHC suggests that CDKN2A may serve as a common diagnostic and prognostic biomarker for these cancer types. However, further research is required to validate these findings and establish their clinical relevance.

Disclosure statement

The authors declare no conflict of interest.

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