Clinical Value and Potential Mechanisms Exploration of GOLGA8B in Hepatocellular Carcinoma: A Comprehensive Investigation Based on Real-Time Quantitative Polymerase Chain Reaction, RNA-seq, Microarray and Immunohistochemistry

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Abstract

Introduction Recent studies found that GOLGA8B plays an essential role in different cancers. However, the role GOLGA8B plays in the carcinogenesis and development of hepatocellular carcinoma (HCC) remains unclear. This study explores the clinical significance and prospective mechanisms of GOLGA8B in HCC.

Materials and methods The expression of GOLGA8B was detected in tissues of HCC and non-HCC controls. A real-time quantitative polymerase chain reaction analysis was performed to evaluate the mRNA expression of GOLGA8B. RNA-sequencing data and microarray chip data were obtained for further analysis. The role GOLGA8B plays in patients with HCC was also evaluated. An immunohistochemistry (IHC) analysis was also performed to evaluate the protein expression of GOLGA8B. The different GOLGA8B expression resources, including mRNA and protein expression, were integrated by calculating standard mean difference (SMD) and summary of the receiver operator characteristic (sROC). Genes co-expressed GOLGA8B were predicted. Enrichment analyses including Gene ontology (GO) and biological pathway were performed to investigate the essential molecular mechanisms. Hub genes were screened out by a protein-protein interactions network. MicroRNAs which target GOLGA8B at a posttranscriptional level were also predicted.

Results According to different resources, GOLGA8B manifested a higher expression in tissues of HCC than in non-HCC controls and exhibited clinical values for HCC. The RT-qPCR analysis revealed an increasing trend of GOLGA8B expression in HCC. GOLGA8B expression was significantly increased in RNA-sequencing and 7 of 13 microarray chip. IHC analysis also revealed significantly higher expression of GOLGA8B protein. Moreover, GOLGA8B expression was correlated with pathologic tumors and stages according to RNA-sequencing data and IHC analysis. The integrated SMD and sROC of different resources was 0.893 ($P=0.004$) and 0.79. A total 1303 co-expressed genes were gathered to perform enrichment analyses. The most significant biological pathways of co-expressed genes were spliceosome and mitogen-activated protein kinase signalling (MAPK). Four hub genes (SF3B1, HNRNPA2B1, HNRNPA1 and SRRM2) and five miRNAs (miR-369-3p, miR-203a, miR-374b-5p, miR-139-5p and miR-144-3p) were screened out.

Conclusion GOLGA8B expression was increased in HCC and may serve as a novel target for HCC diagnosis and treatment.

Background

Liver cancer is one of the most prevalent malignant cancers in the world, and it contributes to several cancer-related deaths. The estimated numbers of new cases and deaths of liver cancer are 42,030 and 31,780 in 2019 in the United States [1–2]. Hepatocellular carcinoma (HCC) is the most common class of liver cancer (85–90% liver cancer cases are HCC cases) [3]. Several risk factors—including alcohol consumption, xenobiotics, hepatitis B and C infections, primary biliary cirrhosis, diabetes and genetic disorders—contribute to HCC development and progression [4]. The mortality rate of HCC in the United States is increasing, but significant advances have been made in diagnosis and treatment [5]. However, the accurate mechanism of HCC development and progression remains unclear. Therefore, exploring the molecular mechanisms underlying HCC and seeking to innovate methods for HCC early diagnosis and treatment are necessary.

Golgin A8 family member B (GOLGA8B) is located at 15q14. The protein it encodes is a highly coiled-coil protein called golgin-67, with a molecular weight of 67 kDa. GOLGA8B belongs to the golgin family and functions as maintaining structural integrity of the Golgi complex [6–9]. Previous studies have demonstrated the relationships between golgins and variant cancers [10–12]. As for GOLGA8B, Baine et al. (2011) found that its expression level was decreased between pancreatic cancer patients and healthy controls through the whole genome cDNA microarray analysis [13]. Sugarbaker et al. (2008) found that GOLGA8B had mutations in the malignant pleural mesothelioma tumours [14]. However, the role GOLGA8B plays in cancers still remains unknown, and there are no any relevant studies about GOLGA8B and HCC.

We hope this study will clear the potential molecular mechanisms and contribute to the clinical values of GOLGA8B. To achieve this goal, resected tissues from HCC cases were obtained from the Department of Pathology, First Affiliated Hospital of Guangxi Medical University. Real-time quantitative polymerase chain reaction (RT-qPCR) analysis, together with data excavation of different databases, was used to verify the mRNA expression of GOLGA8B. Sequencing data obtained from the Cancer Genome Atlas (TCGA) and Oncomine and microarray chip data obtained from Gene Expression Omnibus (GEO) and ArrayExpress were integrated for further analyses. Gene Expression Profiling Interactive Analysis (GEPIA) was used to perform the survival analysis. We also conducted the immunohistochemistry (IHC) analysis to detect the protein expression level and clinical significance of GOLGA8B. Furthermore, coexpressed genes of GOLGA8B were chosen to perform enrichment analyses—including gene ontology (GO) enrichment, biological pathway and the protein-protein interactions (PPI) network analysis to investigate potential pathways and hub genes. MicroRNAs (miRNAs) which may target GOLGA8B were also predicted.

In summary, based on the detection of mRNA and protein expression, our study used multiple methods and a large sample size to evaluate the expression and clinical value of GOLGA8B. We hope this study may discover the clinical significance of GOLGA8B and clarify its underlying molecular mechanism. The flowchart of this study is shown in Figure 1.

Methods

Clinical Samples Collection

We collected formalin-fixed, paraffin-embedded clinical samples from the Department of Pathology, First Affiliated Hospital of Guangxi Medical University (Nanning, Guangxi, China) from January 2014 to July 2016—including 384 HCC tissues, 162 adjacent nontumorous tissues and 26 normal tissues. The Ethics Committee of the First Affiliated Hospital of Guangxi Medical University approved the research protocol of this study, and all the participants signed the informed consent.

mRNA Expression

1. RT-qPCR analysis of GOLGA8B mRNA expression.

Tissues with HCC and non-HCC control collected from 102 cases were used for RT-qPCR analysis. The total RNA was extracted by the miRNeasy FFPE Tissue Kit (QIAGEN, Shanghai, China). The NanoDrop 2000 (ThermoScientific, USA) was utilised to ensure the purity and concentration of the RNA extracted from sample tissues. The specific primers were as follows: 5'-TGCAAGACCTGAGGAAGGAGC-3', reverse primer: 5'-CCTCTCCTTTTGCCACGGT-3'; ACTB (internal control) forward primer: 5'-CCTCGCCTTTGCCGATCC-3', reverse primer: 5'-TTGCACATGCCGGAGCC-3'. The expression difference was calculated by the formula $2^{-\Delta\text{Cq}}$ in this study.

2. Data excavation.

The RNA-sequencing data and microarray chip data were obtained to determine the association between GOLGA8B expression and clinical features. mRNA expression RNAseq and clinical features of HCC samples were downloaded from TCGA through UCSC xena (<https://xena.ucsc.edu/>), with 373 HCC samples and 50 adjacent nontumorous samples contained, and each sample was $\log_2(x+1)$ transformed RSEM normalised. Data obtained from Oncomine (<https://www.oncomine.org/>) was adopted to confirm the GOLGA8B mRNA expression. The GOLGA8B-related microarrays were downloaded from GEO and ArrayExpress, with the following keywords used for the searches for HCC (hepatocellular OR hepatic OR liver OR HCC) AND (carcinoma OR tumour OR malignan* OR neoplas* OR cancer OR tumour). The expression data of GOLGA8B was extracted from microarrays. The prognosis evaluation of GOLGA8B was performed by GEPIA (<http://gepia.cancer-pku.cn/>).

Protein Expression

IHC analysis of the GOLGA8B protein expression in HCC.

To identify the expression pattern of GOLGA8B in HCC cells, 282 HCC tissues, 60 para-carcinoma tissues and 26 normal liver tissues were utilised to perform IHC analysis. The GOLGA8B antibody provided by Santa Cruz Biotechnology (Heidelberg, Germany) was utilised for IHC detection. Two pathologists independently identified the staining intensity and positive ratio of GOLGA8B in HCC cells through blind evaluations. The staining intensity and proportion of positively stained cells of GOLGA8B were accessed: (0), (1), (2), (3) for negative, weak, moderate and strong staining, respectively, and (0), (1), (2), (3), (4) represent 0%, 1–25%, 26–50%, 51–75% and 75–100% positively stained cells, respectively. The staining intensity multiplied by the proportion of the positively stained cells was considered as the result of the IHC analysis.

Statistical Analysis

Statistical Product and Service Solutions 22.0 (SPSS 22.0) was applied to analyse the data we obtained. The expression differences between different groups were analysed by independent sample t-test or one-way analysis of variance (ANOVA). Mean $\pm$ standard deviation (SD) was adopted to present all the data. The area under the curve (AUC) was calculated by plotting receiver operating characteristic (ROC) curves. Stata statistical software version 12.0 (StataCorp, Colleges Station, TX, USA) was used to integrate the

results of different resources, which were accessed by the standard mean difference (SMD). The heterogeneity of microarray datasets was assessed by the I² statistic and Q test. We pooled the SMD by fixed effects when small heterogeneity existed ($P \geq 0.05$, $I^2 < 50\%$); otherwise, a random-effect model would have been chosen. Egger's and Begg's tests were adopted to estimate publication bias. To access the diagnostic value of GOLGA8B, we obtained the summary of the receiver operator characteristic (sROC), diagnostic odds ratio (DOR), sensitivity, specificity, positive likelihood ratio (PLR) and negative likelihood ratio (NLR) based on each resource.

Gene functional enrichment and network analysis

1. Enrichment analyses.

Coexpressed genes were obtained from the Multi Experiment Matrix (<http://biit.cs.ut.ee/mem/index.cgi>), cBioPortal (<http://www.cbioportal.org/>) and GEPIA. Genes obtained from cBioportal were selected only when their Spearman score was higher than 0.4. The overlapped genes were removed. Then coexpressed genes were pooled together for further analysis. FunRich 3.0 was utilised to perform enrichment analyses [16]. The Search Tool for the Retrieval Interacting Genes (STRING) (<http://www.string-db.org/>) database and Cytoscape were used for PPI construction and hub genes identification [17]. Hub genes were selected by calculating the degrees of the nodes and regarded as the key coexpressed genes of GOLGA8B in HCC. A combined score over 0.9 was adopted to access the associations among proteins. The RNA-sequencing expression data of hub genes were downloaded from UCSC xena to evaluate their differences between HCC and paracarcinoma tissues. The correlations between GOLGA8B and these hub genes were also conducted. Moreover, IHC of hub genes were obtained from the Human Protein Atlas (HPA) (<https://www.proteinatlas.org/>).

2. The prediction of miRNAs.

The miRWalk 2.0 (<http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/>) was used to predict the potential miRNAs which could influence GOLGA8B expression. To explore the relationship between miRNAs and GOLGA8B, $\log_2(\text{total_RPM} + 1)$ transformed miRNA-sequencing expression data of selected miRNAs were downloaded from UCSC xena for further analysis.

Results

mRNA Expression Data

According to the RT-qPCR analysis, GOLGA8B exhibited higher mRNA expression in HCC tissues than in noncancerous tissues. (0.6800 ± 4.0804 vs 0.1105 ± 0.1932 , $P = 0.161$). As for the correlation between GOLGA8B expression clinicopathological features, no significant difference was determined (Table 1). Among 373 HCC patients obtained from TCGA, the expression level of GOLGA8B was significantly higher (8.9607 ± 1.1649 vs 7.1845 ± 1.2512 , $P = 0.000$ (Fig. 2a, Table 2). The ROC curve presented a meaningful result of GOLGA8B for HCC ($AUC = 0.9165$, $P = 0.000$) (Fig. 2b). With regard to the correlation between

GOLGA8B and clinicopathological features, the RNA-sequencing data manifested a significant difference with pathologic tumour groups ($F = 5.853$, $P = 0.001$) (Fig. 2c, Table 2). As for Oncomine, three available studies were acquired for further verification [18–19] Chen et al. (2002) discovered that expression of GOLGA8B was significantly higher in HCC tissues (-0.2270 ± 0.06918 vs -0.6244 ± 0.07905 , $P = 0.000$) and the AUC was 0.7719 ($P < 0.0001$) (Fig. 3a and 3e)[18]. The rest of the studies did not manifest significant differences between HCC and non-HCC control (Fig. 3b, 3c, 3f and 3g) [19]. A total of 13 relevant microarray datasets (GSE45436, GSE84005, GSE76427, GSE74656, GSE64041, GSE62232, GSE60502, GSE59259, GSE57957, GSE39791, GSE36376, GSE14520 and GSE12941), which contained the expression of GOLGA8B in 801 HCC samples and 565 non-HCC samples, were identified and downloaded for further analysis (Fig. 4 and Fig. 5). In addition, the GEPIA database was used to evaluate the role GOLGA8B may play in HCC patients' survival. However, no correlation was found between GOLGA8B expression and the survival of HCC patients (Fig. 6a and 6b).

Protein Expression Data

The IHC of HCC and normal liver tissues is shown in Figure 7. The staining intensity of GOLGA8B exhibited strongly in HCC tissues (Fig. 7a and 7b). In contrast, GOLGA8B exhibited no staining in the normal liver tissues (Fig. 7c and 7d). As shown in Table 3, GOLGA8B staining exhibited higher scores in HCC tissues than in non-HCC controls (11.8688 ± 0.6593 vs 5.8989 ± 1.8589 , $P = 0.000$). Moreover, IHC staining of GOLGA8B in HCC tissues was stronger in the late stages than in the early stages ($P = 0.000$).

Expression integration

Data from different resources were integrated to confirm the GOLGA8B expression. Nineteen studies with 1783 HCC cases and 987 noncancerous controls were included. The SMD of our PCR analysis was 0.20 (95% CI: -0.08–0.47) and showed an upregulation trend. Most of the other expression resources at the mRNA level were significantly overexpressed in HCC, which confirmed our experimental data. The SMD of IHC analysis was 4.28 (95% CI: 3.98–4.58), which further elucidated the overexpression of GOLGA8B in HCC. A random-effects model was used since major heterogeneity existed ($I^2 = 98.4\%$, $P = 0.000$). The combined effects of GOLGA8B expression was higher in HCC tissues with the SMD = 0.893 (95% CI: 0.279–1.506, $P = 0.004$) (Fig. 8a). The result of Begg's test ($z = 1.33$, $P = 0.184$) and Egger's test ($t = 0.08$, $P = 0.937$) showed no statistical significance (Fig. 8b). The sROC was 0.79 (95% CI: 0.75–0.82). The combined DOR, sensitivity, specificity, PLR and NLR were 6.29 (95% CI: 3.55–11.16), 0.65 (95% CI: 0.53–0.76), 0.82 (95% CI: 0.69–0.90), 2.52 (95% CI: 1.76–3.60) and 0.48 (95% CI: 0.37–0.62), respectively (Fig. 9a–f).

Gene functional enrichment and miRNA prediction

1. Analyses of coexpressed genes

Three different databases were utilised for GOLGA8B coexpressed genes selection, and a total of 1303 genes were screened out for further enrichment analyses (Fig. 10a). Additionally, the PPI network was

constructed (Fig. 10b). As for the biological process (BP), genes were mainly gathered in peptide metabolism, mitosis and the regulation of cell cycle. In the cellular component (CC), genes were majorly enriched in the nucleus, nucleolus and cytoplasm. Regarding molecular function, the coexpressed genes were involved in RNA binding, transcription regulator activity and DNA binding. And for biological pathway, genes coexpressed with GOLGA8B were majorly gathered in spliceosome and the mitogen-activated protein kinase (MAPK) signalling pathway (Fig. 11a–d). Hub genes—including SF3B1, HNRNPA2B1, HNRNPA1 and SRRM2—were discovered according to the degrees and interactions. HNRNPA2B1 (13.65 ± 0.01848 vs 13.41 ± 0.03188) and HNRNPA1 (12.17 ± 0.03196 vs 11.50 ± 0.04929) were increased significantly in HCC tissues ($P = 0.000$). SF3B1 (12.03 ± 0.02576 vs 11.91 ± 0.05114) and SRRM2 (12.57 ± 0.03430 vs 12.42 ± 0.04990) showed an upregulated trend in HCC tissues. ($P = 0.0932$ and 0.1186). However, there was no correlation between GOLGA8B and four hub genes (Fig. 12a–h). According to the HPA database, SF3B1 and SRRM2 showed no obvious staining in both normal liver tissues and HCC tissues (antibodies HPA050275 and HPA041411). HNRNPA2B1 staining was medium in normal liver tissues but strong in HCC tissues (antibody CAB12403). HNRNPA1 indicated strong staining in HCC tissues than in normal tissues, which showed weak staining (HPA001666) (Fig. 13a–h).

2. The prediction of miRNAs

In our research, the potential target miRNAs of GOLGA8B were predicted by 12 target prediction algorithms of miRWalk. The miRNAs predicted by more than 6 algorithms were selected as the target candidate miRNAs, and 15 miRNAs were screened out. According to the miRNA-sequencing expression data analysis, miR-203a, miR-139-5p, miR-144-3p, miR-369-3p and miR-374b-5p exhibited significant lower expressions in HCC. (Fig. 14).

Discussion

In spite of the advances in diagnosis and treatment, HCC patients are still diagnosed at a late stage and possess a poor overall survival rate [20–21]. Therefore, more strategies are needed for HCC early diagnosis and treatment. A growing body of articles has explored numerous molecules year on year, hoping to clear the underlying molecular mechanisms of HCC and contribute to its diagnosis and treatment. Some of them have manifested vital roles in HCC carcinogenesis, such as metadherin [22–24]. However, so far, there has been no valid target protein—especially a protein located in the Golgi complex which could be the target for HCC diagnosis and treatment, making it necessary to be explored. Recent studies have concentrated on proteins of the Golgi apparatus in HCC. Yang et al. (2014) focused on the Golgi apparatus proteomes in HCC tissues and adjacent liver tissues; proteins related to the different BPs were found [25]. It is reported that GP73 acts as a key oncogene in HCC by regulating metastasis. For HCC diagnosis, the accuracy of Golgi protein 73 was higher than alpha-fetoprotein, which is used for clinical diagnosis [26–27]. Liu et al. (2018) suggested that GOLGPH3 acts as an oncogene in HCC development and progression by activating the mTOR pathway, which is also a potential target for HCC diagnosis and therapy [28]. Lee et al. (2018) indicated that the Golgi transmembrane protein TME165 contributes to the progression of HCC [29]. GOLGA8B—a gene that encodes protein golgin-67, which is

located in the Golgi complex to maintain its structure—may also serve as a key character in HCC carcinogenesis.

We were the first group to focus on GOLGA8B in HCC. Some previous studies discovered that GOLGA8B manifested different expressions in variant cancers through different methods [11–15]. However, there has been no research so far that has directly studied the relationship between GOLGA8B and cancers. To investigate the accurate function and underlying molecular mechanisms of GOLGA8B, we identified the expression and clinical significance of GOLGA8B, at mRNA level, in HCC by different data combination. IHC was also performed to access the expression of GOLGA8B at the protein level. Consequently, potential functions and pathways were explored.

First, we investigated the role GOLGA8B plays in HCC by an RT-qPCR analysis. Big data which contained RNA-sequencing data and microarray chip data were also utilised for GOLGA8B expression elucidation. There was an higher expression of GOLGA8B in HCC, according to different resources. Based on RNA-sequencing data, GOLGA8B expression was higher in the late pathologic tumour stages (T3–T4) than the early pathologic tumour stages (T1–T2), indicating GOLGA8B might serve as an oncogene in HCC carcinogenesis. However, no correlations were found between GOLGA8B expression and the survival of HCC patients. The expression of GOLGA8B in IHC revealed a similar trend with mRNA expression. GOLGA8B protein exhibited significantly higher expressions in HCC tissues. GOLGA8B protein exhibited higher expressions in late stages (III–IV) than in early stages (I–II), which further elucidated its tumour promotor role in HCC development and progression.

We integrated the expression data obtained from different resources at mRNA and protein level. The results showed SMD = 0.893 (95% CI: 0.279–1.506, $P = 0.004$). A significantly higher expression in HCC at mRNA and protein level further confirmed the function of GOLGA8B in tumour promotion. Moreover, GOLGA8B might be of clinical value due to the result of sROC with an AUC of 0.79. However, a study with larger samples is still necessary for GOLGA8B expression and clinical value elucidation.

Enrichment analyses also reveal the vital functions and pathways of GOLGA8B in HCC. Within all the biological pathways, the spliceosome pathway was the most significantly enriched term. The spliceosome includes more than 150 different proteins; it yields mature mRNA by removing introns and joining exons of pre-mRNA, which is responsible for gene expression [30–31]. The spliceosome pathway could participate in the carcinogenesis, cancer development and chemoresistance [32]. By the regulation of mRNA and RNA splicing, phosphorylation of spliceosome proteins may also take effect in the metastasis of HCC [33]. Besides, the spliceosome pathway also takes part in the tumour progression from cirrhosis to HCC [34]. Many genes associated with spliceosome, such as the MYC genes, manifested different expressions in HCC samples compared to the corresponding adjacent healthy liver samples. A number of studies have found that spliceosome could be a target for many anticancer drugs [35].

In addition, classic tumour suppressor phosphatase and tensin homologue could affect proteins on the Golgi apparatus to play a role in tumour suppression by interacting with spliceosome [36]. However, no relevant study related to GOLGA8B and spliceosome was found. In this study, we hypothesised GOLGA8B

could be a vital element in the spliceosome pathway, but the potential molecular mechanism of GOLGA8B in HCC needs further confirmation. In addition, the MAPK pathway, which consists of extracellular signal-regulated kinase, functions as regulating fundamental cellular process, which includes survival, proliferation, progression and migration [37]. As reported, in 50–100% of HCC cases, the MAPK pathway is activated and is related to poor prognosis [38]. Carbohydrate-responsive element-binding protein, which acts an important role in lipid and glucose metabolism in liver, takes effect in the development of HCC by regulating the MAPK pathway [39].

Factors such as anticancer drugs act on HCC regulation through triggering the MAPK pathway. This indicates the MAPK pathway might be a potential therapeutic target [40–41]. The MAPK pathway also participates in liver-disease progression—initiated from inflammation, followed by fibrosis, cirrhosis and HCC [42]. A number of studies have elucidated that MAPK pathway plays a significant role in HCC. In this study, we assumed that GOLGA8B is a vital factor in the MAPK pathway. However, no studies related to GOLGA8B and the MAPK pathway could be found; more experiments are necessary to verify this hypothesis.

Hub genes SF3B1, HNRNPA2B1, HNRNPA1 and SRRM2 were selected for further analyses according to degrees and interactions. SF3B1 was overexpressed in HCC tissues and was identified to be a target antigen of HCC-associated antibodies. Also, SF3B1 was significantly mutated through DNA sequencing and mutation analyses [43–44]. Both HNRNPA2B2 and HNRNPA1 belong to the hnRNP A/B family, which is a subset of hnRNP proteins [45]. HNRNPA2B1 was overexpressed in HCC tissues. The expression of HNRNPA2B1 was significantly correlated with tumour differentiation, microvascular invasion and survival rate. As a splicing factor, HNRNPA2B1 participates in cancer development by different pathways—including the MAPK pathway and NF- κ B pathway, which provides an interplay between HNRNPA2B1 and GOLGA8B. Furthermore, HNRNPA2B1 interacts with factors like long noncoding RNA and human telomerase reverse transcriptase to serve as an important marker in HCC [45–46]. Similarly, HNRNPA1 expression was upregulated in HCC cell lines and tissues. Through the regulation of CD44v6, overexpression of HNRNPA1 promotes HCC invasion; this indicates shorter overall survival and a higher tumour recurrence rate [47].

SRRM2 is a factor of spliceosome. The mutation in SRRM2 predisposes people to thyroid carcinoma by affecting the alternative splicing of downstream target genes [48]. However, studies related to SRRM2 and HCC have not been found. These four hub genes were all overexpressed in HCC based on TCGA data and showed an inverse trend with GOLGA8B expression. The expression of GOLGA8B and hub genes in HCC informed us that GOLGA8B may target these hub genes to hinder their expression through different mechanisms or signalling pathways. Further explorations are necessary to elucidate the relationship between GOLGA8B and hub genes in HCC.

miRNAs are endogenous, non-coding small RNAs which participate in posttranscriptional regulation by targeting the 3'-untranslated region (UTR) of mRNAs, causing degradation or translation suppressing of mRNAs, and act as suppressors or oncogenes in tumorigenesis [49–50]. In this study, miRNAs which may

influence GOLGA8B expression were selected only when they were predicted by six algorithms. The expression of those selected miRNAs were obtained for further analysis. miR-203a, miR-139-5p, miR-144-3p, miR-369-3p and miR-374b-5p were significantly decreased in HCC tissues. It is reported that miR-203a is downregulated in HCC and is associated with the prognosis of HCC patients[51]. Wang et al. (2018) found that miR-203a could suppress the metastasis, migration, invasion and angiogenesis of HCC cells[52]. miR-139-5p exhibited lower expressions in HCC and HCC cell lines. miR-139-5p also plays a role as a tumour suppressor via inhibiting migration, invasion and growth of HCC cells[53-54]. Wu et al. (2017) found that miR-144-3p could suppress the migration and growth of HCC cells[55]. Moreover, the downregulated miR-144-3p was correlated to poor disease-free survival. As for miR-369-3p and miR-374b-5p, they were dysregulated in various types of cancers, but no study has been conducted to explore their functions and mechanisms in HCC[56-60]. These five miRNAs may target GOLGA8B to function as tumour suppressors. However, in vivo and in vitro experiments are necessary for further elucidation.

Conclusions

This study indicates overexpressed GOLGA8B may play an important role in HCC carcinogenesis and is related to pathologic tumor stages. The potential clinical value and underlying molecular mechanisms, however, require further elucidation.

Declarations

Authors' Contribution

GZ, FM, HW, QS, ZH, GC and YD: study design, data analysis, writing of the article, and final approval of the article. GZ, FM: data collection, data analysis, writing of the article and final approval of the article. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All data generated or analysed during the present study are included in this published article.

Consent for publication

All the participants provided signed informed consent before the study.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University.

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References

1. Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Pineros M, et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. *Int J Cancer*. 2019;144:1941-53.
2. Hung YT, Wu CF, Liang TH, Chou SS, Chen GL, Wu PN, et al. Developing a decision-aid website for breast cancer surgery: an action research approach. *J Med Internet Res*. 2019;21:e10404.
3. Wang HL, Mo DC, Zhong JH, Ma L, Wu FX, Xiang BD, et al. Systematic review of treatment strategy for recurrent hepatocellular carcinoma: Salvage liver transplantation or curative locoregional therapy. *Medicine (Baltimore)*. 2019;98: e14498.
4. Ren T, Zhu L, Cheng M. CXCL10 accelerates EMT and metastasis by MMP-2 in hepatocellular carcinoma. *Am J Transl Res*. 2017; 9:2824-37.
5. Siegel RL, Miller KD, Jemal A.. Cancer statistics, 2018. *CA Cancer J Clin*. 2018; 68:7-30.
6. Jakymiw A, Raharjo E, Rattner JB, Eystathioy T, Chan EK, Fujita DJ. Identification and characterization of a novel Golgi protein, golgin-67. *J Biol Chem*. 2000;275:4137-44.
7. Munro S. The golgin coiled-coil proteins of the Golgi apparatus. *Cold Spring Harb Perspect Biol*. 2011;3.
8. Eystathioy T, Jakymiw A, Fujita DJ, Fritzler MJ, Chan EK. Human autoantibodies to a novel Golgi protein golgin-67: high similarity with golgin-95/gm 130 autoantigen. *J Autoimmun*. 2000;14:179-87.
9. Nozawa K, Casiano CA, Hamel JC, Molinaro C, Fritzler MJ, Chan EK. Fragmentation of Golgi complex and Golgi autoantigens during apoptosis and necrosis. *Arthritis Res*. 2002;4:R3.
10. Zhao J, Yang C, Guo S, Wu Y. GM130 regulates epithelial-to-mesenchymal transition and invasion of gastric cancer cells via snail. *Int J Clin Exp Pathol*. 2015; 8:10784-91.
11. Baschieri F, Uetz-von Allmen E, Legler DF, Farhan H. Loss of GM130 in breast cancer cells and its effects on cell migration, invasion and polarity. *Cell Cycle*. 2015; 14:1139-47.

12. Chang SH, Hong SH, Jiang HL, Minai-Tehrani A, Yu KN, Lee JH, et al. GOLGA2/GM130, cis-Golgi matrix protein, is a novel target of anticancer gene therapy. *Mol Ther*. 2012;20:2052-63.
13. Baine MJ, Chakraborty S, Smith LM, Mallya K, Sasson AR, Brand RE, et al. Transcriptional profiling of peripheral blood mononuclear cells in pancreatic cancer patients identifies novel genes with potential diagnostic utility. *PLoS One*. 2011;6: e17014.
14. Sugarbaker DJ, Richards WG, Gordon GJ, Dong L, De Rienzo A, Maulik G, et al. Transcriptome sequencing of malignant pleural mesothelioma tumors. *Proc Natl Acad Sci U S A*. 2008;105:3521-26.
15. He R, Gao L, Ma J, Peng Z, Zhou S, Yang L, et al. The essential role of MTDH in the progression of HCC: a study with immunohistochemistry, TCGA, meta-analysis and in vitro investigation. *Am J Transl Res*. 2017;9:1561-79.
16. Pathan M, Keerthikumar S, Ang CS, Gangoda L, Quek CY, Williamson NA, et al. FunRich: An open access standalone functional enrichment and interaction network analysis tool. *Proteomics*. 2015;15:2597-601.
17. Shannon P, Markiel A, Ozier O, Balig NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res*. 2003;13(11):2498-2504. doi: 10.1101/gr.1239303.
18. Chen X, Cheung ST, So S, Fan ST, Barry C, Higgins J, et al. Gene expression patterns in human liver cancers. *Mol Biol Cell*. 2002; 13:1929-39.
19. Guichard C, Amaddeo G, Imbeaud S, Ladeiro Y, Pelletier L, Maad IB, et al. Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma. *Nat Genet*. 2012;44:694-8.
20. De Toni EN, Schlesinger-Raab A, Fuchs M, Schepp W, Ehmer U, Geisler F, et al. Age independent survival benefit for patients with hepatocellular carcinoma (HCC) without metastases at diagnosis: a population-based study. *Gut*. 2019; Mar 16. pii: gutjnl-2018-318193.
21. Elmoghazy W, Ahmed K, Vijay A, Kamel Y, Elaffandi A, El-Ansari W, et al. Hepatocellular carcinoma in a rapidly growing community: epidemiology, clinico-pathology and predictors of extrahepatic metastasis. *Arab J Gastroenterol*. 2019; Mar 7. pii: S1687-1979(19)30006-1.
22. Huang Y, Li LP. Progress of cancer research on astrocyte elevated gene-1/Metadherin (Review). *Oncol Lett*. 2014;8:493-501.
23. Vasuri F, Visani M, Acquaviva G, Brand T, Fiorentino M, Pession A, et al. Role of microRNAs in the main molecular pathways of hepatocellular carcinoma. *World J Gastroenterol*. 2018;24:2647-60.
24. Chen E, Xu X, Liu R, Liu T. Small but heavy role: microRNAs in hepatocellular carcinoma progression. *Biomed Res Int*. 2018;2018:6784607.
25. Yang Y, Luo Y, Li X, Yi Y.. Differential expression analysis of Golgi apparatus proteomes in hepatocellular carcinomas and the surrounding liver tissues. *Hepatol Res*. 2014;44:542-50.
26. Dai M, Chen X, Liu X, Peng Z, Meng J, Dai S. Diagnostic value of the combination of Golgi protein 73 and alpha-fetoprotein in hepatocellular carcinoma: a meta-analysis. *PLoS One*. 2015;10:e0140067.

27. Yang Y, Liu Q, Li Z, Zhang R, Ji C, Yang Z, et al. GP73 promotes epithelial-mesenchymal transition and invasion partly by activating TGF-beta1/Smad2 signaling in hepatocellular carcinoma. *Carcinogenesis*. 2018;39:900-10.
28. Liu H, Wang X, Feng B, Tang L, Li W, Zheng X, et al. Golgi phosphoprotein 3 (GOLPH3) promotes hepatocellular carcinoma progression by activating mTOR signaling pathway. *BMC Cancer*. 2018;18:661.
29. Lee JS, Kim MY, Park ER, Shen YN, Jeon JY, Cho EH, et al. TMEM165, a Golgi transmembrane protein, is a novel marker for hepatocellular carcinoma and its depletion impairs invasion activity. *Oncol Rep*. 2018;40:1297-306.
30. Hepburn LA, McHugh A, Fernandes K, Boag G, Proby CM, Leigh IM, et al. Targeting the spliceosome for cutaneous squamous cell carcinoma therapy: a role for c-MYC and wild-type p53 in determining the degree of tumour selectivity. *Oncotarget*. 2018;9:23029-46.
31. Wu G, Fan L, Edmonson MN, Shaw T, Boggs K, Easton J, et al. Inhibition of SF3B1 by molecules targeting the spliceosome results in massive aberrant exon skipping. *RNA*. 2018;24:1056-66.
32. Iguchi T, Komatsu H, Masuda T, Nambara S, Kidogami S, Ogawa Y, et al. Increased copy number of the gene encoding SF3B4 indicates poor prognosis in hepatocellular carcinoma. *Anticancer Res*. 2016;36:2139-44.
33. Tian M, Cheng H, Wang Z, Su N, Liu Z, Sun C, et al. Phosphoproteomic analysis of the highly-metastatic hepatocellular carcinoma cell line, MHCC97-H. *Int J Mol Sci*. 2015;16:4209-25.
34. Wang Y, Li J, Chen J, Liu L, Peng Z, Ding J, et al. From cirrhosis to hepatocellular carcinoma in HCV-infected patients: genes involved in tumor progression. *Eur Rev Med Pharmacol Sci* 2012;16:995-1000.
35. Xu W, Huang H, Yu L, Cao L. Meta-analysis of gene expression profiles indicates genes in spliceosome pathway are up-regulated in hepatocellular carcinoma (HCC). *Med Oncol*. 2015;32:96.
36. Shen SM, Ji Y, Zhang C, Dong SS, Yang S, Xiong Z, et al. Nuclear PTEN safeguards pre-mRNA splicing to link Golgi apparatus for its tumor suppressive role. *Nat Commun*. 2018;9:2392.
37. Achkar IW, Abdulrahman N, Al-Sulaiti H, Joseph JM, Uddin S, Mraiche F. Cisplatin based therapy: the role of the mitogen activated protein kinase signaling pathway. *J Transl Med*. 2018;16:96.
38. Delire B, Starkel P. The Ras/MAPK pathway and hepatocarcinoma: pathogenesis and therapeutic implications. *Eur J Clin Invest*. 2015;45:609-23.
39. Ribback S, Che L, Pil MG, Cigliano A, Latte G, Pes GM, et al. Oncogene-dependent addiction to carbohydrate-responsive element binding protein in hepatocellular carcinoma. *Cell Cycle*. 2018;17:1496-512.
40. Chen X, Wang L, Zhao Y, Yuan S, Wu Q, Zhu X, et al. ST6Gal-I modulates docetaxel sensitivity in human hepatocarcinoma cells via the p38 MAPK/caspase pathway. *Oncotarget*. 2016;7:51955-64.
41. Chen C, Wang G. Mechanisms of hepatocellular carcinoma and challenges and opportunities for molecular targeted therapy. *World J Hepatol*. 2015;7:1964-70.

42. Ranneh Y, Akim AM, Hamid HA, Khazaai H, Fadel A, Mahmoud AM. Stingless bee honey protects against lipopolysaccharide induced-chronic subclinical systemic inflammation and oxidative stress by modulating Nrf2, NF-kappaB and p38 MAPK. *Nutr Metab (Lond)*. 2019;16:15.
43. Hwang HM, Heo CK, Lee HJ, Kwak SS, Lim WH, Yoo JS, et al. Identification of anti-SF3B1 autoantibody as a diagnostic marker in patients with hepatocellular carcinoma. *J Transl Med*. 2018;16:177.
44. Ally A, Balasundaram M, Carlsen R, Chuah E, Clarke A, Dhalla N, et al. Comprehensive and integrative genomic characterization of hepatocellular carcinoma. *Cell*. 2017;169:1327-41 e23.
45. Shilo A, Ben Hur V, Denichenko P, Stein I, Pikarsky E, Rauch J, et al. Splicing factor hnRNP A2 activates the Ras-MAPK-ERK pathway by controlling A-Raf splicing in hepatocellular carcinoma development. *RNA*. 2014;20:505-15.
46. Wang H, Liang L, Dong Q, Huan L, He J, Li B, et al. Long noncoding RNA miR503HG, a prognostic indicator, inhibits tumor metastasis by regulating the HNRNPA2B1/NF-kappaB pathway in hepatocellular carcinoma. *Theranostics*. 2018;8:2814-29.
47. Zhou ZJ, Dai Z, Zhou SL, Fu XT, Zhao YM, Shi YH, et al. Overexpression of HnRNP A1 promotes tumor invasion through regulating CD44v6 and indicates poor prognosis for hepatocellular carcinoma. *Int J Cancer*. 2013;132: 1080-89.
48. Tomsic J, He H, Akagi K, Liyanarachchi S, Pan Q, Bertani B, et al. A germline mutation in SRRM2, a splicing factor gene, is implicated in papillary thyroid carcinoma predisposition. *Sci Rep*. 2015;5:10566.
49. Ahmed EK, Fahmy SA, Effat H, Wahab AHA. Circulating miR-210 and miR-1246 as potential biomarkers for differentiating hepatocellular carcinoma from metastatic tumors in the liver. *J Med Biochem*. 2019;38:109-17.
50. Zhang Z, Han Y, Sun G, Liu X, Jia X, Yu X. MicroRNA-325-3p inhibits cell proliferation and induces apoptosis in hepatitis B virus-related hepatocellular carcinoma by down-regulation of aquaporin 5. *Cell Mol Biol Lett*. 2019;24:13.
51. Liu D, Wu J, Liu M, Yin H, He J, Zhang B. Downregulation of miRNA-30c and miR-203a is associated with hepatitis C virus core protein-induced epithelial-mesenchymal transition in normal hepatocytes and hepatocellular carcinoma cells. *Biochem Biophys Res Commun*. 2015;464:1215-21.
52. Wang L, Tong D, Guo Q, Wang X, Wu F, Li Q, et al. HOXD3 targeted by miR-203a suppresses cell metastasis and angiogenesis through VEGFR in human hepatocellular carcinoma cells. *Sci Rep*. 2018;8:2431.
53. Qiu G, Lin Y, Zhang H, Wu D. miR-139-5p inhibits epithelial-mesenchymal transition, migration and invasion of hepatocellular carcinoma cells by targeting ZEB1 and ZEB2. *Biochem Biophys Res Commun*. 2015;463:315-21.
54. Li P, Xiao Z, Luo J, Zhang Y, Lin L. MiR-139-5p, miR-940 and miR-193a-5p inhibit the growth of hepatocellular carcinoma by targeting SPOCK1. *J Cell Mol Med*. 2019; Apr;23(4):2475-2488

55. Wu M, Huang C, Huang X, Liang R, Feng Y, Luo X. MicroRNA-144-3p suppresses tumor growth and angiogenesis by targeting SGK3 in hepatocellular carcinoma. *Oncol Rep.* 2017;38:2173-81.
56. Li P, Dong M, Wang Z. Downregulation of TSPAN13 by miR-369-3p inhibits cell proliferation in papillary thyroid cancer (PTC). *Bosn J Basic Med Sci.* 2018; Aug 2
57. Hao GJ, Ding YH, Wen H, Li XF, Zhang W, Su HY, et al. Attenuation of deregulated miR-369-3p expression sensitizes non-small cell lung cancer cells to cisplatin via modulation of the nucleotide sugar transporter SLC35F5. *Biochem BiophysRes Commun.* 2017;488:501-8.
58. Wang S, Zhang G, Zheng W, Xue Q, Wei D, Zheng Y, et al. MiR-454-3p and miR-374b-5p suppress migration and invasion of bladder cancer cells through targetting ZEB2. *Biosci Rep.* 2018;38.
59. Li H, Liang J, Qin F, Zhai Y. MiR-374b-5p-FOXP1 feedback loop regulates cell migration, epithelial-mesenchymal transition and chemosensitivity in ovarian cancer. *Biochem Biophys Res Commun.* 2018;505:554-60.
60. Sun D, Wang X, Sui G, Chen S, Yu M, Zhang P. Downregulation of miR-374b-5p promotes chemotherapeutic resistance in pancreatic cancer by upregulating multiple anti-apoptotic proteins. *Int J Oncol.* 2018. Mar 14.

Tables

Table 1. The RT-qPCR data of GOLGA8B.

Variable	Category	N	Mean $\pm$ SD	T/F	P
Tissue	HCC	248	1.5565 $\pm$ 2.3644	-5.851	0
	Adjacent noncancerous	247	2.8040 $\pm$ 2.3791		
	liver tissue				
Age	<50	126	1.6202 $\pm$ 2.3735	0.43	0.667
	$\geq$ 50	122	1.4907 $\pm$ 2.3629		
Gender	male	215	1.6600 $\pm$ 2.4093	1.766	0.079
	female	33	0.8826 $\pm$ 1.9469		
HBV infection	+	167	1.5753 $\pm$ 2.4227	0.179	0.858
	-	81	1.5178 $\pm$ 2.2536		
Size	$\leq$ 5 cm	173	1.6348 $\pm$ 2.4891	0.791	0.43
	> 5 cm	75	1.3761 $\pm$ 2.0521		
Vascular invasion	+	88	1.4806 $\pm$ 2.4433	-0.374	0.708
	-	160	1.5983 $\pm$ 2.3266		
Lymph node metastasis	+	17	0.6020 $\pm$ 2.3719	-1.724	0.086
	-	230	1.6243 $\pm$ 2.3585		
Embolus	+	136	1.3365 $\pm$ 2.2854	-1.644	0.102
	-	111	1.8328 $\pm$ 2.4497		
Pathological grading	I	10	1.8179 $\pm$ 2.1113	F = 0.173	0.952
	II	119	1.4451 $\pm$ 2.2571		
	III	106	1.6556 $\pm$ 2.4905		
	IV	11	1.4667 $\pm$ 2.6808		
AFP	+	180	1.4647 $\pm$ 2.3613	-0.995	0.321
	-	68	1.7996 $\pm$ 2.3730		

*HCC: hepatocellular carcinoma; HBV: hepatitis B virus; AFP: alpha-fetoprotein; N: number; t: t-test; SD: standard deviation; F: ANOVA; P: p-value

Table 2. The GOLGA8B expression data based on TCGA.

Variable	Category	N	Mean $\pm$ SD	T/F	P
Tissue	HCC	373	8.9607 $\pm$ 1.1649	10.035	0.000
	Adjacent noncancerous	50	7.1845 $\pm$ 1.2512		
	liver tissues				
Gender	Male	252	8.9708 $\pm$ 1.1121	0.232	0.817
	Female	121	8.9395 $\pm$ 1.2724		
Age	≤ 60	168	9.0520 $\pm$ 1.0979	1.413	0.159
	> 60	204	8.8807 $\pm$ 1.2153		
Pathologic tumour I (T)	T1	182	9.0702 $\pm$ 1.0866	F = 5.853	0.001
	T2	95	8.8597 $\pm$ 1.2131		
	T3	80	9.0508 $\pm$ 1.1544		
	T4	13	7.7433 $\pm$ 1.3951		
Pathologic tumour II	T1 + T2	277	8.9980 $\pm$ 1.1338	0.928	0.354
	T3 + T4	93	8.8680 $\pm$ 1.2673		
Pathologic node	NX	115	9.0018 $\pm$ 1.1693	0.488	0.626
	N0	253	8.9383 $\pm$ 1.1504		
Metastasis	MX	102	9.0356 $\pm$ 1.1389	0.64	0.522
	M0	267	8.9495 $\pm$ 1.1602		
TNM stage I	I	172	9.0616 $\pm$ 1.1019	F = 1.579	0.194
	II	87	8.8353 $\pm$ 1.1839		
	III	85	8.9866 $\pm$ 1.1809		
	IV	5	8.1642 $\pm$ 1.7591		
TNM stage II	I	172	9.0616 $\pm$ 1.1019	1.397	0.163
	II + III + IV	177	8.8890 $\pm$ 1.2007		
Vascular invasion	Yes	110	9.0106 $\pm$ 1.1660	-0.995	0.321
	No	207	8.9029 $\pm$ 1.1575		

*HCC: hepatocellular carcinoma; N: number; t: t-test; SD: standard deviation; F: ANOVA; P: p-value

Table 3. IHC analysis of GOLGA8B.

Variable	Category	N	Mean $\pm$ SD	T/F	P
Tissue	HCC	282	11.8688 $\pm$ 0.6593	29.713	0.000
	Noncancerous liver tissues	89	5.8989 $\pm$ 1.8589		
Age	≤ 50	172	11.9012 $\pm$ 0.5789	0.823	0.411
	> 50	124	11.8387 $\pm$ 0.7256		
Gender	Male	241	11.9170 $\pm$ 0.5257	1.603	0.411
	Female	57	11.7018 $\pm$ 0.9813		
Grade	I	50	11.9400 $\pm$ 0.4242	F = 1.453	0.236
	II	197	11.8832 $\pm$ 0.6157		
	III	38	11.7105 $\pm$ 1.0109		
Pathologic tumour (T)	I	13	12.0000 $\pm$ 0.0000	F = 0.450	0.718
	II	163	11.8650 $\pm$ 0.6984		
	III	96	11.9438 $\pm$ 0.6700		
	IV	17	12.0000 $\pm$ 0.0000		
Pathologic node	N0	264	11.8712 $\pm$ 0.6572	-0.027	0.978
	N1	24	11.8750 $\pm$ 0.6123		
Stage	I	13	10.3077 $\pm$ 1.9315	F = 36.686	0.000
	II	103	11.9085 $\pm$ 0.5173		
	III	164	12.0000 $\pm$ 0.0000		
	IV	11	12.0000 $\pm$ 0.0000		

*HCC: hepatocellular carcinoma; N: number; t: t-test; SD: standard deviation; F: ANOVA; P: p-val

Figures

Figure 1

The flow diagram exhibited the main design of this study.

Figure 2

The expression and clinical features of GOLGA8B based on TCGA data. a. The expression of GOLGA8B based on TCGA. b. The ROC curve of GOLGA8B expression based on TCGA. c. The expression of GOLGA8B between TNM stages I–IV based on 373 HCC tissues.

Figure 3

The expression and clinical value of GOLGA8B based on Oncomine data. a–c. The expression of GOLGA8B based on Oncomine. d–f. The ROC curves of GOLGA8B expression based on Oncomine.

Figure 4

GOLGA8B expression in HCC according to GEO.

Figure 5

ROC curves of GOLGA8B expression according to GEO.

Figure 6

Survival analysis of GOLGA8B. a. The overall survival of patients based on GEPIA. b. The disease-free survival of patients based on GEPIA.

Figure 7

The expression patterns of GOLGA8B in liver tissues. a, b. GOLGA8B staining in HCC tissues. c, d. GOLGA8B in normal liver tissues (x 400).

Figure 8

Meta-analyses of GOLGA8B expression in HCC. a. The forest plot of GOLGA8B expression in HCC (SMD = 0.893 95%: 0.279 to 1.506). b. The funnel plot of different datasets related to GOLGA8B.

Figure 9

SROC and forest plots showing a. sROC b. Diagnostic odds ratio. c. Sensitivity. d. Specificity. e. Positive likelihood ratio. f. Negative likelihood ratio.

GEPIA

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Figure 11

Enrichment analyses of coexpressed genes. a. Biological process. b. Cellular component. c. Molecular function. d. Biological pathway.

Figure 12

Hub genes expression based on TCGA. a-d. Hub genes expression in HCC and non-HCC control. e-h. Correlations between hub genes and GOLGA8B.

Figure 13

The IHC analysis of four hub genes obtained from HPA. a-d. The expression of SF3B1, HNRNPA2B1, HNRNPA1 and SRRM2 in HCC tissues (antibody HPA050275, CAB012403, HPA001666 and HPA041411). e-h. The expression of SF3B1, HNRNPA2B1, HNRNPA1 and SRRM2 in normal tissues (antibody HPA050275, CAB012403, HPA001666 and HPA041411).

Figure 14

The heatmap showing the different expression patterns of miRNAs which target GOLGA8B.