Identification of IncRNA FAM99A gene as a prognostic biomarker of hepatocellular carcinoma

CURRENT STATUS: UNDER REVIEW

BMC Medical Genetics ▪ BMC Series

Manyi Sun
Tianjin Union Medical Center & Tianjin People's Hospital

✉ qwe_11344@163.comCorresponding Author
ORCID: https://orcid.org/0000-0002-9669-1832

Shuhua Lv
Tianjin Union Medical Center

Jin Zhong
Tianjin Union Medical Center

DOI:
10.21203/rs.3.rs-16068/v1

SUBJECT AREAS
Medical Genetics Cancer Biology

KEYWORDS
FAM99A, IncRNA, methylation, hepatic cancer
Abstract

Background The complicated pathogenesis of hepatic cancer involves multiple clinical prognosis-associated oncogenes.

Methods We utilized the bioinformatics approach to analyze the data from hepatic cancer cases collected by TCGA repository.

Results We first found that the FAM99A (Family With Sequence Similarity 99 Member A) gene, a long non-coding RNA (lncRNA), is lowly expressed in hepatocellular carcinoma and closely related to clinical prognosis. We further analyzed the underlying molecular mechanism from the perspectives of copy number variation (CNV), DNA methylation, immune cell infiltration, and related cellular pathway. Even though we did not observe a strong correlation between the FAM99A expression and the CNV or immune cell infiltration, the high methylation levels of the five methylated probe sites (cg24218935, cg01745044, cg04353359, cg04938738, cg25356611) were found to be negatively correlated with low expression level of FAM99A. Besides, we performed the enrichment analysis to screen out a group of FAM99A-correlated genes and molecular pathways, such as complement cascade, RNA metabolism, drug metabolic process, PPAR signaling pathway, or cell cycle.

Conclusions The liver-specific FAM99A gene was first identified as a prognosis marker of hepatocellular carcinoma, and the underlying molecular mechanism involves DNA methylation and a series of cellular pathways.

Background

Emerging evidence supports the correlation between long non-coding RNAs (lncRNAs) and the pathogenesis of clinical hepatocellular carcinoma (HCC), the most notable lethal malignancy [1–4]. However, the reported data remains limited. As a public funded project,
TCGA (The Cancer Genome Atlas) archives the multiple-genomics data from more than thirteen types of cancer, including expression level, mutation, copy number variation (CNV), genome methylation of lncRNA genes, and clinical information, etc. [5, 6]. It helps to identify the prognosis-associated lncRNA oncogenes. Herein, we aimed to first analyze the potential role of the lncRNA FAM99A gene in the pathogenesis and prognosis of hepatic cancer.

FAM99A (Family With Sequence Similarity 99 Member A) gene, namely Entrez Gene: 387742, is affiliated with the lncRNA class (https://www.genecards.org/cgi-bin/carddisp.pl?gene=FAM99A&keywords=FAM99A) and a fetal imprinted gene [7]. After database retrieval, there are only three reports regarding the association between lncRNA FAM99A and pregnancy [7–9]. However, there are still no reports investigating the role of lncRNA FAM99A and other clinical disorders, especially cancers.

In this study, we first identified that the lncRNA FAM99A is primarily expressed in liver cancer, based on the data of TCGA. Also, we explored the possible molecular mechanisms of lncRNA FAM99A in hepatic carcinogenesis from the perspectives of gene expression, copy number variation (CNV), DNA methylation, immune cell infiltration, and enrichment analysis of FAM99A-correlated genes.

Materials And Methods

Expression analysis

We analyzed the expression profile of FAM99A gene in the different cancer tissues and corresponding control tissues in the TCGA project by the GEPIA 2 (http://gepia2.cancer-pku.cn/#analysis) [10]. The boxplot data and the expression levels of FAM99A gene by pathological stage in the TCGA-LIHC (Liver hepatocellular carcinoma) and TCGA-CHOL (Cholangio carcinoma) cohorts were provided, respectively.

Survival curve analysis
We utilized the Kaplan-Meier plotter (http://kmplot.com/analysis/index.php?p=service&cancer=liver_rnaseq) to perform the overall survival (OS), relapse free survival (RFS), progress free survival (PFS), disease specific survival (DSS) analyses by the expression level of the *FAM99A* gene in the hepatic cancer cases [11]. Auto select best cutoff was set. The clinical factors, including the pathologic stages, grade, AJCC_T, gender, vascular invasion, race, sorafenib treatment, alcohol consumption, hepatitis virus, were also considered.

**Copy number variation analysis**

Based on the GSCALite (http://bioinfo.life.hust.edu.cn/web/GSCALite/) [12], we performed the copy number variation (CNV) analysis of lncRNA *FAM99A* in the hepatic cancer cases of TCGA-LIHC cohort. The CNV pie distribution, CNV profile (homozygous amplification, homozygous deletion, heterozygous amplification, heterozygous deletion), and the Pearson correlation between CNV and expression level were provided, respectively.

**DNA methylation analysis**

We analyzed the DNA methylation status of lncRNA *FAM99A* in the hepatic cancer cases of the TCGA-LIHC cohort through the MEXPRESS [13, 14]. The correlation between DNA methylation and expression level of *FAM99A* gene was analyzed by Pearson’s test. The correlation coefficients (r) and Benjamini-Hochberg-adjusted P values targeting the different methylation probes, including cg24218935, cg01745044, cg04353359, cg04938738, cg25356611, were provided, respectively.

**Immune cell infiltration analysis**

We utilized the GEPIA 2 approach to conduct the pair-wise gene correlation analysis between lncRNA *FAM99A* expression and the signatures of the following immune cells: central memory T cell; Effector memory T cell; Effector T cell; Effector Treg T cell; Exhausted T cell; Native T cell; Th1 like cell; Resting Treg T cell.
Enrichment analysis of FAM99A-correlated genes

We performed the cluster analysis of the lncRNA FAM99A-correlated significant genes, through LinkedOmics (https://www.biostars.org/p/287820/) [15]. The heat map targeting the FAM99A positively or negatively correlated significant genes, and GSEA (Gene Set Enrichment Analysis) profiles for the enrichment category of reactome pathway were provided, respectively. In addition, we performed the GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analysis. Weighted set cover was utilized for the redundancy reduction. The data was visualized by the bar chart, DAG (Directed Acyclic Graph) or volcano plot.

Result

Expression analysis data

First, we analyzed the expression profile of lncRNA FAM99A gene in the different cancers, enrolled in the TCGA project. As shown in Fig.1A, FAM99A was specifically expressed in the tissue samples of TCGA-LICH or TCGA-CHOL cohorts. Then, we compared the expression difference between the tumor tissues and corresponding controls in the TCGA-LICH/CHOL cohorts. The data indicated the reduced expression level of FAM99A gene in the hepatocellular carcinoma (Fig.1B, \( P<0.05 \)) or cholangio carcinoma (Fig.1C, \( P<0.05 \)) tissues, compared with the control tissues. Moreover, we observed the correlation between the FAM99A expression and the pathological stages of liver hepatocellular carcinoma cases (Fig.1D), but not cholangio carcinoma cases (Fig.1E). Therefore, these suggested the potential role of lncRNA FAM99A gene in the etiology of hepatic cancer or cholangio carcinoma.

Survival curve analysis data

Next, we tried to analyze the association between FAM99A expression status and clinical prognosis for hepatocellular carcinoma and cholangio carcinoma. Due to the lack of
survival data for the cholangio carcinoma cases, we only focused on the hepatocellular carcinoma. We observed the lower rates of overall survival (Fig.2A, HR=0.56, \( P=0.0014 \)), relapse free survival (Fig.2B, HR=0.63, \( P=0.011 \)), progress free survival (Fig.2C, HR=0.62, \( P=0.0035 \)), disease specific survival (Fig.2D, HR=0.56, \( P=0.015 \)), in the FAM99A high expression group, compared with the high expression group. In addition, we fully considered the effect of different clinical factors, such as the pathologic stages, grade, vascular invasion, or sorafenib treatment, in the above correlation. We performed survival curve analysis after grouping the samples by different clinical factors. As shown in Table 1 and Table S1-S3, we observed the relationship between FAM99A low expression and the worse survival in the subgroups of “pathologic stage 3”, “grade 3”, “AJCC_T3”, and “male” (all HR<1, \( P<0.05 \)), but not female subgroup (all \( P>0.05 \)). These results provide evidence regarding the association between FAM99A low expression and poor clinical outcomes of hepatocellular carcinoma, which warrants a more in-depth molecular mechanism investigation.

**CNV analysis data**

Herein, we analyzed the CNV status of the IncRNA FAM99A gene. IncRNA FAM99B was also examined. As shown in Fig.3A-B, we did not observe the copy number variations in the majority of hepatic cancer cases, and the heterozygous amplification/heterozygous deletion in the limited cancer cases. Furthermore, we did not detect a strong correlation between CNV and expression of the IncRNA FAM99A gene (Fig.3C). Thus, copy number variations of the FAM99A gene may not play an essential role in hepatic tumorigenesis.

**DNA methylation analysis data**

We attempted to exploit the potential molecular mechanism from the point of FAM99A DNA methylation. Based on the methylation data of TCGA-LIHC, we found that FAM99A gene expression were negatively correlated with the methylation signal values of five
methylation probe sites, including cg24218935 (Fig.4, r=-0.397, P<0.001), cg01745044 (r=-0.359, P<0.001), cg04353359 (r=-0.564, P<0.001), cg04938738 (r=-0.421, P<0.001), cg25356611 (r=-0.395, P<0.001). This suggested the potential role of FAM99A DNA methylation in the hepatic tumorigenesis.

**Immune cell infiltration analysis data**

Also, we aimed to investigate whether the FAM99A gene is involved in the etiology of hepatic cancer through immune cell infiltration by GEPIA2 tool. As shown in Fig.5, the expression of FAM99A gene was slightly correlated with the infiltration level of native T cells (P=3.8e-05, r=-0.20), Th1 like cells (P=0.0074, r=-0.13), native T cells (P=0.0038, r=-0.14), but not others.

**Enrichment analysis data**

Finally, we utilized the LinkedOmics approach to screen out a group of FAM99A expression-correlated negatively genes (e.g., SLC2A1, BZW2, TSN, KIAA0114, and CCT4, etc.) and positively related genes (e.g., FAM99B, SLC22A7, HSD17B13, C14orf68, HAO2, etc.) in Fig.6A. We then performed the GSEA for the category of reactome pathway. As shown in Fig.6B, positively related pathways (e.g., complement cascade, fatty acid metabolism, etc.) and negatively related pathways (e.g., metabolism of RNA, M phase, etc.) were obtained. Enrichment plots of complement cascade and metabolism of RNA were shown in Fig.6C as examples.

Furthermore, GO analysis data (Fig.7) presented a series of FAM99A-correlated issues of biological process (e.g., protein activation cascade, drug metabolic process, etc.), cellular component (e.g., extracellular organelle, mitochondrion, etc.), and molecular function (e.g., oxidoreductase activity, RNA binding, etc.). KEGG analysis (Fig.8) further showed the enriched pathways, such as metabolic pathways, PPAR signaling pathway, cell cycle.

**Discussion**
Based on the available data sets of hepatic cancer cases collected by TCGA, for the first time,

We discovered that IncRNA FAM99A is mainly expressed in liver-related tumors, namely hepatocellular carcinoma and cholangio carcinoma. When compared with the adjacent controls, IncRNA FAM99A is lowly expressed in the hepatocellular carcinoma or cholangio carcinoma, suggesting that FAM99A may be a liver-specific tumor suppressor gene. Nevertheless, there are only a total of 36 cholangio carcinoma tissues and 5 adjacent control tissues in the TCGA-CHOL project. Also, we did not obtain a positive result in clinical prognostic analysis. Therefore, in this study, we only focus on the correlation between IncRNA FAM99A and hepatocellular carcinoma. Despite this, we do not rule out the potential regulatory role of IncRNA FAM99A in the initiation and progression of cholangio carcinoma, considering the link between the Noncoding RNAs (ncRNAs) and cholangio carcinoma [16]. More sample sizes, clinical and basic experimental data are needed for an in-depth investigation.

With regards to the hepatocellular carcinoma, we reported a statistical correlation between low expression of FAM99A gene and poorer prognosis status of overall survival, relapse free survival, progress free survival, and disease specific survival. There existed the statistical expression reference of FAM99A among different pathological stages (stage I-IV) as well. When hepatic cancer samples were grouped according to the clinical information, the positive association between lowly-expressed FAM99A and poor survival outcomes exists in the subgroups of “pathologic stage 3”, “grade 3”, “AJCC_T3”. Besides, it is important to note that we observed the correlation between FAM99A gene expression and the clinical prognosis of male hepatic cases, but not female cases. These suggest that the prognostic warning ability of lowly expressed FAM99A gene may increase with the tumor differentiation process or pathological state in the male patients with hepatic
LncRNA FAM99A rs1489945 was reported to be linked to the maternal mean arterial blood pressures in a Cambridge birth cohort [7]. Therefore, we also explored the mutation and CNV status FAM99A gene in cancers. Our findings showed a very low genetic mutation frequency of FAM99A in cancers, which is not statistically significant correlated with gene expression or clinical prognosis (data no shown). We also did not observe a high frequency of CNV, and a strong correlation between the FAM99A expression and the CNV. In addition, considering the links between cellular immune responses and hepatocellular carcinoma [17], we also analyzed the correlation between the LncRNA FAM99A expression and the signatures of the following immune cells: central memory T cell; Effector memory T cell; Effector T cell; Effector Treg T cell; Exhausted T cell; Native T cell; Th1 like cell; Resting Treg T cell. However, we still did not observe a strong correlation.

DNA methylation status of RNA was closely related to the gene expression and the carcinogenesis of hepatic cancer [18, 19]. Eukaryotic LncRNA also take part in the metastasis and prognosis of hepatocellular carcinoma, through regulating the chromatin remodeling and methylation [20, 21]. The high methylation levels of the five methylated probe sites (cg24218935, cg01745044, cg04353359, cg04938738, cg25356611) were found to be negatively correlated with low expression levels of FAM99A. And we found that the cg24218935 and cg04353359 sites are located in the promoter region, while cg01745044, cg04938738, and cg25356611 are in the non-promoter region. It is worthwhile to further explore the synergy role of different methylation sites of FAM99A in the expression level and survival prognosis of hepatic cancer cases.

As a downregulated gene in preeclampsia, FAM99A takes part in the regulation of invasion, migration and apoptosis of trophoblast cells [8]. We analyzed a series of genes related to FAM99A expression. Among them, we observed a high degree of expression
consistency between FAM99A and FAM99B (Family With Sequence Similarity 99 Member B). Regarding FAM99B, only one article was reported by searching that FAM99B is also a liver-specific IncRNA, which can inhibit cell proliferation, migration, and invasion of cells [22]. Such cellular function attribute may also be involved in the role of FAM99A in hepatic tumorigenesis and progression. In addition, we performed a series of enrichment analyses based on FAM99A expression-related genes. FAM99A gene is related to numerous biological events such as completion cascade, fatty acid metabolism, metabolism of RNA, drug metabolic process, oxidoreductase activity, and RNA binding, which provides possible research directions for in-depth molecular research. The molecular mechanism regarding the role of DNA methylation or ceRNA (competing endogenous RNAs) networks of FAM99A in the above biological activities merits further experiments.

Conclusion
Based on the liver cancer cases within TCGA-LIHC cohorts, we first identified the lowly expressed liver-specific IncRNA FAM99A as a prognostic gene for the hepatocellular carcinoma. High DNA methylation of FAM99A is associated with low expression of the FAM99A gene. In addition, a series of cellular pathway may contribute to the role of FAM99A in the hepatic tumorigenesis. These merit further in-depth cell molecular experiments.

Abbreviations
FCGR2A: Fc fragment of IgG receptor IIa; AIDS: immune deficiency syndrome; SLE: systemic lupus erythematosus; IPF: idiopathic pulmonary fibrosis; NSIP: nonspecific interstitial pneumonia; CAP: community-acquired pneumonia; IIP: idiopathic interstitial pneumonia; FIP: familial interstitial pneumonia; SNP: single nucleotide polymorphism; PRISMA: preferred reporting items for systematic reviews and meta-analyses; WOS: Web of
Science; NOS: Newcastle-Ottawa quality assessment Scale; CI: confidence interval; GWAS: genome-wide association studies.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent to publish
Not applicable.

Availability of data and materials
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Funding
Not applicable.

Authors’ contributions
Manyi Sun conceived and designed the study. Manyi Sun and Shuhua Lv performed the expression, survival curve analysis, CNV analysis. Manyi Sun and Jin Zhong performed the DNA methylation, immune cell infiltration, and enrichment analysis. Manyi Sun drafted the manuscript. All authors reviewed the manuscript before submission. All the authors approved the final version of the manuscript.

Acknowledgments
Not applicable.

Supplementary Information

Supplementary Table S1: Correlation of FAM99A expression and RFS of hepatic cancer cases. Supplementary Table S2: Correlation of FAM99A expression and PFS of hepatic
cancer cases. Supplementary Table S3: Correlation of FAM99A expression and DSS of hepatic cancer cases.

References

1. Mai H, Zhou B, Liu L, Yang F, Conran C, Ji Y, Hou J, Jiang D. Molecular pattern of IncRNAs in hepatocellular carcinoma. J Exp Clin Cancer Res. 2019;38(1):198.

2. Chi Y, Wang D, Wang J, Yu W, Yang J. Long Non-Coding RNA in the Pathogenesis of Cancers. Cells. 2019;8(9).

3. Shi H, Xu Y, Yi X, Fang D, Hou X. Current Research Progress on Long Noncoding RNAs Associated with Hepatocellular Carcinoma. Anal Cell Pathol (Amst). 2019;2019:1534607.

4. Liu J, Li W, Zhang J, Ma Z, Wu X, Tang L. Identification of key genes and long non-coding RNA associated ceRNA networks in hepatocellular carcinoma. PeerJ. 2019;7:e8021.

5. Wang Z, Jensen MA, Zenklusen JC. A Practical Guide to The Cancer Genome Atlas (TCGA). Methods Mol Biol. 2016;1418:111-41.

6. Tomczak K, Czerwinska P, Wiznerowicz M. The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge. Contemp Oncol (Pozn). 2015;19(1a):A68-77.

7. Petry CJ, Koulman A, Lu L, Jenkins B, Furse S, Prentice P, Matthews L, Hughes IA, Acerini CL, Ong KK, et al. Associations between the maternal circulating lipid profile in pregnancy and fetal imprinted gene alleles: a cohort study. Reprod Biol Endocrinol. 2018;16(1):82.

8. He T, Qiao Y, Lv Y, Wang J, Hu R, Cao Y. IncRNA FAM99A is downregulated in preeclampsia and exerts a regulatory effect on trophoblast cell invasion, migration and apoptosis. Mol Med Rep. 2019;20(2):1451-8.

9. Petry CJ, Sanz Marcos N, Pimentel G, Hayes MG, Nodzenski M, Scholtens DM, Hughes
IA, Acerini CL, Ong KK, Lowe WL, Jr., et al. Associations Between Fetal Imprinted Genes and Maternal Blood Pressure in Pregnancy. Hypertension. 2016;68(6):1459-66.

10. Tang Z, Kang B, Li C, Chen T, Zhang Z. GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. Nucleic Acids Res. 2019;47(W1):W556-w60.

11. Menyhart O, Nagy A, Gyorffy B. Determining consistent prognostic biomarkers of overall survival and vascular invasion in hepatocellular carcinoma. R Soc Open Sci. 2018;5(12):181006.

12. Liu CJ, Hu FF, Xia MX, Han L, Zhang Q, Guo AY. GSCALite: a web server for gene set cancer analysis. Bioinformatics. 2018;34(21):3771-2.

13. Koch A, De Meyer T, Jeschke J, Van Criekinge W. MEXPRESS: visualizing expression, DNA methylation and clinical TCGA data. BMC Genomics. 2015;16:636.

14. Koch A, Jeschke J, Van Criekinge W, van Engeland M, De Meyer T. MEXPRESS update 2019. Nucleic Acids Res. 2019;47(W1):W561-w5.

15. Modhukur V, Iljasenko T, Metsalu T, Lokk K, Laisk-Podar T, Vilo J. MethSurv: a web tool to perform multivariable survival analysis using DNA methylation data. Epigenomics. 2018;10(3):277-88.

16. Salati M, Braconi C. Noncoding RNA in Cholangiocarcinoma. Semin Liver Dis. 2019;39(1):13-25.

17. Schmidt N, Neumann-Haefelin C, Thimme R. Cellular immune responses to hepatocellular carcinoma: lessons for immunotherapy. Dig Dis. 2012;30(5):483-91.

18. Raggi C, Invernizzi P. Methylation and liver cancer. Clin Res Hepatol Gastroenterol. 2013;37(6):564-71.

19. Yuan SX, Zhang J, Xu QG, Yang Y, Zhou WP. Long noncoding RNA, the methylation of genomic elements and their emerging crosstalk in hepatocellular carcinoma. Cancer
Lett. 2016;379(2):239-44.

20. Abastabar M, Sarfi M, Golestani A, Khalili E. IncRNA involvement in hepatocellular carcinoma metastasis and prognosis. Excli j. 2018;17:900-13.

21. Lim LJ, Wong SYS, Huang F, Lim S, Chong SS, Ooi LL, Kon OL, Lee CG. Roles and Regulation of Long Noncoding RNAs in Hepatocellular Carcinoma. Cancer Res. 2019;79(20):5131-9.

22. Mo M, Liu S, Ma X, Tan C, Wei L, Sheng Y, Song Y, Zeng X, Huang D, Qiu X. A liver-specific lncRNA, FAM99B, suppresses hepatocellular carcinoma progression through inhibition of cell proliferation, migration, and invasion. J Cancer Res Clin Oncol. 2019;145(8):2027-38.

Table

Table 1: Correlation of FAM99A expression and overall survival of hepatic cancer cases

| Factor          | Group | Sample size | HR  | 95% CI   | logRank_P |
|-----------------|-------|-------------|-----|----------|-----------|
| Stage           | Stage 1 | 171     | 0.5 | 0.27~0.95 | 0.03      |
|                 | Stage 2 | 86      | 1.97| 0.88~4.45 | 0.095     |
|                 | Stage 3 | 85      | 0.44| 0.23~0.84 | 0.011     |
| Grade           | Grade 1 | 55      | 0.28| 0.11~0.73 | 0.0054    |
|                 | Grade 2 | 177     | 0.7 | 0.39~1.24 | 0.22      |
|                 | Grade 3 | 122     | 0.43| 0.23~0.79 | 0.0047    |
| AJCC_T          | T1     | 181     | 0.5 | 0.28~0.90 | 0.019     |
|                 | T2     | 94      | 1.83| 0.86~3.88 | 0.11      |
|                 | T3     | 80      | 0.43| 0.22~0.83 | 0.0095    |
| Gender          | Female | 121     | 1.39| 0.79~2.44 | 0.25      |
|                 | Male   | 250     | 0.41| 0.26~0.54 | 6.4e-05   |
| Vascular invasion | None | 205     | 0.48| 0.29~0.81 | 0.0045    |
|                 | micro  | 93      | 0.72| 0.29~1.79 | 0.47      |
|                 | White  | 184     | 0.57| 0.36~0.9  | 0.014     |
|                 | Asian  | 158     | 0.39| 0.21~0.72 | 0.0017    |
| Sorafenib treatment | treated | 30  | 0.46| 0.14~1.51 | 0.19      |
| Alcohol consumption | Yes  | 117     | 0.49| 0.25~0.95 | 0.032     |
|                 | none   | 205     | 0.49| 0.31~0.79 | 0.0024    |
| Hepatitis virus | Yes    | 153     | 0.46| 0.23~0.89 | 0.018     |
|                 | none   | 169     | 0.57| 0.36~0.91 | 0.017     |

HR, hazard ratio; CI, confidence interval; AJCCAmerican Joint Committee on Cancer.

Figures

14
Figure 1

Expression analysis of IncRNA FAM99A. (A) The expression profile of FAM99A gene in the different cancer tissues and corresponding control tissues in the TCGA project was analyzed by the GEPIA2 tool. The boxplot data in the TCGA-LIHC (B) or TCGA-CHOL (C) cohort were provided, respectively. *P<0.05. In addition, the expression levels of FAM99A gene by pathological stage were also analyzed through GEPIA 2 (D-E).
Survival curve analysis of IncRNA FAM99A for the hepatic cancer cases. We performed the overall survival (OS) (A), relapse free survival (RFS) (B), progress free survival (PFS) (C), disease specific survival (DSS) (D) analyses, according to the expression level of FAM99A gene, using Kaplan-Meier Plotter. HR, hazard ratio.
Figure 3

CNV analysis of lncRNA FAM99A and FAM99B. The CNV pie distribution (A), CNV profile (B), and the correlation between CNV and expression level (C) of lncRNA FAM99A and FAM99B was analyzed, respectively. Hete Amp, heterozygous amplification; Homo Amp, homozygous amplification; Hete Del, heterozygous deletion; Homo Del, homozygous deletion.
Correlation analysis between DNA methylation status and expression level of FAM99A for the hepatic cancer. The detailed information on the methylation probe was provided. Pearson correlation coefficients (r) and Benjamini-Hochberg-adjusted P values for the comparison were shown as well.
Figure 5

Correlation between IncRNA FAM99A expression and infiltration level of immune cell. (A) central memory T cell; (B) Effector memory T cell; (C) Effector T cell; (D) Effector Treg T cell; (E) Exhausted T cell; (F) Native T cell; (G) Th1 like cell; (H) Resting Treg T cell.
The cluster analysis of the lncRNA FAM99A correlated significant genes. (A) The heat map targeting the FAM99A positively or negatively correlated significant genes. (B-C) GSEA data for the enrichment category of reactome pathway was provided. FDR, false discovery rate.
The GO analysis of the lncRNA FAM99A correlated significant genes. The DAG data for the biological process (A), cellular component (B), and molecular function (C) were provided, respectively.
The KEGG analysis of the IncRNA FAM99A correlated significant genes. Volcano plot was provided. FDR, false discovery rate.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

Table S1-S3.docx