Pathology - Research and Practice 215 (2019) 152570

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

Identification of DNA methylation-regulated differentially-expressed genes and related pathways using Illumina 450K BeadChip and bioinformatic analysis in gastric cancer

Yu Liang, Cheng Zhang, Dong-Qiu Dai*

Department of Gastrointestinal Surgery, The Fourth Affiliated Hospital of China Medical University, Shenyang, 110032, China

ARTICLE INFO

Keywords:
Gastric cancer
DNA methylation
Differentially-expressed genes
Bioinformatic analysis
Overall survival

ABSTRACT

In the current research, we aimed to identify and analyze methylation-regulated differentially-expressed genes (MeDEGs) and related pathways using bioinformatic methods. We downloaded RNA-seq, Illumina Human Methylation 450 K BeadChip and clinical information of gastric cancer (GC) from The Cancer Genome Atlas (TCGA) project. Differentially-expressed genes (DEGs) were identified using the edgeR package. Then, we performed Spearman's correlation analysis between DEG expression levels and methylation levels. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed in the DAVID database. We then conducted Kaplan–Meier survival analysis to explore the relationship between methylation, expression and prognosis. The protein–protein interaction networks were further analyzed using the STRING database. A total of 204 down-regulated DEGs and 164 up-regulated DEGs were identified as MeDEGs. GO and KEGG pathway analyses showed that MeDEGs were enriched in multiple cancer-related terms. Kaplan–Meier survival analysis showed that eight up-regulated MeDEGs (CAMKV, COMP, FGF3, FGF19, FOXL2, IGF2BP1, IGFBP1 and NPPB) and five down-regulated MeDEGs (ALDH3B2, CALML3, FLRT1, G6PC and HRASLS2) were associated with prognosis of GC patients. In addition, PPI networks and KEGG pathway analyses further confirmed the critical role of prognosis-related MeDEGs. In conclusion, methylation plays a critical role in GC progression. Multiple MeDEGs are related to prognosis, suggesting that they may be potential targets in tumor treatment.

1. Introduction

According to Global cancer statistics, there were approximately 1.03 million new cases and 0.78 million deaths from gastric cancer (GC) in 2018 [2]. The incidence and mortality were ranked third and fifth worldwide, respectively. Epidemiological studies have confirmed that the occurrence of GC is related to *Helicobacter pylori* (*H. pylori*) [36] infection, smoking, alcohol abuse, and intake of salted and high-fat food [47]. However, the initial symptoms of GC are not typical and the prognosis of patients in an advanced stage is relatively poor [57]. Hence, research into specific biomarkers and therapeutic targets is still of great value and significance for the prevention and treatment of GC.

In recent years, the function of epigenetic modifications in the initiation and progression of GC has drawn a lot of attention. DNA methylation, acknowledged as a key modification in the field of epigenetics, regulates gene expression at the transcriptional level. Aberrant cytosine-phosphate-guanine (CpG)-island methylation in DNA regulatory regions could upregulate oncogenes and downregulate tumor-suppressor genes without changing the sequences. For instance, DNMT family has been verified as a critical DNA methyltransferase in generating the aberrant DNA methylation in oocytes [24]. TET family inhibited solid tumors and hematological malignancies progression by mediating active DNA demethylation [20,27]. However, studies of methylation in the control of gene expression are still inadequate. Zouridis et al. [61] reported that approximately three quarters of gene expression showed a negative correlation with methylation. Therefore, identification of methylation-regulated differentially-expressed genes (MeDEGs) will be helpful in further clarifying the role of methylation and identifying candidate targets for future research.

In the last few years, with the development of high-throughput sequencing platforms, increasing numbers of differentially-expressed genes (DEGs) and epigenetic alterations have been revealed in diverse

* Corresponding author at: Department of Gastrointestinal Surgery, The Fourth Affiliated Hospital of China Medical University, No. 4 Chongshan East Road, Shenyang, 110032, China.
E-mail address: daidq63@163.com (D.-Q. Dai).

https://doi.org/10.1016/j.prp.2019.152570
Received 25 April 2019; Received in revised form 16 July 2019; Accepted 26 July 2019

malignancies. For instance, Ge et al. [14] applied an Illumina Human Methylation 450 K BeadChip to detect methylation expression profiles and corresponding methylation expression in five early-onset GC patients and seven advanced patients. The results demonstrated that hypermethylation of EIF4E was associated with age at diagnosis and poor survival of GC patients. Bure et al. [3] also utilized a Human Methylation 450 K BeadChip to analyze the DNA methylation level in gastrointestinal stromal tumor (GISTs) cell lines. Researchers identified two CpG sites that control CD34 expression. Moreover, studies into the differential methylation and expression of CD34 provide new evidence for the idea that GISTs arise from different cell subpopulations.

The Illumina Human Methylation 450 K BeadChip can detect almost 450,000 methylation sites and covers 96% of the CpG islands in the entire human genome [42]. Furthermore, it can detect differential tissue methylation sites of normal tissues and tumors, miRNA promoter regions and 90% of the sites on the Human Methylation 27 BeadChip [40].Previous studies have shown that the Illumina Human Methylation 450 K BeadChip has important applications in research into methylation. However, conjoint analysis of GC methylation in large cohorts has not been performed. In our study, we obtained *in silico* data and clinical information of GC from The Cancer Genome Atlas (TCGA, http://cancergenome.nih.gov) project [46]. MeDEGs and related enrichment analysis were then identified. Furthermore, prognosis-related MeDEGs and protein–protein interaction (PPI) networks were analyzed. We aimed to identify new specific biomarkers and therapeutic targets for GC patients based on the Illumina Human Methylation 450 K BeadChip.

2. Materials and methods

2.1. Sample collection

We downloaded GC transcriptome profiles and clinical information from TCGA through the Genomic Data Commons (GDC) Data Transfer Tool 1.3.0 [46]. As of September 2018, the public database included the expression profiles of 375 GC tissues and 32 normal tissues derived by RNA-seq. Then, we found that 338 out of 375 GC tissues had also been examined in the Illumina Human Methylation 450 K BeadChip platform and we obtained corresponding methylation data using GDC software. According to the publication guidelines (2015) provided by TCGA (https://cancergenome.nih.gov/publications/publicationguidelines), our study does not require the approval of an ethics committee.

2.2. Data processing for the identification of DEGs and MeDEGs

We constructed an RNA matrix containing the transcriptome profiles using PERL software. The coding RNAs were extracted and then the official gene ID was converted to the gene name according to Ensembl (Homo sapiens) (http://asia.ensembl.org/index.html). DEGs were identified with the edgeR package with a threshold |log2 fold change (FC)| > 2.0 and *P*-value < 0.01. Additionally, we analyzed the correlation between methylation data and DEG expression using Spearman's correlation analysis. The DEGs were classified as MeDEGs when they satisfied the cut-off criteria including correlation coefficient < -0.2 and *P*-value < 0.01. The volcano plot and scatter plots were mapped by R software.

2.3. Functional enrichment analyses

To explore the function of the MeDEGs in GC tumorigenesis and metastasis, gene ontology (GO) [15] and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways [10] were analyzed using DAVID (https://david.ncifcrf.gov/), which is a web server that annotates an input set of genes with enrichment analyses based on human disease databases.

2.4. Association analysis between MeDEGs and GC patient prognosis

First, we divided the 338 GC patients into two groups according to the median methylation value of each MeDEG. Additionally, GC patients were also divided into a hypermethylation and low-expression MeDEG (Hyper-LG) group and a hypomethylation and high expression MeDEG (Hypo-HG) group according to the median value of methylation and expression of MeDEGs. Comparison of the overall survival between each two groups was then analyzed. Kaplan–Meier's method and the log-rank test were performed to assess survival rate. Differences with *P*-value < 0.05 were regarded as statistically significant.

2.5. PPI network construction of prognosis-related MeDEGs

PPI analysis was performed to reveal the molecular mechanisms of the prognosis-related MeDEGs in GC. We utilized the STRING protein database v10.5 (http://string-db.org/) to construct the PPI networks. An interaction score > 0.4 was regarded as the cut-off criterion.

3. Results

3.1. Identification of DEGs and MeDEGs in GC

The flowchart of our research was shown in Fig. 1 [30]. We identified DEGs between 375 GC and 32 normal tissue expression profiles from TCGA. As a result, we identified 858 up-regulated and 778 down-regulated DEGs using the edgeR package. The DEGs are shown as a volcano plot in Supplementary Fig. 1. Then, we analyzed the correlation between DEG expression and methylation value. In total, 164 up-regulated and 204 down-regulated DEGs were identified as MeDEGs that satisfied the cut-off criteria. The list of MeDEGs was shown in Supplementary Table 1. The top 10 up-regulated and down-regulated genes with the highest Spearman's correlation coefficients are shown in Fig. 2. Among them, multiple genes have been verified mediating the function of DNMT family and TET family. For instance, TET-induced oxidation of 5-methylcytosine of LEFTY1 and reversed the methylation effect of DNMT3A and DNMT3B in early body formation [7]. SALL4 was a 5hmC binder and promoted 5hmC oxidation by stabilizing TET2 association in mouse embryonic stem cells [48].

3.2. Functional enrichment analyses of MeDEGs

To further reveal the function of MeDEGs in GC, we subjected the 368 MeDEGs to GO and KEGG pathway analysis using DAVID 6.8. The enrichment analyses of biological processes are shown in Fig. 3A. Among them, negative regulation of transcription from the RNA polymerase II promoter, proteolysis and cell differentiation are associated with cancer. Furthermore, cell component analysis indicated that MeDEGs are mainly enriched in the extracellular exosome, extracellular region and extracellular space (Fig. 3B). As for molecular function (Fig. 3C), sequence-specific DNA binding was the most enriched term. KEGG pathway enrichment analysis suggested that MeDEGs predominantly participated in the cancer-related pathways including the PI3K-Akt signaling pathway, chemical carcinogenesis, glycolysis, gastric acid secretion and PPAR signaling pathway (Fig. 3D).

3.3. MeDEGs related to the prognosis of GC patients

We preformed Kaplan–Meier curve analysis to identify the MeDEGs related to overall survival. First, we analyzed the relationship between MeDEG methylation data and prognosis. The Kaplan–Meier curves showed that hypermethylation of eight up-regulated MeDEGs (CAMKV, COMP, FGF3, FGF19, FOXL2, IGF2BP1, IGFBP1 and NPPB) and hypomethylation of five down-regulated MeDEGs (ALDH3B2, CALML3, FLRT1, G6PC and HRASLS2) were positively correlated with overall survival (Fig. 4).

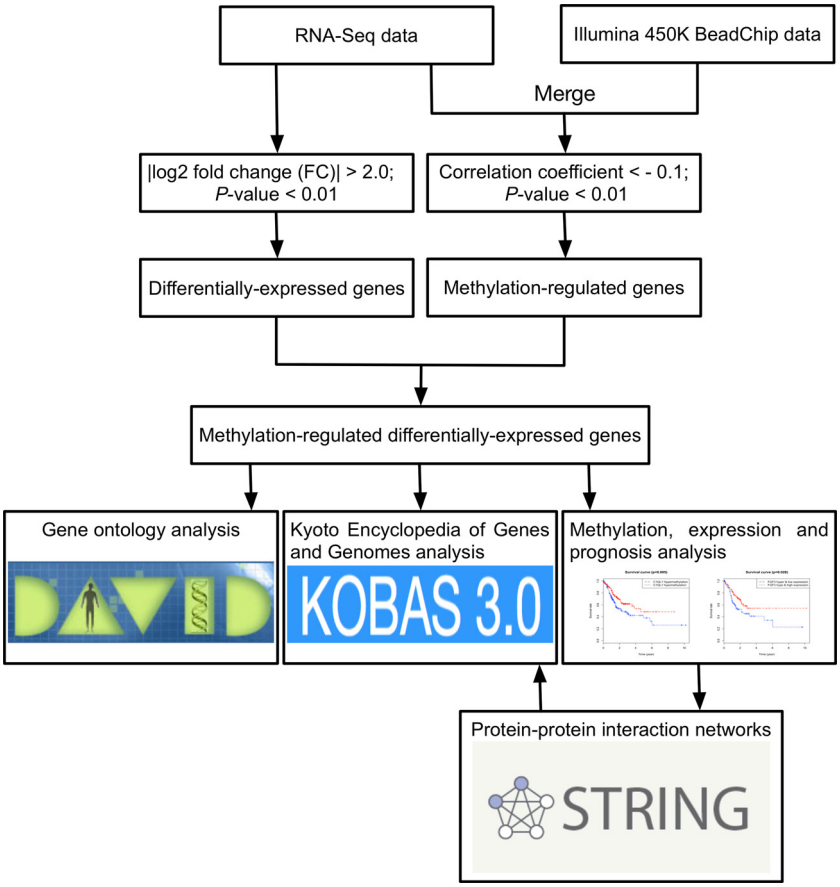

Fig. 1. The flow chart of bioinformatics analysis in the present research.

Next, we further compared the prognosis of the above 13 MeDEGs between the Hyper-LG group and the Hypo-HG group. As shown in Fig. 5, the results showed that Hypo-HGs of eight up-regulated MeDEGs (CAMKV, COMP, FGF3, FGF19, FOXL2, IGF2BP1, IGFBP1 and NPPB) and Hyper-LGs of five down-regulated MeDEGs (ALDH3B2, CALML3, FLRT1, G6PC and HRASLS2) were significantly related to poor survival of GC patients (Fig. 5).

3.4. PPI network construction of prognosis-related MeDEGs

We utilized the STRING protein database to analyze the PPI networks of prognosis-related MeDEGs. The PPI enrichment *P*-value was 2.02×10^{-8} (Supplementary Fig. 2). Furthermore, we performed KEGG pathway enrichment analysis of the genes involved in the PPI networks. It was worth noting that 12 out of the top 20 KEGG pathways were closely related to cancer, including the PI3K-Akt signaling pathway, Rap1 signaling pathway, Ras signaling pathway, prostate cancer, pathways in cancer, MAPK signaling pathway, proteoglycans in cancer, glycolysis, AMPK signaling pathway, FoxO signaling pathway, central carbon metabolism in cancer and chemical carcinogenesis (Table 1).

4. Discussion

So far, a series of DNA sequence modifications including 5-Methylcytosine, N6-methyladenine and N4-methylcytosine have been found in the process of tumorigenesis through bioinformatics analysis [5,18]. However, DNA methylation plays a critical role in the regulation of cancer-related gene expression [33]. Furthermore, DNA methylation is recognized as a biomarker [60] and therapeutic target for GC [6]. Thus, there is an urgent need to identify and analyze MeDEGs based on a large sample size of whole-genome sequences.

In the present research, we identified a total of 164 up-regulated and 204 down-regulated MeDEGs according to the results of Spearman's correlation analysis. We further performed functional enrichment analysis to clarify the role of methylation in GC. Negative regulation of transcription from the RNA polymerase II promoter, proteolysis, cell differentiation and cell – cell signaling related to cancer were enriched in biological processes. Negative regulation of transcription was consistent with the function of methylation [21]. Deregulation of proteolysis contributed to the induction and progression of *H. pylori*-induced GC [37].

Cell differentiation is another important process in the development and prognosis of GC. Cellular component analysis showed that a majority of MeDEGs were expressed in the extracellular exosome, extracellular region and extracellular space. This indicated that MeDEGs might exert their biological functions by regulating the microenvironment of GC. Enrichment of molecular function included sequence-specific DNA binding which was related to promoter binding. Furthermore, receptor binding [28], cytokine activity [38] and growth factor activity [29] were also important mechanisms in GC. KEGG pathway analysis further clarified the function of MeDEGs. The PI3K-Akt signaling pathway was one of the most critical mechanisms promoting GC migration, invasion and epithelial–mesenchymal transition (EMT) [19]. Aerobic glycolysis, also known as the Warburg effect, could accumulate lactate and promote development of GC [55]. Furthermore, the PPAR signaling pathway has been reported to be down-regulated in the malignant transformation of intestinal-type early GC [34].

In this study, we applied Kaplan–Meier curve analysis to identify the methylation and expression of MeDEGs that correlated with clinical prognosis among GC patients. Ultimately, eight up-regulated MeDEGs (CAMKV, COMP, FGF3, FGF19, FOXL2, IGF2BP1, IGFBP1 and NPPB)

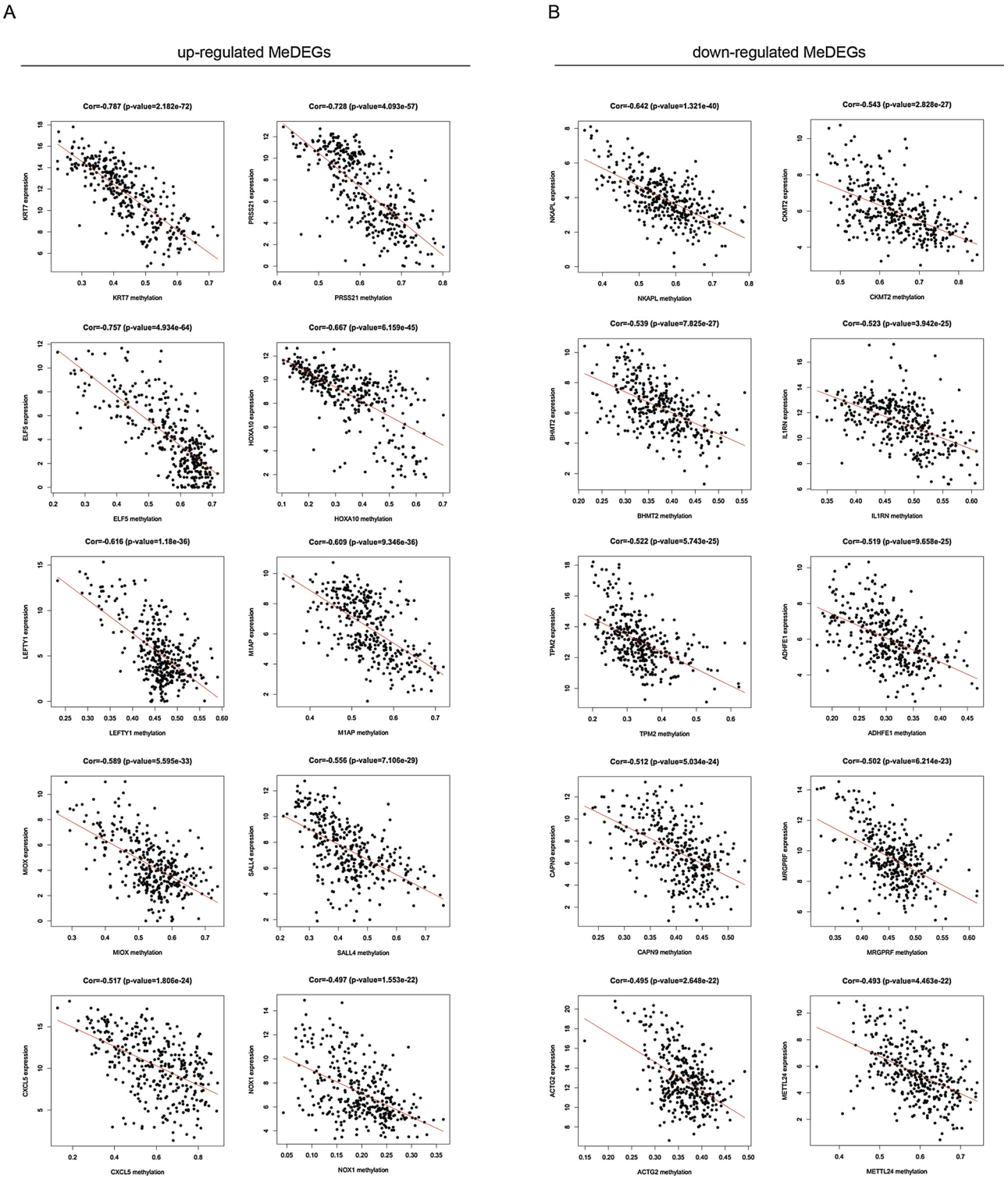

Fig. 2. The up-regulated and down-regulated differentially-expressed genes with the top 10 correlation coefficients.
(A) Spearman's correlation analysis between methylation of up-regulated genes (horizontal axis) and expression (vertical axis); (B) Spearman's correlation analysis between methylation of down-regulated genes (horizontal axis) and expression (vertical axis). Spearman's correlation coefficient and *P*-values are shown in each plot. MeDEGs, methylation-regulated differentially-expressed genes.

were detected as prognosis-related indicators. CAMKV is a member of the cyclin-dependent kinase family and previous research has mainly focused on nervous system disorders [26,43]. COMP is a soluble glycoprotein expressed in cartilage. Englund et al. [11] reported that COMP is up-regulated in breast cancer tissues and it has been confirmed by *in vitro* and *in vivo* experiments that it promotes invasiveness and viability of breast cancer cells. Furthermore, COMP has been verified as a driver of the progression of prostate cancer by interfering with Ca^{2+} homeostasis and lactate production [12]. Zhou et al. [59]. indicated that COMP correlated with recurrence of stage III and IV GC patients who were treated with surgery and chemoradiotherapy. Li et al. [25] revealed that methylation of FGF3 was confirmed as a biomarker for

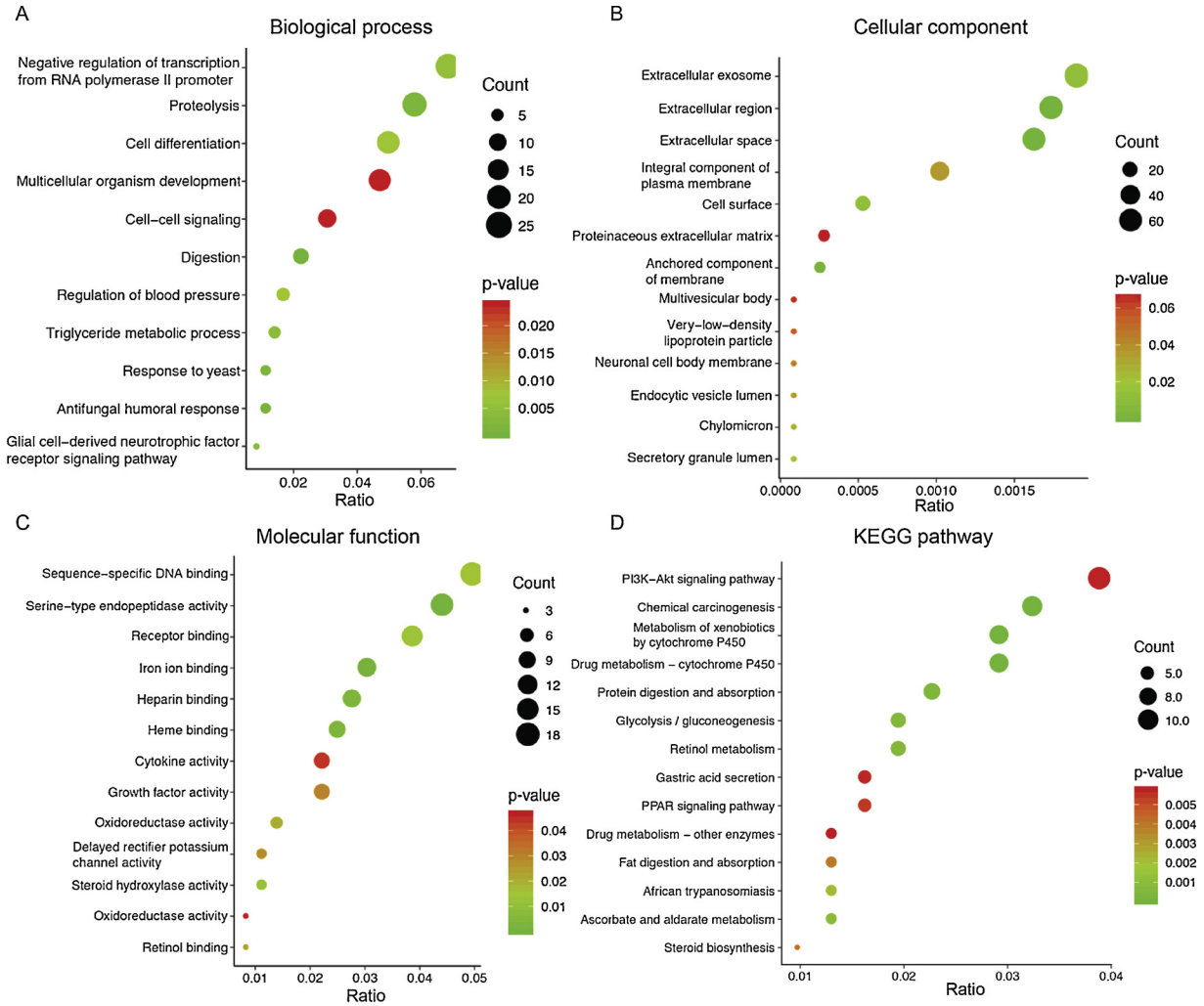

Fig. 3. Gene ontology and KEGG pathway enrichment analysis of all of the MeDEGs.
(A) Biological process terms of MeDEGs; (B) Cell component terms of MeDEGs; (C) Molecular function terms of MeDEGs; (D) KEGG pathway enrichment terms of MeDEGs. The "Count" represents the number of genes. MeDEGs, methylation-regulated differentially-expressed genes; KEGG, Kyoto Encyclopedia of Genes and Genomes.

detecting oral squamous cell cancer. Moreover, Sun et al. [44] revealed that FGF 3 hypomethylation could serve as a potential biomarker for oral squamous cell cancer which occurred in the early stage of oral carcinogenesis. Salem et al. [41] recently reported that FGF3 was up-regulated in GC. However, little is known about the function and regulatory mechanism of CAMKV, COMP and FGF3 in GC. Studies have shown that epigenetic modification could directly lead to the dysregulation of FGF19 [8,52]. Wang et al. [45] reported that FGF19 is up-regulated in GC tissues and cell lines, and enhances the migration and invasion abilities of GC cells. FOXL2 is regarded as an oncogene in GC due to its actions in promoting proliferation, metastasis and EMT [9,54]. Interestingly, the promoter of FOXL2 has been found to be hypermethylated in multiple cancers, including non-small cell lung cancer [58] and esophageal adenocarcinoma [16]. Therefore, it will be necessary to explore the methylation of FOXL2 in GC in future research. IGF2BP1, which belongs to a family of RNA-binding proteins, could promote the stability of c-Myc mRNA by direct binding in GC [50]. Luo et al. demonstrated that IGFBP1 was up-regulated in *H. pylori*-infected GC cell lines. However, it played a protective role in the migration of *H. pylori*-induced GC by inhibiting MMP9 [31]. NPPB is a cardiac hormone which is regarded as a diagnostic biomarker for acute heart failure. In recent years, NPPB has been identified as an oncogene and candidate biomarker for malignant cancers, including ovarian cancer [23], colorectal cancer [13] and osteosarcoma [22]. In GC, NPPB was involved in the *H. pylori*-induced PI3K/Akt pathway. Nevertheless, research into the role of NPPB in tumorigenesis and progression of GC is still rare.

Moreover, we identified five down-regulated MeDEGs (ALDH3B2, CALML3, FLRT1, G6PC and HRASLS2) that were associated with overall survival. Yang et al. [51] revealed that ALDH3B2 is down-regulated in gefitinib-resistant cell lines in lung adenocarcinoma, while Yin et al. [53] demonstrated that ALDH3B2 gene polymorphism may be a risk factor for esophageal squamous cancer carcinogenesis [53]. CALML3 has been identified as a novel biomarker and therapeutic target for breast cancer [39], lung adenocarcinoma [56] and hepatocellular cancer [49]. CALML3 has also been verified as a tumor suppressive gene which is up-regulated in metformin-treated GC-associated fibroblasts [4]. FLRT1 has been identified as a long-term survival-related DEG in glioblastoma which was consistent with our study. G6PC is involved in glucose mechanism by gluconeogenesis, and is hypermethylated in the proximal tubules during the process of diabetic kidney disease [32]. However, G6PC has been found to be up-regulated in ovarian cancer [17] and glioblastoma [1], findings which are contradictory to our results. Therefore, the function of G6PC in GC should be clarified in future research. Moreover, HRASLS2 has been identified as a potential biomarker to predict the preoperative sensitivity to

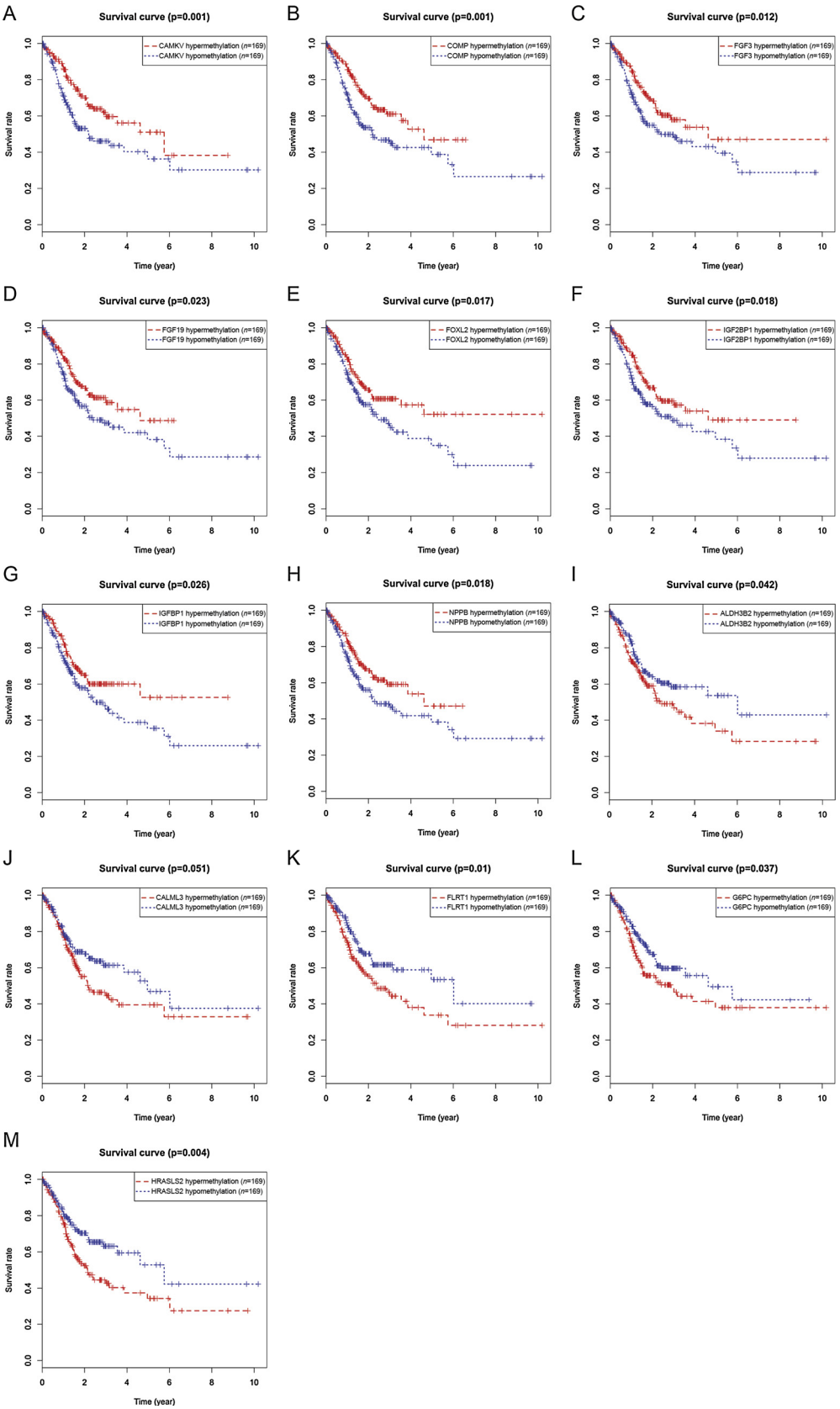

Fig. 4. Kaplan-Meier curves for the methylation of MeDEGs that are associated with overall survival.
MeDEGs were ranked by the median of methylation and then scored for each gastric cancer patient in accordance with high- or low-level methylation value. The horizontal axis represents the overall survival time and the vertical axis represents survival function. MeDEGs, methylation-regulated differentially-expressed genes.

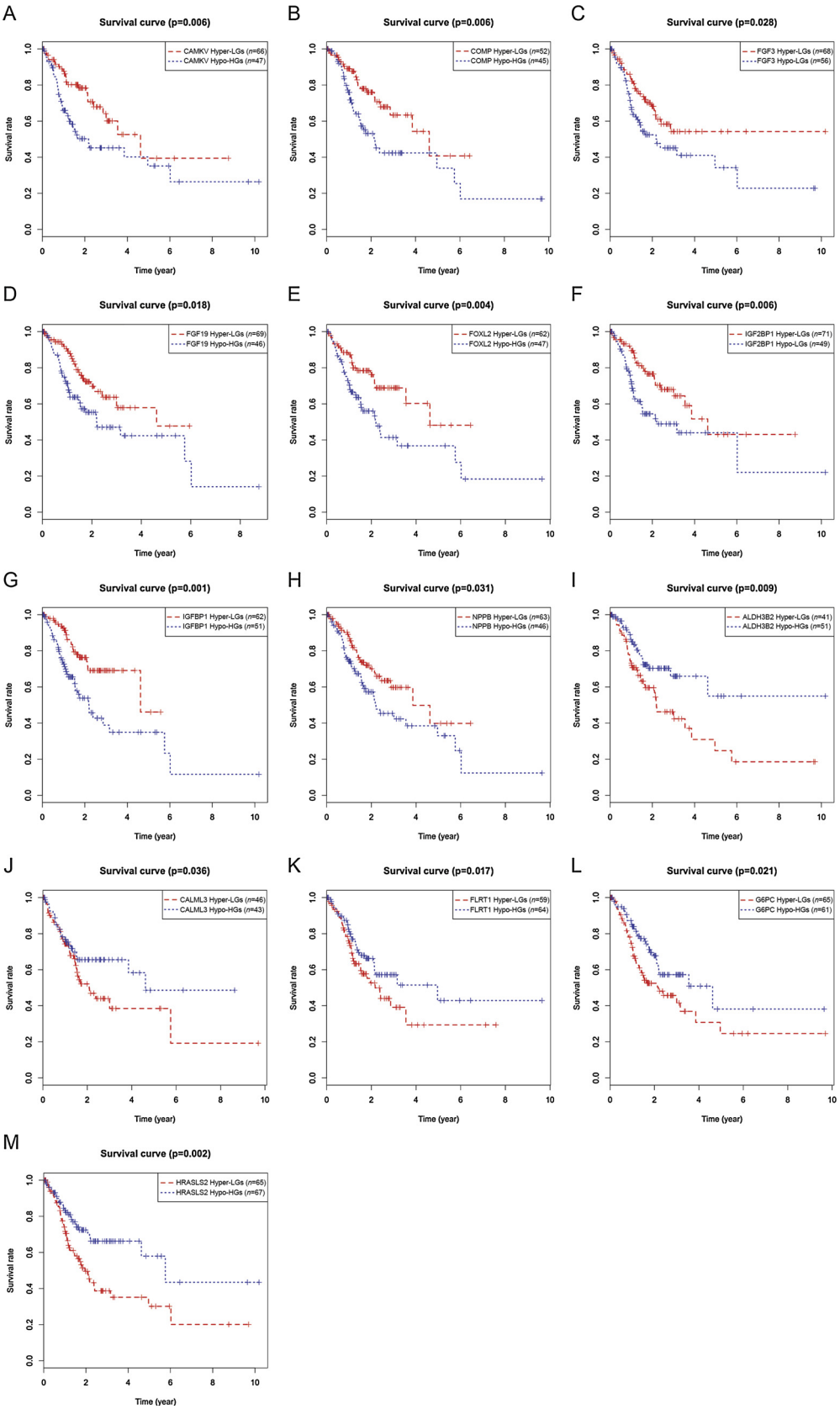

Fig. 5. Kaplan-Meier curves for the methylation-expression of MeDEGs that are associated with overall survival.
MeDEGs were ranked by the median of methylation and expression and then scored for each gastric cancer patient in accordance with high- or low-level methylation value and high or low-level expression value. The horizontal axis represents the overall survival time and the vertical axis represents survival function. MeDEGs, methylation-regulated differentially-expressed genes; Hyper-LGs, hypermethylation and low expression genes MeDEGs; Hypo-HGs, hypomethylation and high expression MeDEGs.

Table 1
The top 20 KEGG pathways enriched by the genes involved in the protein–protein interaction network.

ID	Description	p-value	Gene name
hsa04151	PI3K-Akt signaling pathway	2.67E-15	ADH71, IGF1, INS, FGF19, G6PC, FGF3, FGFR2, FGFR1, FGFR3, FGFR4
hsa04015	Rap1 signaling pathway	3.13E-15	IGF1, INS, FGF19, FGF3, CALML3, FGFR2, FGFR1, FGFR3, FGFR4
hsa04014	Ras signaling pathway	6.17E-15	IGF1, INS, FGF19, FGF3, CALML3, FGFR2, FGFR1, FGFR3, FGFR4
hsa04810	regulation of actin cytoskeleton	3.48E-11	INS, FGF19, FGF3, FGFR2, FGFR1, FGFR3, FGFR4
hsa05215	prostate cancer	2.15E-09	FOXO1, FGFR1, IGF1, INS, FGFR2
hsa05200	pathways in cancer	2.25E-09	FOXO1, IGF1, FGF19, FGF3, FGFR2, FGFR1, FGFR3
hsa04010	MAPK signaling pathway	6.92E-09	FGF19, FGF3, FGFR2, FGFR1, FGFR3, FGFR4
hsa04550	signaling pathways regulating pluripotency of stem cells	2.05E-08	FGFR2, FGFR1, FGFR3, IGF1, FGFR4
hsa00010	glycolysis / gluconeogenesis	8.04E-08	ADH7, ADH6, G6PC, ALDH3B2
hsa05218	melanoma	1.00E-07	FGF19, FGFR1, FGF3, IGF1
hsa04152	AMPK signaling pathway	8.91E-07	FOXO1, G6PC, IGF1, INS
hsa04068	FoxO signaling pathway	1.17E-06	FOXO1, G6PC, IGF1, INS
hsa04910	insulin signaling pathway	1.34E-06	CALML3, FOXO1, G6PC, INS
hsa00350	tyrosine metabolism	1.41E-06	ADH7, ADH6, ALDH3B2
hsa04213	longevity regulating pathway	7.89E-06	FOXO1, IGF1, INS
hsa05230	central carbon metabolism in cancer	9.01E-06	FGFR2, FGFR1, FGFR3
hsa00982	drug metabolism - cytochrome P450	9.81E-06	ADH7, ADH6, ALDH3B2
hsa00980	metabolism of xenobiotics by cytochrome P450	1.15E-05	ADH7, ADH6, ALDH3B2
hsa01521	EGFR tyrosine kinase inhibitor resistance	1.56E-05	FGFR2, FGFR3, IGF1
hsa04151	chemical carcinogenesis	1.62E-05	ADH7, ADH6, ALDH3B2

KEGG: Kyoto Encyclopedia of Genes and Genomes.

chemoradiotherapy in advanced rectal cancer [35]. From the above, the methylation state and function of five down-regulated MeDEGs in GC have not been well elucidated.

We constructed the PPI networks that involved prognosis-related MeDEGs. Then, KEGG pathway analysis was performed to clarify the function of the identified genes in the PPI networks. The results showed that multiple cancer-associated pathways were identified which verified that prognosis-related MeDEGs play critical roles in the progression of GC. Thus, additional research is needed to explore the biological and molecular mechanisms of these MeDEGs in GC.

In conclusion, we identified MeDEGs by analyzing the expression profiles and methylation data of GC samples from TCGA. Functional enrichment analyses further confirmed the role of MeDEGs in GC. Moreover, we identified eight up-regulated MeDEGs and five down-regulated MeDEGs that were related to overall survival. PPI networks and KEGG pathway analyses further clarified the function of prognosis-related MeDEGs. Our study deepens the understanding of methylation and provides novel therapeutic targets and prognosis-related biomarkers for further research.

Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

Acknowledgements

Publication of this article is funded by the National Natural Science Foundation of China (Grant No: 30572162), and Natural Science Foundation of Liaoning Province (Grant No: 201602817).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prp.2019.152570.

References

[1] S. Abbadi, J.J. Rodarte, A. Abutaleb, E. Lavell, C.L. Smith, W. Ruff, J. Schiller, A. Olivi, A. Levchenko, H. Guerrero-Cazares, A. Quinones-Hinojosa, Glucose-6-phosphatase is a key metabolic regulator of glioblastoma invasion, Mol. Cancer Res. 12 (2014) 1547–1559.
[2] F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre, A. Jemal, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA Cancer J. Clin. 68 (2018) 394–424.
[3] I. Bure, A. Braun, C. Kayser, H. Geddert, I.-M. Schaefer, S. Cameron, M.B. Ghadimi, P. Ströbel, M. Werner, A. Hartmann, S. Wiemann, A. Agaimy, F. Haller, E.A. Moskalev, The expression of hematopoietic progenitor cell antigen CD34 is regulated by DNA methylation in a site-dependent manner in gastrointestinal stromal tumours, Int. J. Cancer 141 (2017) 2296–2304.
[4] G. Chen, C. Yu, Z. Tang, S. Liu, F. An, J. Zhu, Q. Wu, J. Cao, Q. Zhan, S. Zhang, Metformin suppresses gastric cancer progression through calmodulinlike protein 3 secreted from tumorassociated fibroblasts, Oncol. Rep. 41 (2019) 405–414.
[5] W. Chen, H. Lv, F. Nie, H. Lin, i6mA-Pred: identifying DNA N6-methyladenine sites in the rice genome, Bioinformatics (2019), https://doi.org/10.1093/bioinformatics/btz015.
[6] S.J. Choi, S.W. Jung, S. Huh, Y.S. Chung, H. Cho, H. Kang, Alteration of DNA methylation in gastric cancer with chemotherapy, J. Microbiol. Biotechnol. 27 (2017) 1367–1378.
[7] H.Q. Dai, B.A. Wang, L. Yang, J.J. Chen, G.C. Zhu, M.L. Sun, H. Ge, R. Wang, D.L. Chapman, F. Tang, X. Sun, G.L. Xu, TET-mediated DNA demethylation controls gastrulation by regulating Lefty-Nodal signalling, Nature 538 (2016) 528–532.
[8] A. Dallol, A. Buhmeida, A. Merdad, J. Al-Maghrabi, M.A. Gari, M.M. Abu-Elmagd, A. Elaimi, M. Assidi, A.G. Chaudhary, A.M. Abuzenadah, T. Nedjadi, E. Ermiah, S.S. Alkhayyat, M.H. Al-Qahtani, Frequent methylation of the KLOTHO gene and overexpression of the FGFR4 receptor in invasive ductal carcinoma of the breast, Tumour Biol. 36 (2015) 9677–9683.
[9] J. Dong, R. Wang, G. Ren, X. Li, J. Wang, Y. Sun, J. Liang, Y. Nie, K. Wu, B. Feng, Y. Shang, D. Fan, HMGA2-FOXL2 Axis regulates metastases and epithelial-to-mesenchymal transition of chemoresistant gastric cancer, Clin. Cancer Res. 23 (2017) 3461–3473.
[10] S. Draghici, P. Khatri, A.L. Tarca, K. Amin, A. Done, C. Voichita, C. Georgescu, R. Romero, A systems biology approach for pathway level analysis, Genome Res. 17 (2007) 1537–1545.
[11] E. Englund, M. Bartoschek, B. Reitsma, L. Jacobsson, A. Escudero-Esparza, A. Orimo, K. Leandersson, C. Hagerling, A. Aspberg, P. Storm, M. Okroj, H. Mulder, K. Jirström, K. Pietras, A. Blom, Cartilage oligomeric matrix protein contributes to the development and metastasis of breast cancer, Oncogene 35 (2016) 5585–5596.
[12] E. Englund, G. Canesin, K.S. Papadakos, N. Vishnu, E. Persson, B. Reitsma, A. Anand, L. Jacobsson, L. Helczynski, H. Mulder, A. Bjartell, A.M. Blom, Cartilage oligomeric matrix protein promotes prostate cancer progression by enhancing invasion and disrupting intracellular calcium homeostasis, Oncotarget 8 (2017) 98298–98311.
[13] F.U. Ertem, W. Zhang, K. Chang, W. Mohaiza Dashwood, P. Rajendran, D. Sun, A. Abudayyeh, E. Vilar, M. Abdelrahim, R.H. Dashwood, Oncogenic targets Mmp7, S100a9, Nppb and Aldh1a3 from transcriptome profiling of FAP and Pirc adenomas are downregulated in response to tumor suppression by Clotam, Int. J. Cancer 140 (2017) 460–468.
[14] Y. Ge, Q. Wu, G. Ma, W. Shao, H. Liu, Q. Zhang, J. Xin, Y. Xue, M. Du, Q. Zhao, M. Wang, H. Chu, Z. Zhang, Hypermethylation of EIF4E promoter is associated with early onset of gastric cancer, Carcinogenesis 39 (2018) 66–71.
[15] C. Gene Ontology, The Gene Ontology (GO) project in 2006, Nucleic Acids Res. 34 (2006) D322–326.
[16] I. Guilleret, L. Losi, S.T. Chelbi, S. Fonda, S. Bougel, S. Saponaro, G. Gozzi, L. Alberti, R. Braunschweig, J. Benhattar, DNA methylation profiling of esophageal adenocarcinoma using Methylation Ligation-dependent Macroarray (MLM), Biochem. Biophys. Res. Commun. 479 (2016) 231–237.
[17] T. Guo, T. Chen, C. Gu, B. Li, C. Xu, Genetic and molecular analyses reveal G6PC as a key element connecting glucose metabolism and cell cycle control in ovarian cancer, Tumor Biol. 36 (2015) 7649–7658.

[18] W. He, C. Jia, Q. Zou, 4mCPred: machine learning methods for DNA N4-methylcytosine sites prediction, Bioinformatics 35 (2019) 593–601.
[19] V. Henderson, B. Smith, L.J. Burton, D. Randle, M. Morris, V.A. Odero-Marah, Snail promotes cell migration through PI3K/AKT-dependent Rac1 activation as well as PI3K/AKT-independent pathways during prostate cancer progression, Cell Adh. Migr. 9 (2015) 255–264.
[20] Y. Huang, A. Rao, Connections between TET proteins and aberrant DNA modification in cancer, Trends Genet. 30 (2014) 464–474.
[21] S. Kumar, B.C. Karmakar, D. Nagarajan, A.K. Mukhopadhyay, R.D. Morgan, D.N. Rao, N4-cytosine DNA methylation regulates transcription and pathogenesis in Helicobacter pylori, Nucleic Acids Res. 46 (2018) 3429–3445.
[22] S.U. Lauvrak, E. Munthe, S.H. Kresse, E.W. Stratford, H.M. Namløs, L.A. Meza-Zepeda, O. Myklebost, Functional characterisation of osteosarcoma cell lines and identification of mRNAs and miRNAs associated with aggressive cancer phenotypes, Br. J. Cancer 109 (2013) 2228–2236.
[23] K. Lawrenson, B. Grun, N. Lee, P. Mhawech-Fauceglia, J. Kan, S. Swenson, Y.G. Lin, T. Pejovic, J. Millstein, S.A. Gayther, NPPB is a novel candidate biomarker expressed by cancer-associated fibroblasts in epithelial ovarian cancer, Int. J. Cancer 136 (2015) 1390–1401.
[24] Y. Li, Z. Zhang, J. Chen, W. Liu, W. Lai, B. Liu, X. Li, L. Liu, S. Xu, Q. Dong, M. Wang, X. Duan, J. Tan, Y. Zheng, P. Zhang, G. Fan, J. Wong, G.L. Xu, Z. Wang, H. Wang, S. Gao, B. Zhu, Stella safeguards the oocyte methylome by preventing de novo methylation mediated by DNMT1, Nature 564 (2018) 136–140.
[25] Y.F. Li, Y.H. Hsiao, Y.H. Lai, Y.C. Chen, Y.J. Chen, J.L. Chou, M.W. Chan, Y.H. Lin, Y.A. Tsou, M.H. Tsai, C.K. Tai, DNA methylation profiles and biomarkers of oral squamous cell carcinoma, Epigenetics 10 (2015) 229–236.
[26] Z. Liang, Y. Zhan, Y. Shen, C.C. Wong, J.R. Yates 3rd, F. Plattner, K.O. Lai, N.Y. Ip, The pseudokinase CaMKv is required for the activity-dependent maintenance of dendritic spines, Nat. Commun. 7 (2016) 13282.
[27] D. Liu, G. Li, Y. Zuo, Function determinants of TET proteins: the arrangements of sequence motifs with specific codes, Brief. Bioinform. (2018), https://doi.org/10.1093/bib/bby053.
[28] D.-R. Liu, Q.-L. Guan, M.-T. Gao, L. Jiang, H.-X. Kang, Mannose receptor as a potential biomarker for gastric cancer: a pilot study, Int. J. Biol. Markers 32 (2017) 278–283.
[29] R. Liu, W. Tang, X. Han, R. Geng, C. Wang, Z. Zhang, Hepatocyte growth factor-induced mesenchymal-epithelial transition factor activation leads to insulin-like growth factor 1 receptor inhibitor unresponsiveness in gastric cancer cells, Oncol. Lett. 16 (2018) 5983–5991.
[30] C. Long, W. Li, P. Liang, S. Liu, Y. Zuo, Transcriptome comparisons of multi-species identify differential genome activation of mammals embryogenesis, IEEE Access 7 (2019) 7794–7802.
[31] C. Luo, F. Sun, H. Zhu, Y. Ni, J. Fang, Y. Liu, S. Shao, H. Shen, J. Hu, Insulin-like growth factor binding protein-1 (IGFBP-1) upregulated by Helicobacter pylori and is associated with gastric cancer cells migration, Pathol. Res. Pract. 213 (2017) 1029–1036.
[32] T. Marumo, S. Yagi, W. Kawarazaki, M. Nishimoto, N. Ayuzawa, A. Watanabe, K. Ueda, J. Hirahashi, K. Hishikawa, H. Sakurai, K. Shiota, T. Fujita, Diabetes induces aberrant DNA methylation in the proximal tubules of the kidney, J. Am. Soc. Nephrol. 26 (2015) 2388–2397.
[33] A.A. Mello, M.F. Leal, J.A. Rey, G.R. Pinto, L.M. Lamarao, R.C. Montenegro, A.P. Alves, P.P. Assumpcao, N. Borges Bdo, M.C. Smith, R.R. Burbano, Deregulated expression of SRC, LYN and CKB kinases by DNA methylation and its potential role in gastric cancer invasiveness and metastasis, PLoS One 10 (2015) e0140492.
[34] B.H. Min, J. Hwang, N.K. Kim, G. Park, S.Y. Kang, S. Ahn, S. Ahn, S.Y. Ha, Y.K. Lee, R. Kushima, M. Van Vrancken, M.J. Kim, C. Park, H.Y. Park, J. Chae, S.S. Jang, S.J. Kim, Y.H. Kim, J.I. Kim, K.M. Kim, Dysregulated Wnt signalling and recurrent mutations of the tumour suppressor RNF43 in early gastric carcinogenesis, J. Pathol. 240 (2016) 304–314.
[35] T. Nakao, T. Iwata, M. Hotchi, K. Yoshikawa, J. Higashijima, M. Nishi, C. Takasu, S. Eto, H. Teraoku, M. Shimada, Prediction of response to preoperative chemoradiotherapy and establishment of individualized therapy in advanced rectal cancer, Oncol. Rep. 34 (2015) 1961–1967.
[36] M. Plummer, S. Franceschi, J. Vignat, D. Forman, C. de Martel, Global burden of gastric cancer attributable to Helicobacter pylori, Int. J. Cancer 136 (2015) 487–490.
[37] G. Posselt, J.E. Crabtree, S. Wessler, Proteolysis in Helicobacter pylori-induced gastric cancer, Toxins 9 (2017) 134.
[38] T.L. Putoczki, S. Thiem, A. Loving, R.A. Busuttil, N.J. Wilson, P.K. Ziegler, P.M. Nguyen, A. Preaudet, R. Farid, K.M. Edwards, Y. Boglev, R.B. Luwor, A. Jarnicki, D. Horst, A. Boussioutas, J.K. Heath, O.M. Sieber, I. Pleines, B.T. Kile, A. Nash, F.R. Greten, B.S. McKenzie, M. Ernst, terleukin-11 is the dominant IL-6 family cytokine during gastrointestinal tumorigenesis and can be targeted therapeutically, Cancer Cell 24 (2013) 257–271.
[39] S. Qin, J.N. Ingle, M. Liu, J. Yu, D.L. Wickerham, M. Kubo, R.M. Weinshilboum, L. Wang, Calmodulin-like protein 3 is an estrogen receptor alpha coregulator for gene expression and drug response in a SNP, estrogen, and SERM-dependent fashion, Breast Cancer Res. 19 (2017) 95.
[40] J. Roessler, O. Ammerpohl, J. Gutwein, B. Hasemeier, S.L. Anwar, H. Kreipe, U. Lehmann, Quantitative cross-validation and content analysis of the 450k DNA methylation array from Illumina, Inc. BMC Res. Notes 5 (2012) 210.
[41] M.E. Salem, A. Puccini, J. Xiu, D. Raghavan, H.J. Lenz, W.M. Korn, A.F. Shields, P.A. Philip, J.L. Marshall, R.M. Goldberg, Comparative molecular analyses of esophageal squamous cell carcinoma, esophageal adenocarcinoma, and gastric adenocarcinoma, Oncologist 23 (2018) 1319–1327.
[42] J. Sandoval, H. Heyn, S. Moran, J. Serra-Musach, M.A. Pujana, M. Bibikova, M. Esteller, Validation of a DNA methylation microarray for 450,000 CpG sites in the human genome, Epigenetics 6 (2011) 692–702.
[43] K. Shah, S. Rossie, Tale of the good and the bad Cdk5: remodeling of the actin cytoskeleton in the brain, Mol. Neurobiol. 55 (2017) 3426–3438.
[44] Y.W. Sun, K.M. Chen, Y. Imamura Kawasawa, A.C. Salzberg, T.K. Cooper, C. Caruso, C. Aliaga, J. Zhu, K. Gowda, S. Amin, K. El-Bayoumy, Hypomethylated Fgf3 is a potential biomarker for early detection of oral cancer in mice treated with the tobacco carcinogen dibenzo[def,p]chrysene, PLoS One 12 (2017) e0186873.
[45] S. Wang, D. Zhao, R. Tian, H. Shi, X. Chen, W. Liu, L. Wei, FGF19 contributes to tumor progression in gastric cancer by promoting migration and invasion, Oncol. Res. 23 (2016) 197–203.
[46] Z. Wang, M.A. Jensen, J.C. Zenklusen, A practical guide to the cancer genome atlas (TCGA), Methods Mol. Biol. (Clifton, N.J.) 1418 (2016) 111–141.
[47] M.J. Wiseman, Nutrition and cancer: prevention and survival, Br. J. Nutr. (2018) 1–7.
[48] J. Xiong, Z. Zhang, J. Chen, H. Huang, Y. Xu, X. Ding, Y. Zheng, R. Nishinakamura, G.L. Xu, H. Wang, S. Chen, S. Gao, B. Zhu, Cooperative action between SALL4A and TET proteins in stepwise oxidation of 5-methylcytosine, Mol. Cell 64 (2016) 913–925.
[49] B. Yang, M. Li, W. Tang, W. Liu, S. Zhang, L. Chen, J. Xia, Dynamic network biomarker indicates pulmonary metastasis at the tipping point of hepatocellular carcinoma, Nat. Commun. 9 (2018) 678.
[50] F. Yang, X. Xue, L. Zheng, J. Bi, Y. Zhou, K. Zhi, Y. Gu, G. Fang, Long non-coding RNA GHET1 promotes gastric carcinoma cell proliferation by increasing c-Myc mRNA stability, FEBS J. 281 (2014) 802–813.
[51] T. Yang, J. Gu, T. Liu, H. Ma, X. Ma, J. Tao, Y. Jin, X. Liang, Expression of acetaldehyde dehydrogenase in gefitinib-resistant human lung adenocarcinoma HCC-827/GR cells, Zhongguo fei ai za zhi = Chin. J. Lung Cancer 21 (2018) 431–436.
[52] C. Ye, R. Tao, Q. Cao, D. Zhu, Y. Wang, J. Wang, J. Lu, E. Chen, L. Li, Whole-genome DNA methylation and hydroxymethylation profiling for HBV-related hepatocellular carcinoma, Int. J. Oncol. 49 (2016) 589–602.
[53] J. Yin, W. Tang, T. Long, H. Pan, J. Liu, L. Lv, C. Liu, Y. Shi, J. Zhu, Y. Sun, A. Shao, Q. Zhou, Z. Ren, G. Ding, S. Chen, Y. Liu, J. Yao, H. Ding, Y. Yan, H. Gu, C. Qian, L. Wang, Q. Wang, L. Tan, Association of ALDH3B2 gene polymorphism and risk factors with susceptibility of esophageal squamous cell carcinoma in a Chinese population: a case-control study involving 2,358 subjects, Oncotarget 8 (2017) 110153–110165.
[54] L. Yu, J. Chen, Y. Liu, Z. Zhang, S. Duan, MicroRNA-937 inhibits cell proliferation and metastasis in gastric cancer cells by downregulating FOXL2, Cancer Biomark. 21 (2017) 105–116.
[55] L.W. Yuan, H. Yamashita, Y. Seto, Glucose metabolism in gastric cancer: the cutting-edge, World J. Gastroenterol. 22 (2016) 2046–2059.
[56] C. Zhan, L. Yan, L. Wang, Y. Sun, X. Wang, Z. Lin, Y. Zhang, Y. Shi, W. Jiang, Q. Wang, Identification of immunohistochemical markers for distinguishing lung adenocarcinoma from squamous cell carcinoma, J. Thorac. Dis. 7 (2015) 1398–1405.
[57] X.Y. Zhang, P.Y. Zhang, Gastric cancer: somatic genetics as a guide to therapy, J. Med. Genet. 54 (2017) 305–312.
[58] Y. Zhao, H. Zhou, K. Ma, J. Sun, X. Feng, J. Geng, J. Gu, W. Wang, H. Zhang, Y. He, S. Guo, X. Zhou, J. Yu, Q. Lin, Abnormal methylation of seven genes and their associations with clinical characteristics in early stage non-small cell lung cancer, Oncol. Lett. 5 (2013) 1211–1218.
[59] Y.Y. Zhou, Y.T. Kang, C. Chen, F.F. Xu, H.N. Wang, R. Jin, Combination of TNM staging and pathway based risk score models in patients with gastric cancer, J. Cell. Biochem. 119 (2018) 3608–3617.
[60] L. Zong, N. Hattori, Y. Yoda, S. Yamashita, H. Takeshima, T. Takahashi, M. Maeda, H. Katai, S. Nanjo, T. Ando, Y. Seto, T. Ushijima, Establishment of a DNA methylation marker to evaluate cancer cell fraction in gastric cancer, Gastric Cancer 19 (2016) 361–369.
[61] H. Zouridis, N. Deng, T. Ivanova, Y. Zhu, B. Wong, D. Huang, Y.H. Wu, Y. Wu, I.B. Tan, N. Liem, V. Gopalakrishnan, Q. Luo, J. Wu, M. Lee, W.P. Yong, L.K. Goh, B.T. Teh, S. Rozen, P. Tan, Methylation subtypes and large-scale epigenetic alterations in gastric cancer, Sci. Transl. Med. 4 (2012) 156ra140.