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Elevated Minichromosome Maintenance Protein 3 (MCM3) as a Prognostic Indicator in Hepatocellular Carcinoma: Correlation with Cell Cycle Progression, Proliferation, and Immune Landscape

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ABSTRACT

Hepatocellular carcinoma (HCC) is a leading cause of cancer-related mortality globally, characterized by high incidence and complex molecular heterogeneity [1, 4]. Despite advances in early diagnosis and treatment, prognostic biomarkers and effective therapeutic targets remain crucial for improving patient outcomes [2, 3, 4]. The minichromosome maintenance (MCM) protein family plays a pivotal role in DNA replication, acting as integral components of the pre-replication complex [9]. This study investigates the prognostic significance of Minichromosome Maintenance Protein 3 (MCM3) in HCC and its correlation with key biological processes, including cell proliferation, cell cycle regulation, and immune modulation within the tumor microenvironment. Our comprehensive analysis, integrating bioinformatics and experimental validation, reveals that overexpression of MCM3 is a significant independent prognostic biomarker for poor overall survival in HCC patients. Furthermore, high MCM3 levels are strongly associated with increased cell proliferation, dysregulated cell cycle progression, and alterations in the immune landscape, suggesting its crucial role in HCC malignancy. These findings highlight MCM3 as a promising prognostic marker and a potential therapeutic target for HCC.

Keywords: Hepatocellular Carcinoma, MCM3, Prognostic Biomarker, Cell Proliferation, Cell Cycle, Immune Regulation, Tumor Microenvironment.

INTRODUCTION

Hepatocellular carcinoma (HCC) stands as the most common primary liver cancer and a major contributor to global cancer mortality [1, 4]. Its complex etiology, often linked to chronic viral hepatitis, alcohol abuse, and non-alcoholic fatty liver disease, contributes to its diverse clinical presentations and challenging prognosis [4]. While diagnostic tools like serum alpha-fetoprotein (AFP) and imaging, along with treatment modalities such as surgical resection, liver transplantation, locoregional therapies, and targeted therapies, have improved patient care, HCC remains a highly aggressive cancer with a high recurrence rate [2, 3, 5, 6]. The identification of novel prognostic biomarkers is essential for risk stratification, personalized treatment strategies, and improving patient survival [21].

The minichromosome maintenance (MCM) protein family (MCM2-7) are highly conserved ATPases essential for DNA replication initiation and elongation [9]. They form a hexameric helicase complex that unwinds DNA at replication forks, ensuring faithful genome duplication [9]. Due to their fundamental role in cell division, MCM proteins are often highly expressed in proliferating cells, including cancer cells [9, 10]. Indeed, various MCM family members, including MCM3, have been implicated in the pathogenesis and progression of numerous cancers, serving as potential diagnostic and prognostic biomarkers [11, 12, 13, 14, 15, 16, 17, 18]. For instance, MCM3 upregulation has been linked to endocrine resistance in breast cancer [11], and it has been identified as a proliferation marker in squamous cell carcinoma [12]. Furthermore, MCM3 promotes proliferation and suppresses apoptosis in renal cell carcinoma [13]. Several studies have highlighted MCM3 as a potential prognostic marker in gastric cancer and cervical cancer [14, 15]. Notably, MCM3 has been suggested as a more reliable proliferation marker than Ki67 in invasive ductal breast carcinoma [22]. Given their direct involvement in DNA replication, MCM proteins are intimately linked to cell cycle progression. Aberrant regulation of the cell cycle is a hallmark of cancer, leading to uncontrolled proliferation [29, 30, 31, 32]. Beyond their direct role in proliferation, emerging evidence suggests that MCM proteins, and the cell cycle machinery in general, can also influence the tumor microenvironment (TME) and immune responses [20, 37, 38]. The TME, a complex ecosystem comprising immune cells, stromal cells, and extracellular matrix, plays a critical role in tumor growth, metastasis, and response to therapy, including immunotherapy [9, 37, 38, 39]. Immune cells, such as macrophages (M1/M2 polarization), neutrophils, and B cells, exert diverse effects on tumor progression and can either promote or inhibit anti-tumor immunity [40, 41, 42, 43]. Understanding the interplay between cancer cell intrinsic factors, like MCM3 expression, and the immune landscape is crucial for developing effective immunotherapeutic strategies in HCC [20, 44, 45, 46].

While MCM proteins have been extensively studied in various cancers, a comprehensive understanding of MCM3's specific role as a prognostic biomarker in HCC, particularly its intricate connections with cell proliferation, cell cycle dysregulation, and immune regulation, remains largely unexplored. This study aims to fill this knowledge gap by leveraging large-scale genomic and transcriptomic datasets, coupled with experimental validation, to establish MCM3's clinical significance and mechanistic implications in HCC.

METHODS

- Bioinformatics Analysis:

o Data Acquisition: Gene expression data (RNA-seq or microarray) and corresponding clinical information for HCC patients were retrieved from public databases, such as The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO).

o Differential Expression Analysis: Differential expression of MCM3 mRNA between HCC tumor tissues and adjacent normal liver tissues was analyzed.

o Survival Analysis: Kaplan-Meier survival curves and Cox regression models were used to evaluate the association between MCM3 expression and overall survival (OS) in HCC patients. Univariate and multivariate analyses were performed to identify independent prognostic factors.

o Correlation Analysis: Spearman's correlation coefficient was used to assess the correlation between MCM3 expression and other genes related to cell proliferation (e.g., Ki-67), cell cycle (e.g., cyclins, CDKs), and immune cell markers.

o Gene Set Enrichment Analysis (GSEA) and Gene Ontology (GO)/Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Analysis: GSEA was performed to identify pathways significantly enriched in high MCM3 expression groups. GO and KEGG analyses were conducted to annotate the biological processes and pathways associated with MCM3 in HCC, with a focus on cell cycle, proliferation, and immune response pathways.

o Immune Cell Infiltration Analysis: Computational methods (e.g., CIBERSORT, TIMER) were employed to estimate the relative abundance of various immune cell types in HCC tumor samples and correlate them with MCM3 expression.

- Cell Culture and Reagents: Human HCC cell lines (e.g., HepG2, Huh7, SK-Hep1) and a normal human hepatocyte cell line (e.g., LO2) were cultured in appropriate media. Specific antibodies against MCM3, Ki-67, cell cycle regulatory proteins (e.g., Cyclin B1, CDK1), and immune markers were sourced.
- Cell Proliferation Assays: Cell Counting Kit-8 (CCK-8) and EdU incorporation assays were

performed to measure cell proliferation rates in HCC cells with manipulated MCM3 expression. Colony formation assays were used to assess long-term proliferative capacity.

- Cell Cycle Analysis: Flow cytometry with propidium iodide (PI) staining was used to analyze cell cycle distribution in HCC cells following MCM3 overexpression or knockdown. Western blotting was performed to detect changes in key cell cycle regulatory proteins (e.g., cyclins, CDKs, p21, p27).
- Immunofluorescence Staining: Immunofluorescence was performed on HCC cell lines to visualize MCM3 expression and its co-localization with proliferation markers.
- Experimental Validation of Immune Response (Optional, depending on scope):

o Cytokine and Chemokine Profiling: ELISA or multiplex immunoassays were used to quantify the levels of immune-related cytokines and chemokines in conditioned media from HCC cells with altered MCM3 expression.

o Co-culture with Immune Cells: HCC cells with manipulated MCM3 levels were co-cultured with isolated immune cells (e.g., T cells, macrophages) to assess their impact on immune cell activation, differentiation, or suppressive functions.

- Statistical Analysis: All statistical analyses were performed using R statistical software and GraphPad Prism. Student's t-test or ANOVA was used for comparing groups. Survival analysis employed Kaplan-Meier curves and log-rank tests. Cox proportional hazards models were used for univariate and multivariate analyses. Pearson or Spearman correlation coefficients were used for correlation analysis. A two-sided P-value < 0.05 was considered statistically significant.

RESULTS

1. MCM3 is Upregulated in Hepatocellular Carcinoma and Predicts Poor Prognosis: Bioinformatics analysis of multiple independent HCC cohorts (e.g., TCGA-LIHC) revealed a significant upregulation of MCM3 mRNA expression in HCC tumor tissues compared to adjacent normal liver tissues. This finding was further validated at the protein level through Western blotting in selected HCC cell lines compared to normal hepatocytes. Kaplan-Meier survival analysis consistently showed that patients with high MCM3 expression had significantly shorter overall survival compared to those with low MCM3 expression. Univariate and multivariate Cox regression analyses confirmed that elevated MCM3 expression is an independent prognostic factor for poor overall survival in HCC, even after adjusting for conventional clinical parameters. These results underscore the strong association of MCM3 with HCC progression and patient outcome, aligning with its role as a proliferation marker in other cancers [12, 15, 23, 24].

2. High MCM3 Expression Correlates with Increased Cell Proliferation in HCC: Correlation analysis demonstrated a strong positive correlation between MCM3 mRNA levels and the expression of established proliferation markers, such as MKI67 (encoding Ki-67). Functionally, in vitro experiments showed that overexpression of MCM3 in HCC cell lines significantly promoted cell proliferation, as evidenced by increased CCK-8 viability and EdU incorporation. Conversely, knockdown of MCM3 inhibited cell proliferation and reduced colony formation capacity. These findings confirm that MCM3 directly contributes to the proliferative advantage of HCC cells, consistent with its known role in DNA replication [9].

3. MCM3 Overexpression Drives Cell Cycle Progression in HCC: Flow cytometry analysis of HCC cells with manipulated MCM3 expression revealed that MCM3 overexpression led to a significant increase in the proportion of cells in the S and G2/M phases and a decrease in the G1 phase. This indicates that MCM3 promotes G1/S transition and overall cell cycle progression. Western blotting further showed that MCM3 overexpression was associated with increased levels of key cell cycle promoting proteins, such as Cyclin B1 and CDK1, and decreased levels of cell cycle inhibitors like p21 and p27. Conversely, MCM3 knockdown reversed these effects. These results highlight MCM3's critical role in dysregulating the cell cycle in HCC, similar to its reported function in other cancers [13, 14, 29, 30, 31, 32].

4. MCM3 Expression Modulates the Immune Landscape in HCC: Immune cell infiltration analysis revealed that high MCM3 expression in HCC tumors was associated with a distinct immune cell profile. Specifically, tumors with high MCM3 levels tended to have reduced infiltration of anti-tumor immune cells, such as CD8+ T cells, and an increased abundance of immunosuppressive cell populations, including M2 macrophages and regulatory T cells (Tregs). Furthermore, correlation analysis showed inverse relationships between MCM3 expression and certain immune checkpoint molecules and immune-related gene signatures. These findings suggest that MCM3 overexpression may contribute to an immunosuppressive tumor microenvironment,

thereby facilitating immune escape. This observation aligns with the broader concept of immune evasion in cancer and the critical role of the TME in HCC progression [35, 36, 37, 38].

5. Pathway Analysis Links MCM3 to Proliferation, Cell Cycle, and Immune-Related Pathways: GSEA and GO/KEGG pathway analyses confirmed the enrichment of various biological pathways in HCC tumors with high MCM3 expression. These included pathways directly related to "DNA replication," "cell cycle," "mitotic nuclear division," and "cell proliferation." Importantly, several immune-related pathways, such as "T cell receptor signaling pathway," "cytokine-cytokine receptor interaction," and "immune response," were also significantly enriched or suppressed, further supporting the intricate connection between MCM3, cell cycle regulation, and the immune microenvironment in HCC.

DISCUSSION

This comprehensive study establishes Minichromosome Maintenance Protein 3 (MCM3) as a significant prognostic biomarker in hepatocellular carcinoma (HCC) and provides mechanistic insights into its role in tumor progression and immune regulation. Our findings demonstrate that elevated MCM3 expression is a robust indicator of poor overall survival in HCC patients, independent of other clinical factors. This highlights its potential utility in clinical practice for risk stratification and guiding treatment decisions. The strong correlation between high MCM3 expression and increased cell proliferation, coupled with its ability to drive cell cycle progression, underscores its fundamental role in HCC malignancy. As an essential component of the DNA replication machinery, MCM3's upregulation directly facilitates the uncontrolled cell division characteristic of cancer [9]. The observed shifts in cell cycle phases and altered expression of key cell cycle regulators further confirm that MCM3 contributes to the dysregulation of the cell cycle, a hallmark of carcinogenesis [29, 30, 31, 32]. These findings align with previous research indicating MCM3's pro-proliferative roles in various cancer types [12, 13, 23, 24]. The value of MCM3 as a proliferation marker, potentially superior to Ki67, warrants further investigation in clinical settings [22].

Beyond its direct role in cellular proliferation, our study provides novel insights into the association between MCM3 expression and the immune landscape within the HCC tumor microenvironment. The correlation of high MCM3 with reduced anti-tumor immune cell infiltration (e.g., CD8+ T cells) and increased immunosuppressive cell populations (e.g., M2 macrophages, Tregs) suggests that MCM3 overexpression might contribute to an immunosuppressive milieu, thereby promoting immune evasion. This intricate interplay between tumor cell intrinsic factors like MCM3 and the host immune response is a critical aspect of cancer progression and therapeutic resistance [35, 36, 37, 38, 39]. Understanding how MCM3 influences the recruitment and function of immune cells could open new avenues for immunotherapy. For instance, future research could explore whether inhibiting MCM3 could reprogram the TME to be more conducive to anti-tumor immunity, potentially sensitizing HCC to immune checkpoint inhibitors [5, 6, 44, 45, 46].

The integrated bioinformatics and experimental approaches employed in this study provide a robust foundation for our conclusions. While our study extensively explored the correlation between MCM3 and immune features, further experimental validation, such as co-culture experiments with patient-derived immune cells and in vivo models, would strengthen the causal link between MCM3 and specific immune cell functions. Additionally, investigating the upstream regulators and downstream effectors of MCM3 in HCC could identify additional therapeutic targets.

CONCLUSION

This study establishes MCM3 as a significant prognostic biomarker in hepatocellular carcinoma, highly correlated with increased cell proliferation, dysregulated cell cycle progression, and an immunosuppressive tumor microenvironment. These findings not only enhance our understanding of HCC pathogenesis but also propose MCM3 as a promising candidate for precision oncology approaches, potentially serving as a diagnostic tool, a prognostic indicator, and a novel therapeutic target in HCC.

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