Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

A detailed multi-omics analysis of GNB2 gene in human cancers

Uma análise multiômica detalhada do gene GNB2 em cânceres humanos

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Abstract

The Guanine-nucleotide binding protein 2 (GNB2) encodes for β2 subunit (Gβ2) of the G-protein complex. Keeping in view the increased demand of reliable biomarkers in cancer, the current study was planned to extensively explored GNB2 expression variation and its roles in different cancers using online available databases and diverse methodology. In view of our results, the GNB2 was notably up-regulated relative to corresponding controls in twenty three cancer types. As well, the elevated expression of GNB2 was found to be associated with the reduced overall survival (OS) of the Liver Hepatocellular Carcinoma (LIHC) and Rectum Adenocarcinoma (READ) only out of all analyzed cancer types. This implies GNB2 plays vital role in the tumorigenesis of LIHC and READ. Several additional analysis also explored six critical pathways and few important correlations related to GNB2 expression and different other parameters such as promoter methylation, tumor purity, CD8+ T immune cells infiltration, and genetic alteration, and chemotherapeutic drugs. In conclusion, GNB2 gene has been identified in this study as a shared potential biomarker (diagnostic and prognostic) of LIHC and READ.

Keywords: GNB2, tumorigenesis, LIHC, biomarker, READ.

Resumo

A proteína 2 de ligação de nucleotídeos de guanina (GNB2) codifica a subunidade β2 (Gβ2) do complexo da proteína G. Tendo em vista o aumento da demanda de biomarcadores em câncer, o presente estudo foi planejado para explorar extensivamente a variação da expressão de GNB2 e seus papéis em diferentes cânceres usando bancos de dados on-line disponíveis e metodologia diversificada. Em vista de nossos resultados, o GNB2 foi notavelmente regulado para cima em relação aos controles correspondentes em 23 tipos de câncer. Além disso, a expressão elevada de GNB2 foi associada à redução da sobrevida global (OS) do carcinoma hepatocelular do fígado (LIHC) e do adenocarcinoma do reto (READ) apenas em todos os tipos de câncer analisados. Isso implica que GNB2 desempenha um papel vital na tumorigênese de LIHC e READ. Várias análises adicionais também exploraram seis vias críticas e poucas correlações relacionadas à expressão de GNB2 e diferentes outros parâmetros, como metilação do promotor, pureza do tumor, infiltração de células T CD8+, alteração genética e drogas quimioterápicas. Em conclusão, o gene GNB2 foi identificado neste estudo como um potencial biomarcador compartilhado (diagnóstico e prognóstico) de LIHC e READ.

Palavras-chave: GNB2, tumorigênese, LIHC, biomarcador, READ.

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Received: January 18, 2022 – Accepted: May 7, 2022

1. Background

Cancer is the main cause of mortality, serious social and economic issues worldwide (Nagai and Kim, 2017; Sung et al., 2021). As per the cancer statistics 2020 of Cancer Registry, Health Research Institute (HRI) Pakistan, a total of new 148,000 cases of cancer and around 100,000 deaths due to cancer were reported in Pakistan. Similarly in China, a total of 2,205,200 (including 1,425,700 male and 779,500 female) cancer-related deaths were reported in 2017, accounting for 22.40% of the total deaths (Nagai and Kim, 2017). These huge numbers of cancer-related cases and deaths indicated the lack of appropriate biomarkers for the management of disease. Furthermore, in addition to conventional cancer treatment strategies such as surgery, radiotherapy, and chemotherapy, the tyrosine kinase inhibitors (TKIs) have provided a new reliable treatment options for cancer patients. The usage of TKIs targeting different mutational hotspots in different cancers, for example, anaplastic lymphoma kinase (ALK) is growing rapidly (Koivunen et al., 2008). However, drug resistance remains a major obstacle in the TKIs treatment strategy (Pearson et al., 2022; Song et al., 2021). In addition to secondary drug resistance caused by heterogeneity in cancer (Zhou et al., 2009), the primary drug resistance also restricts the TKIs treatment efficiency and ultimately results in the mismanagement of the disease (Shih et al., 2005). Therefore, its urgent to identify the high-quality molecular biomarkers and therapeutics targets that could help to diagnosis, and treatment of cancer patients.

Guanine nucleotide-binding protein subunit beta-2 (GNB2) is a member of the guanine nucleotide-binding proteins family and encodes of the GNB2 protein (Cai et al., 2021; Fjær et al., 2021). This protein is an essential regulator of G-protein alpha subunit, as well as of various other signal transduction pathways (Malerba et al., 2019; Liu et al., 2021; Ofoe, 2021). Best to our knowledge, the studies relating GNB2 to human cancer are very limited. Kotani et al. (Kotani et al., 2019) have reported that mutation and up-regulation of GNB2 could cause leukemogenesis, furthermore, in that study, it was also indicated that decreased GNB2 expression could reduce the proliferation of tumor cells. Furthermore, Yoda et al. (Yoda et al., 2015) have reported that different types of mutations in GNB2 can activate numerous canonical signaling pathways and confer resistance to targeted kinase inhibitors in several cancer types including acute myeloid leukemia and melanoma.

At present, the identification of crucial cancer-associated biomarkers and therapeutics targets using cancer-based gene expression databases and bioinformatics analysis has become reliable (Rung and Brazma, 2013; Xu et al., 2020). In our study, we briefly analyzed the GNB2 expression and its association with prognostic values of different cancer patients using cancer-based gene expression databases and Bioinformatics analysis. Findings of this study predicted that GNB2 gene is a reliable biomarker (diagnostic and prognostic) of Liver Hepatocellular Carcinoma (LIHC), and Rectum Adenocarcinoma (READ).

2. Methods

2.1. UALCAN

The UALCAN portal (http://ualcan.path.uab.edu/analysis-prot.html), an interactive web resource for analyzing cancer Omics data, allowed us to conduct protein expression analysis of the CPTAC (Clinical proteomic tumor analysis consortium) dataset (Chandrashekar et al., 2017). Herein, we explored the expression level of the total pro UALCAN (http://ualcan.path.uab.edu/analysis-prot.html), is an excellent and easy-to-use web resource for exploring TCGA cancer Omics data, thus, facilitated scientists to perform the mRNA and protein expression analysis of gene of interest (Chandrashekar et al., 2017). Herein, this database was used differential expression and methylation analysis of GNB2 in different cancer types..

2.2. KM plotter

The GNB2 gene symbol was queued in the KM plotter (http://kmplot.com/analysis/index.php?p=service) (Maciejczyk et al., 2013) tool to obtain the overall survival (OS) curves across different cancer types.

2.3. GEPIA and Human Protein Atlas (HPA)

GEPIA (http://gepia.cancer-pku.cn/) is a cancer transcriptomics data analysis webserver (Tang et al., 2017) while HPA webserver (https:/www.proteinatlas.org/) is a source of immunohistochemistry (IHC) based proteomic data obtained from the tissues or cells of the different cancer subtypes (Uhlén et al., 2015). In our study we used GEPA and HPA for the cross validation of GENB2 expression at both mRNA and protein level using new independent cohorts.

2.4. cBioPortal

After opening the cBioPortal web port (https://www.cbioportal.org/) (Cerami et al., 2012), we selected the "TCGA Pancancer Atlas, LIHC and COAD" datasets in the "Quick select" section and entered "GNB2" in the query box to identify the copy number variations (CNVs) and genetic alterations of GNB2 in LIHC and COAD patients. As a result, the CNVs and mutations information were papered as horizontal bars in the schematic diagram.

2.5. STRING, Cytoscape, and DAVID

STRING (http://string-db.org/) (von Mering et al., 2003) was conducted to obtain GNB2 PPI network. Furthermore, Cytoscape (Shannon et al., 2003) was also conducted to analyze GNB2 PPI network. Finally, pathway enrichment was done via DAVID (Huang et al., 2007).

2.6. TIMER

In this study, we utilized the "Immune-Gene" module from the TIMER2 database (https://cistrome.shinyapps.io/timer/) to evaluate the association between Tumor purity, CD8+ T immune cells infiltration, GNB2 expression across LIHC and READ (Li et al., 2020).

2.7. Screening of GNB2-associated chemotherapeutic drugs

GNB2 was supposed to be a promising therapeutic target, therefore, the Comparative Toxicogenomics Database (CTD, http://ctdbase.org/) (Mattingly et al., 2003) was queued in the current study to find GNB2-associated chemotherapeutic drugs.

3. Results

3.1. GNB2 expression profiling

We analyzed the GNB2 transcription gene expression level across 24 types of human cancer sample paired with normal controls through Pan-cancer analysis feature of UALCAN. Results demonstrated that GNB2 was significantly down-regulated in Kidney Chromophobe (KICH) samples as compared to the normal control, while significantly up-regulated in 23 types of other cancer samples relative to controls including LIHC, and READ (Figure 1).

3.2. GNB2 prognostic importance

The KM analysis has helped us to evaluate GNB2 prognostic importance across 24 types of cancers across TCGA datasets. Results of the analysis shown that higher GNB2 expression level predicted significantly ($p<0.05$) poor prognosis (OS duration) in LIHC and READ patients (Figure 2). Taken together, these data suggested that GNB2 might have a significant contribution to the development and progression of LIHC and READ, thus the next part of our study will mainly focus on the unique role of GNB2 in these two types of cancers.

3.3. Association between GNB2 and clinical variables

Via this analysis, GNB2 expression variations were analyzed in normal and LIHC and READ samples of different clinicopathological parameters including different individual cancer stage, patient race, patient gender, and patient body weight. Results have shown that GNB2 was also significantly ($p<0.05$) overexpressed in LIHC and READ patients with different clinicopathological variable relative to the corresponding control samples (Figure 3).

3.4. GNB2 expression validation

We further validated the GNB2 expression at both mRNA and protein level via GEPIA and HPA databases using new independent cohorts of LIHC and READ. In view

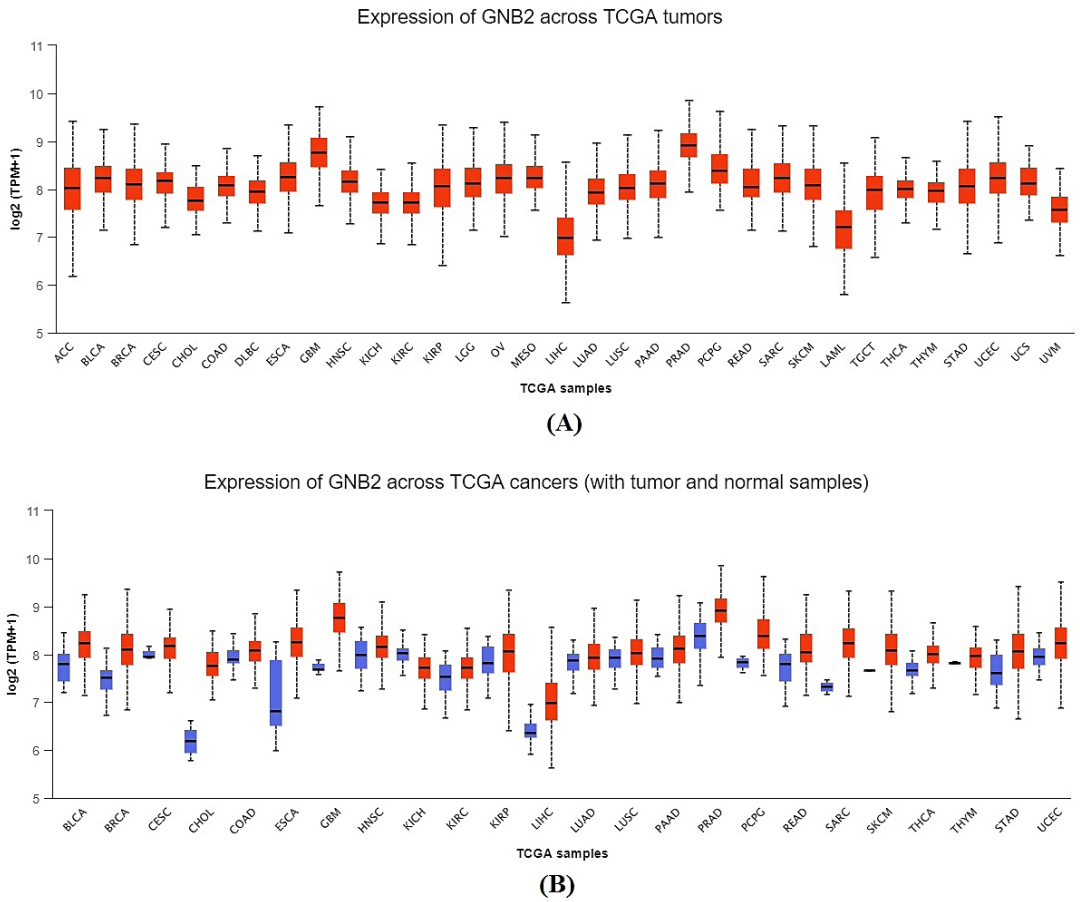

Figure 1. GNB2 mRNA expression across various cancers. (A) GNB2 mRNA expression across cancer subjects only, and (B) GNB2 mRNA expression across cancer subjects and corresponding controls. P-value less than 0.05 = significant difference.

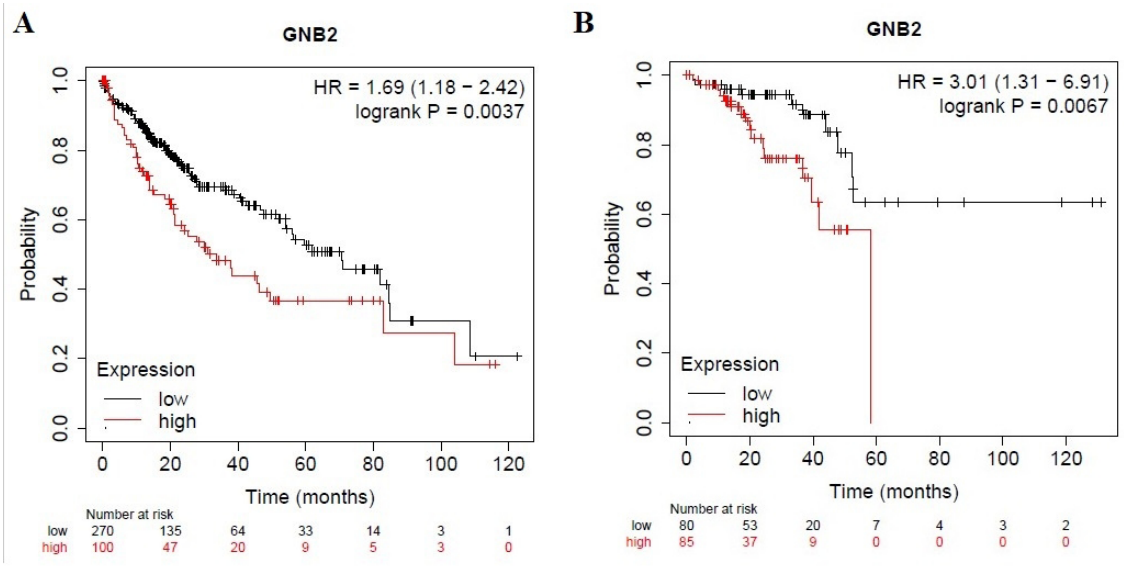

Figure 2. This figure shows the KM plotter-based GNB2 OS analysis results. (A) across LIHC, and (B) across READ. P-value less than 0.05 = significant difference.

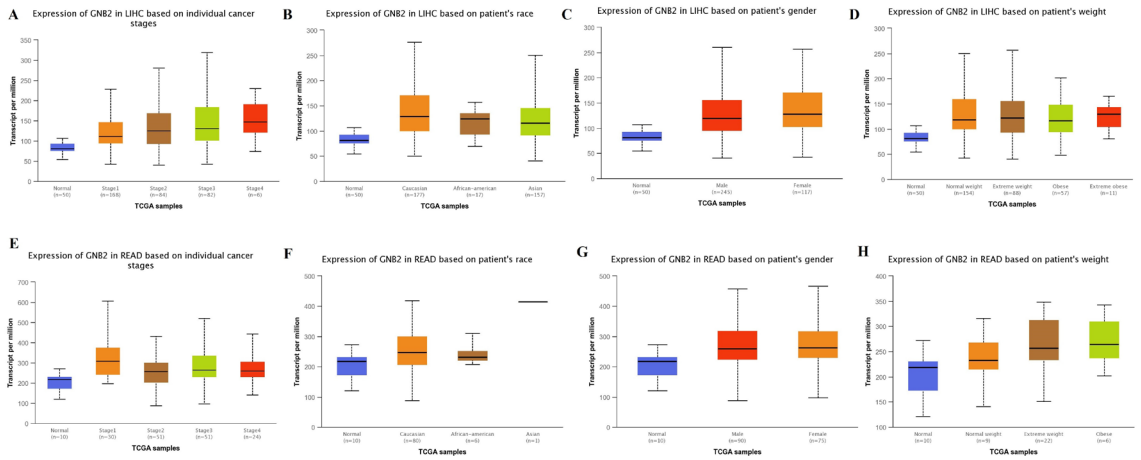

Figure 3. Clinical variable-wise GNB2 expression. (A) Cancer stage-wise GNB2 expression across LIHC, (B) Patient race-wise GNB2 expression across LIHC, (C) Patient gender-wise GNB2 expression across LIHC, (D) Patient body weight-wise GNB2 expression across LIHC, (E) Cancer stage-wise GNB2 expression across READ, (F) Patient race-wise GNB2 expression across READ, (G) Patient gender-wise GNB2 expression across READ, (H) Patient body weight-wise GNB2 expression across READ. P-value less than 0.05 = significant difference.

of our results, we also observed the significant ($p<0.05$) up-regulation of GNB2 at both mRNA and protein level in the LIHC and READ patients of new independent cohorts (Figure 4). Taken together, these analyses have further validated our findings.

3.5. GNB2 prompter methylation

Via this analysis, we analyzed the correlations among GNB2 expression and its promoter methylation in LIHC and READ using UALCAN. Results of the analysis revealed the significant ($p<0.05$) negative correlations among GNB2 expression and its promoter methylation in LIHC and READ (Figure 5).

3.6. Genetic alterations in GNB2

GNB2 genetic alterations related data across LIHC and READ samples were obtained from different TCGA datasets including, LIHC (TCGA, Pancancer Atlas consisting of 372 cancerous samples), and READ (TCGA, Pancancer Atlas consisting of 594 cancerous samples). Results revealed that GNB2 harbors genetic alterations in 1.2%, 1.1% samples of the LIHC and READ, respectively, with maximum deep amplification abnormalities (Figure 6).

3.7. GNB2 PPI network

GNB2 PPI network that was obtained via STRING database and visualized through Cytoscape has revealed

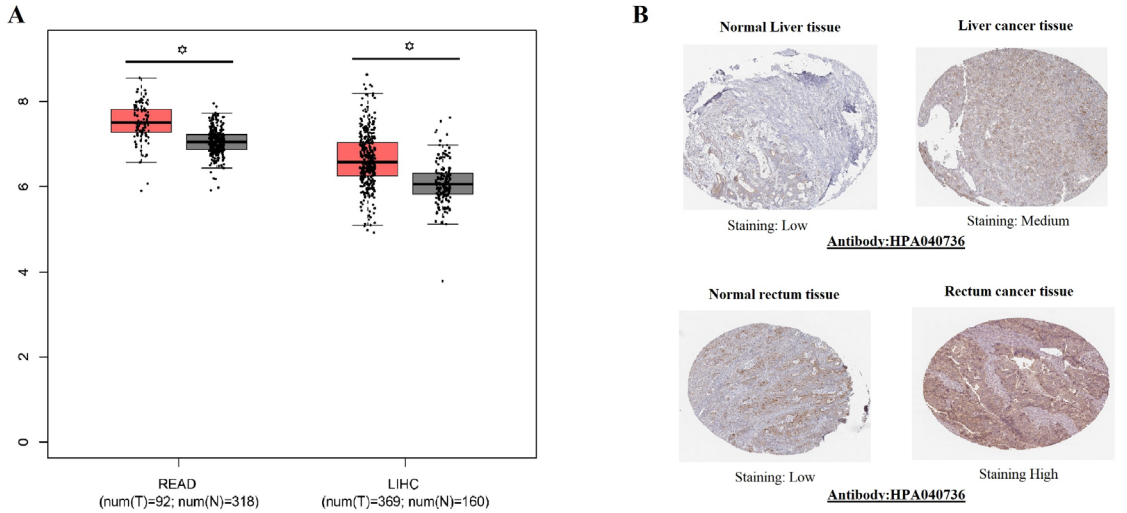

Figure 4. GEPIA and HPA-based mRNA and protein expression validation of GNB2. (A) GEPIA-based mRNA expression validation of GNB2 across LIHC and READ, and (B) HPA based protein expression validation of GNB2 in liver and rectum cancer tissues. In HPA, normal liver and rectum tissues have displayed the down-regulation (showing low level of staining) of GNB2, however, in cancerous liver and rectum tissues, GNB2 was found overexpressed (showing medium and high level of staining, respectively). P-value less than 0.05 = significant difference.

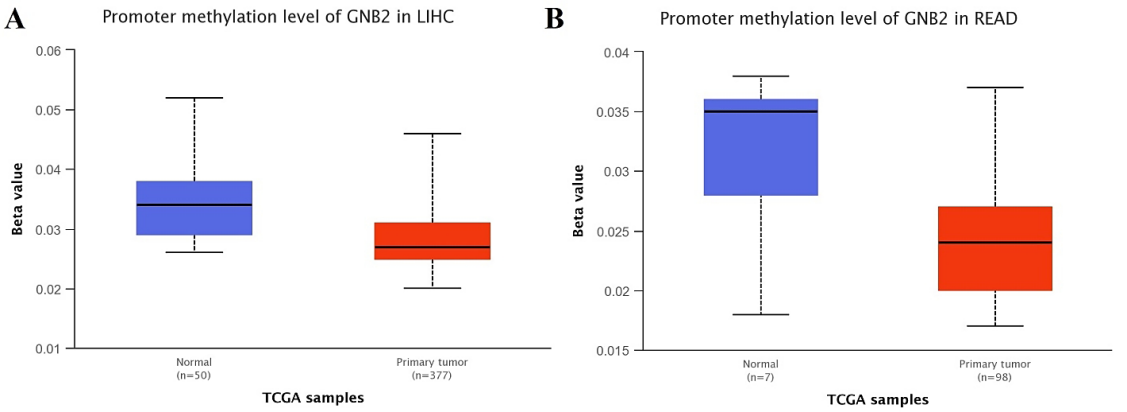

Figure 5. DNA promoter methylation level of GNB2. (A) across LIHC, and (B) across READ. P-value less than 0.05 = significant difference.

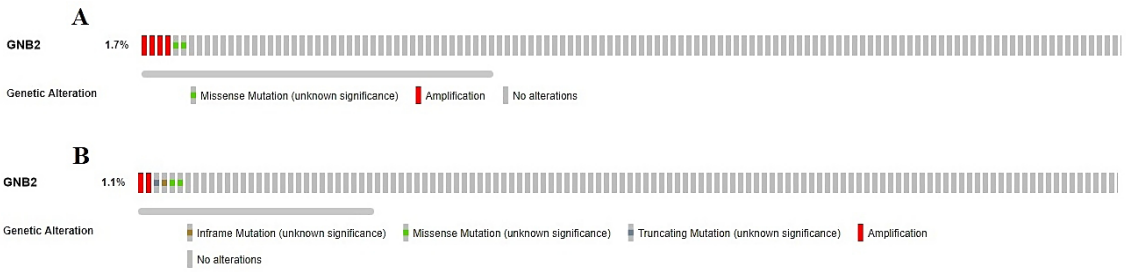

Figure 6. TCGA datasets-based genetic alterations information of GNB2. (A) information taken from LIHC dataset, and (B) information taken from READ dataset.

in total 10 GNB2-associated genes. Furthermore, the pathway enrichment of these genes has shown the significant involvement of these genes in a variety of pathways including “Carbon metabolism”, “Glycolysis / Gluconeogenesis”, “Biosynthesis of amino acids”, and “Biosynthesis of antibioics” (Figure 7; Table 1).

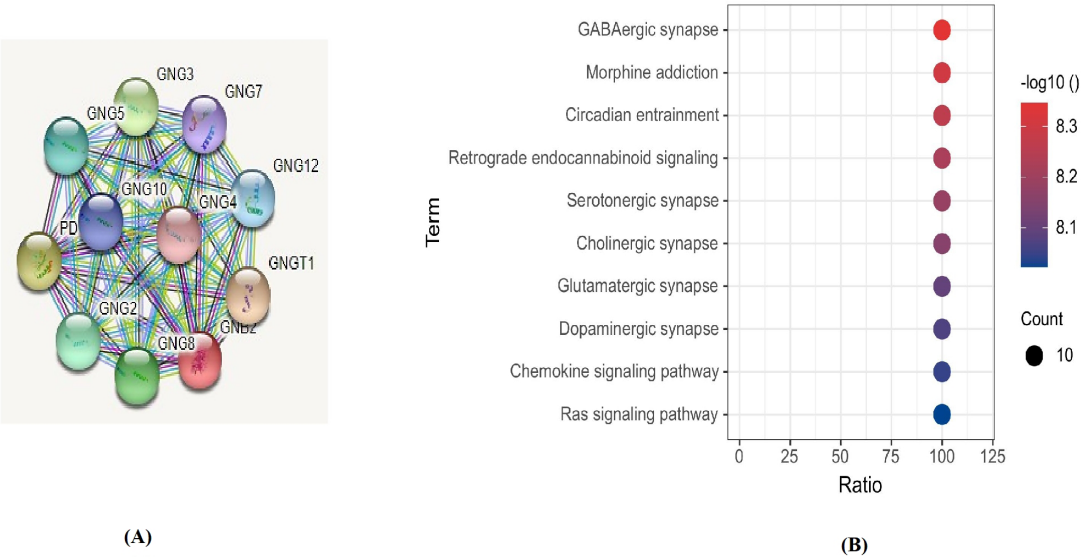

Figure 7. A PPI network and KEGG pathway enrichment of GNB2 linked genes. (A) A PPI network of GNB2 linked genes, and (B) KEGG pathway enrichment of GNB2 linked genes.

Table 1. KEGG enrichment of GNB2 linked genes.

ID number	Pathway	Involved genes count	P-value	Genes involved
04727	GABAergic synapse	10	< 0.05	GNGT1, GNG3, GNG10, GNG2, GNG5, GNG4, GNG7, GNB2, GNG8, GNG12
05032	Morphine addiction	10	< 0.05	GNGT1, GNG3, GNG10, GNG2, GNG5, GNG4, GNG7, GNB2, GNG8, GNG12
04713	Circadian entrainment	10	< 0.05	GNGT1, GNG3, GNG10, GNG2, GNG5, GNG4, GNG7, GNB2, GNG8, GNG12
04723	Retrograde endocannabinoid signaling	10	< 0.05	GNGT1, GNG3, GNG10, GNG2, GNG5, GNG4, GNG7, GNB2, GNG8, GNG12
04726	Serotonergic synapse	10	< 0.05	GNGT1, GNG3, GNG10, GNG2, GNG5, GNG4, GNG7, GNB2, GNG8, GNG12
04725	Cholinergic synapse	10	< 0.05	GNG3, GNG10, GNG2, GNG5, GNG4, GNG7, GNB2, GNG8, GNG12
04724	Glutamatergic synapse	10	< 0.05	GNGT1, GNG3, GNG10, GNG2, GNG5, GNG4, GNG7, GNB2, GNG8, GNG12
04728	Dopaminergic synapse	10	< 0.05	GNGT1, GNG3, GNG10, GNG2, GNG5, GNG4, GNG7, GNB2, GNG8, GNG12
04062	Chemokine signaling pathway	10	< 0.05	GNGT1, GNG3, GNG10, GNG2, GNG5, GNG4, GNG7, GNB2, GNG8, GNG12
04014	Ras signaling pathway	10	< 0.05	GNGT1, GNG3, GNG10, GNG2, GNG5, GNG4, GNG7, GNB2, GNG8, GNG12

KEGG = Kyoto Encyclopedia of Genes and Genomes

3.8. GNB2 have correlations with tumor purity and CD8+ T immune cells infiltration

Correlations between CD8+ T immune cells infiltration and genes expression have laid the foundation of improved cancer immunotherapies at present (Ziai et al., 2018). So, in our study, we figured out the correlations among CD8+ T immune cells infiltration and GNB2 in READ and LIHC using TIMER. Results revealed a significant (p>0.05) positive correlation between CD8+ T immune cells infiltration and GNB2 expression in LIHC while a significant (p>0.05) negative correlation in READ (Figure 8A). Moreover, we have also observed a significant (p>0.05) positive correlation

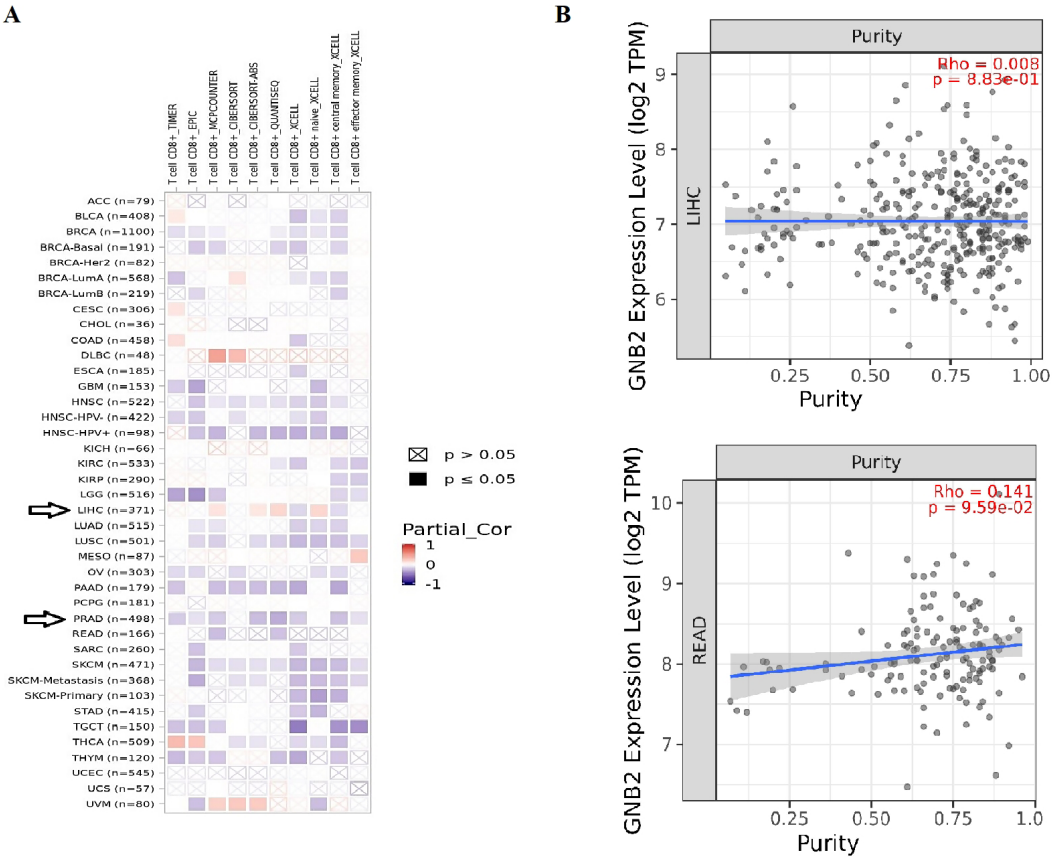

Figure 8. Correlations among tumor purity, CD8+ T immune cells, and GNB2 expression across LIHC and READ. (A) Correlations between CD8+ T immune cells, and GNB2 expression across LIHC and READ, and (B) Correlations between tumor purity, and GNB2 expression across LIHC and READ. P-value less than 0.05 = significant difference.

between tumor purity and GNB2 expression in LIHC and READ (Figure 8B).

3.9. Screening of GNB2-associated chemotherapeutic drugs

By considering the gene-drug interaction network constructed via CTD and Cytoscape, we noticed that GNB2 expression can be regulated by different drugs drugs. For example, bisphenol A and gefitnib could overexpress GNB2 while clofibrate and undecane could suppress GNB2 expression level (Figure 9).

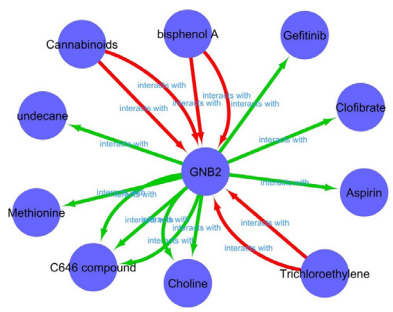

Figure 9. CTD-based GNB2 gene-drug interaction network. Red arrows: chemotherapeutic drugs relevant to higher GNB2 expression, green arrows: chemotherapeutic drugs relevant to lower GNB2 expression, and count of arrows: specific interaction supported numbers of studies in medical literature.

4. Discussion

Expression analysis-based study is an ideal approach to analyze the cancer-associated biomarkers (Su et al., 2021). In the current study we applied an integrated bioinformatics approach to analyze the significance of GNB2 gene as potential biomarker (Gautam et al., 1998).

So far, GNB2 has been not been studied extensively as a potential candidate gene involved in cancer initiation and progression with controversial results. For example, Kotani et al. (Kotani et al., 2019) have reported that mutation and up-regulation of GNB2 as a causative agent of leukemogenesis, and they also indicated through a series of experiments that decreased GNB2 expression could reduce the proliferation of tumor cells. Yoda et al. (Yoda et al., 2015) reported that different types of mutations in GNB2 can activate numerous canonical signaling pathways and confer resistance to targeted kinase inhibitors in several cancer types including acute myeloid leukemia and melanoma.

Petrovski et al. (Petrovski et al., 2016) have reported few other pathogenic variants of GNB2 including Gly77 residue, which were mainly clustered in the Gα-Gβγ binding region. According to their functional impact prediction, the p.Gly77Arg variant may possibly affect the inhibition or activation of various downstream effector proteins. Recently, another GNB2 variant (p.Arg52Leu) was reported in a 3 generation family with dysfunctioning of sinus node and atrioventricular conduction (Stallmeyer et al., 2017). In another report, a novel variant and down-regulation of GNB2 has also been reported in a sporadic case of global developmental delay and intellectual disability (Fukuda et al., 2020).

We revealed that GNB2 was up-regulated in twenty three types of human cancers and its overexpression was significantly ($p<0.05$) correlated with the decreased OS durations of LIHC and READ patients. Altogether these results give us clues that GNB2 play a key role in tumorigenesis of LIHC and READ. We next also documented the significant overexpression of GNB2 across LIHC and READ patients with various clinicopathological variables.

As genetic abnormalities and DNA promoter methylation level are the prime reasons of abnormal gene expression variations, GNB2 was analyzed for to check the participation of these two parameters in the up-regulation of GNB2 across LIHC and READ samples. In view of our analysis results, GNB2 was found harboring genetic abnormalities in the least proportions (1.2%, 1.1% cases) of the analyzed LIHC and READ patients, respectively. Therefore, we speculate that genetic abnormities do not affect the GNB2 expression. In addition, we also explored the expected negative correlations between GNB2 expression and its promoter methylation level across LIHC and READ samples. These observed negative correlations confirmed the role of promoter hypomethylation in the up-regulation of GNB2 gene across LIHC and READ.

At present, dysregulation of different genes such as ALB, CCNB1, CDC20, CCNA2, CDK1, MAD2L1, AURKA, TOP2A, EZH2, CCNB2, TYMS, KIF11, BUB1B, ESR1, BUB1 have been reported as the potential biomarkers of LIHC (Zou et al., 2020; Ameri et al., 2022). However, none out of these or any other biomarkers have been generalized so far across LIHC subjects with different clinicopathological variables. In the present study, we revealed the significant overexpression of GNB2 expression in LIHC patients with different clinicopathological variables (cancer stage, patient race, patients gender, and patients body weight) relative to controls. Furthermore, we also shown that GNB2 overexpression decrease the OS duration of LIHC subjects.

To date, various molecular biomarkers have been investigated for the early detection and prognosis of READ, recently Kim et al. and collogues published a review article in which they compared the results of different studies which extensively investigated the different READ molecular biomarkers including KRAS pathway gene mutations, BRAF gene mutations, EGFR pathway gene mutations, stool biomarkers, CpG Island Methylator Phenotype of TP53 and BRAF, RNAs and miRNAs expression of various DNA repair and tumor suppressor genes such as TP53, BRCA1 and PTEN. The results of their brief analysis showed that the findings of different research groups concerning the prognostic or predictive value were not always consistent (Gonzalez-Pons and Cruz-Correa, 2015). Moreover, none out of these or any other biomarker have been generalized so far in READ patients with different clinicopathological features. Furthermore, we also shown that GNB2 overexpression decrease the OS duration of READ subjects.

Tumor purity and CD8+ T immune cells infiltration are important parameters to consider while designing the anticancer immunotherapy (Deng et al., 2021; Yuan et al., 2021). The interesting positive and negative correlations documented in this study between tumor purity, CD8+ T immune cells and GNB2 expression may open-up a new avenue for the treatment of LIHC and READ patients with respect to GNB2 expression.

Pathway enrichment of GNB2 associated genes highlighted the involvement of few genes in different key signaling pathways such as “Carbon metabolism”, “Glycolysis / Gluconeogenesis”, “Biosynthesis of amino acids”, and “Biosynthesis of antibioics”. Finally, using CTD and Cytoscape, we also identified few GNB2 expression regulatory drugs that could be useful in designing the appropriate chemotherapy for LIHC and READ patients.

5. Conclusion

In conclusion, based on the results of our detailed analysis, GNB2 is an important therapeutic target and molecular biomarker for predicting the diagnosis and prognosis of READ and LIHC patients. However, a voluminous testing is recommended prior to clinical implication.

Acknowledgement

The authors extend their appreciation on the Research Supporting Project number (RSP2022R470), King Saud University, Riyadh, Saudi Arabia.

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