Trends in Immunotherapy 2024, 8(2), 6804.
https://doi.org/10.24294/ti.v8.i2.6804

Article

Prognostic evaluation of glycogen synthase kinase 3A (*GSK3A*) mRNA expression in colon cancer patients

Neha Sylvia Walter, Jasmeet Kaur*

Department of Biophysics, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh 160012, India
*** Corresponding author:** Jasmeet Kaur, kaur.jasmeet@pgimer.edu.in, jasmeet23k@gmail.com

CITATION

Walter NS, Kaur J. Prognostic evaluation of glycogen synthase kinase 3A (*GSK3A*) mRNA expression in colon cancer patients. Trends in Immunotherapy. 2024; 8(2): 6804. https://doi.org/10.24294/ti.v8.i2.6804

ARTICLE INFO

Received: 31 May 2024
Accepted: 10 July 2024
Available online: 26 July 2024

Abstract: Introduction: GSK3, a multifunctional serine/threonine kinase regulates cell-cycle progression, differentiation and apoptosis and its inhibition can have a tumor suppressor/promoter effect, depending on the cell type. There are conflicting reports of GSK3 in cell growth, but most studies have focused on GSK3β and very few on GSK3α in cancer. GSK3α regulates proliferation of melanoma and pancreatic and colon cancer cells, but the predictive role of *GSK3A* is not known in colon cancer. **Material and methods:** The prognostic role of *GSK3A* was assessed in colon cancer patients employing Kaplan-Meier plotter (KM plotter) database. Online ROC plotter tool was used to compare the *GSK3A* gene expression in colorectal cancer patients receiving any form of chemotherapy. **Results:** Current results show that higher *GSK3A* mRNA expression is significantly related to poorer Relapse Free Survival (RFS) in colon cancer patients. Assessment of *GSK3A* mRNA for different clinicopathological features like clinical stages, TP53 mutation, stage T and stage N highlighted the critical prognostic value of *GSK3A* mRNA in colon cancer. **Discussion and conclusion:** *GSK3A* will help to better predict colon cancer prognosis and to develop better treatment strategies for colon cancer patients and will be beneficial in combating the heterogeneity and complexity of colon cancer.

Keywords: colon cancer; *GSK3A*; relapse-free survival; Kaplan-Meier plotter; ROC plotter

1. Introduction

Colon cancer is one of the most widespread and fatal neoplasms in the world and second leading cause of cancer deaths globally [1]. It is the third most common cancer in the world after breast and lung cancers. The disease is highly heterogeneous in terms of biology and clinical features which result in varied treatment outcomes in different individuals. Thus, the evaluation of molecular mechanisms causing occurrence and progression of colon cancer is required to identify novel prognostic biomarkers for drug targets. This will improve the clinical outcome of colon cancer patients by predicting colon cancer recurrence for personalized treatments regimens.

Glycogen Synthase Kinase 3 (GSK3) as a serine/threonine protein kinase primarily regulates glycogen metabolism [2,3] by inhibiting glycogen synthase. GSK3 has two highly homologous forms in mammals *i.e GSK3A* (51 kDa) and *GSK3B* (47 kDa) [4] having 87% overall identity. Both isoforms are 98% identical in their ATP binding pocket, but differ in their N- and C-terminal domains [2] with distinctive functions/roles in different physiological processes [5]. GSK3 regulates activity of many proteins [4] by altering their

stability by degradation and has a role in cancer, Alzheimer's disease and diabetes [6]. Due to its ability to phosphorylate pro-and anti-oncogenic molecules and availability of small molecule GSK3 inhibitors [7]; GSK3 is a therapeutic target in pancreatic cancer [8], parenchymal renal diseases [9] and HIV-1-associated dementia [10]. As phosphorylation by GSK3 can either suppress or activate a protein, GSK can act either as a tumor promoter or suppressor based on the cell type.

Either redundant or distinct functions of *GSK3A* and *GSK3B* are seen in cell survival, [11–13], but distinct functions are identified during developmental and differentiation processes [14] and in transcriptional activation [15], which varies for the cell type. *GSK3A* null mice are viable with increased sensitivity to glucose and insulin, and decreased fat mass that could not be curbed by β-isoform [16]. Thus, very few studies address the significance of *GSK3A* in cancer development and progression and most studies focus on GSK3β's [17] role in various diseases and cancers. *GSK3A* inhibition regulated drug-resistance and necroptosis induced by chemotherapy in drug-resistant colon carcinoma cells [18]. A recent study has implicated the role of *GSK3A* in colon cancer employing proteomics and phosphoproteomics [19]. Another report by Guil-Luna *et al*. has provided evidence of the clinical importance of GSK-3 expression and tumor budding grade in risk stratification of colorectal cancer patients with inhibition of GSK-3 a potential therapy for colorectal cancer [20]. Another study has also highlighted the role of *GSK3A* in non-small-cell lung cancer (NSCLC) tumorigenesis by activating HIF1/VEGFA signaling [21]. Our previous study reported the distinct prognostic effect of *GSK3A* mRNA expression in breast cancer patients [22]. However more studies are warranted to establish the prognostic role of *GSK3A* in different cancers.

In view of the emerging evidence of *GSK3A* expression in colon cancer, in the present study KM plotter (Kaplan-Meier plotter) was used to evaluate the prognostic role of *GSK3A*'s mRNA in 1342 colon cancer patients. KM plotter provided analysis of *GSK3A* mRNA for Relapse Free Survival (RFS), overall (OS) and post-progression survival (PPS) in colon cancer patients using Gene Expression Omnibus (GEO-www.ncbi.nlm.nih.gov/geo/) database [23]. In addition, ROC analysis was also carried out using gene expression data from ROC plotter database.

2. Materials and methods

2.1. Kaplan-Meier survival analysis

Kaplan-Meier plotter (https://kmplot.com/analysis/index.php?p=service&cancer=colon) was used to assess the prognostic value of *GSK3A* mRNA expression on relapse free survival (RFS) (n = 1342), overall survival (OS) (n = 551) and post-progression survival (PPS) (n = 145) in colon cancer patients till July 2023. Cancer Biomedical Informatics Grid (caBIG, http://cabig.cancer.gov/, microarray samples are published in the caArray project), the Gene Expression

Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) and The Cancer Genome Atlas (TCGA, http://cancergenome.nih.gov) cancer datasets were used to identify cancer patients in KM plotter [24], which is an online survival analysis tool. For colon cancer patients, the mRNA expression and survival data of 1342 (RFS), 551 (OS) and 145 (PPS) colon cancer patients were downloaded from the KM-Plotter [23].

For the analysis the following criteria and steps were involved in the data selection. On the home page of KM-Plotter mRNA data of Colon Cancer was selected. In the colon cancer page, the *GSK3A* gene was typed for Affy ID/Gene symbol, the patients were split by 'Median', under survival 'RFS, OS and PPS were selected one by one and for the probe set options 'all probe sets per gene' was selected to perform the analysis for all patients for a 240-month follow-up threshold. The option of all probes sets per gene' was selected to avoid bias and to accomplish high sensitivity and specificity as some differences in the correlation with gene expression were observed for both the Affymetric ID's. This system was introduced by Affymetrix to employ a series of specific and non-specific gene probe sets so as to improve the accuracy of distinguishing between random hybridization and a genuine signal [25].

Likewise, keeping the aforementioned options constant, we analyzed the subtypes (clinical stages, tumor grades, location, site, BRAF mutation, KRAS mutation, TP53 mutation, MSI, Stage M, Stage N, Stage T) and selected cohorts (Gender, CMS and adjuvant chemotherapy). Under plot options, the data was downloaded as 'text file' and then exported to Graphpad PRISM 8 to perform Kaplan Meier survival analysis. The samples were divided into low and high expression groups based on median expression of *GSK3A*. The median expression was employed to divide the patients in comparison to other options to provide almost similar sample numbers in both the groups with less bias.

For survival analysis Logrank P, 95% confidence intervals and Hazard ratio (HR) were calculated wherein P value < 0.05 was statistically significant. Moreover, the number-at-risk has been illustrated beneath the survival plot. Clinical trials use hazards ratios to compare the survival rates of a group of patients receiving a certain treatment to a control group receiving no treatment at all or a different treatment at any given period. If the rates of survival in two groups are equal, then the hazard ratio is one while a higher or lower hazard ratio indicates that one of the groups had a higher chance of survival. Log-rank p-value is calculated during Kaplan Meier survival analysis to test the null hypothesis that there is no difference in the populations' probabilities of an event (death) occurring at any given time. On the other hand, the calculation of confidence intervals is based on the standard error of measurement for instance one has a 5% probability of being incorrect with a 95% confidence interval.

2.2. ROC analysis

Online ROC plotter tool was used to compare the *GSK3A* gene expression in colorectal cancer patients (https://www.rocplot.com/colorectal) receiving any form of chemotherapy [23]. It is the first transcriptome-level validation tool for predictive biomarkers available online. The significance of ROC plotter is that it is capable of associating gene expression and therapy response employing the transcriptome level data of different cancers. On the ROC plotter, the following steps were carried out for data selection *viz.* ROC plotter tool was selected from KM-Plotter homepage and on the subsequent webpage the tab 'ROC plotter for colorectal cancer' was selected under **'**ROC plot using patient data'. Under the gene symbol *GSK3A*-4616 gene was typed and response based on RECIST criteria was selected, for treatment 'any chemotherapy' was clicked, the optional filter was not selected and under settings 'no outliers' was clicked as this option; this would exclude outliers in the box plot. Using the aforementioned selections, the calculations were performed and the ROC data and Box Plot data of 805 patients was exported from the ROC plotter as 'text', followed by ROC analysis on Graphpad PRISM 8. The ROC plot was made and the Area under the curve (AUC) and ROC p-value was determined. Box and Whisker plots were made to compare the *GSK3A* gene expression between chemotherapy responders (n = 451) and non-responders (n = 354). Mann Whitney U test was also carried out to ascertain the significance and $p < 0.05$ was termed statistically significant.

3. Results

3.1. Kaplan-Meier survival analysis

Current study assessed the prognostic significance of *GSK3A* mRNA expression using www.kmplot.com. There are two available Affymetrix IDs (202210_x_at and 632_at) for *GSK3A*. Survival curves for RFS (Relapse free Survival) (n = 1342), OS (Overall survival) (n = 551) and PPS (post-progression survival) (n = 145) were plotted for colon cancer patients. Higher *GSK3A* mRNA expression significantly correlated to poorer RFS (**Figure 1A,B**) for all colon cancer patients till 240 months of follow-up threshold. Moreover, enhanced expression of this gene was not significantly correlated with OS (**Figure 1C,D**) and PPS (**Figure 1E,F**) for all colon cancer patients.

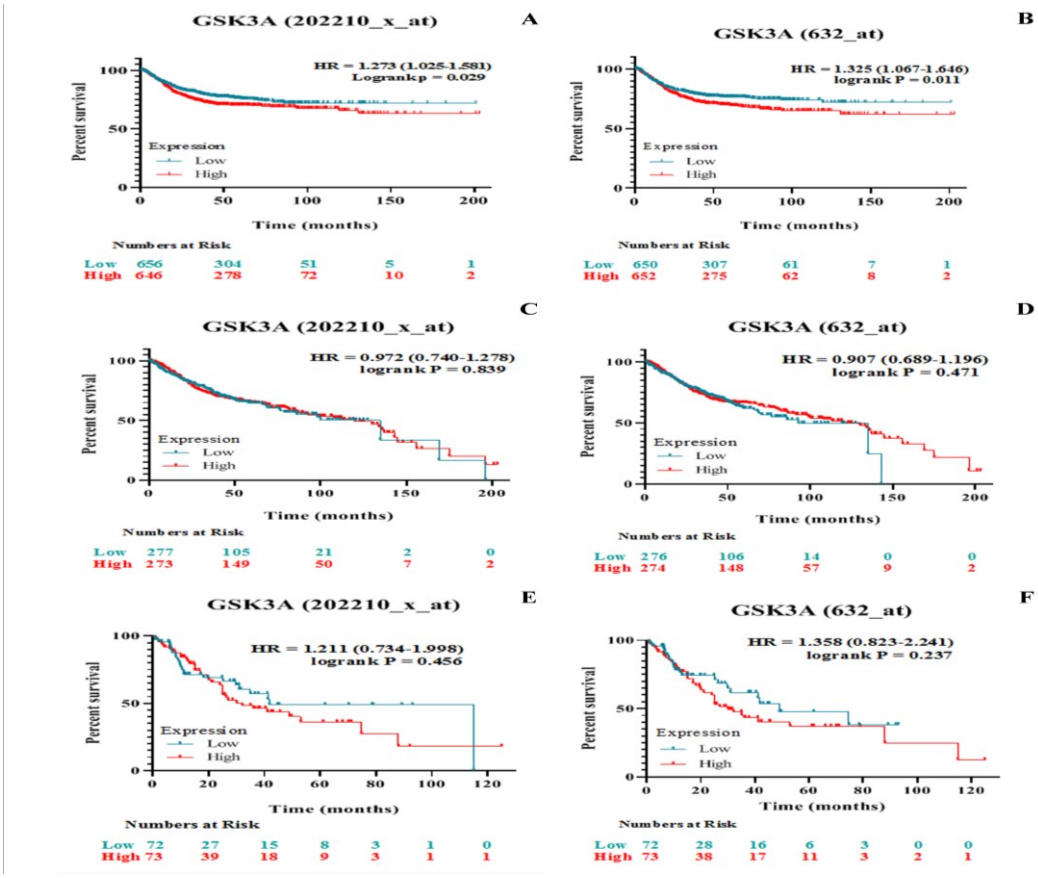


Figure 1. Kaplan-Meier plot showing effect of *GSK3A* mRNA expression on relapse free survival (**A,B**), overall survival (**C,D**), and post progression survival (**E,F**) in all colon cancer patients.

GSK3A higher mRNA expression was further assessed for its correlation to other clinicopathological features: with clinical stages, tumor grades, location, site, BRAF mutation, KRAS mutation, TP53 mutation, MSI, Stage M, Stage N, Stage T, Gender, CMS and adjuvant chemotherapy of colon cancer patients.

Assessment of *GSK3A* mRNA expression (Affy ID: 632_at) in different clinical stages for colon cancer patients (**Table 1**) showed that higher *GSK3A* mRNA expression is significantly correlated to poorer RFS only for stage 2 (Affy ID: 632_at), stage 1 + 2 + 3 (Affy ID: 632_at) and stage 2 + 3 + 4 (Affy ID: 202210_x_at; Affy ID: 632_at) of colon cancer patients.

Table 1. Correlation of high *GSK3A* mRNA expression with different clinical stages of colon cancer patients.

Affy ID: 202210_x_at				
Survival	**Clinical stage**	**Cases**	**HR (95% CI)**	***P* value**
RFS	1	124	0.488 (0.098–2.418)	0.397
	2	542	1.464 (0.969–2.215)	0.071
	1 + 2	666	1.320 (0.884–1.970)	0.174
	3	486	1.009 (0.755–1.347)	0.952
	1 + 2 + 3	1152	1.206 (0.953–1.524)	0.117
	4	56	1.458 (0.684–3.107)	0.333

Table 1. (*Continued*).

Affy ID: 202210_x_at				
Survival	**Clinical stage**	**Cases**	**HR (95% CI)**	***P* value**
RFS	**2 + 3 + 4**	**1084**	**1.295 (1.033–1.623)**	**0.025**
	3 + 4	542	1.107 (0.844–1.449)	0.461
OS	1	83	0.554 (0.177–1.738)	0.307
	2	166	0.821 (0.354–1.900)	0.633
	1 + 2	249	0.648 (0.327–1.283)	0.191
	3	166	0.684 (0.399–1.174)	0.131
	1 + 2 + 3	415	0.745 (0.488–1.137)	0.150
	4	135	0.800 (0.556–1.153)	0.221
	2 + 3 + 4	467	0.962 (0.725–1.275)	0.783
	3 + 4	301	0.891 (0.660–1.204)	0.440
PPS	1	9	Sample number too low for meaningful analysis	
	2	41	2.122 (0.519–8.664)	0.311
	1+2	50	1.385 (0.400–4.789)	0.585
	3	72	0.600 (0.296–1.216)	0.124
	1 + 2 + 3	122	0.893 (0.489–1.631)	0.706
	4	23	1.084 (0.423–2.774)	0.861
	2 + 3 + 4	136	1.268 (0.762–2.112)	0.366
	3 + 4	95	0.833 (0.478–1.453)	0.506
Affy ID: 632_at				
Survival	**Clinical stage**	**Cases**	**HR (95% CI)**	***P* value**
RFS	1	124	1.781 (0.358–8.8)	0.498
	2	**542**	**1.518 (1.004–2.295)**	**0.049**
	1 + 2	666	1.461 (0.979–2.180)	0.065
	3	486	0.979 (0.734–1.308)	0.890
	1 + 2 + 3	**1152**	**1.320 (1.044–1.668)**	**0.020**
	4	56	1.221 (0.516–2.890)	0.660
	2 + 3 + 4	**1084**	**1.448 (1.155–1.815)**	**0.001**
	3 + 4	542	1.005 (0.767–1.317)	0.968
OS	1	83	0.842 (0.244–2.909)	0.770
	2	166	1.069 (0.444–2.570)	0.873
	1 + 2	249	0.947 (0.460–1.949)	0.874
	3	166	0.653 (0.379–1.125)	0.090
	1 + 2 + 3	415	0.763 (0.495–1.175)	0.178
	4	135	1.022 (0.711–1.468)	0.905
	2 + 3 + 4	467	0.949 (0.715–1.259)	0.707
	3 + 4	301	0.947 (0.702–1.278)	0.713

Table 1. (*Continued*).

Affy ID: 202210_x_at				
Survival	**Clinical stage**	**Cases**	**HR (95% CI)**	***P* value**
PPS	1	9	Sample number too low for meaningful analysis	
	2	41	3.390 (0.773–14.86)	0.200
	1 + 2	50	2.921 (0.832–10.24)	0.133
	3	72	1.087 (0.551–2.143)	0.808
	1 + 2 + 3	122	1.012 (0.555–1.844)	0.968
	4	23	1.486 (0.589–3.745)	0.383
	2 + 3 + 4	136	1.394 (0.838–2.320)	0.206
	3 + 4	95	1.102 (0.637–1.903)	0.728

In addition, higher *GSK3A* mRNA expression did not significantly correlate with RFS, OS and PPS for any tumor grades (**Table 2**) as well as for location (**Table 3**) and site (**Table 4**) in colon cancer patients.

Table 2. Correlation of high *GSK3A* mRNA expression with tumor grades of colon cancer patients.

Affy ID: 202210_x_at				
Survival	**Tumor grade**	**Cases**	**HR 95% CI**	***P* value**
RFS	1	23	1.785 (0.307–10.37)	0.518
	2	165	0.602 (0.304–1.192)	0.149
	3	46	0.864 (0.278–2.689)	0.800
OS	1	17	Sample number too low for meaningful analysis	
	2	197	0.773 (0.480–1.244)	0.291
	3	61	1.099 (0.509–2.373)	0.805
PPS	1	**7**	Sample number too low for meaningful analysis.	
	2	68	0.799 (0.339–1.884)	0.606
	3	14	Sample number too low for meaningful analysis.	
Affy ID: 632_at				
Survival	**Tumor grade**	**Cases**	**HR 95% CI**	***P* value**
RFS	1	23	0.525 (0.089–3.072)	0.469
	2	165	1.813 (0.916–3.588)	0.094
	3	46	0.980 (0.316–3.039)	0.972
OS	1	17	Number too low for meaningful analysis	
	2	197	1.139 (0.707–1.832)	0.589
	3	61	0.956 (0.443–2.063)	0.909
PPS	1	7	Sample number too low for meaningful analysis.	
	2	68	0.826 (0.351–1.947)	0.662
	3	14	Sample number too low for meaningful analysis.	

Table 3. Correlation of high *GSK3A* mRNA expression with location in colon cancer patients.

Affy ID: 202210_x_at				
Survival	**Location**	**Cases**	**HR (95% CI)**	***P* value**
RFS	Distal	552	1.345 (0.983–1.839)	0.063
	Proximal	384	1.187 (0.8000–1.760)	0.393
OS	Distal	179	0.805 (0.498–1.302)	0.372
	Proximal	110	1.182 (0.671–2.080)	0.557
PPS	Distal	48	1.013 (0.460–2.231)	0.973
	Proximal	35	1.123 (0.345–3.651)	0.839
Affy ID: 632_at				
Survival	**Location**	**Cases**	**HR (95% CI)**	***P* value**
RFS	Distal	552	1.162 (0.849–1.589)	0.346
	Proximal	384	1.256 (0.847–1.863)	0.255
OS	Distal	179	0.922 (0.569–1.494)	0.733
	Proximal	110	0.667 (0.378–1.177)	0.154
PPS	Distal	48	1.534 (0.693–3.395)	0.310
	Proximal	35	1.239 (0.398–3.850)	0.712

Table 4. Correlation of high *GSK3A* mRNA expression with site in colon cancer patients.

Affy ID: 202210_x_at				
Survival	**Site**	**Cases**	**HR (95% CI)**	***P* value**
RFS	Left Colon	188	1.193 (0.692–2.055)	0.524
	Right Colon	196	1.236 (0.706–2.164)	0.459
	Rectum	83	0.490 (0.189–1.270)	0.149
OS	Left Colon	105	1.158 (0.640–2.093)	0.625
	Right Colon	110	1.182 (0.671–2.080)	0.557
	Rectum	74	1.514 (0.667–3.437)	0.304
PPS	Left Colon	37	0.946 (0.342–2.616)	0.914
	Right Colon	35	1.123 (0.345–3.651)	0.839
	Rectum	11	Sample number too low for meaningful analysis	
Affy ID: 632_at				
Survival	**Site**	**Cases**	**HR (95% CI)**	***P* value**
RFS	Left Colon	188	1.426 (0.827–2.456)	0.201
	Right Colon	196	1.075 (0.614–1.882)	0.799
	Rectum	83	1.619 (0.624–4.198)	0.322
OS	Left Colon	105	1.148 (0.634–2.076)	0.644
	Right Colon	110	0.667 (0.378–1.177)	0.154
	Rectum	74	1.115 (0.486–2.553)	0.791

Table 4. (*Continued*).

Affy ID: 202210_x_at				
Survival	**Site**	**Cases**	**HR (95% CI)**	***P* value**
PPS	Left Colon	37	1.145 (0.412–3.179)	0.796
	Right Colon	35	1.239 (0.398–3.850)	0.712
	Rectum	11	Sample number too low for meaningful analysis	

Tables 5 and **6** shows that higher *GSK3A* mRNA expression was only significantly correlated with poorer RFS for wild type (Affy ID: 632_at) and not with BRAF and KRAS mutation while the sample number was too low for meaningful analysis in case of OS and PPS. On the contrary higher *GSK3A* mRNA expression significantly correlated with poorer RFS for TP53 mutation (Affy ID: 202210_x_at) and not in case of the wild type (**Table 7**) whereas for Affy ID: 632_at, higher *GSK3A* mRNA expression significantly correlated with poorer RFS in both TP53 mutated as well as wild type (**Table 7**). Moreover, the sample number was too low for meaningful analysis in case of OS and PPS.

Table 5. Correlation of high *GSK3A* mRNA expression with BRAF mutation in colon cancer patients.

Affy ID: 202210_x_at				
Survival	**BRAF Mutation**	**Cases**	**HR (95% CI)**	***P* value**
RFS	Mutated	49	1.161 (0.350–3.847)	0.804
	Wild Type	509	1.391 (0.971–1.991)	0.070
OS	Mutated	0	Sample number too low for meaningful analysis	
	Wild Type	0	Sample number too low for meaningful analysis	
PPS	Mutated	0	Sample number too low for meaningful analysis	
	Wild Type	0	Sample number too low for meaningful analysis	
Affy ID: 632_at				
Survival	**BRAF Mutation**	**Cases**	**HR (95% CI)**	***P* value**
RFS	Mutated	49	0.595 (0.182–1.941)	0.400
	Wild Type	**509**	**1.622 (1.133–2.324)**	**0.0079**
OS	Mutated	0	Sample number too low for meaningful analysis	
	Wild Type	0	Sample number too low for meaningful analysis	
PPS	Mutated	0	Sample number too low for meaningful analysis	
	Wild Type	0	Sample number too low for meaningful analysis	

Table 6. Correlation of high *GSK3A* mRNA expression with KRAS mutation in colon cancer patients.

Affy ID: 202210_x_at				
Survival	**KRAS Mutation**	**Cases**	**HR (95% CI)**	***P* value**
RFS	Mutated	230	1.579 (0.981–2.541)	0.055
	Wild Type	361	1.230 (0.795–1.902)	0.350
OS	Mutated	0	Sample number too low for meaningful analysis	
	Wild Type	0	Sample number too low for meaningful analysis	
PPS	Mutated	0	Sample number too low for meaningful analysis	
	Wild Type	0	Sample number too low for meaningful analysis	
Affy ID: 632_at				
Survival	**KRAS Mutation**	**Cases**	**HR (95% CI)**	***P* value**
RFS	Mutated	230	1.419 (0.884–2.277)	0.144
	Wild Type	**361**	**1.835 (1.185–2.841)**	**0.006**
OS	Mutated	0	Sample number too low for meaningful analysis	
	Wild Type	0	Sample number too low for meaningful analysis	
PPS	Mutated	0	Sample number too low for meaningful analysis	
	Wild Type	0	Sample number too low for meaningful analysis	

Table 7. Correlation of high *GSK3A* mRNA expression with TP53 mutation in colon cancer patients.

Affy ID: 202210_x_at				
Survival	**TP53 Mutation**	**Cases**	**HR (95% CI)**	***P* value**
RFS	**Mutated**	**226**	**1.656 (1.036–2.647)**	**0.035**
	Wild Type	186	0.998 (0.573–1.739)	0.996
OS	Mutated	0	Sample number too low for meaningful analysis	
	Wild Type	0	Sample number too low for meaningful analysis	
PPS	Mutated	0	Sample number too low for meaningful analysis	
	Wild Type	0	Sample number too low for meaningful analysis	
Affy ID: 632_at				
Survival	**TP53 Mutation**	**Cases**	**HR (95% CI)**	***P* value**
RFS	**Mutated**	**226**	**2.521 (1.574–4.038)**	**0.0002**
	Wild Type	**186**	**1.842 (1.057–3.210)**	**0.032**
OS	Mutated	0	Sample number too low for meaningful analysis	
	Wild Type	0	Sample number too low for meaningful analysis	
PPS	Mutated	0	Sample number too low for meaningful analysis	
	Wild Type	0	Sample number too low for meaningful analysis	

Higher *GSK3A* mRNA expression significantly correlated with poor RFS (Affy ID: 202210_x_at) for stable or low MSI (microsatellite instability) and not with stable and high MSI (**Table 8**). In case of Affy ID: 632_at, higher *GSK3A* mRNA expression also significantly correlated with poor RFS for stable or low MSI and not for stable and high MSI (**Table 8**). In case of OS, no significant correlation between high *GSK3A* mRNA expression and MSI

was observed while in case of PPS the sample number was too low for meaningful analysis.

Table 8. Correlation of high *GSK3A* mRNA expression with MSI of colon cancer patients.

Affy ID: 202210_x_at				
Survival	**MSI**	**Cases**	**HR (95% CI)**	***P* value**
RFS	Stable	156	1.339 (0.714–2.509)	0.363
	Stable or Low	**471**	**1.697 (1.216–2.368)**	**0.001**
	High	99	1.250 (0.438–3.567)	0.673
OS	Stable	117	1.035 (0.633–1.691)	0.889
	Stable or Low	32	1.949 (0.678–5.602)	0.231
	High	35	2.255 (0.802–6.337)	0.091
PPS	Stable	26	1.224 (0.539–2.776)	0.614
	Stable or Low	6	Sample number too low for meaningful analysis	
	High	2	Sample number too low for meaningful analysis	
Affy ID: 632_at				
Survival	**MSI**	**Cases**	**HR (95% CI)**	***P* value**
RFS	Stable	156	1.898 (1.013–3.556)	0.050
	Stable or Low	**471**	**1.499 (1.074–2.092)**	**0.016**
	High	99	1.252 (0.439–3.572)	0.671
OS	Stable	117	0.932 (0.571–1.523)	0.780
	Stable or Low	32	0.764 (0.257–2.270)	0.600
	High	35	0.438 (0.159–1.210)	0.106
PPS	Stable	26	1.689 (0.740–3.853)	0.201
	Stable or Low	6	Sample number too low for meaningful analysis	
	High	2	Sample number too low for meaningful analysis	

High *GSK3A* mRNA expression did not correlate with RFS, OS and PPS for stage M (**Table 9**) (Affy ID: 202210_x_at) whereas in case of Affy ID: 632_at, high *GSK3A* mRNA expression was significantly correlated with poor RFS for sub- stage 0 of stage M (**Table 9**).

Table 9. Correlation of high *GSK3A* mRNA expression with Stage M of colon cancer patients.

Affy ID: 202210_x_at				
Survival	**Stage M**	**Cases**	**HR (95% CI)**	***P* value**
RFS	0	218	1.038 (0.523–2.058)	0.913
	1	21	1.707 (0.618–4.712)	0.244
OS	0	228	1.052 (0.633–1.748)	0.841
	1	81	1.141 (0.714–1.825)	0.572
PPS	0	42	1.537 (0.675–3.494)	0.316
	1	17	Sample number too low for meaningful analysis.	

Table 9. (*Continued*).

Affy ID: 632_at				
Survival	**Stage M**	**Cases**	**HR (95% CI)**	***P* value**
RFS	**0**	**218**	**2.230 (1.124–4.425)**	**0.030**
	1	21	1.060 (0.397–2.827)	0.900
OS	0	228	0.784 (0.457–1.345)	0.335
	1	81	0.894 (0.558–1.431)	0.633
PPS	0	42	1.351 (0.579–3.148)	0.487
	1	17	Sample number too low for meaningful analysis.	

As far as stage N is concerned, high *GSK3A* mRNA expression (Affy ID: 632_at) significantly correlated with poor RFS in sub-stage 1 and 2 of stage N and poor PPS in sub-stage 0 of stage N (**Table 10**). For Affy ID: 202210_x_at high *GSK3A* mRNA expression correlated with neither with RFS, OS nor PPS for stage N (**Table 10**).

Table 10. Correlation of high *GSK3A* mRNA expression with Stage N of colon cancer patients.

Affy ID: 202210_x_at				
Survival	**Stage N**	**Cases**	**HR (95% CI)**	***P* value**
RFS	0	125	0.441 (0.153–1.268)	0.128
	1	54	2.297 (0.882–5.979)	0.089
	2	28	1.295 (0.485–3.450)	0.604
OS	0	162	1.010 (0.550–1.854)	0.973
	1	86	1.430 (0.798–2.563)	0.218
	2	62	1.277 (0.697–2.338)	0.420
PPS	0	20	2.419 (0.600–9.749)	0.223
	1	22	1.231 (0.443–3.420)	0.686
	2	17	Sample number too low for meaningful analysis	
Affy ID: 632_at				
Survival	**Stage N**	**Cases**	**HR (95% CI)**	***P* value**
RFS	0	125	2.014 (0.702–5.777)	0.216
	1	**54**	**2.973 (1.143–7.736)**	**0.030**
	2	**28**	**0.342 (0.123–0.949)**	**0.026**
OS	0	162	1.644 (0.878–3.077)	0.127
	1	86	1.300 (0.727–2.323)	0.372
	2	62	0.617 (0.333–1.143)	0.103
PPS	**0**	**20**	**-**	**0.006**
	1	22	1.293 (0.468–3.567)	0.616
	2	17	Sample number too low for meaningful analysis.	

Further, high *GSK3A* mRNA expression (Affy ID: 202210_x_at) was found to be significantly correlated with decreased RFS in sub-stage 2 and poor OS in sub-stage 3 of stage T (**Table 11**). There was no significant correlation between PPS and any of the sub-stages of stage T. For Affy ID: 632_at high *GSK3A* mRNA expression significantly correlated with poor RFS in sub-stage 3 of stage T (**Table 11**). A non-significant correlation was found between OS and PPS for all the sub-stages of stage T.

Table 11. Correlation of high *GSK3A* mRNA expression with Stage T of colon cancer patients.

Affy ID: 202210_x_at				
Survival	**Stage T**	**Cases**	**HR (95% CI)**	***P* value**
	2	**48**	**Undefined**	**0.013**
RFS	3	145	1.497 (0.786–2.853)	0.222
	4	12	Sample number too low for meaningful analysis	
	2	58	0.445 (0.147–1.342)	0.142
OS	**3**	**227**	**1.530 (1.045–2.239)**	**0.027**
	4	22	2.239 (0.683–7.333)	0.162
	2	3	Sample number too low for meaningful analysis.	
PPS	3	48	1.974 (0.962–4.047)	0.070
	4	8	Sample number too low for meaningful analysis.	
Affy ID: 632_at				
Survival	**Stage T**	**Cases**	**HR (95% CI)**	***P* value**
	2	48	0.633 (0.084–4.736)	0.626
RFS	**3**	**145**	**1.973 (1.035–3.759)**	**0.045**
	4	12	Sample number too low for meaningful analysis	
	2	58	0.707 (0.217–2.301)	0.519
OS	3	227	1.178 (0.801–1.731)	0.389
	4	22	1.411 (0.429–4.631)	0.557
	2	3	Sample number too low for meaningful analysis.	
PPS	3	48	1.275 (0.603–2.696)	0.519
	4	8	Sample number too low for meaningful analysis.	

GSK3A expression further correlated with gender of colon cancer patients. It was found that high *GSK3A* mRNA expression (Affy ID: 632_at) was significantly correlated to poorer RFS for male colon cancer patients (**Table 12**) while there was no significant correlation between gender and OS or PPS. Interestingly, there was no significant correlation between gender and RFS, OS or PPS for Affy ID: 202210_x_at (**Table 12**).

Table 12. Correlation of high *GSK3A* mRNA expression with Gender of colon cancer patients.

Affy ID: 202210_x_at				
Survival	**Gender**	**Cases**	**HR (95% CI)**	***P* value**
RFS	Female	607	1.225 (0.907–1.655)	0.184
	Male	550	1.282 (0.916–1.794)	0.148
OS	Female	249	0.898 (0.613–1.317)	0.575
	Male	232	1.060 (0.703–1.596)	0.778
PPS	Female	74	0.775 (0.391–1.537)	0.453
	Male	65	1.878 (0.894–3.944)	0.106
Affy ID: 632_at				
Survival	**Gender**	**Cases**	**HR (95% CI)**	***P* value**
RFS	Female	607	0.977 (0.723–1.321)	0.882
	Male	**550**	**1.407 (1.005–1.969)**	**0.048**
OS	Female	249	1.008 (0.687–1.477)	0.966
	Male	232	0.764 (0.504–1.158)	0.177
PPS	Female	74	1.780 (0.908–3.487)	0.098
	Male	65	0.918 (0.432–1.951)	0.819

Higher *GSK3A* mRNA expression (Affy ID: 202210_x_at) was also significantly correlated to poor RFS for subtype metabolic of CMS (consensus molecular subtype). Likewise, high expression of this gene also worsened OS for subtype canonical and mesenchymal of CMS in colon cancer patients (**Table 13**). Moreover, there no significant correlation between RFS, OS or PPS and any of the CMS subtypes for Affy ID: 632_at (**Table 13**).

Table 13. Correlation of high *GSK3A* mRNA expression with CMS of colon cancer patients.

Affy ID: 202210_x_at				
Survival	**CMS**	**Cases**	**HR (95% CI)**	***P* value**
RFS	Canonical	327	1.360 (0.836–2.212)	0.216
	Mesenchymal	298	0.927 (0.642–1.339)	0.686
	Metabolic	**216**	**2.200 (1.262–3.833)**	**0.006**
	Microsatellite Unstable	188	1.126 (0.635–1.995)	0.682
OS	**Canonical**	**141**	**1.779 (1.016–3.115)**	**0.045**
	Mesenchymal	**117**	**0.588 (0.359–0.963)**	**0.025**
	Metabolic	85	1.055 (0.520–2.137)	0.880
	Microsatellite Unstable	83	0.906 (0.462–1.775)	0.770
PPS	Canonical	36	1.454 (0.462–4.574)	0.523
	Mesenchymal	39	1.025 (0.466–2.256)	0.948
	Metabolic	24	1.275 (0.345–4.711)	0.697
	Microsatellite Unstable	25	0.695 (0.197–2.451)	0.557

Table 13. (*Continued*).

Affy ID: 632_at				
Survival	**CMS**	**Cases**	**HR (95% CI)**	***P* value**
RFS	Canonical	327	1.218 (0.748–1.980)	0.427
	Mesenchymal	298	1.105 (0.765–1.595)	0.593
	Metabolic	216	1.049 (0.602–1.826)	0.865
	Microsatellite Unstable	188	1.065 (0.601–1.887)	0.827
OS	Canonical	141	1.157 (0.656–2.039)	0.606
	Mesenchymal	117	0.690 (0.425–1.121)	0.128
	Metabolic	85	1.406 (0.694–2.846)	0.347
	Microsatellite Unstable	83	0.827 (0.419–1.634)	0.559
PPS	Canonical	36	1.254 (0.392–4.012)	0.701
	Mesenchymal	39	1.342 (0.612–2.940)	0.451
	Metabolic	24	2.302 (0.621–8.531)	0.197
	Microsatellite Unstable	25	0.611 (0.170–2.194)	0.430

High *GSK3A* mRNA expression was significantly correlated with worst RFS in the absence of adjuvant chemotherapy (**Table 14**) for both Affy ID: 202210_x_at and Affy ID: 632_at. However, *GSK3A* mRNA expression did not correlate with OS and PPS in colon cancer patients in the presence or absence of adjuvant chemotherapy (**Table 14**).

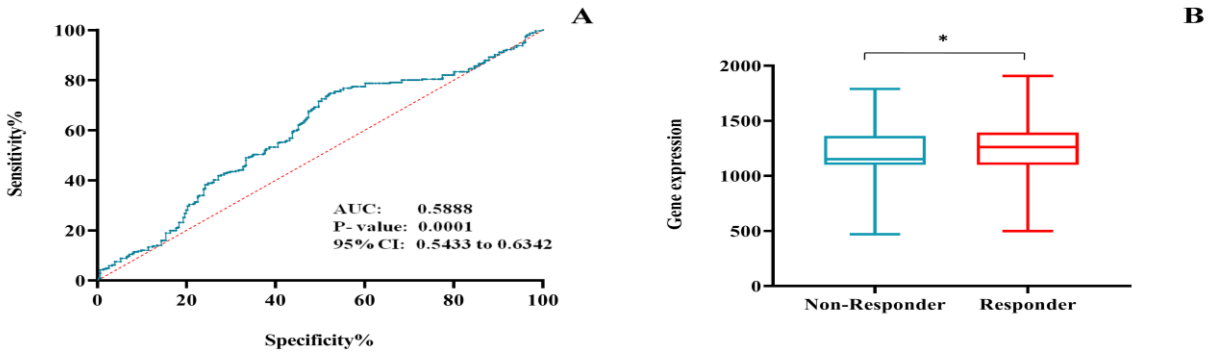
Table 14. Correlation of high *GSK3A* mRNA expression with adjuvant chemotherapy of colon cancer patients.

Affy ID: 202210_x_at				
Survival	**Adjuvant chemotherapy**	**Cases**	**HR (95% CI)**	***P* value**
RFS	**0**	**382**	**2.021 (1.283–3.184)**	**0.002**
	1	266	1.131 (0.749–1.706)	0.555
OS	0	27	1.674 (0.505–5.548)	0.368
	1	38	2.159 (0.754–6.182)	0.155
PPS	0	4	Sample number too low for meaningful analysis.	
	1	15	Sample number too low for meaningful analysis.	
Affy ID: 632_at				
Survival	**Adjuvant chemotherapy**	**Cases**	**HR (95% CI)**	***P* value**
RFS	**0**	**382**	**1.677 (1.065–2.642)**	**0.025**
	1	266	1.178 (0.781–1.777)	0.431
OS	0	27	1.169 (0.358–3.811)	0.784
	1	38	1.055 (0.369–3.008)	0.920
PPS	0	4	Sample number too low for meaningful analysis.	
	1	15	Sample number too low for meaningful analysis.	

3.2. ROC analysis

The data of 805 patients receiving any form of chemotherapy was downloaded from ROC plotter followed by ROC analysis using Graphpad PRISM 8. *GSK3A* gene expression data from responders (n = 451) and non-responders (n = 354) was then compared. Mann Whitney U test was also carried out to ascertain the significance and box and whisker plots were prepared and $p < 0.05$ was termed statistically significant.

In patients with receiving chemotherapy *GSK3A* gene expression was significantly associated with better prognosis as illustrated by ROC analysis (**Figure 2A**). In addition, there was a significant difference of *GSK3A* gene expression between responders and non-responders ($p = 0.0005$) as ascertained by Mann Whitney U test (**Figure 2B,C**).



C

	CHEMOTHERAPY		
	N	Median	P value
Non-responders	354	1152	0.0005
Responders	451	1261	

Figure 2. ROC plot (**A**) and Box plot (**B**) showing *GSK3A* gene expression significantly (**C**) associated with better prognosis in colorectal cancer patients receiving chemotherapy.

4. Discussion

Colon cancer ranks second in terms of cancer-related mortality and is the third most frequent type of cancer globally [1]. The prevalence of this cancer is increasing despite improvements in the colorectal cancer (CRC) screening methods, early detection and advanced therapeutic strategies. The rise in incidence of colon cancer in young adults is specifically related to lifestyle factors, viz. racial background, personal or family history of inflammatory bowel disease or colorectal cancer or polyps, and inherited syndromes. These factors also include unhealthy eating habits, excessive alcohol consumption, unhealthy lifestyle choices, and reduced physical activity [26]. Studies have estimated about 22% of colorectal cancers to be metastatic during initial diagnosis while there is a 70% chance to develop metastatic relapse later in

patients [27]. Moreover, there is poor prognosis in patients with metastatic CRC with 14% relative 5-year survival rate in comparison patients with regional (71%) and localized (90%) CRC. This scenario highlights critical need to find new prognostic markers for better targeted therapy and improved outcomes for colon cancer patients.

GSK3 regulates cell-cycle progression, differentiation and apoptosis [28,29] through phosphorylation of its targets. It is usually active in normal cells either as α and β isoform and depending on the cell type, act as either a tumor suppressor or promoter by its inactivation [30]. GSK-3 has a ubiquitous expression and its isoform have been found in varied concentrations in human tissues. The enzyme has been found to play a crucial role in several cellular pathways for instance Ras/Raf/MEK/ERK and PI3K/PTEN/Akt/mTORC1 [31]. GSK-3 is also implicated in the Wnt and Hedgehog (HH) pathways wherein it plays a role in cell morphology and survival. The development of different human cancers has been associated with GSK-3 dysregulation in these pathways [32]. It has been reported to promote cell survival via the NF-kB pathway and through the Wnt and HH pathways it in part affects the development and progression of diverse human tumors. The development of hepatocellular carcinoma, melanoma, prostate, ovarian, pancreatic and colorectal cancers have been linked with GSK3 expression [33].

In colon cancer, a sequence of molecular events leads to the neoplastic transformation which includes mutation in adenomatous polyp coli tumor-suppressor gene (APC) contributing to the development of polyps. This is followed by mutations in K-ras oncogene leading to the adenomatous phase of polyp development followed by malignant transformation via TP53 mutations. In addition, alteration in the TGF-β/SMAD and DNA mismatch repair pathway also contributes to colon cancer [33]. Studies have found APC to regulate several signaling pathways by increasing GSK-3 activity in colorectal cancer cells [34]. Furthermore, nuclear concentrations of GSK-3 as well as Wnt signaling in colon cancer cells are regulated by P53, the effect of which are not known. Inactivation of GSK3 by phosphorylation was found to be defective in colorectal tumor cells but not in preserved in normal cells [35].

GSK3 inactivation inhibited neuroblastoma, pancreatic and neuroendocrine cancers [36–40], but it still remains elusive as which GSK3 isoform regulates carcinogenesis. There are contrasting reports on role of isoforms of GSK3 in the cell growth regulation [13,41] and very few reports addressed role *GSK3A* in cancer [42]. The studies about role of *GSK3A* in cancer growth and development are limited; hence *GSK3A* prognostic role in colon cancer patients remains unknown. The present study assessed the prognostic and predictive significance of *GSK3A* mRNA in colon cancer patients.

Studies have highlighted the impact of GSK3 over growth, invasion, angiogenesis, and metastasis of tumors making it an attractive therapeutic target [43]. Recent data have indicated the important role of GSK-3 in the immune response to cancer as well as in the regulation of the functioning of the immune cells [44]. For instance, the GSK-3β knockdown or its inhibition

using specific inhibitors has been reported to increase the activation function of NK cells. Likewise, inactivation of GSK-3 activity is crucial for the T cells function and activity of induced regulatory Treg (iTreg). These findings are suggestive of the implication of GSK-3 inhibitors as potential anticancer agents that are capable of enhancing the antitumor activity of T cells [45]. GSK3 has also been reported to be involved in the regulation of NF-κB pathway [46], which plays a role in immunity, inflammation and cell survival. Therefore, inhibitors of GSK3 can regulate the activation of NF-κB, resulting in decreased expression of pro-survival and pro-inflammatory genes thereby inhibiting cancer growth and metastasis.

GSK3A inhibition helped p53-null colon carcinoma cells to overcome resistance to DNA-damaging chemotherapy without affecting cell proliferation or cell cycle by raising a necroptotic response [18]. *GSK3A* inhibition detained melanoma growth by inducing programmed cell death, cell cycle arrest in G0/G1 phase of [47] and sensitizing melanoma cells to ISC-4 (Akt pathway inhibitor) and staurosporine [48]. *GSK3A* reduced phosphorylation by AR-A014418, as compared to GSK3β which slowed down pancreatic cancer cell lines growth [42].

In the current study results indicate *GSK3A* higher mRNA expression to significantly correlate with poor RFS in patients with colon cancer. Moreover, male colon cancer patients showed significantly poorer RFS with higher *GSK3A* mRNA expression. However, there was no correlation of higher *GSK3A* mRNA expression with OS and PPS with the gender of the colon cancer patients.

GSK3A mRNA expression was also evaluated in various clinicopathological conditions. Higher *GSK3A* mRNA expression worsened RFS in stage 2, stage 1+2+3 and stage 2+3+4 in colon cancer patients (Affy ID: 632_at). Interestingly, higher *GSK3A* mRNA expression was not significantly correlated with RFS, OS and PPS for any tumor grades, location and site in colon cancer patients.

Genome analyses showed the development as well as progression of colorectal cancer to be associated with genetic mutations in driver genes *viz. APC*, *KRAS*, *TGFBR2*, and *TP53* [49]. Of these TP53 mutation has been observed in ~60% of CRC patients. The current study illustrated high *GSK3A* mRNA expression to be significantly correlated with worst RFS in patients with TP53 mutation (Affy ID: 202210_x_at) and not in case of the wild type. However, for Affy ID: 632_at, high *GSK3A* mRNA expression significantly correlated with poorer RFS in both TP53 mutated as well as wild type (**Table 7**). *KRAS* is a proto-oncogene that encodes a 21-kD GTP (guanosine triphosphate)/ GDP (guanosine diphosphate) that binds the protein implicated in regulating the cellular response to external stimuli [50]. This gene is found to be mutated in about 35%-45% of CRCs and is linked with reduced response to selective chemotherapeutics [51]. *BRAF* on the other hand is a serine/threonine protein kinase implicated in the MAPK (mitogen-activated protein kinase) signaling pathway. This pathway is involved in angiogenesis, survival, migration, differentiation and cell proliferation and its dysregulation

results in tumor development [52]. These mutations are found in about 10% of CRC patients [53]. In the present analysis, high *GSK3A* mRNA expression was significantly correlated with poorer RFS for wild type (Affy ID: 632_at) and not in patients with BRAF and KRAS mutation which agrees with literature findings as only 10 % colon cancer patients harbor these mutations. Staging is a method to illustrate location, spread of the cancer and if it is impacting other parts of the body. The TNM (Tumor (T), Node (N) and Metastasis (M)) system of staging is employed by doctors and physicians to describe the various stages of colorectal cancer to plan the best treatment regimen for the patients. In the current analysis, high *GSK3A* mRNA expression (Affy ID: 632_at) was significantly correlated with poor RFS for sub- stage 0 of stage M, poor RFS in sub-stage 1 and 2 and poor PPS in sub-stage 0 of stage N and poor RFS (Affy ID: 202210_x_at) in sub-stage 2 and poor OS in sub-stage 3 of stage T as well as poor RFS (Affy ID: 632_at) in sub-stage 3 of stage T which further highlights the prognostic significance of high *GSK3A* mRNA expression.

Microsatellite instability (MSI) is a molecular signature in case of some colorectal cancers wherein short tandem repeats are subject to mutations concomitant with DNA sequences on account of the deficiency in DNA-mismatch-repair system**.** This deficiency occurs due to a somatic or germline mutation in MMR (mismatch-repair) genes [54]. MSI can be employed to categorize the colorectal cancers as microsatellite stable, MSI-low (MSI-S) and MSI-high (MSI-H) tumors and studies indicate MSI testing to be a good prognostic marker for colon cancer [55]. In the current analysis, enhanced expression of *GSK3A* mRNA was found to be correlated significantly with poor RFS for stable or low MSI (Affy ID: 202210_x_at and Affy ID: 632_at) and not with stable and high MSI. This suggests MSI-high as a prognostic and diagnostic marker for the colon cancer in accordance with the previous reports.

The consensus molecular subtypes (CMSs) can be employed to illustrate tumor heterogeneity at the gene-expression level for colorectal cancer. There are four broad consensus molecular subtypes for colorectal cancer *viz.* Microsatellite Unstable, Canonical, Mesenchymal and Metabolic. The CMS classification is the most reliable method for colorectal cancer having unambiguous biological interpretability and can serves as the foundation for upcoming clinical stratification and subtype-based targeted therapies [56]. In the present study, high *GSK3A* mRNA expression (Affy ID: 202210_x_at) was found to correlate significantly with poor RFS for subtype metabolic of CMS. Likewise, high expression of this gene also worsened OS for subtype canonical and mesenchymal in colon cancer patients. Interestingly, there no significant correlation between RFS, OS or PPS and any of the CMS subtypes for Affy ID: 632_at. These findings highlight the prognostic significance of high *GSK3A* expression to worsen RFS for subtype metabolic and OS for subtypes canonical and mesenchymal.

In addition, the high *GSK3A* mRNA expression significantly correlated with worst RFS in the absence of adjuvant chemotherapy. It is interesting to

note that there was no correlation of high *GSK3A* mRNA expression with OS and PPS in the presence or absence of adjuvant chemotherapy.

Furthermore, ROC analysis was also carried out in the present study. This analysis provides an assessment of a biomarker's overall diagnostic performance using the ROC plot. In addition to determining the AUC and ROC p-value it also helps to calculate the strongest cut-off which can differentiate between chemotherapy responder and non-responders. In the present study ROC analysis illustrated *GSK3A* gene expression in patients receiving chemotherapy to be significantly associated with better prognosis. In addition, there was a significant difference of the gene expression between chemotherapy responders and non-responders which further affirms the prognostic value of *GSK3A* gene in colon cancer.

At length, evaluation of prognostic role of *GSK3A* in colon cancer patients through KM plotter illustrated that *GSK3A* high mRNA expression significantly worsens RFS in colon cancer patients. This observation is in agreement with another study which highlighted the increased *GSK3A* levels to be predictive of poor prognosis in colon cancer patients with THRAP3 phosphorylation at S248 by *GSK3A* and to be a key factor responsible for promoting migration of cancer cells [19]. Moreover, emerging reports that highlight the crucial role of inhibition of GSK3 in anticancer therapy as well in regulating the function of NK cells, T cells and iTegs [44,45] which further support this observation. Further assessment of *GSK3A* mRNA in different clinic-pathological features including clinical stages, MSI, TP53 mutation, stage T, stage N and CMS showed that there is a critical prognostic value of *GSK3A* in colon cancer patients. This observation is further supported by ROC analysis.

Considering the significant role played by GSK-3 in tumor development, its regulators and inhibitors can be employed for the treatment of cancer. Several GSK3 inhibitors like lithium, AR-A014418 9-ING-41 and ABC1183 have illustrated promising inhibitory activity against numerous cancer cell lines. Lithium has been reported to exhibit inhibitory activity against GSK-3β. Studies have illustrated lithium to increase the efficacy of gemcitabine synergistically against PDA cells via targeting the hedgehog-GLI signaling pathway [57]. Treatment with AR-A014418 illustrated potential inhibitory activity on growth and apoptosis on pancreatic cells [58]. 9-ING-41, a GSK3β specific inhibitor is undergoing clinical trials for pancreatic cancer has shown promising efficacy [59]. ABC1183 acts by arresting cell cycle at G2/M phase and has illustrated inhibitory activity against numerous cancer cell lines [60]. Selective inhibitors of COX-2 have been found to illustrate beneficial effect on colon cancer patients [33]. Recently, computational tools are also being employed to identify potential GSK-3 inhibitors. These inhibitors can be used in combination to act synergistically with existing chemotherapy or immunotherapy to treat colon cancer.

5. Conclusion

GSK-3 has been found to play an important role in tumor initiation and proliferation as a Wnt/β-catenin pathway mediator. The present study highlights *GSK3A* as a predictive and prognostic biomarker for colon cancer. High *GSK3A* mRNA expression has been found to significantly worsen RFS in colon cancer patients. ROC analysis revealed *GSK3A* gene expression in patients receiving chemotherapy to be significantly associated with better prognosis. Thus, role of *GSK3A* in predicting the prognosis will help develop more accurate treatment strategies for combating the heterogeneity and complexity of colon cancer. Moreover, considering the effect of GSK-3 on both cancer as well as the immune cells of the tumor microenvironment identification of novel GSK-3 inhibitors would be fruitful strategy to treat colon cancer.

6. Clinical significance

The manuscript illustrates the prognostic role of *GSK3A's* mRNA in 1342 colon cancer patients through KM plotter wherein it was found that that high *GSK3A* mRNA expression significantly worsens RFS in colon cancer patients. These observations are also supported by ROC analysis. The role *GSK3A* in predicting the prognosis will be useful in developing accurate treatment strategies in order to combat the heterogeneity and complexity of colon cancer.

7. Limitations of the study

Despite the overall interesting correlations provided by ample sample size and follow-up time, sample sizes for *GSK3A* mRNA in some groups of colon cancer patients were too low to reach a significant correlation and therefore further studies are needed to reach a sufficient sample size.

Author contributions: Study conception and design, JK; data collection, NSW; analysis and interpretation of results, NSW and JK; draft manuscript preparation, NSW and JK. All authors have read and agreed to the published version of the manuscript.

Data availability statement: The survival data analyzed in this study was obtained from the KM plotter database https://kmplot.com/analysis/index.php?p=service&cancer=colon) and the data for ROC analysis was obtained from ROC plotter (https://www.rocplot.com/colorectal).

Conflict of interest: The authors declare no conflict of interest.

References

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians. 2018; 68(6): 394-424. doi: 10.3322/caac.21492

2. Woodgett JR. Molecular cloning and expression of glycogen synthase kinase-3/factor A. The EMBO Journal. 1990; 9(8): 2431-2438. doi: 10.1002/j.1460-2075.1990.tb07419.x
3. Welsh GI, Proud CG. Glycogen synthase kinase-3 is rapidly inactivated in response to insulin and phosphorylates eukaryotic initiation factor eIF-2B. Biochemical Journal. 1993; 294(3): 625-629. doi: 10.1042/bj2940625
4. Cohen P, Frame S. The renaissance of GSK3. Nature Reviews Molecular Cell Biology. 2001; 2(10): 769-776. doi: 10.1038/35096075
5. Duda P, Akula SM, Abrams SL, et al. Targeting GSK3 and Associated Signaling Pathways Involved in Cancer. Cells. 2020; 9(5): 1110. doi: 10.3390/cells9051110
6. Jope RS, Johnson GVW. The glamour and gloom of glycogen synthase kinase-3. Trends in Biochemical Sciences. 2004; 29(2): 95-102. doi: 10.1016/j.tibs.2003.12.004
7. Medina M, Castro A. Glycogen synthase kinase-3 (GSK-3) inhibitors reach the clinic. Curr Opin Drug Discov Devel. 2008; 11:533-43.
8. Garcea G, Manson M, Neal C, et al. Glycogen Synthase Kinase-3 Beta; A New Target in Pancreatic Cancer? Current Cancer Drug Targets. 2007; 7(3): 209-215. doi: 10.2174/156800907780618266
9. Obligado SH, Ibraghimov-Beskrovnaya O, Zuk A, et al. CDK/GSK-3 inhibitors as therapeutic agents for parenchymal renal diseases. Kidney International. 2008; 73(6): 684-690. doi: 10.1038/sj.ki.5002731
10. Dewhurst S, Maggirwar SB, Schifitto G, et al. Glycogen Synthase Kinase 3 Beta (GSK-3β) as a Therapeutic Target in NeuroAIDS. Journal of Neuroimmune Pharmacology. 2006; 2(1): 93-96. doi: 10.1007/s11481-006-9051-1
11. MacAulay K, Doble BW, Patel S, et al. Glycogen Synthase Kinase 3α-Specific Regulation of Murine Hepatic Glycogen Metabolism. Cell Metabolism. 2007; 6(4): 329-337. doi: 10.1016/j.cmet.2007.08.013
12. Piazza F, Manni S, Tubi LQ, et al. Glycogen Synthase Kinase-3 regulates multiple myeloma cell growth and bortezomib-induced cell death. BMC Cancer. 2010; 10(1). doi: 10.1186/1471-2407-10-526
13. Wilson W, Baldwin AS. Maintenance of Constitutive IκB Kinase Activity by Glycogen Synthase Kinase-3α/β in Pancreatic Cancer. Cancer Research. 2008; 68(19): 8156-8163. doi: 10.1158/0008-5472.can-08-1061
14. Forde JE, Dale TC. Glycogen synthase kinase 3: A key regulator of cellular fate. Cellular and Molecular Life Sciences. 2007; 64(15): 1930-1944. doi: 10.1007/s00018-007-7045-7
15. Liang MH, Chuang DM. Differential Roles of Glycogen Synthase Kinase-3 Isoforms in the Regulation of Transcriptional Activation. Journal of Biological Chemistry. 2006; 281(41): 30479-30484. doi: 10.1074/jbc.m607468200
16. Hoeflich KP, Luo J, Rubie EA, et al. Requirement for glycogen synthase kinase-3β in cell survival and NF-κB activation. Nature. 2000; 406(6791): 86-90. doi: 10.1038/35017574
17. Borden BA, Baca Y, Xiu J, et al. The Landscape of Glycogen Synthase Kinase-3 Beta Genomic Alterations in Cancer. Molecular Cancer Therapeutics. 2021; 20(1): 183-190. doi: 10.1158/1535-7163.mct-20-0497
18. Grassilli E, Ianzano L, Bonomo S, et al. GSK3A Is Redundant with GSK3B in Modulating Drug Resistance and Chemotherapy-Induced Necroptosis. Condorelli G, ed. PLoS ONE. 2014; 9(7): e100947. doi: 10.1371/journal.pone.0100947
19. Gao L, Lu Y, Chen HN, et al. Deciphering the Clinical Significance and Kinase Functions of GSK3α in Colon Cancer by Proteomics and Phosphoproteomics. Molecular & Cellular Proteomics. 2023; 22(5): 100545. doi: 10.1016/j.mcpro.2023.100545
20. Guil-Luna S, Rivas-Crespo A, Navarrete-Sirvent C, et al. Clinical significance of glycogen synthase kinase 3 (GSK-3) expression and tumor budding grade in colorectal cancer: Implications for targeted therapy. Biomedicine & Pharmacotherapy. 2023; 167: 115592. doi: 10.1016/j.biopha.2023.115592
21. Cao X, Wu W, Wang D, et al. Glycogen synthase kinase GSK3α promotes tumorigenesis by activating HIF1/VEGFA signaling pathway in NSCLC tumor. Cell Communication and Signaling. 2022; 20(1). doi: 10.1186/s12964-022-00825-3
22. Kaur J. Prognostic Effect of Glycogen Synthase Kinase 3A (GSK3A) mRNA Expression in Breast Cancer Patients. American Journal of Biomedical Sciences. Published online April 2022: 58-71. doi: 10.5099/aj220200058
23. Kovács SA, Fekete JT, Győrffy B. Predictive biomarkers of immunotherapy response with pharmacological applications in solid tumors. Acta Pharmacologica Sinica. 2023; 44(9): 1879-1889. doi: 10.1038/s41401-023-01079-6

24. Győrffy B. Discovery and ranking of the most robust prognostic biomarkers in serous ovarian cancer. GeroScience. 2023; 45(3): 1889-1898. doi: 10.1007/s11357-023-00742-4
25. Liu H. Microarray probes and probe sets. Frontiers in Bioscience. 2010; E2(1): 325-338. doi: 10.2741/e93
26. Siegel RL, Miller KD, Goding Sauer A, et al. Colorectal cancer statistics, 2020. CA: A Cancer Journal for Clinicians. 2020; 70(3): 145-164. doi: 10.3322/caac.21601
27. Ansa B, Coughlin S, Alema-Mensah E, et al. Evaluation of Colorectal Cancer Incidence Trends in the United States (2000–2014). Journal of Clinical Medicine. 2018; 7(2): 22. doi: 10.3390/jcm7020022
28. Ali A, Hoeflich KP, Woodgett JR. Glycogen Synthase Kinase-3: Properties, Functions, and Regulation. Chemical Reviews. 2001; 101(8): 2527-2540. doi: 10.1021/cr000110o
29. Luo J. Glycogen synthase kinase 3β (GSK3β) in tumorigenesis and cancer chemotherapy. Cancer Letters. 2009; 273(2): 194-200. doi: 10.1016/j.canlet.2008.05.045
30. McCubrey JA, Davis NM, Abrams SL, et al. Diverse roles of GSK-3: Tumor promoter–tumor suppressor, target in cancer therapy. Advances in Biological Regulation. 2014; 54: 176-196. doi: 10.1016/j.jbior.2013.09.013
31. McCubrey JA, Fitzgerald TL, Yang LV, et al. Roles of GSK-3 and microRNAs on epithelial mesenchymal transition and cancer stem cells. Oncotarget. 2016; 8(8): 14221-14250. doi: 10.18632/oncotarget.13991
32. Doble BW, Woodgett JR. GSK-3: tricks of the trade for a multi-tasking kinase. Journal of Cell Science. 2003; 116(7): 1175-1186. doi: 10.1242/jcs.00384
33. Vidri RJ, Fitzgerald TL. GSK-3: An important kinase in colon and pancreatic cancers. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research. 2020; 1867(4): 118626. doi: 10.1016/j.bbamcr.2019.118626
34. Valvezan AJ, Zhang F, Diehl JA, et al. Adenomatous Polyposis Coli (APC) Regulates Multiple Signaling Pathways by Enhancing Glycogen Synthase Kinase-3 (GSK-3) Activity. Journal of Biological Chemistry. 2012; 287(6): 3823-3832. doi: 10.1074/jbc.m111.323337
35. Shakoori A, Ougolkov A, Yu ZW, et al. Deregulated GSK3β activity in colorectal cancer: Its association with tumor cell survival and proliferation. Biochemical and Biophysical Research Communications. 2005; 334(4): 1365-1373. doi: 10.1016/j.bbrc.2005.07.041
36. Ougolkov AV, Fernandez-Zapico ME, Bilim VN, et al. Aberrant Nuclear Accumulation of Glycogen Synthase Kinase-3β in Human Pancreatic Cancer: Association with Kinase Activity and Tumor Dedifferentiation. Clinical Cancer Research. 2006; 12(17): 5074-5081. doi: 10.1158/1078-0432.ccr-06-0196
37. Ougolkov AV, Bone ND, Fernandez-Zapico ME, et al. Inhibition of glycogen synthase kinase-3 activity leads to epigenetic silencing of nuclear factor κB target genes and induction of apoptosis in chronic lymphocytic leukemia B cells. Blood. 2007; 110(2): 735-742. doi: 10.1182/blood-2006-12-060947
38. Kunnimalaiyaan M, Vaccaro AM, Ndiaye MA, et al. Inactivation of glycogen synthase kinase-3β, a downstream target of the raf-1 pathway, is associated with growth suppression in medullary thyroid cancer cells. Molecular Cancer Therapeutics. 2007; 6(3): 1151-1158. doi: 10.1158/1535-7163.mct-06-0665
39. Adler JT, Cook M, Luo Y, et al. Tautomycetin and tautomycin suppress the growth of medullary thyroid cancer cells via inhibition of glycogen synthase kinase-3β. Molecular Cancer Therapeutics. 2009; 8(4): 914-920. doi: 10.1158/1535-7163.mct-08-0712
40. Carter YM, Kunnimalaiyaan S, Chen H, et al. Specific glycogen synthase kinase-3 inhibition reduces neuroendocrine markers and suppresses neuroblastoma cell growth. Cancer Biology & Therapy. 2014; 15(5): 510-515. doi: 10.4161/cbt.28015
41. Ougolkov AV, Fernandez-Zapico ME, Savoy DN, et al. Glycogen Synthase Kinase-3β Participates in Nuclear Factor κB–Mediated Gene Transcription and Cell Survival in Pancreatic Cancer Cells. Cancer Research. 2005; 65(6): 2076-2081. doi: 10.1158/0008-5472.can-04-3642
42. Kunnimalaiyaan S, Clark Gamblin T, Kunnimalaiyaan M. Glycogen synthase kinase-3 inhibitor AR-A014418 suppresses pancreatic cancer cell growth via inhibition of GSK-3-mediated Notch1 expression. HPB. 2015; 17(9): 770-776. doi: 10.1111/hpb.12442
43. Thapa R, Gupta G, Bhat AA, et al. A review of Glycogen Synthase Kinase-3 (GSK3) inhibitors for cancers therapies. International Journal of Biological Macromolecules. 2023; 253: 127375. doi: 10.1016/j.ijbiomac.2023.127375

44. Sahin I, Eturi A, De Souza A, et al. Glycogen synthase kinase-3 beta inhibitors as novel cancer treatments and modulators of antitumor immune responses. Cancer Biology & Therapy. 2019; 20(8): 1047-1056. doi: 10.1080/15384047.2019.1595283
45. Augello G, Emma MR, Cusimano A, et al. The Role of GSK-3 in Cancer Immunotherapy: GSK-3 Inhibitors as a New Frontier in Cancer Treatment. Cells. 2020; 9(6): 1427. doi: 10.3390/cells9061427
46. Walz A, Ugolkov A, Chandra S, et al. Molecular Pathways: Revisiting Glycogen Synthase Kinase-3β as a Target for the Treatment of Cancer. Clinical Cancer Research. 2017; 23(8): 1891-1897. doi: 10.1158/1078-0432.ccr-15-2240
47. Madhunapantula SV, Sharma A, Gowda R, et al. Identification of glycogen synthase kinase 3α as a therapeutic target in melanoma. Pigment Cell & Melanoma Research. 2013; 26(6): 886-899. doi: 10.1111/pcmr.12156
48. Sharma A, Sharma AK, Madhunapantula SV, et al. Targeting Akt3 Signaling in Malignant Melanoma Using Isoselenocyanates. Clinical Cancer Research. 2009; 15(5): 1674-1685. doi: 10.1158/1078-0432.ccr-08-2214
49. Nakayama M, Oshima M. Mutant p53 in colon cancer. Journal of Molecular Cell Biology. 2018; 11(4): 267-276. doi: 10.1093/jmcb/mjy075
50. Schubbert S, Shannon K, Bollag G. Hyperactive Ras in developmental disorders and cancer. Nature Reviews Cancer. 2007; 7(4): 295-308. doi: 10.1038/nrc2109
51. Dinu D, Dobre M, Panaitescu E. et al. Prognostic significance of KRAS gene mutations in colorectal cancer--preliminary study. J Med Life. 2014; 7: 581-7.
52. Caputo F, Santini C, Bardasi C, et al. BRAF-Mutated Colorectal Cancer: Clinical and Molecular Insights. International Journal of Molecular Sciences. 2019; 20(21): 5369. doi: 10.3390/ijms20215369
53. Tejpar S, Bertagnolli M, Bosman F, et al. Prognostic and Predictive Biomarkers in Resected Colon Cancer: Current Status and Future Perspectives for Integrating Genomics into Biomarker Discovery. The Oncologist. 2010; 15(4): 390-404. doi: 10.1634/theoncologist.2009-0233
54. Neumann JHL, Jung A, Kirchner T. Molekulare Pathologie des kolorektalen Karzinoms. Der Pathologe. 2015; 36(2): 137-144. doi: 10.1007/s00292-015-0005-3
55. Zeinalian M, Hashemzadeh-Chaleshtori M, Salehi R, Emami MH. Clinical Aspects of Microsatellite Instability Testing in Colorectal Cancer. Adv Biomed Res. 2018; 7:28. doi: 10.4103/abr.abr_185_16.
56. Guinney J, Dienstmann R, Wang X, et al. The consensus molecular subtypes of colorectal cancer. Nature Medicine. 2015; 21(11): 1350-1356. doi: 10.1038/nm.3967
57. Peng Z, Ji Z, Mei F, et al. Lithium Inhibits Tumorigenic Potential of PDA Cells through Targeting Hedgehog-GLI Signaling Pathway. Xie J, ed. PLoS ONE. 2013; 8(4): e61457. doi: 10.1371/journal.pone.0061457
58. Acikgoz E, Güler G, Camlar M, et al. Glycogen synthase kinase-3 inhibition in glioblastoma multiforme cells induces apoptosis, cell cycle arrest and changing biomolecular structure. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2019; 209: 150-164. doi: 10.1016/j.saa.2018.10.036
59. Park R, Coveler AL, Cavalcante L, et al. GSK-3β in Pancreatic Cancer: Spotlight on 9-ING-41, Its Therapeutic Potential and Immune Modulatory Properties. Biology. 2021; 10(7): 610. doi: 10.3390/biology10070610
60. Schrecengost RS, Green CL, Zhuang Y, et al. In Vitro and In Vivo Antitumor and Anti-Inflammatory Capabilities of the Novel GSK3 and CDK9 Inhibitor ABC1183. Journal of Pharmacology and Experimental Therapeutics. 2018; 365(1): 107-116. doi: 10.1124/jpet.117.245738