Expression and prognosis of microliposome maintenance proteins family in liver cancer

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Abstract

Cancer is the leading cause of death worldwide, despite advances in the treatment of cancer, mortality of liver cancer remains high. The Microliposome maintenance (MCM) family has been reported to be involved in a variety of diseases. However, little is known about its expression and prognostic value in liver cancer. In this study, we analyzed the expression of the MCM family in liver cancer tissues and normal liver tissues. Oncomine and GEPIA were used to analyze the mRNA expression level of MCM family in liver cancer and other cancers. We observed that the mRNA expression levels of MCM1-8 and MCM10 are higher than normal tissues in most cancers. In addition, the expression levels of MCM1-10 were highly expressed in liver cancer cell lines, analyzed by Cancer Cell Line Encyclopedia (CCLE) and European Bioinformatics Institute (EMBL-EBI) databases. Furthermore, LinkedOmics and GEPIA databases were used to evaluate correlation between MCM1-10 and overall survival of patients. In the LinkedOmics-based survival analysis, high expressions of MCM2-8 and MCM10 were associated with worse patient prognosis (OS). The results showed that there was no correlation between the expression levels of MCM1 and MCM9 and prognosis of patients' prognosis. The GEPIA dataset was also used for overall survival analysis, and the results also support the results of LinkedOmics. In conclusion, the MCM family has the potential to become a biomarker, especially MCM2-8 and MCM10 are probably the most promising biomarkers for liver cancer prognosis.

Background

Over the past few decades, the incidence of primary liver cancer has gradually increased globally, with East Asia (China, Japan, and South Korea) as one of the regions with the highest incidence. Primary liver malignancies, hepatocellular carcinoma (HCC) is the main type, is the sixth most prevalent cancer worldwide and the third leading cause of cancer-related death[1]. The major causes of poor prognosis in HCC patients are the high rates of tumor metastasis, invasion and recurrence and untimely diagnosis. Treatment options for liver cancer are limited. The standard drugs for the treatment of advanced HCC are the multikinase inhibitors sorafenib and regorafenib, but they have shown limited efficacy, so they obviously cannot meet the medical needs of new drug targets for HCC. In recent years, despite the continuous improvement and development of diagnostic and treatment protocols, the prognosis is still poor, especially for patients with HCC. Therefore, it is necessary to actively explore effective tumor biomarkers to predict the prognosis of patients and improve the survival rate. Currently, databases based on large-scale, genome-wide association studies are helping to explore new biomarkers for cancer diagnosis and treatment. In recent years, many genes have been discovered, which are expected to become tumor biomarkers and have important significance for the diagnosis and treatment of tumors.

Microliposome maintenance (MCM) proteins are conserved in eukaryotes and are significant proteins for initiation and progression of DNA replication[2]. Ten protein family members make up the MCM protein family: MCM1 (also known as SRF), MCM2, MCM3, MCM4, MCM5, MCM6, MCM7, MCM8, MCM9 and MCM10[3,4]. MCM1 and MCM10 regulate gene expression by interacting with co-factors that bind to different DNA sequences[5]. The MCM1 protein indirectly affects replication by linking directly to the

replication initiation site and by regulating the expression of pre-RC complex factors (such as Cdc6 and MCM 2-7) [6]. In contrast, MCM 10 appears to be directly related to binding of DNA polymerase enzyme α /primase to chromatin and the stability of this binding [7]. MCM2–7 proteins adopt a hexameric ring-like structure large enough to accommodate single-stranded DNA or dsDNA and are considered the prime candidates for the eukaryotic replicative helicase [8]. Among these proteins, MCM2-7 forms a hexamer called the MCM complex, which has a partial homologous sequence. MCM complexes play an important role in the initiation of DNA replication. In G1 phase, the MCM2-7 replication helicase binds to double-stranded DNA (dsDNA) in the form of an inactive dimer. During the S phase, the active MCM2-7 double hexamer (MCM DH) was synthesized bidirectional DNA at the eukaryotic origin. MCM 8 is a protein found in human liver cells [9]. The MCM 8 gene has been implicated in the carcinogenesis of liver cancer. Although MCM 8 protein was found to be completely independent of MCM 2-7, it had similar properties to the MCM family [10]. MCM 8 does not participate in the composition of MCM 2-7 complex. Instead, by attaching the MCM 2-7 complex to chromatin, it can also attach to chromatin [11]. The MCM 9 gene and its encoded protein have been reported to be active in various types of mice and human liver tissues, and its role in DNA synthesis has not been explained. Numerous studies have confirmed that this protein family is closely related to a variety of cancers and can be regarded as a biomarker for diagnosis and prognosis [11,12]. However, a detailed analysis of the clinical significance of MCM1-10 expression levels in HCC requires further study.

At present, our research aims to explore the possibility of MCM1-10 protein as a biomarker in liver hepatocellular carcinoma (LIHC) by using whole-genome RNA sequencing (RNA-seq) data sets and gene and protein analysis tools.

Results

Transcriptional Levels of MCMs in Patients with Hepatocellular Carcinoma

The presence of ten factors of the MCM protein family in mammals has been confirmed. We used data from the ONCOMINE database to compare the expression levels of MCMs protein in normal liver tissue and liver cancer (Figure 1). Except for the expression level of MCM 9 protein, the expression level of other MCMs proteins in hepatocellular carcinoma tissues were higher than normal liver tissues (Table 1). It is worth noting that the expression levels of minichromosome maintenance (MCM) 2–7 were significantly increased in hepatocellular carcinoma tissues compared with normal liver tissues (Table 1). In addition, MCM protein family expression was elevated in multiple tumors, such as esophageal cancer, gastric cancer, colorectal cancer and breast cancer. Compared with normal tissues, in Roessler Liver's dataset [13], MCM1 protein expression level was increased in hepatocellular carcinoma tissues, and fold change could reach 1.086 (Table 1). In Roessler Liver 2 dataset [13], MCM1 is also overexpressed in hepatocellular carcinoma with a fold change of 1.053 (Table 1). In the data of Roessler's Liver 2 [13], MCM2-7 genes are all expressed highly in hepatocellular carcinoma than normal liver tissue, and the fold change

> 2[1]. The data of other researchers also showed the same trend, in the datasets of Wurmbach Liver[14], Mas Liver[15], Roessler Liver[13] and Chen Liver[16], the fold changes > 1[1]. The result of Wurmbach Liver[14] showed that the MCM8 was highly in hepatocellular carcinoma, and the fold change was 2.295[1]. The MCM8 is overexpression also found in hepatocellular carcinoma with a fold change of 1.523 in Chen Liver's dataset[16][1]. In the results about MCM9, P values of Chen liver[16] and Wurmbach's liver[14] were all greater than 0.05, so we could not judge the expression level of MCM9[1]. In Wurmbach Liver's dataset[14], MCM10 is overexpressed in hepatocellular carcinoma with a fold change of 2.116[1]. Although fold changes did not reach 2 in the datasets about MCM10 of other researchers, these fold change were still higher than 1[1].

Relationship between the mRNA Levels of MCMs and the Clinicopathological Parameters of Patients with Hepatocellular Carcinoma

Using the GEPIA (Gene Expression Profiling Interactive Analysis) dataset (<http://gepia.cancer-pku.cn/>), we compared the mRNA expression level of MCM protein family between hepatocellular carcinoma and normal tissue of liver. The results showed that the expression of MCM1 and MCM9 genes in hepatocellular carcinoma was not different from that in normal liver tissue samples. However, the mRNA expression levels of MCM2-8 and MCM10 genes in hepatocellular carcinoma were higher than those in normal liver tissues (Figs. 2A–2L).

MCM Translational Factors Expression in Cell Lines of Liver Cancer

By assembling the Cancer Cell Line Encyclopedia (CCLE), we have expanded the process of detailed annotation of preclinical human cancer models (<https://www.broadinstitute.org/ccle>).

We found that MCM1-10 were all highly expressed in cell lines of liver cancer (Fig. 3A). Moreover, the European Bioinformatics Institute (EMBL-EBI) bioinformatics website (<https://www.ebi.ac.uk/gxa/home>) was also used to test the expression of MCM translational factors in liver cancer cell lines, and results indicated that MCM1-10 were increased in most cell lines of liver cancer (Fig. 3B).

The Prognostic Values of MCMs in Liver Cancer

We used LinkedOmics and GEPIA databases to explore the prognostic analysis of MCM1-10 in liver cancer. The results showed changes in MCM1 and MCM9 levels were not associated with poor overall survival (OS) for HCC. However, in the LinkedOmics dataset, increased MCM2-8 and MCM10 were associated with poor OS in liver cancer (Fig. 4A). Notably, MCM1 and MCM9 expression levels, obtained from the GEPIA database, were also not correlated with OS of HCC patients (Fig. 4B).

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Co-expressed Genes of MCMs, and the Correction between MCMs in Liver Cancer

For intuitive observation, data from The Cancer Genome Atlas (TCGA) (<https://portal.gdc.cancer.gov/>) database were collected and the heat map was drawn based on the co-expression values of every two genes between MCM1-10 (Fig. 5A). Genes co-expressed with MCM1 were analyzed in Jia Liver [17], and we found MCM1 was positively corrected with TBC1D19, IPO11, CCDC123, CASP5, ATP2C2, ZNF438, CCL23, EPB41L3, ZHX1, GTPBP2, and TMEM59L (Fig. 5B). Genes co-expressed with MCM2 were analyzed in WOO Liver [18], and we found MCM2 was positively corrected with GINS1, DTL, ZWINT, CDK1, RACGAP1, KIAA0101, NUSAP1, BUB1B, KIF18A, HJURP, and UBE2C (Fig. 5B). Genes co-expressed with MCM3 were analyzed in Chiang Liver [19], and we found MCM3 was positively corrected with RACGAP1, KIF15, C12orf48, HMGB2, LMNB1, HELLS, CCNA2, CENPK, FBXO5, DEPDC1B, and CKS2 (Fig. 5B). Genes co-expressed with MCM4 were analyzed in Hoshida Liver [20], and we found MCM4 was positively corrected with TYMS, HIST1H2AD, ZWINT, E2F1, MCM6, NDC80, MELK, NUSAP1, KIAA0101, CCNB1, and TOP2A (Fig. 5B). Genes co-expressed with MCM5 were analyzed in Hoshida Liver [20], and we found MCM5 was positively corrected with KIF23, ORC1L, CDC45, CCNF, PLK4, CDC20, MKI67, BRCA1, CDK2, WDR67 and TRIP13 (Fig. 5B). Genes co-expressed with MCM6 were analyzed in Chen Liver [16], and we found MCM6 was positively corrected with IFIT1, DTL, MLF1IP, ASF1B, NUDT1, CDC45, E2F1, NUF2, NEK2, ARL4A, and TRIP13 (Fig. 5B). Genes co-expressed with MCM7 were analyzed in Chiang Liver [19], and we found MCM7 was positively corrected with RNASEH2A, BIRC5, NEK2, UBE2T, CENPH, TYM5, and C4orf46 (Fig. 5B). Genes co-expressed with MCM8 were analyzed in Wurmbach Liver [14], and we found MCM8 was positively corrected with FANCG, ORC6L, KNTC1, UHRF1, STIL, CENPN, ASPM, AURKB, and SHCBP1 (Fig. 5B). Genes co-expressed with MCM9 were analyzed in Jia Liver [17], and we found MCM9 was positively corrected with METTL9, SIAE, SLC36A1, SAP30L, MED21, ALG6, UTP14C, ASH2L, COMMD10, TCFL5, and ZNF207 (Fig. 5B). Genes co-expressed with MCM10 were analyzed in Wurmbach Liver [14], and we found MCM10 was positively corrected with C12orf48, CENPH, PLK1, SKA1, C5orf34, DEPDC1B, DEPDC1, KIF2C, GPSM2, MKI67, and KIF15 (Fig. 5B). We also analyzed the correlation between MCM1-10 through LinkedOmics database, and found that except for the following three pairs of genes, including: MCM1 and MCM6, MCM1 and MCM8, MCM1 and MCM9, the correlation between other MCM protein family members was positive (Supplement Fig. 1). In addition, we used GEPIA dataset to verify the above results; The co-expression relationships between each two genes of MCM1-10 were positive and the statistical results were significantly (Supplement Fig. 2). Therefore, in liver cancer, except for MCM1 and

MCM6, MCM1 and MCM8, and MCM1 and MCM9, the co-expression of other gene members is positively correlated.

Discussion

The literatures indicated that changes in MCM factor expression levels have been reported in multiple tumors[21-41]. This study is the first to investigate the mRNA expression, prognostic(OS) value, and correlation between different MCM factors in liver cancer. We hope that the results of our research will help to integrate the existing data, so as to design a more reasonable treatment plan and improve the accuracy of the prognosis of liver cancer patients.

As previously reported in the literature, cancer cell proliferation was often accompanied by an imbalance in DNA replication, and chemotherapeutic drugs that target the replication mechanism have been widely used in cancer treatment[31]. MCM1-10 are necessary molecules for initiation and elongation of DNA replication, and changes in MCM are more frequent in tumor cells than in non-tumor cells. A study of transcription factors involved in cell cycle regulation of gene expression puts MCM1 at the top of a list of regulatory networks[42]. MCM1 also plays a direct role in DNA replication by binding to the replication origin[43]. One study showed that MCM1 binds to multiple sites in the minimal functional domain of the origin of replication *in vitro*[44]. *In vivo*, chromatin immunoprecipitation also confirmed the association between MCM1 and the origin of replication[45]. Therefore, MCM1 may regulate the process of DNA replication and the expression of DNA replicating genes. Roessler et al.[13] reported that MCM1 may be associated with disease-free survival and early recurrence rates in patients with liver cancer. In hepatocellular carcinoma tissues, the increased level of MCM1 enhances its expression level as a key gene for cell proliferation, which may be related to the occurrence of hepatocellular carcinoma. In our study, the Oncomine dataset and the GEPIA dataset showed that the expression of MCM1 in human liver cancer tissues was higher than that in normal tissues, but the difference was not significant. CCLE and EMBL-EBI databases showed that MCM1 was highly expressed in human hepatocellular carcinoma cell lines. Since the above results could not provide strong support, we determined that MCM1 was not associated with prognosis in HCC patients using GEPIA and LinkedOmics data sets. Therefore, we did not correlate the expression level of MCM1 with the prognosis of patients.

Among these proteins, MCM2-7 forms hexamers called MCM complexes, all of which are AAA+ ATPases and share part of the homologous sequence. MCM complexes play an important role in the initiation of DNA replication. In G1 phase, the MCM2-7 replication helicase binds to double-stranded DNA (dsDNA) in the form of an inactive head-to-head dimer. During the S phase, the active MCM2-7 duplex hexamer was synthesized bidirectional DNA at the origin of eukaryotes. In addition, the MCM2-7 complex contributes to the replication, cohesion, condensation, transcription, and recombination of DNA molecules. Therefore, it is more reasonable to use MCM2-7 as a complex to analyze its role in liver cancer. It has been proved that the expression level of MCM2-7 can be used as an indicator to predict the prognosis of breast cancer patients[41]. MCM2-7 can also be used as an independent marker of Merkel cell carcinoma (MCC) [28]. Furthermore, MCM immunohistochemistry may be a useful tool for detecting MCC tumor cell

proliferation. Roessler[13] and Wurmbach et al.[14] showed that MCM2-7 were overexpressed in hepatocellular carcinoma. CCLE and EMBL-EBI databases indicated that MCM2-7 were overexpressed in human hepatocellular carcinoma cell lines. In GEPIA and LinkedOmics datasets, MCM2-7 associated with prognosis in HCC patients. We could correlate expression levels of MCM2-7 with the prognosis of patients.

MCM8 and its physical partner MCM9, have been reported to be implicated in DNA replication[46]. Besides it has reported that it is overexpressed in several cancers. However, the mechanistic basis of MCM9's role in DNA repair needs more explore. Sloane et al. [47] conducted a comprehensive screening of mutations and deletions of MCM9 in patients with Lynch-like syndrome, but found that this rare mutation and deletion was difficult to correlate with the cancer susceptibility of patients with Lynch-like syndrome. Although MCM8 and MCM9 are closely related, their expression levels in tumor tissues and liver cancer cell lines are complete inconsistent. Our data from the oncomine database found that MCM8 is highly expressed in a variety of tumors, but MCM9 is only highly expressed in ovarian cancer. According to the data in the CCLE and GEPIA databases, MCM8 is highly expressed in various liver cancer cell lines, which is also inconsistent with the expression level of MCM9. Moreover, there was no statistical correlation between the overexpression of MCM9 and overall survival in patients with liver cancer. Therefore, even if they are physical partners, MCM8 has more potential to become a liver cancer biomarker.

DNA replication is a crucial process in cell division, MCM10 plays a vital role in this process and abnormalities in this process can lead to tumors[48]. It has been shown that in Ewing's sarcoma, MCM10 overexpression was caused by EWS RNA-binding protein 1 (EWS) / Fli-1 proto-oncogene, ETS transcription factor (FLI1) -nuclear receptor subfamily 0 Group B member 1 -DAX1 interaction-driven[49]. In addition, Zhang et al[39]. Found that MCM10 promotes lung cancer cell proliferation by up-regulating CCND1. The results of our study show that although the expression level of MCM10 in multiple liver cancer cell lines does not increase significantly, it is highly expressed in liver cancer tissue levels, and the high expression of MCM10 is significantly related to the overall survival of liver cancer patients.

Conclusions

We systematically analyzed the expression and prognostic value of MCM1-10 in liver cancer, and made a deep understanding of the molecular biology heterogeneity and complexity of liver cancer. Our results indicate that increased expression of MCM2-7, MCM8, and MCM10 in liver cancer may play an important role in the occurrence and development of liver cancer. High MCM2-7, MCM8 and MCM10 expression may be used as biomarkers to predict the prognosis of liver cancer. Furthermore, MCM2-7, MCM8, and MCM10 play important roles in cell proliferation, these eight genes also have the potential to become targets for drug therapy.

Materials And Methods

Ethics Statement

The study was approved by the Academic Committee of Changzhou Second People's Hospital affiliated to Nanjing Medical University and was conducted in accordance with the principles expressed in the Helsinki Declaration. All datasets are from published literature, so it can be confirmed that all written informed consent has been obtained.

ONCOMINE Analysis

ONCOMINE gene expression array datasets (<https://www.oncomine.org/>), an online cancer microarray database, is used to analyze the transcription level of the MCM gene family in different cancers. The Student's t test was used to calculate the P value to verify whether the difference between the expression level of the MCM gene family in clinical liver cancer samples and normal liver tissue was meaningful. The cut-off values for p value and fold change are defined as 0.01 and 2, respectively.

TCGA Datasets

The Cancer Genome Atlas (TCGA) is a project jointly launched by the National Cancer Institute (NCI) and the National Human Genome Research Institute (NHGRI) in 2006. It collects clinical data of various human cancers, including genomic variation, mRNA expression, miRNA expression, methylation and other data are important data sources for cancer researchers. We downloaded the co-expression data about MCM1-10 through the TCGA database and produced a heat map (<http://cancergenome.nih.gov/>).

GEPIA Datasets

The Gene Expression Profiling Interactive Analysis (GEPIA) is a newly web-based tool for gene expression analysis between the tumor and normal data from the Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx), applying a standard processing pipeline (<http://gepia.cancer-pku.cn/>). It provides customizable functions such as tumor and normal differential expression analysis, and we could demonstrate the expression of MCM1-10 in LIHC tissues and normal ones. GEPIA possesses key variable and interactive functions, including profiling plotting, differential expression analysis, patient survival analysis, similar gene detection and dimensionality reduction analysis.

LinkedOmics Dataset

LinkedOmics (<http://www.linkedomics.org/login.php>) is a new and unique tool in the software ecosystem for disseminating data from all 32 TCGA cancer types. It can be used to access, analyze, and compare multiomics data within and across tumor types. We performed a prognosis analysis for the MCM gene family using the LinkedOmics LIHC databases.

CCLC Dataset

The CCLC (<https://www.broadinstitute.org/>) project is a collaborative project between the Broad Institute and Novartis Institute of Biomedicine and its Novartis Genomics Institute. The CCLC database provides public access to genomic data, analysis and visualization and contains approximately 1,000 cell lines. We confirmed the expression of the MCM gene family in tumor cell lines by using the CCLC dataset.

EMBL-EBI Dataset

Since 1998, EMBL-EBI (<https://www.ebi.ac.uk>) has provided a series of free and open access bioinformatics applications for sequence analysis. The EMBL-EBI dataset validated the expression of the MCM family in LIHC cell lines.

Declarations

AUTHOR CONTRIBUTIONS

Cui XH were responsible for the conception and design of the study. Cui XH, Zhu Bei, Fu Li and Qin XH were responsible for statistical and bioinformatic analysis. Cui XH, Lao YX and Zhu CF were responsible for paper elaboration and revision. All authors were involved in manuscript writing and provided final approval of the manuscript.

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Availability of data and materials

The data and database usage used in this study are mentioned in the Materials and Methods section.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interest.

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Supplementary Figure Caption

Supplement Figure 1 and 2: The correlation between MCMs in Liver Cancer, analyzed by LinkedOmics and GEPIA.

Figures

Figure 1

The Transcription Levels of MCM Factors in Different Types of Cancers (ONCOMINE)

Figure 2

The Expression of MCMs in Liver cancer (GEPIA) (A-J) The expression of MCMs in pan-cancer. (K-L) The expression of MCMs in LIHC.

A

B

Figure 3

The Expression of MCMs in Liver Cancer Cell Lines (CCLE and EMBL-EBI) (A) The expression of MCM1-10 in Liver Cancer Cell Lines, analyzing by CCLE. (B) The expression of MCM1-10 in Liver Cancer Cell Lines, analyzed by EMBL-EBI.

A

B

Figure 4

The Prognostic Value of mRNA Level of MCM Factors in Liver Cancer Patients (LinkedOmics and GEPIA)

(A) The prognostic value of mRNA level of MCM Factors in Liver Cancer Patients, analyzed by LinkedOmics. (B) The prognostic value of mRNA level of MCM Factors in Liver Cancer Patients, analyzed by GEPIA.

A

B

Figure 5

Co-expressed Genes of MCMs, and the Correction between MCMs in Liver Cancer (TCGA,ONCOMINE, LinkedOmics, and GEPIA) (A)The correction between MCMs in Liver Cancer, analyzed by TCGA. (B)Co-expressed genes of MCMs in HCC, analyzed by ONCOMINE .(C-D)The correction between MCMs in Liver Cancer, analyzed by LinkedOmics and GEPIA.

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