Clinical Values and Functional Analysis Of KMT2A/B/C/D/E/F in Gastric Cancer by Integrated Bioinformatics Analysis

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Abstract

KMT2A/B/C/D/E/F have been identified be involved in the tumor progression of gastric cancer (GC). However, the clinical significance and function of the 6 KMT2s have not yet been analyzed. Here, we investigated the transcriptional and survival data of KMT2s family genes in GC based on integrated bioinformatics analysis. cBioPortal was used to analyze the alteration frequency and functional networks of KMT2s in GC. And GO and KEGG pathways were also identified. Gene enrichment analysis was performed to investigate the networks of kinase, miRNAs and transcription factor target. We found that KMT2s were highly expressed in GC, and associated with tumor stages and grades. Besides, higher KMT2s expression were significantly associated with shorter overall survival (OS), progression-free survival (FP) and post-progression survival (PPS). Also, promoter methylation levels of KMT2s were positively associated with KMT2s expression and clinical data. Moreover, high mutation rate of KMT2s (40%) was also observed in GC patients. Functional networks analysis suggested that KMT2s might regulate histone lysine methylation and chromatin organization via PI3K/AKT and MAPK signaling pathways involving in some kinase, miRNA and transcription factor. Taken together, these findings suggested that KMT2A/B/C/D/E/F may be prognostic and therapeutic biomarkers of patients with GC.

Introduction

Gastric cancer (GC) is the fifth most common cancer and the third leading cause of cancer-related death worldwide[1, 2]. In advanced GC patients, the purpose of chemotherapy is to shrink tumor size, reduce tumor growth, and inhibit tumor metastasis. The majority of patients with advanced GC are initially responsive to the combined chemotherapy. However, the patients eventually experience tumor recurrence or metastasis partially due to drug resistance, and the 5 year survival rate is only 30% in advanced GC patients[3]. Fortunately, some of those patients are responsive to cancer-targeted therapy. Thus, identifying novel cancer-related genes is essential to optimizing current therapeutic strategies.

The lysine methyltransferase 2 (KMT2, also known as mixed lineage leukemia (MLL)) family methylates histone H3 lysine 4 (H3K4) to promote genome accessibility and transcription[4, 5]. The KMT2 family of histone modifying proteins play essential roles in regulating developmental pathways, and mutations in the genes encoding these proteins have been identified being involving in various human malignancies[6], including breast cancer[7], bladder cancer[8], lung cancer[9], blood cancer[10], large intestinal cancer[11] and kidney cancer[12].

To date, seven KMT2 family proteins have been identified in human genes (KMT2A, KMT2B, KMT2C, KMT2D, KMT2E, KMT2F, SETD1A and SETD1B). Those of KMT2 proteins reside in large, multi-subunit complexes composed of unique sets of interacting proteins[13–16]. KMT2s have been reported to be associated with the carcinogenesis of gastric cancer. Koh J et al. reported that KMT2A served as a hypermutated status in the II and III GC of tumor microenvironment immune type, which suggests its effect on immune checkpoint therapy[17]. Howard L. McLeod et al. found that KMT2A mutation occurred in GC, and significant differences in protein-altering somatic mutation frequency in diverse geographic populations[18]. According to one study of next-generation sequencing (NGS) for advanced GC, the results show that KMT2B and KMT2D functioned as oncogenic drivers with the somatic mutation frequency was up to 11.5% and 15.4%, respectively[19]. Meanwhile, KMT2D was examined to upregulated in GC tissues and depletion of KMT2D suppressed cell proliferation and induced apoptosis, which indicated KMT2D as an oncogene in GC[20]. In addition, highly somatic mutation frequency of KMT2C gene is reported to be occurred in GC but its effects on metastasis was different in some researches[21]. Sam S. Yoon et al. reported that KMT2C was frequently mutated in certain populations with diffuse-type gastric adenocarcinomas (DGA). KMT2C loss in DGA promoted epithelial-to-mesenchymal transition (EMT) and was associated with worse overall survival. Jaffer A Ajani et al. found that KMT2C mutation was the common cancer driver in primary gastric adenocarcinoma (DCA), and promoted peritoneal invasion of GCA especial for DGA via using whole-exome sequencing (WES) and whole transcriptome sequencing (RNA-seq) on 44 peritoneal carcinomatosis (PC) specimens (43 patients with PC) [22]. KMT2E is widely reported to be interaction with the development and progression in acute myeloid leukemia (AML) but its role in GC remains unknown[23]. Recent studies have revealed that KMT2F is involved in breast cancer and liver cancer development. Kwang Won Jeong et al. found that KMT2F was upregulated in breast cancer, and depletion of KMT2F in MCF-7 cells significantly decreased cell proliferation and migration, repressing tamoxifen-resistant via regulating miR-1915-13p[24]. Dongdong Lu et al. reported that long noncoding RNA CUDR cooperated with KMT2F to promote liver cancer growth and hepatocyte-like stem cell malignant transformation epigenetically[25]. Combined with the above findings, we recognize that KMT2s may be a significant oncogene or suppressor gene in various cancers. However, the underlying mechanism by which KMT2s are activated or inhibited, as well as the distinct functions of the KMT2s in GC have yet to be fully elucidated. Most of those genes are found with highly somatic mutation frequencies in GC based on the results of NGS but expression of KMT2s in tissues, the correlations with clinicopathological features and prognosis and the functional networks have not been reported in human GC.

Here, we examined the correlation between the different KMT2s and GC based on bioinformatics analyses to explore their clinical significance and the functional networks in human GC. Expression of KMT2s in GC tissues and normal tissues, Correlation between KMT2s expression with clinicopathological characters and prognosis were examined by some databases including the Human Protein Atlas, UALCA, GEPIA and KMPLLOT. Next, we analyzed the correlation between of KMT2s methylation with its expression and clinical data in GC. Finally, we further explore he mutations of different KMT2s in patients with GC to determine the functions, predicting the downstream kinase, miRNA and transcription factor targets.

Results

Over-expression of different KMT2s family members in patients with GC

To examine the potential correlation between the different KMT2s members and GC, we compared the TCGA data by using GEPIA database (<http://gepia.cancer-pku.cn/>) to explore the expression of KMT2s in cancer tissues and the normal tissues. We found that transcriptional levels of KMT2A/B/C/D had a trend to be higher in cancer tissues than the normal tissues, while transcriptional levels of KMT2E/F were significantly higher in cancer tissues (Figure 1A-B).

Having examined the mRNA expression patterns of KMT2s in GC, we tried to explore the protein expression patterns of KMT2s in GC by the Human Protein Atlas. As showed in Figure 2A-F, the expressions of KMT2A/B/C/D/E/F proteins were also higher in cancer tissues than the normal tissues. In summary, our results showed that transcriptional and proteomic expressions of KMT2s were overexpressed in patients with GC.

Correlation between mRNA expressions of different KMT2s family members with the clinicopathological parameters of patients with GC

Relationship between mRNA expressions of different KMT2s with clinicopathological features of GC patients were analyzed by using UALCAN (<http://ualcan.path.uab.edu>) including patients' individual cancer stages and tumor grades. Our results showed that KMT2A/B/C/D/E/F mRNA expressions were significantly related with patients' individual cancer stages, and patients with advanced stages or grades tended to express higher mRNA of KMT2s. As showed in Figure 3, the highest mRNA expression of different KMT2s were found in stage 3. The reason why mRNA expressions of KMT2A/B/C/D/E/F in stage 3 seemed to be higher than that in stage 4 may be due to the small sample size. Consistent with the analysis of patients' individual cancer stages, our work found that mRNA expression of different KMT2s family members were also correlated with tumor grades. As tumor grades increased, the expression levels of KMT2s mRNA tended to be higher. As showed in Figure 4 A-F, the highest expression of KMT2A/B/D/F were all found in grade 3, while the highest expression of KMT2C/E were in grade 2. Taken together, the results above indicated that mRNA expressions of 6 KMT2s family members were significantly related with clinicopathological characteristics in GC patients.

Correlation between methylation of KMT2s with its expression and clinical data in GC

DNA methylation is a common heritable epigenetic modification in the genome of eukaryotic cells, plays an important role in regulating cell proliferation, differentiation, and individual development, and abnormal DNA methylation levels are closely related to tumor development. We used MEXPRESS to analyze the correlation between methylation of KMT2s with its expression and clinical data in GC. As showed in Figure 5A, KMT2A expression was positively related with KMT2A promoter methylation ($r=0.448^{***}$), tumor stage ($p=0.01$) and histological type ($p=4.311e-5$). In Figure 5B, KMT2B promoter methylation was positively related with KMT2B expression ($r=0.479^{***}$), tumor stage ($p=0.002$) and histological type ($p=0.002$). In Figure 5C, KMT2C promoter methylation was positively related with KMT2C expression ($r=0.429^{***}$), tumor stage ($p=0.018$). In Figure 5D, KMT2D promoter methylation was positively related with KMT2D expression ($r=0.303^{***}$). In Figure 5E, KMT2CE promoter methylation was positively related with KMT2E expression ($r=0.403^{***}$), tumor stage ($p=0.014$). In Figure 5F, KMT2F (SETD1A) promoter methylation was positively related with KMT2E expression ($r=0.357^{***}$), tumor stage ($p=0.021$) and histological type ($p=0.047$). These findings indicated that high promoter methylation levels of KMT2A/B/C/D/E/F contributed normal expression of oncogenes (KMT2A/B/C/D/E/F) to induce the development and progression of GC.

Prognostic values of expression levels of KMT2s mRNA in patients with GC

We further explore the critical efficiency of KMT2s family members in the survival of patients with GC. Kaplan-Meier plotter (<http://kmplot.com/analysis/>) was performed to analyze the association between KMT2s mRNA expressions with the survival including overall survive (OS), progression-free survival (FP) and post-progression survival (PPS). The Kaplan-Meier curve and log rank test analyses revealed that patients of GC with higher of KMT2A/B/C/D/E/F mRNA expression levels have a shorter OS, FP and PPS in 150 months' follow up (Figure 6), $P < 0.05$. Thus, our results demonstrated that increased KMT2A/B/C/D/E/F expression were predicted to be associated with poor prognosis in GC.

Genetic alteration of KMT2s family members and the network in GC

We used the cBioPortal online tools (<https://www.cbioportal.org/>) to analyze the KMT2s alterations, correlations and networks based on sequencing data from GC patients in the TCGA database. KMT2s were altered in 117 of 293 (40%) GC patients. These alterations of KMT2A/B/C/D/E/F were in the following (Table 1): KMT2A: amplification (2%), mutation (10.6%); KMT2B: amplification (2.4%), homozygous (1.7%) and mutation (7.5%); KMT2C: amplification (0.7%), homozygous (1.7%) and mutation (15.4%); KMT2D: amplification (0.3%), homozygous (1%) and mutation (21.8%); KMT2E: amplification (2.7%), homozygous (0.7%) and mutation (5.8%); KMT2F: amplification (0.3%) and mutation (5.5%). We also calculated the co-expression of KMT2s with each other for stomach adenocarcinoma, and Pearson's correlation was included. We found significant and positive correlations in the followings KMT2s: KMT2A with KMT2C, KMT2D and KMT2E; KMT2B with KMT2D and KMT2F; KMT2C with KMT2A and KMT2E; KMT2D with KMT2A, KMT2B and KMT2F; KMT2E with KMT2A and KMT2C; KMT2F with KMT2B and KMT2D (Figure 7C). Next, we constructed the network for KMT2s and the neighboring genes with the alteration frequency >5% (Figure 7D).

Predicted functions and pathways of the changes in KMT2s and their frequently altered neighboring genes in patients with GC

The functions of KMT2s and the genes significantly correlated with KMT2s alterations were predicted by analyzing GO and KEGG pathways in the database for STRING (<https://string-db.org/>). To address this, we used the KMT2s-neighboring genes that were altered at frequencies > 5% in the GC TCGA database (Figure 7D and Table 1). As showed in Figure 8, analysis of significantly enriched GO terms suggested that those proteins were mainly located in (GO:0005654) nucleoplasm, (GO:0005694) chromosome, (GO:0035097) histone methyltransferase complex, (GO:0043231) intracellular membrane-bounded organelle, (GO:0044451) nucleoplasm part and (GO:0043232) intracellular non-membrane-bounded organelle. These proteins are primarily involved in (GO:0034968) histone lysine methylation, (GO:0006325) chromatin organization, (GO:0018193) peptidyl-amino acid modification, (GO:0006355) regulation of transcription, DNA-templated and (GO:0051252) regulation of RNA metabolic process. while they also serve as (GO:0018024) histone-lysine N-methyltransferase activity, (GO:0042800) histone methyltransferase activity (H3-K4 specific), (GO:0140100) transferase activity, (GO:0140110) transcription regulator activity and (GO:0003677) DNA binding. Moreover, KEGG pathway analysis showed enrichment in (hsa00310) lysine degradation, (hsa05226) gastric cancer such as MAPK and PI3K/AKT pathways, (hsa05210) colorectal cancer, (hsa04550) signaling pathways regulating pluripotency of stem cells and (hsa04934) Cushing's syndrome. Among these significant pathways, pathways of lysine degradation and gastric cancer were regulated by KMT2s in GC (Figure 9A-B).

KMT2s networks of kinase, miRNA and transcription factor targets in GC

To further predict the targets of KMT2s in GC, we analyzed the kinase, miRNA and transcription factor targets generated by Gene Set Enrichment Analysis (GSEA). The top 5 most significant target networks of KMT2A/B/C/D/E/F were included in Table 2, respectively. Importantly, we found that all of the 6 KMT2s had common kinase targets: AKT and MAPK1, which suggested KMT2s might be involved in pathways such as MAPK and PI3K/AKT signaling pathway.

Discussion

Cancer genetic and epigenetic alterations are reported to play an important role in the development and progression of gastric cancer (GC) [26]. KMT2s family of genes mutations are involved in many cancers. Although the role of KMT2s as oncogenic drivers of several cancers have been partially confirmed, its clinical significance and functional networks in GC are known with limitation in literature. In this study, we used bioinformatics analysis to elaborate the effects of KMT2A/B/C/D/E/F on the tumorigenesis and prognosis of GC.

Previous studies have identified KMT2A and KMT2B (KMT2A/B) as oncogenes in several cancers including breast cancer, colorectal cancer, pancreatic cancer, and acute myeloid leukemia[27]. Thus, KMT2A/B are of great significance in the cancer research. Somatic mutation frequency of KMT2A/B was found in GC through sequencing method, but its clinical value and biological function of KMT2A/B in GC has not been reported. Our result that KMT2A/B were upregulated in GC, and their overexpression were negatively related with tumor stages and grades. Patients with lower KMT2A/B benefited with good prognosis in OS, FP and PPS. The above results first revealed the clinical significance of KMT2A/B in GC, expanding the knowledge of KMT2A/B applied for clinical transformation. Also, our work analyzed GO and KEGG pathway of KMT2A/B in GC, and predicted the kinase, miRNA and transcription factor targets in the development of GC. These findings indicated the potential mechanism of KMT2A/B in regulating the development and progression of GC.

Among KMT2s, KMT2C and KMT2D (KMT2C/D) are the 2 most studied in gastric cancer. Silencing KMT2C in non-transformed HFE-145 gastric epithelial cells promoted EMT, while KMT2C re-expression in the DGA cell lines MKN-45 and SNU-668 could inhibit the EMT[21]. However, another paper published on "GUT" performed whole-exome sequencing (WES) and whole transcriptome sequencing (RNA-seq) on 44 PC specimens (43 patients with PC), and found that alterations associated with aggressive PC phenotypes emerged with increased

mutation in KMT2C[22]. In our study, we also identified KMT2C as hypermutation in GC, and patients with increased KMT2C expression suffered poor OS, FP and FFP. These results were consistent with the paper published in “GUT”, but more experiments in details needed to be performed. KMT2D was found to be highly expressed in GC tissues, and related with poor survival. Depletion of KMT2D suppressed cell proliferation and induced apoptosis. Similarity to the above results, we further confirmed the clinical value of KMT2D in GC through bioinformatics analysis, suggesting KMT2D as a novel oncogene for prognosis and optimal treatment of GC patients.

Up to now, little was known about the expression and role of KMT2E and KMT2F (KMT2E/F) in GC. In our report, the expression levels of KMT2E and KMT2F in gastric cancer tissues were significantly higher than that in normal tissues. Advanced tumor stages and grades tended to express higher KMT2E/F mRNA levels. Interestingly, high KMT2E and KMT2F expression were significantly correlated with poor OS, FP and PPS in all of the patients with gastric cancer, indicating the role of these KMT2s as oncogenic drivers in GC. No studies have yet reported the function networks of KMT2E and KMT2F in the development of GC. Through bioinformatics analysis, we revealed the GO and KEGG pathway of those KMT2s family proteins and also predicted the potential kinase, miRNA and transcription factor targets in GC. These findings provide the knowledge about the mechanism of KMT2s in GC. However, further studies needed to be carried via cellular or animal experiments to verify the ideas.

DNA methylation is an important epigenetic modification, mainly occurring in the CpG island of DNA and its changes at the single gene level and genome level play an important role in tumor development. Increased DNA methylation levels of oncogenes or decreased DNA methylation levels of tumor suppressor genes may lead to tumorigenesis[28]. In this study, we found that the promoter methylation levels of KMT2A/B/C/D/E/F were significantly associated with KMT2A/B/C/D/E/F expression in GC, and also found to be related with tumor stage. The above results revealed that the hypermethylation levels of oncogenes predicted the high risk for patients with higher KMT2A/B/C/D/E/F expression in the development of GC, and improve the therapeutic strategies for patients as well.

Significantly, KMT2A/B/C/D/E/F were predicted to have two common meaningful kinases targets: AKT and MAPK, which indicated that KMT2s were involved in MAPK and PI3K/AKT signaling pathways. Consistent with the above results, we also found that KMT2A/B/C/D/E/F were markedly associated with gastric cancer pathway such as MAPK and PI3K/AKT pathways through GEAS analysis. Thus, KMT2A/B/C/D/E/F might regulate MAPK and PI3K/AKT pathways by interacting with common targets of AKT and MAPK to inhibit GC occurrence.

In conclusion, results of our study showed that increased expression of mRNA and protein were found in all of the 6 KMT2s family of genes in GC tissues, and KMT2s mRNA expression were related with patients' individual cancer stages and tumor grades in patients with GC. Meanwhile, the promoter methylation levels of KMT2s were found to be significantly positively related with KMT2s expression in GC. In addition, patients with higher KMT2A/B/C/D/E/F expression were associated with shorter OS, FP and PPS. And high somatic mutation frequency (40%) of KMT2s were observed in GC. Furthermore, we used the significant neighboring genes of KMT2s to explore the GO and KEGG pathway, the results showed that biological process such as histone lysine methylation and chromatin organization, cellular component such as nucleoplasm and chromosome, molecular function such as histone-lysine N-methyltransferase activity and DNA binding, KEGG pathway such as lysine degradation and gastric cancer such as PI3K/AKT and MAPK pathways were markedly regulated by KMT2s mutation in GC. Finally, we predicted the network of kinase AKT and MAPK targets commonly regulated by KMT2s in GC, which also suggested that KMT2s may regulate PI3K/AKT and MAPK pathways to influence the development and progression of GC. Thus, our study demonstrated that data mining efficiently revealed information about the clinical value of KMT2s and potential regulatory networks in GC, laying a foundation for further study of the role KMT2s in carcinogenesis.

Materials And Methods

GEPIA Database

GEPIA (<http://gepia.cancer-pku.cn/>) is an online tool for analyzing the RNA sequencing expression data of 9,736 tumors and 8,587 normal samples from the Cancer Genome Atlas and the Genotype Tissue Expression (GTEx) projects[29]. GEPIA provides customizable functions such as tumor/normal differential expression analysis, profiling according to cancer types or pathological stages, patient survival analysis, similar gene detection, correlation analysis and dimensionality reduction analysis.

The Human Protein Atlas

The Human Protein Atlas (<https://www.proteinatlas.org>) is a publicly available database, which provides tissue and cell distribution information containing all 24,000 human proteins[30]. We analyzed the protein expression of KMT2s in GC tissues and the normal tissues via immunohistochemistry (IHC) analysis.

UALCAN Analysis

UALCAN is publicly available at <http://ualcan.path.uab.edu>. It provides easy access to publicly available cancer OMICS data (TCGA and MET500) from 31 cancer types, which contains graphs and plots depicting gene expression and patient survival information based on gene expression[31]. We used this database to analyze the association between KMT2s mRNA expression with individual cancer stages and tumor stages.

The Kaplan-Meier Plotter

The prognostic value of mRNA expression of distinct KMT2s in gastric cancer was analyzed by using Kaplan-Meier plotter (<http://kmplot.com/analysis/>), in which information about association of gene expression with survival of patients of liver cancer, breast cancer, ovarian cancer, lung cancer and gastric cancer could be easily access to. To analyze the OS, FP, and PPS of patients with gastric cancer, patients were divided into high and low expression group based on median values of mRNA expression and validated by K-M survival curves, with the hazard ratio (HR) with 95% confidence intervals (CIs) and log rank p_value. Statically significant difference was considered when a p value < 0.05.

MEXPRESS Analysis

MEXPRESS (<https://mexpress.be/>) is a data visualization tool designed for the easy visualization of TCGA expression, DNA methylation and clinical data, as well as the relationships between them. We used this database to analyze the effect of methylation of KMT2s expression on the development of GC.

cBioPortal Analysis

The cBioPortal (<http://cbioportal.org>), currently containing 225 cancer studies, is an open-access resource for interactive exploration of multidimensional cancer genomics datasets[32]. We used cBioPortal to analyze KMT2s alterations in the TCGA GC dataset, which containing 293 samples. The search parameters included OncoPrint, Cancer Type Summary, Mutations, Co-expression, Network and so on. The tab OncoPrint shows an overview of KMT2s alterations per sample in total 293 GC samples. Network tab reflects the biological functional network of KMT2s interacting with neighboring genes acquired from public pathway databases, with filter options and color-coding based on the frequency of genomic alterations in each gene. Next, we chose KMT2s and the neighboring genes with alteration frequency > 5% to reveal the GO and KEGG pathways in CRC via STRING analysis.

GO and KEGG analysis

Functions of KMT2s mutations and the significant neighboring genes with mutation rates > 5% were chosen to analyze by GO and KEGG in the Database for STRING (<https://string-db.org/>). GO enrichment analysis can predict the functional roles of KMT2s mutations and chosen neighboring genes on the basis of three aspects, including biological processes(BP), cellular components (CC), and molecular functions (MF), while KEGG analysis can define the pathways regulated by KMT2s in GC.

Analysis for The Networks of Kinase, miRNA and Transcription Factor Targets

LinkedOmics is publicly available portal (<http://www.linkedomics.org/login.php>) that includes multi-omics data from all 32 TCGA cancer types. We used the LinkInterpreter module predicted its interacted target networks of kinases, miRNA, and transcription factors though gene set enrichment analysis (GSEA) analysis. The rank criterion was a $P < 0.05$, and 500 simulations were performed.

Declarations

AUTHOR CONTRIBUTIONS

L-Q.Y performed the experiments. L-S.Q, C-Y.O and X-Y analyzed the data. S-X.H wrote the manuscript. W-Y.B is the guarantor of this work, had full access to all data reported in the study, and takes responsibility for the integrity of the data and the accuracy of the data analysis.

CONFLICTS OF INTERESTS

The authors declare no conflicts of interests.

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Tables

Table 1. The Mutations of The KMT2s and their significant Neighboring Genes with Alteration Rates >5% in Gastric Cancer (cBioPortal)

Genes	Total Alteration	Amplification	Homozygous	Mutation
APC	17.4%	0.3%	3.1%	14.3%
ASH1L	11.9%	2.4%	0	9.6%
ATR	6.8%	1.4%	0	5.5%
BCL9	7.2%	0.7%	0	6.5%
BCL9L	8.5%	1%	0	7.8%
CTNNB1	8.2%	1.4%	0.7%	6.1%
EHMT1	6.5%	2%	0	4.4%
EHMT2	7.2%	1.4%	1.7%	4.1%
KMT2A	12.3%	2%	0	10.6%
KMT2B	11.6%	2.4%	1.7%	7.5%
KMT2C	17.4%	0.7%	1.7%	15.4%
KMT2D	23.2%	0.3%	1%	21.8%
KMT2E	9.2%	2.7%	0.7%	5.8%
KMT5B	7.8%	3.8%	0.7%	3.4%
NCOA2	9.2%	3.4%	0.3%	5.8%
NSD1	8.2%	1%	1%	6.1%
NSD2	6.8%	0.3%	1.4%	5.1%
NSD3	7.5%	1%	1%	5.5%
SETD1A	5.8%	0.3%	0	5.5%
SETD2	7.2%	0	1.7%	5.5%
SETDB1	9.2%	4.1%	0	5.1%
SETDB2	5.1%	0.7%	1%	3.4%
TCF	5.5%	0.3%	2%	3.1%
TCFL2	5.5%	1%	0.7%	3.8%

Table 2. Networks of The Top 5 Kinase, miRNA and Transcription Factor Targets of KMT2A/B/C/D/E/F in Gastric Cancer

Genes	Kinase Target	miRNA Target	TF Target	Genes	Kinase Target	miRNA Target	TF Target
KMT2A	Kinase_MAPK1	TGAATGT,MIR-181	V\$GATA6_01	KMT2D	Kinase_GRK3	CTCAGGG,MIR-125	V\$E2F1_Q6_01
	Kinase_AKT1	TGCACTT,MIR-519	V\$GFI1_01		Kinase_CDK2	CTGAGCC,MIR-24	V\$E2F_Q4
	Kinase_GSK3B	TTGCACT,MIR-130	V\$SRY_02		Kinase_AKT1	TTGCCAA,MIR-182	V\$E2F_Q3_01
	Kinase_PRKACB	ATGTACA,MIR-493	V\$SRF_Q6		Kinase_ATM	CCCAGAG,MIR-326	V\$E2F_Q6
	Kinase_PRKX	CAGTGTT,MIR-141	V\$SOX9_B1		Kinase_MAPK1	TGCTTTG,MIR-330	V\$E2F1_Q4_01
KMT2B	Kinase_CDK2	GTGCCTT,MIR-506	V\$ELK1_02	KMT2E	Kinase_GRK3	GCACTTT,MIR-17-5P	V\$NRF1_Q6
	Kinase_AKT1	ACCAAAG,MIR-9	V\$NRF1_Q6		Kinase_AKT1	TTTGCAC,MIR-19	V\$HFH4_01
	Kinase_MAPK1	CTACCTC,MIR-98	V\$AP2_Q6		Kinase_CDK2	CAGTATT,MIR-429	V\$OCT1_03
	Kinase_CHEK1	CACTGCC,MIR-449	V\$OLF1_01		Kinase_MAPK1	TGAATGT,MIR-181	V\$SRY_02
	Kinase_ATM	CTCAGGG,MIR-125	V\$AP2		Kinase_CSNK2A1	TGCACTT,MIR-519	V\$FOXO4_01
KMT2C	Kinase_GRK3	AAGCACA,MIR-218	V\$ELK1_02	KMT2F	Kinase_CDK2	CTCAGGG,MIR-125	V\$AP2GAMMA_01
	Kinase_CSNK2A1	ACACTGG,MIR-199	V\$MEF2_02		Kinase_AKT1	TGAATGT,MIR-181	V\$AP2ALPHA_01
	Kinase_MAPK3	AGCACTT,MIR-93	V\$NRF1_Q6		Kinase_MAPK1	CTGAGCC,MIR-24	V\$E2F1_Q6_01
	Kinase_MAPK1	AGGCACT,MIR-515-3P	V\$MYC_Q2		Kinase_CHEK1	TTTTGAG,MIR-373	V\$SP1_Q2_01
	Kinase_AKT1	ATACTGT,MIR-144	V\$GABP_B		Kinase_ATM	TAGCTTT,MIR-9	V\$SP1_Q6_01

Figures

Figure 1

The Expression Levels of KMT2s Proteins in Gastric Cancer (The Human Protein Atlas). (A-F) Compared with the normal tissues, KMT2A/B/C/D/E/F were highly expressed in cancer tissues in IHC analysis.

Figure 2

The Expression of KMT2s in Gastric Cancer (GEPIA). (A-B) mRNA expression of 6 KMT2s family member were found to be over-expressed in GC tissues compared to the normal tissues in profile and boxplot.

Figure 3

Correlation between mRNA Expression of Distinct KMT2s Family Members and Tumor Grades of GC Patients (UALCAN). mRNA expression of KMT2 A/B/C/D/E/F were found to be associated with patients' tumor grades, patients who were in advanced grades tended to express higher expression of KMT2s. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 4

Relationship between mRNA Expression of Distinct KMT2s Family Members and Individual Cancer Stages of GC Patients (UALCAN). (A-F) mRNA expression of KMT2 A/B/C/D/E/F were found to be associated with patients' individual cancer stages, patients who were in advanced stages tended to express higher expression of KMT2s. *p<0.05, **p<0.01, ***p<0.001.

Figure 5

Correlation between the Promoter Methylation Levels of KMT2s with Its Expression and Clinical Data in GC (MEXPRESS) (A) KMT2A expression was positively related with KMT2A promoter methylation ($r=0.448^{***}$), tumor stage ($p=0.01$) and histological type ($p=4.311e-5$). (B) KMT2B promoter methylation was positively related with KMT2B expression ($r=0.479^{***}$), tumor stage ($p=0.002$) and histological type ($p=0.002$). (C) KMT2C promoter methylation was positively related with KMT2C expression ($r=0.429^{***}$), tumor stage ($p=0.018$). (D) KMT2D promoter methylation was positively related with KMT2D expression ($r=0.303^{***}$). (E) KMT2CE promoter methylation was positively related with KMT2E expression ($r=0.403^{***}$), tumor stage ($p=0.014$). (F) KMT2F (SETD1A) promoter methylation was positively related with KMT2E expression ($r=0.357^{***}$), tumor stage ($p=0.021$) and histological type ($p=0.047$).

Figure 6

The prognostic value of KMT2A/B/C/D/E/F in Gastric Cancer (Kaplan-Meier Plotter). (A-F) Survival analysis revealed that patients with higher KMT2A/B/C/D/E/F mRNA expression had shorter OS, FP and PPS.

Figure 7

KMT2s Gene Expression and Mutation Analysis in Gastric Cancer (cBioPortal). (A and B) KMT2s gene expression and mutation analysis in gastric cancer. (C) Correlation between KMT2s with each other in gastric cancer. (D) The network of KMT2s and its neighboring genes with mutation rates > 5%.

Figure 8

Predicted the Functions of the Mutations in KMT2s and Their Significant Neighboring Genes with Alteration Rates > 5% in Gastric Cancer. (A-C) GO functional enrichment analysis predicted three main functions of KMT2s mutations and their significant altered neighbor genes with alteration rates >5%, including biological process, cellular components and molecular functions. (D) KEGG pathways of KMT2s in GC.

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