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Original Article

Up-regulation of GINS1 highlighted a good diagnostic and prognostic potential of survival in three different subtypes of human cancer

A regulação positiva de GINS1 destacou um bom potencial diagnóstico e prognóstico de sobrevivência em três subtipos diferentes de câncer humano

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Abstract

Cancer is a fatal malignancy and its increasing worldwide prevalence demands the discovery of more sensitive and reliable molecular biomarkers. To investigate the GINS1 expression level and its prognostic value in distinct human cancers using a series of multi-layered *in silico* approach may help to establish it as a potential shared diagnostic and prognostic biomarker of different cancer subtypes. The GINS1 mRNA, protein expression, and promoter methylation were analyzed using UALCAN and Human Protein Atlas (HPA), while mRNA expression was further validated via GENT2. The potential prognostic values of GINS1 were evaluated through KM plotter. Then, cBioPortal was utilized to examine the GINS1-related genetic mutations and copy number variations (CNVs), while pathway enrichment analysis was performed using DAVID. Moreover, a correlational analysis between GINS1 expression and CD8+ T immune cells and a the construction of gene-drug interaction network was performed using TIMER, CDT, and Cytoscape. The GINS1 was found down-regulated in a single subtypes of human cancer while commonly up-regulated in 23 different other subtypes. The up-regulation of GINS1 was significantly correlated with the poor overall survival (OS) of Liver Hepatocellular Carcinoma (LIHC), Lung Adenocarcinoma (LUAD), and Kidney renal clear cell carcinoma (KIRC). The GINS1 was also found up-regulated in LIHC, LUAD, and KIRC patients of different clinicopathological features. Pathways enrichment analysis revealed the involvement of GINS1 in two diverse pathways, while few interesting correlations were also documented between GINS1 expression and its promoter methylation level, CD8+ T immune cells level, and CNVs. Moreover, we also predicted few drugs that could be used in the treatment of LIHC, LUAD, and KIRC by regulating the GINS1 expression. The expression profiling of GINS1 in the current study has suggested it a novel shared diagnostic and prognostic biomarker of LIHC, LUAD, and KIRC.

Keywords: cancer, diagnostic, CD8+ T immune cells, GINS1, OS analysis, prognostic.

Resumo

O câncer é uma doença maligna fatal e sua crescente prevalência mundial exige a descoberta de biomarcadores moleculares mais sensíveis e confiáveis. Investigar o nível de expressão de GINS1 e seu valor prognóstico em cânceres humanos distintos, usando uma série de abordagens *in silico* em várias camadas, pode ajudar a estabelecê-lo como um potencial biomarcador de diagnóstico e prognóstico compartilhado de diferentes subtipos de câncer. O mRNA de GINS1, a expressão da proteína e a metilação do promotor foram analisados usando UALCAN e Human Protein Atlas (HPA), enquanto a expressão de mRNA foi posteriormente validada via GENT2. Os valores prognósticos potenciais de GINS1 foram avaliados por meio do plotter KM. Em seguida, o cBioPortal foi utilizado para examinar as mutações genéticas relacionadas ao GINS1 e as variações do número de cópias (CNVs), enquanto a análise de enriquecimento da via foi realizada usando DAVID. Além disso, uma análise correlacional entre a expressão de GINS1 e células imunes T CD8 + e a construção de uma rede de interação gene-droga foi realizada usando TIMER, CDT e Cytoscape. O GINS1 foi encontrado regulado negativamente em um único subtipo de câncer humano, enquanto comumente

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regulado positivamente em 23 outros subtipos diferentes. A regulação positiva de GINS1 foi significativamente correlacionada com a sobrevida global pobre (OS) de Carcinoma Hepatocelular de Fígado (LIHC), Adenocarcinoma de Pulmão (LUAD) e Carcinoma de Células Claras Renais de Rim (KIRC). O GINS1 também foi encontrado regulado positivamente em pacientes LIHC, LUAD e KIRC de diferentes características clínico-patológicas. A análise de enriquecimento de vias revelou o envolvimento de GINS1 em duas vias diversas, enquanto poucas correlações interessantes também foram documentadas entre a expressão de GINS1 e seu nível de metilação do promotor, nível de células imunes T CD8 + e CNVs. Além disso, também previmos poucos medicamentos que poderiam ser usados no tratamento de LIHC, LUAD e KIRC, regulando a expressão de GINS1. O perfil de expressão de GINS1 no estudo atual sugeriu que é um novo biomarcador de diagnóstico e prognóstico compartilhado de LIHC, LUAD e KIRC.

Palavras-chave: câncer, diagnóstico, células imunes T CD8 +, GINS1, análise de OS, prognóstico.

1. Introduction

Cancer is one of the most common causes of mortality worldwide (Ma and Yu, 2006). In 2020, a total of 19.3 million new cancer cases were reported worldwide (Sung et al., 2021). Cancer developing risk factors include the lack of physical activity, obesity, alcohol intake, hormonal imbalance, getting older, familial history of cancer, and early menstruation, etc. (Ataollahi et al., 2015). Due to mutations and other epigenetic changes (especially change in promoter methylation level), the abnormal expression of different tumor suppressor and DNA repair genes such as BRCA, APC, ATM, and TP53 genes highly increase the chances of cancer development (Lahtz and Pfeifer, 2011). Despite the substantial improvements in cancer diagnosis, prevention, and treatment methods like surgery, chemotherapy, and radiotherapy, oncologists and clinicians are still unable to eradicate this malignancy which kills thousands of people worldwide each year.

The use of molecular biomarkers for the early diagnosis of cancer, measuring prognosis, and monitoring a therapeutic response has increased recently (Goossens et al., 2015). Information obtained from these biomarkers in serum, cancer cells, and tissues can help in the understanding of tumorigenesis, and metastasis processes (Nagpal et al., 2016). For example, Hassan et al. in their recent study have suggested that elevated expression level of MMP3 in lobular and ductal carcinoma could be a potential biomarker for the detection of recurrence (Hassan et al., 2015). In addition, they also revealed that MMP3 expression is significantly correlated with tumor grades (Hassan et al., 2015). Taken together, the development of diagnostic assays based on the molecular biomarkers knowledge in serum, cancer cells, and tissues enables clinicians to make accurate decision-making regarding cancer treatment. Keeping in view the higher cancer-associated death rate worldwide, it is, therefore, necessary and urgent to discover some common diagnostic and prognostic biomarkers of cancer patients to better manage the disease.

GINS1 is an essential component of the GINS complex which is required for the completion of the DNA replication process (Zhang et al., 2015). Previous studies have reported the up-regulation of GINS1 in few human cancers including cancer of the lung and breast (Zhang et al., 2015). However, best to our knowledge, the role of GINS1 in different other subtypes of human cancers is yet to uncover.

In the present study, the GINS1 expression profiling and survival analysis was done in distinct human cancer subtypes through a series of *in silico* analysis. The findings of our study have provided some useful information regarding the correlation between GINS1 expression and its diagnostic and prognostic values in Liver Hepatocellular Carcinoma (LIHC), Lung Adenocarcinoma (LUAD), and Kidney renal clear cell carcinoma (KIRC).

2. Material and Methods

2.1. The UALCAN based analysis

UALCAN (http://ualcan.path.uab.edu/index.html) is an effective tool for analyzing cancer multi-omics data. It has some user-friendly features such as it provides an easy access to cancer multi-omics data (e.g microarray sequencing data), and also drawn graphs and plots showing gene expression (Chandrashekar et al., 2017). We utilized this database for the pan-cancer differential expression analysis and promoter methylation analysis of GINS1 across multiple human cancer subtypes of different clinicopathological features. In UALCAN, the mRNA expression level was normalized as transcript per million (TPM) reads while promoter methylation level was normalized as beta (β) value. Different cut-off β-values were used to demonstrate the hyper-methylation (β-value: 0.17 - 0.15) and hypo-methylation (β value: 0.13 - 0.12). For statistics purpose, a student's t-test was employed in UALCAN, and a value of $P < 0.05$ was considered statistically significant.

2.2. KM plotter based analysis

The prognostic evaluation of GINS1 expression level in distinct cancer subtypes was done using KM plotter databases. The median value of GINS1 expression was used as a cut-off criterion to classify the high or low GINS1 expression patients (Maciejczyk et al., 2013). A p-value, 95% confidence interval (CI), and hazard ratio (HR) were determined and displayed.

2.3. Transcription expression validation of GINS1 using independent cohorts of LIHC, LUAD, and KIRC

Gene Expression Database of Normal and Tumor Tissues 2 (GENT2) was accessed to further validate the transcription expression level of GINS1 (Park et al., 2019). In GENT2, the tissue-wide GINS1 expression profile was obtained from several integrated Affymetrix U133Plus2 TCGA microarray datasets and visualized as boxplots. The

statistically significant difference in GINS1 expression between normal and cancer tissues were evaluated using a two-sample T-test and a p-value of less than 0.05 was considered statistically significant.

2.4. Data mining through Human Protein Atlas (HPA) database

The Human Protein Atlas (HPA) database was launched to analyze all the known human proteins present in the organs, tissue, and cells through Immunohistochemical staining (Uhlén et al., 2015). In the current study, the Immunohistochemical staining of GINS1 in different human cancer subtypes was obtained using HPA. A p-value(<0.05) was considered statistically significant.

2.5. The cBioportal database

The cBioPortal tool was launched to analyze the TCGA multi-omics data (Cerami et al., 2012). In our study, this tool was used to analyze the GINS1-associated copy number variations (CNVs) and genetic alteration in TCGA datasets of distinct cancer subtypes.

2.6. PPI network construction, visualization and pathway enrichment analysis

Search Tool for the Retrieval of Interacting Genes/Proteins database (STRING v10.5) was launched to construct the PPI network of the genes of interest (Von Mering et al., 2003). On given the list of proteins of interest as an input in the query box, the STRING database searches the interacting partners of these proteins and then draws the PPI network of all the interactions between proteins. (Von Mering et al., 2003). In our study, we utilized STRING to construct the PPI network of the GINS1-associated genes. This PPI network was then visualized by Cytoscape software (Shannon et al., 2003). The KEGG pathway analysis of the GINS1 enriched dataset of the genes was performed through an online tool, DAVID (Huang et al., 2007), and a p value<0.05 was considered as significant.

2.7. GINS1 and infiltrating level of CD8+ T cells in LIHC, LUAD, and KIRC patients

TIMER database was launched to provide immune infiltrates analysis across TCGA RNA-seq based expression datasets of different cancers (Li et al., 2020). In our study, TIMER was used to evaluate the Spearman correlation between the GINS1 expression and CD8+ T immune cells in LIHC, LUAD, and KIRC patients. A p value<0.05 was considered as significant.

2.8. GINS1 gene-drug interaction network analysis

The Comparative Toxicogenomics Database (CTD) was used to retrieve the information of chemotherapeutic drugs that could reduce or enhance the mRNA or protein expression levels of the genes of interest (Mattingly et al., 2003). In this study, GINS1 was searched in the CTD database and a gene-drug interaction network was visualized using Cytoscape software to identify the potential drugs that can influence the expression regulation of GINS1.

3. Results

3.1. mRNA expression level analysis of GINS1 in distinct types of human cancers

To find out the differences in GINS1 mRNA expression in tumor and normal tissues, the TCGA expression profiles across tumor samples and their paired normal tissues were utilized through the UALCAN platform. Results demonstrated that GINS1 was down-regulated in kidney chromophobe (KICH) while overexpressed in all the other 23 distinct subtypes of human cancers samples as compared to the normal controls including, Liver Hepatocellular Carcinoma (LIHC), Lung Adenocarcinoma (LUAD), and Kidney renal clear cell carcinoma (KIRC) (Figure 1).

3.2. GINS1 prognostic potential in various types of human cancers

The km plotter online tool was applied to investigate the prognostic potential of GINS1 in different types of cancers. Result revealed that overexpression of GINS1 was significantly (p<0.05) associated with the decreased OS duration of the LIHC (HR = 2.36, 95% CI: 1.64–3.37, P=1.5e-06), LUAD (HR = 1.69, 95% CI: 1.19–2.41, P=0.0033-04), and KIRC patients (HR = 2.09, 95% CI: 1.53–2.85, P=1.8e-06) (Figure 2). Furthermore, the GINS1 dysregulation was also found associated with the OS of patients with distinct other types of cancers but the correlations were insignificant (p>0.05). Altogether, our data suggested that GINS1 might have a significant contribution to the development and progression of LIHC, LUAD, and KIRC, thus the next part of our study will mainly focus on the unique role of GINS1 in these three types of human cancers.

3.3. Re-analysis of GINS1 mRNA expression in cancers showing its significant negative prognostic values

The distinct cancer types (LIHC, LUAD, and KIRC) in which GINS1 has shown a significant (p<0.05) negative correlation with the OS duration were re-analyzed to verify the significance of the GINS1 expression in normal and cancerous samples of different clinicopathological parameters. Results showed that GINS1 was also significantly (p<0.05) overexpressed in all the LIHC, LUAD, and KIRC samples of different clinicopathological parameters including different cancer stages (1, 2, 3 and 4), patients genders (male and female), patients ages (21-40 years, 41-60 years, 61-80 years, and 81-100 years), and nodal metastasis statuses (N0, N1, N2, N3, and N4) as compared to the normal controls (Figure 3-5). In view of these results, we speculated that GINS1 is a clinicopathological parameter-independent biomarker. Furthermore, the clinicopathological distribution of the LIHC, LUAD, and KIRC cohorts are given in Table 1.

3.4. Transcription expression validation of GINS1 using independent cohorts of LIHC, LUAD, and KIRC

GINS1 transcription expression was further validated identified in LIHC, LUAD, and KIRC tissues paired with normal controls via GENT2 databases. In view of our results,

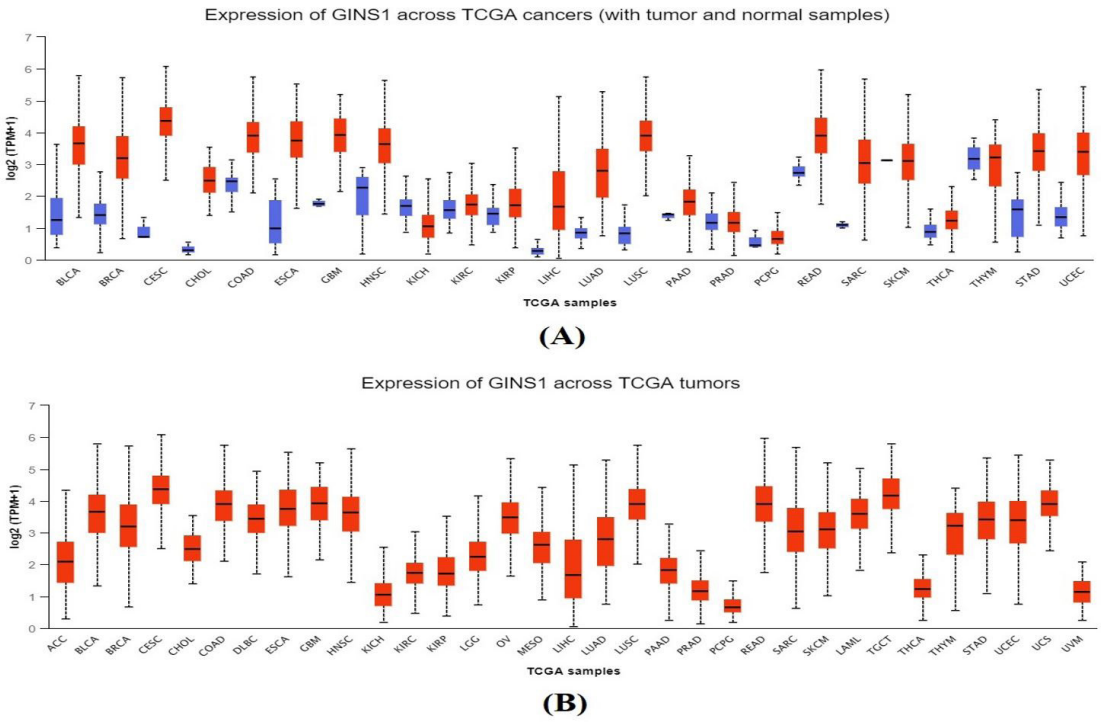

Figure 1. GINS1 expression profile in distinct types of human cancers. (A) with normal controls, (B) without normal controls.

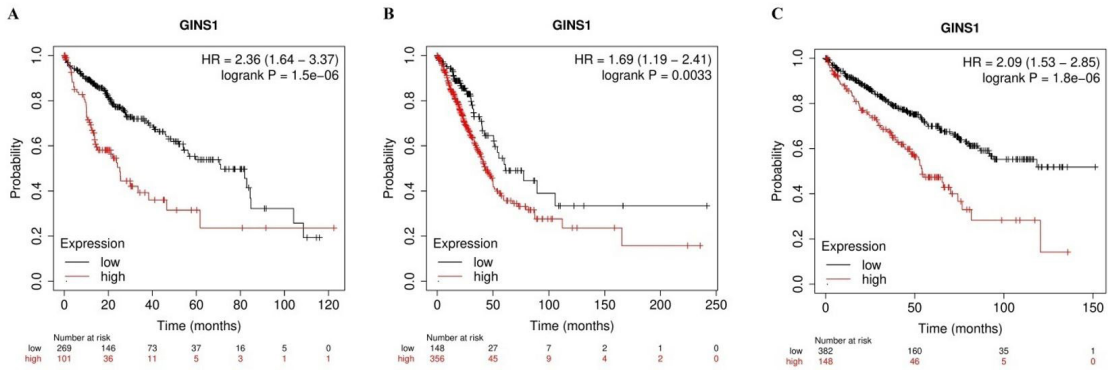

Figure 2. OS analysis of the GINS1 in distinct types of cancers. (A) LIHC, (B) LUAD, and (C) KIRC

after accessing LIHC, LUAD, and KIRC-based microarray data in the GENT2 database, we noticed the significant ($p<0.05$) up-regulation of GINS1 at the transcription level in LIHC, LUAD, and KIRC (Figure 6). Information of the LIHC, LUAD, and KIRC datasets used for GINS1 expression validation is given in Table 2.

3.5. Protein expression level analysis of GINS1 in liver, lung, and kidney cancers

After evaluating the mRNA expression level of GINS1, its proteomics level was accessed using the HPA database. Results revealed that GINS1 was not detected in normal liver tissues whereas its low expression was found in normal lung and kidney tissues. However, relative to normal controls, GINS1 protein was found overexpressed (medium) in liver, lung, and kidney cancerous tissues (Figure 7).

3.6. Promoter methylation analysis of the GINS1 in cancers with its significant overexpression and prognostic values

To assess the level of GINS1 promoter methylation in defined cancer subtypes, we utilized the UALCAN database to investigate the correlation between GINS1 expression and its promoter methylation at the same time. The results have suggested that the promoter methylation level of

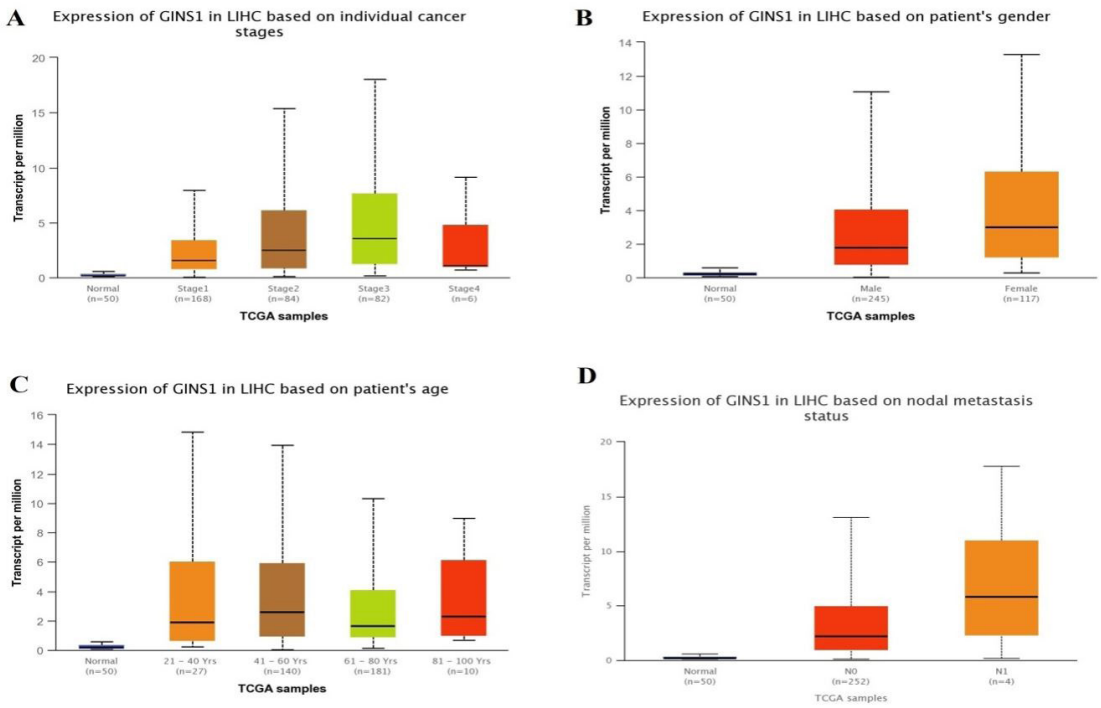

Figure 3. Expression analysis of GINS1 in different clinicopathological parameters of LIHC. (A) Individual cancer stages, (B) Patients gender, (C) Patients age, and (D) Nodal metastasis. A p-value of <0.05 was selected as cutoff criterion.

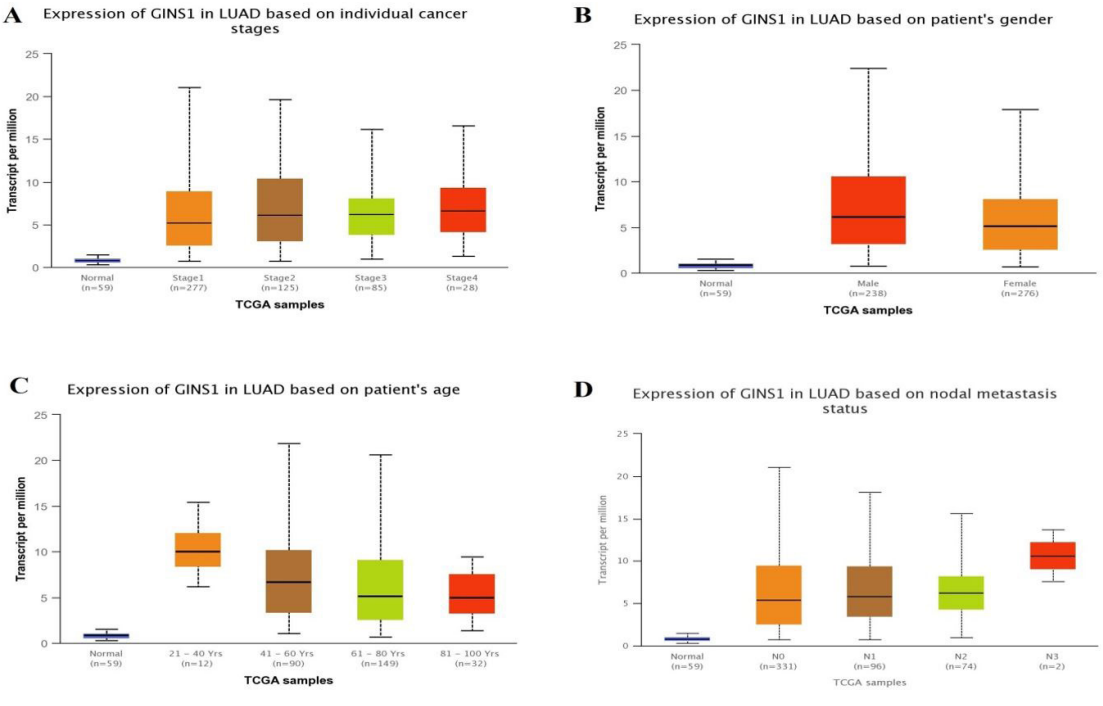

Figure 4. Expression analysis of GINS1 in different clinicopathological parameters of LUAD. (A) Individual cancer stages, (B) Patients gender, (C) Patients age, and (D) Nodal metastasis. A p-value of <0.05 was selected as cutoff criterion.

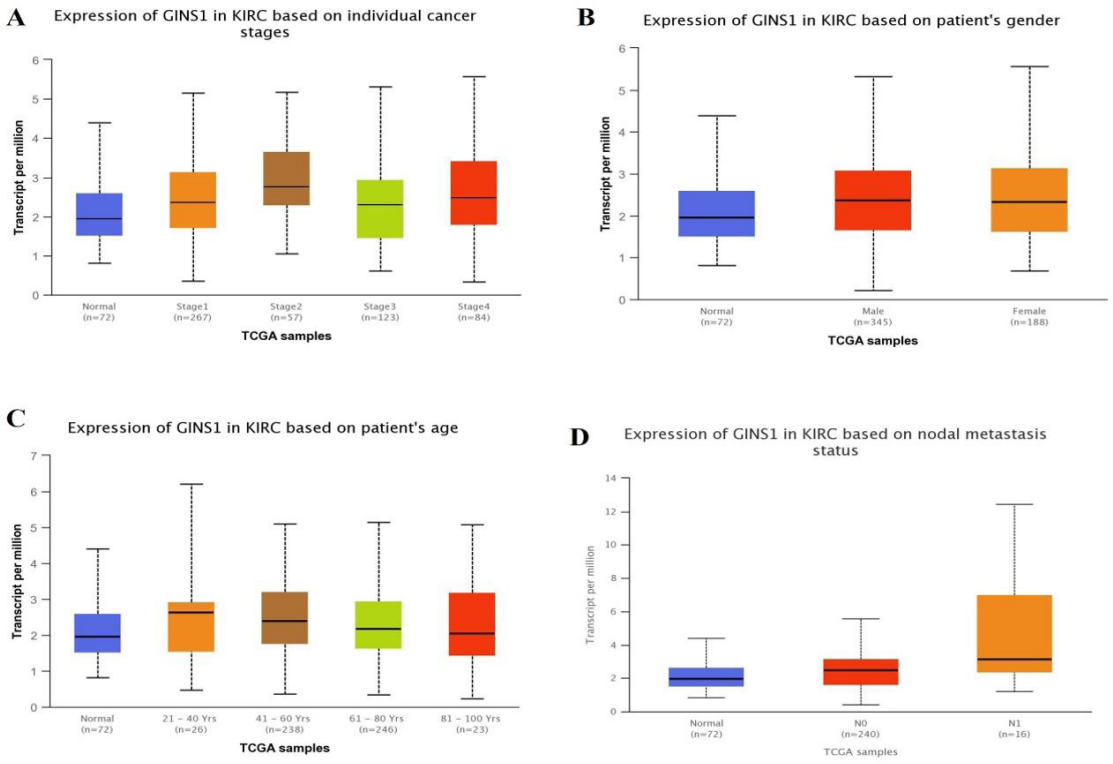

Figure 5. Expression analysis of GINS1 in different clinicopathological parameters of KIRC. (A) Individual cancer stages, (B) Patients gender, (C) Patients age, and (D) Nodal metastasis. A p-value of <0.05 was selected as cutoff criterion.

Table 1. Clinicopathological features of the LIHC and LUAD, KIRC cohorts included in the present study.

Clinicopathological features of the LIHC cohort					
Sr. No	**Clinicopathological Feature**	**No. Samples**	**Total no. of LIHC samples**	**No. Excluded Samples with Missing Information**	**Total No. of Included Samples**
	Cancer stage based distribution				
	Stage 1	168			
	Stage 2	84			
1	Stage 3	82		31	340
	Stage 4	6			
	Gender based distribution				
2	Male	245		9	362
	Female	117	371		
	Age based distribution				
	21-40 years	27			
3	41-60 years	140		13	358
	61-80 years	181			
	81-100 years	10			
	Nodal metastasis based distribution				
4	N0				
	N1	252		115	256
		4			

Table 1. Continued...

Clinicopathological features of the LUAD cohort					
Sr. No	**Clinicopathological Feature**	**No. Samples**	**Total no. of LUAD samples**	**No. Excluded Samples with Missing Information**	**Total No. of Included Samples**
	Cancer stage based distribution				
	Stage 1	277			
	Stage 2	125			
1	Stage 3	85		0	515
	Stage 4	28			
	Gender based distribution		515		
2	Male	238		1	514
	Female	276			
	Age based distribution				
	21-40 years	12			
3	41-60 years	90		232	283
	61-80 years	149			
	81-100 years	32			
	Nodal metastasis based distribution				
	N0	331			
4	N1	96			
	N2	74		13	503
	N3	2			
Clinicopathological features of the KIRC cohort					
Sr. No	**Clinicopathological Feature**	**No. Samples**	**Total no. of KIRC samples**	**No. Excluded Samples with Missing Information**	**Total No. of Included Samples**
	Cancer stage distribution				
1	Stage 1	267			
	Stage 2	57		2	531
	Stage 3	123			
	Stage 4	84			
	Gender distribution				
3	Male	345		0	533
	Female	188	533		
	Age distribution				
	21-40 years	26			
2	41-60 years	238		0	533
	61-80 years	246			
	81-100 years	23			
	Geographical distribution				
4	N0	240		277	256
	N1	16			

GINS1 in LIHC, LUAD, and KIRC samples was significantly ($p>0.05$) lower than normal tissues (Figure 8). Thus, the overall data suggested a negative correlation between GINS1 expression and promoter methylation in LIHC, LUAD, and KIRC samples as compared to the normal controls.

3.7. Copy number variations and mutational analysis of GINS1 in cancers with its significant overexpression and negative prognostic values

Information related to GINS1 genetic alterations including (deep amplification, deep deletion, genetic

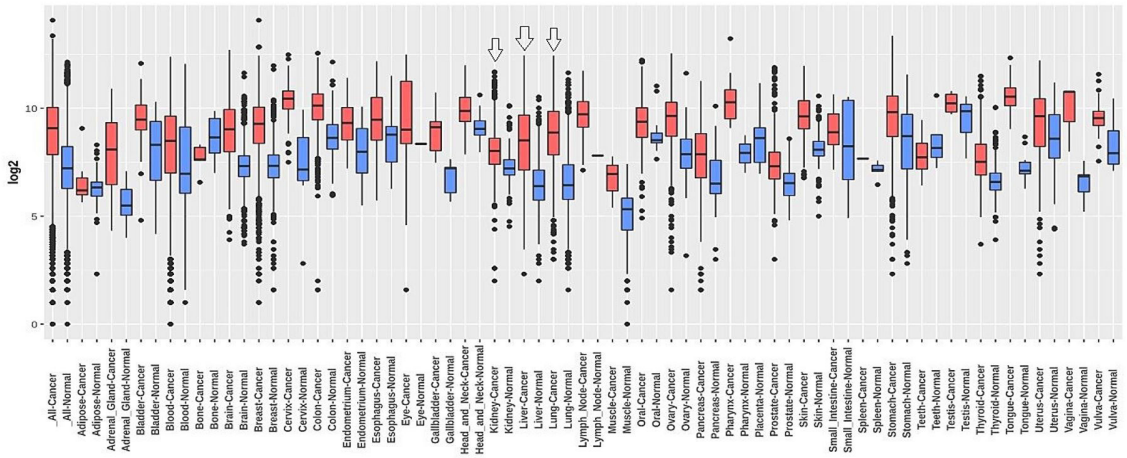

Figure 6. GINS1 transcription expression validation in LIHC, LUAD, KIRC and corresponding normal tissues. GINS1 transcription expression in LIHC, LUAD, KIRC, and normal tissues was accessed using GENT2 database. Red boxplots represent cancer samples, while blue boxplots represent normal samples. A p-value of <0.05 was selected as cutoff criterion.

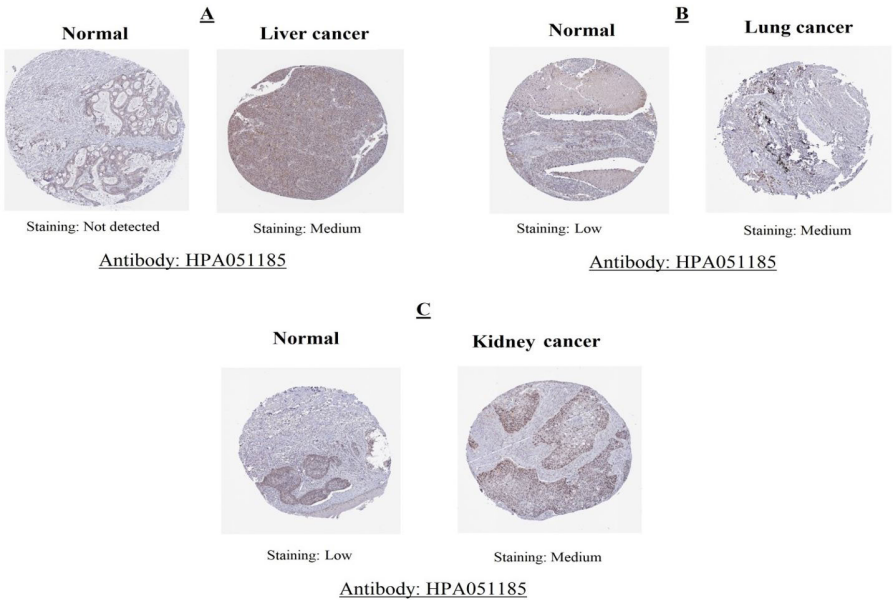

Figure 7. Immunohistochemistry images of GINS1 protein expression in distinct cancer subtypes along with normal controls taken from Human Protein Atlas (HPA) database (×200). (A) Liver cancer, (B) Lung cancer, (C) Kidney cancer.

Table 2. Information of the LIHC, LUAD, and KIRC datasets used for the GINS1 expression validation via GENT2 webserver.

Sr. No	Cancer	Datasets	Source
1	LIHC	GSE45436, GSE49515, GSE2109, GSE58208, GSE6222, GSE62232, GSE6764, GSE75285, GSE9843, GSE40367, and GSE40873, GSE41804	Affymetrix U133A and U133 Plus2 microarray platforms
2	LUAD	GSE40791, GSE37745, GSE2109, GSE43346, GSE43580, GSE50081, GSE30219, GSE63074, GSE64766, GSE77803, GSE10445, GSE19188, GSE27262, GSE33532, GSE40791, GSE5058, and GSE7307	
3	KIRC	GSE2109, GSE46699, GSE47352, GSE53224, GSE53757, GSE7023, GSE68629, GSE7392, GSE8271, GSE11045, GSE11151, GSE12090, GSE12606, GSE14762, GSE19982, GSE22541, GSE36895, GSE53757, and GSE11151	

mutation, and fusion) in LIHC was obtained from a TCGA LIHC (TCGA, Firehose legacy) dataset (consisting of 442 cancerous samples), while in LUAD and KIRC similar information was obtained from TCGA LUAD (TCGA, Firehose legacy) dataset (consisting of 586 cancerous samples), and TCGA KIRC (TCGA, Firehose legacy) dataset (consisting of 538 cancerous samples)

Results revealed that GINS1 harbors genetic alterations (deep amplification) in only 0.3% cases of the LIHC, similarly, in 2.2% cases of LUAD with maximum deep amplification genetic abnormality, and 0.3% cases of KIRC where only 1 case was found positive for truncation mutation (Figure 9).

3.8. PPI network construction, visualization, and pathway enrichment analysis of GINS1

The PPI network of GINS1 was constructed using the STRING database and visualized through Cytoscape software to recognize the set of GINS1 enriched genes. In total one set of 10 GINS1 enriched genes was identified (Figure 10A). We further processed this set of genes for pathway enrichment analysis. The results of pathway enrichment analysis revealed that GINS1 enriched genes were significantly involved in two diverse pathways including "Cell cycle" and "DNA replication" (Figure 10; Table 3).

3.9. GINS1 and infiltrating level of CD8+ T cells in LIHC, LUAD, and KIRC patients

The functions of, and interactions between, the innate and adaptive immune systems are vital for anticancer immunity. Cytotoxic T cells expressing cell-surface CD8+ T are the most powerful effectors in the anticancer immune response and form the backbone of current successful cancer immunotherapies (Ziai et al., 2018). In the current study, the Spearman correlation between the expression of GINS1 and CD8+ T cells has been calculated using the TIMER database. Results revealed a significant ($p>0.05$) positive correlation between the mRNA expression of GINS1 and CD8+ T immune cells level in LIHC, LUAD, and KIRC (Figure 11).

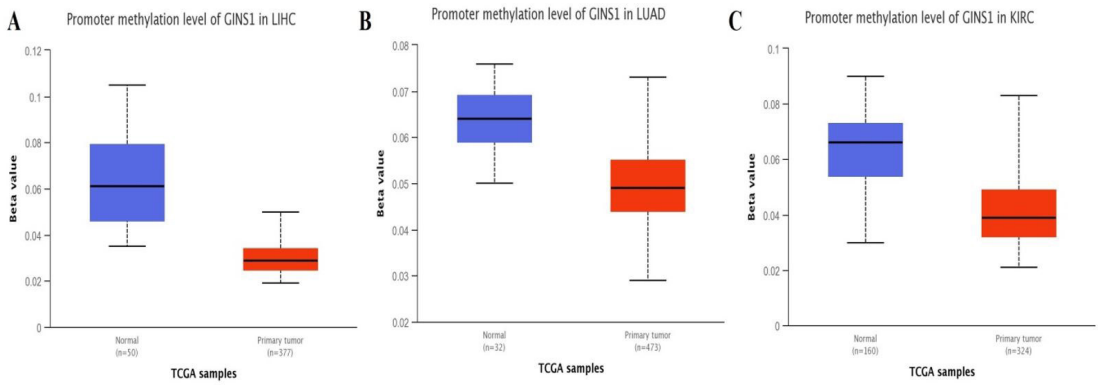

Figure 8. Promoter methylation analysis of GINS1 in LIHC, LUAD, and KIRC. (A) LIHC, (B) LUAD, and (C) KIRC. A p-value of <0.05 was selected as cutoff criterion.

Figure 9. Copy number variations (CNVs) and genetic alterations analysis of the GINS1 in TCGA LIHC, and LUSC datasets, (A) TCGA LIHC dataset, (B) TCGA LUAD dataset, and (C) TCGA KIRC dataset.

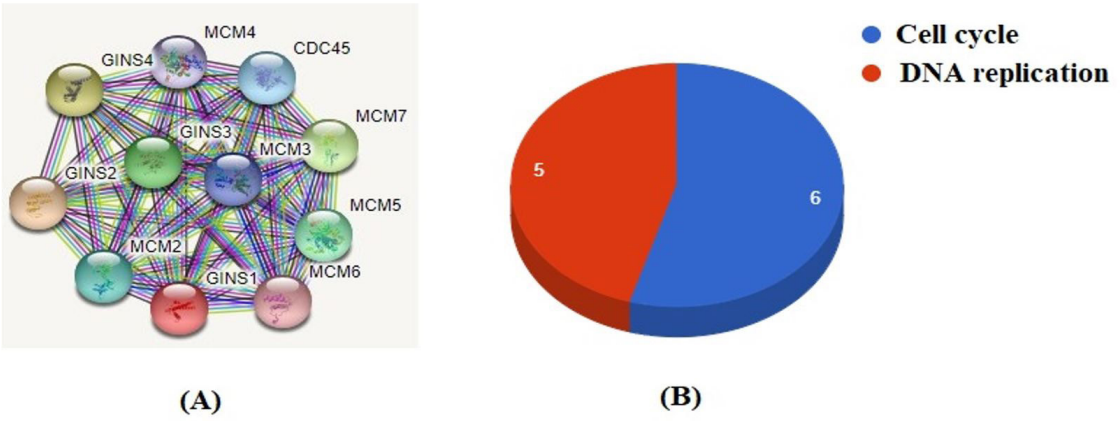

Figure 10. PPI network and Kyoto encyclopedia of genes and genomes (KEGG) pathway analysis of the GINS1 enriched genes. (A) A PPI network of GINS1 enriched genes, (B) KEGG pathway analysis of the GINS1 enriched genes.

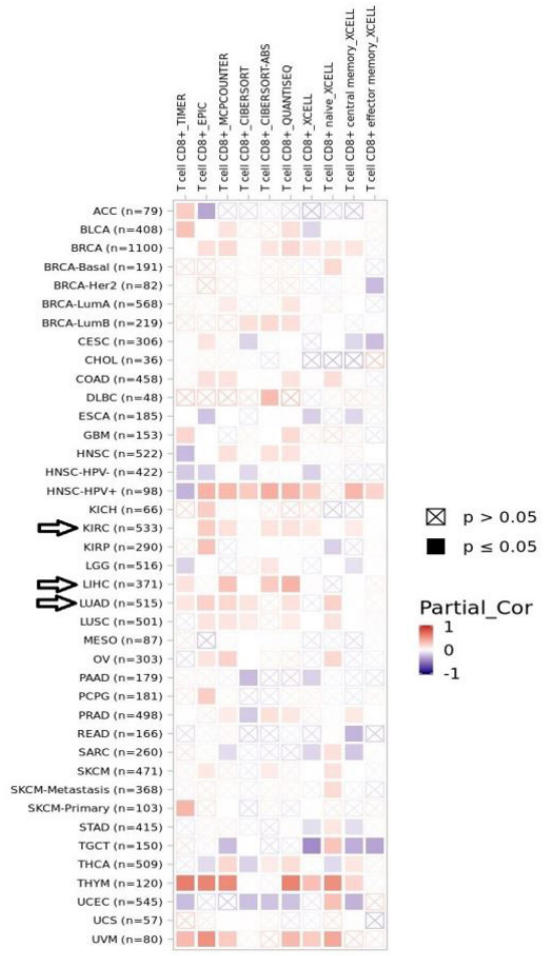

Figure 11. TIMER based Spearman correlational analysis between the GINs1 expression and CD8+ T immune cells level in LIHC, LUAD, and KIRC. A p-value<0.05 was considered to indicate a statistically significant result.

3.10. Gene-drug interaction network analysis of the GINS1

In order to explore the relationship between GINS1 and available cancer therapeutic drugs, a gene-drug interaction network was developed using the CTD database. The expression of GINS1 could potentially influence by a variety of drugs. For example, atrazine and estradiol could elevate the expression level of GINS1 while piroxicam, dasatinib, and cyclosporine could reduce GINS1 expression level (Figure 12).

4. Discussion

Herein, we describe the novel diagnostic and prognostic potential of GINSI in three distinct human cancer subtypes. The GINS1 belongs to the GINS complex which played a key role in the DNA replication process (Chang et al., 2007). Previous studies have shown the major role of GINS1 dysregulation in the development of cancer. For example, Nakahara et al. (2010) have reported that GINS1 overexpressed in breast cancer patients, which was the result of its promoter hypermethylation. Moreover, it was also found that GINS1 strongly relates to various clinical parameters of prostate cancer (PC) including tumor grade, gender, and cancer stages. The GINS1 expression has also been correlated with the poor OS in PC patients by Tahara et al. (2015). Best to our knowledge, until now, GINS1 has not been elucidated in different other cancer subtypes. Therefore, in the present study, we comprehensively analyzed the GINS1 expression and its association with diagnostic and prognostic values of cancer patients through a multi-layered bioinformatics approach.

We revealed that GINS1 was down-regulated in only a single subtype of human cancer while up-regulated in 23 subtypes of human cancers (Figure 1). Results of this study have also shown that overexpression of GINS1 was significantly ($p<0.05$) correlated with the decreased OS duration of LIHC, LUAD, and KIRC patients only. This

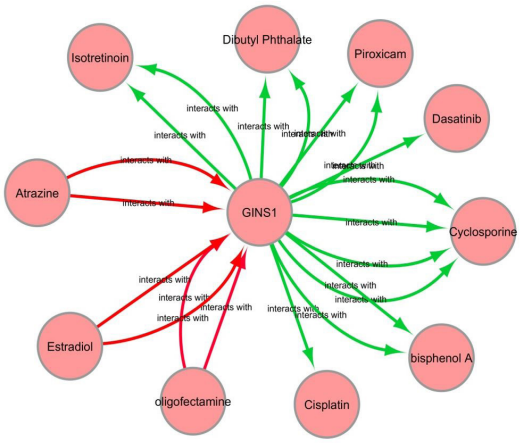

Figure 12. Gene-drug interaction network of the GINS1 and chemotherapeutic drugs. Red arrows: chemotherapeutic drugs increase the expression of GINS1; green arrows: chemotherapeutic drugs decrease the expression of GINS1. The numbers of arrows between chemotherapeutic drugs and key genes in this network represent the supported numbers of literatures by previous reports.

highlighted that GINS1 might have a significant contribution to the development and progression of LIHC, LUAD, and KIRC, thus we only focused on the role of GINS1 in these 3 cancer subtypes.

In view of our results, it was further noticed that GINS1 significantly ($p<0.05$) overexpressed in LIHC, LUAD, and KIRC patients of different clinicopathological features including cancer stages, patient genders, patients ages, and nodal metastasis statuses.

We also tried to explore the possible causes of GINS1 overexpression, for that purpose, we carried out the correlation analysis between GINS1 overexpression and its promoter methylation level, CNVs, and genetic mutations in LIHC, LUAD, and KIRC patients. GINS1 was enriched in the deep amplification abnormality and truncated mutations in insignificant proportion (0.3%, 2.2%, and 0.3% cases) of the LIHC, LUAD, and KIRC patients, respectively. Hence, it is speculated that copy number variations and mutations participate insignificantly in the expression regulation of the GINS1. Furthermore, the results of GINS1 promoter methylation revealed a significantly ($p<0.05$) negative correlation with its expression in LIHC, LUAD, and KIRC patients, this scenario of GINS1 promoter methylation highlighted the significant role of promoter hypomethylation in the overexpression of GINS1.

Early diagnosis is crucial to the patient's survival with LIHC. Currently, various biomarkers including alfa-fetoprotein (AFP), AFP-L3, or Des-γ-carboxyprothrombin (DCP) are being used for LIHC diagnosis and prognosis (Ocker, 2018), however, among all of them serum alfa-fetoprotein (AFP) is the most reliable and commonly used biomarker for LIHC diagnosis; however, its sensitivity and precision are around 50% (Duan et al., 2019). In the present study, we revealed the significant ($p<0.05$) up-regulation of GINS1 expression in LIHC patients of different clinicopathological features (cancer stages, patients genders, patient ages, and nodal metastasis statuses) as compared to the normal controls. We have also shown that GINS1 overexpression is significantly ($p<0.05$) associated with decreased OS of the LIHC patients. Hence, suggested GINS1 up-regulation as a novel diagnostic and prognostic biomarker of LIHC.

Until now, various LUAD specific diagnostic and prognostic biomarkers have been identified by previous studies. For example, Shukla et al. (2016) have carried out the first prognostic biomarkers analysis in LUAD patients through RNA sequencing technique and generated the prognostic feature through the Cox model (Shukla et al., 2016). Subsequently, Li et al. (2017) utilized the RNA sequencing dataset to identify the few immune signatures that can predict the prognosis and OS duration of nonsquamous non-small cell lung cancer patients (Li et al., 2017). Furthermore, Zheng et al. (2017) has used the Cox model and developed an 8-lncRNA diagnostic prognostic signature, which is used as an effective independent prognostic prediction model for LUAD patients (Zheng et al., 2017). In our study, we showed the significant ($p<0.05$) up-regulation of GINS1 expression in LUAD patients of different clinicopathological features (cancer stages, patient genders, patient ages, and nodal metastasis statuses) as compared to the normal controls. Furthermore, GINS1 promoter methylation level and OS information have also proven its useful values as a novel potential biomarker of LUAD patients.

So far, the expression of various genes including ACAA1, ACADSB, ALDH6A1, AUH, HADH, PCCA, and CTLA4 have been significantly correlated with the early diagnosis and survival of the KIRC patients (Zhang et al., 2019; Xiao et al., 2020). In our study, we showed the significant ($p<0.05$) up-regulation of GINS1 expression in KIRC patients of different clinicopathological features (cancer stages, patient genders, patient ages, and nodal metastasis statuses) as compared to the normal controls. Furthermore, GINS1 promoter methylation level and OS information have also proven its useful values as a novel potential diagnostic and prognostic biomarker of KIRC patients.

Immunotherapy has proven very helpful to fight against solid tumors (Chen et al., 2021). For example, chimeric antigen receptor T (CAR-T), dendritic cells (DCs), and CD8+ T cells therapies have shown encouraging results in the personalized cancer treatment (Sabado et al., 2017; Feins et al., 2019; Raskov et al., 2021). Previously Trojan et al. (2004) have successfully used CD8+ T immune cells infiltration for the personalized immunotherapy trials in LSCC (Trojan et al., 2004). However, the drug resistance emergence has instigated us to look for new immune regulatory mechanisms. In this study, GINS1 expression level has shown a positive correlation with CD8+ T cells infiltration. Therefore, it is speculated that GINS1 may contribute to LIHC, LUAD, and KIRC development by regulating immune infiltration in the tumor microenvironment. Taken together, our findings have highlighted the new aspect of the GINS1 in LIHC, LUAD, and KIRC tumorigenesis. Best to our knowledge, this study is the first study to investigate the spearman correlation between the GINS1 expression and CD8+ T immune cells infiltration in LIHC, LUAD, and KIRC. Collectively, these

Table 3. Detail of Kyoto encyclopedia of genes and genomes pathway analysis of the GINS1 enriched genes.

Pathway ID	Pathway Name	Gene count	P-value	Gene name
04110	Cell cycle	6	<0.05	CDC45, MCM7, MCM3, MCM4, MCM5, MCM6
03030	DNA replication	5	<0.05	MCM7, MCM3, MCM4, MCM5, MCM6

findings may bring new ideas for the treatment of LIHC, LUAD, and KIRC patients who do not benefit from the existing immune checkpoint inhibitors/regulators.

In the present study, GINS1 associated genes pathway enrichment analysis revealed their involvement in two important signaling pathways including "Cell cycle" and "DNA replication" (Table 3). Additionally, we also identified few potential drugs that could be useful in the treatment of LIHC, LUAD, and KIRC by regulating the GINS1 expression (Figure 12).

5. Conclusion

In our study, we have reported the unique diagnostic and prognostic potential role of GINS1 in LIHC, LUAD, and KIRC patients. However, further voluminous testing is required to be done in future clinical studies.

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