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·临床研究·

微小染色体维持蛋白3在脑胶质瘤中的表达及其临床意义

闫润芝[1a],慕伟[1a,2],李晋虎[1b],王剑芳[3],刘晓东[1b],范益民[1b](1. 山西医科大学 a. 第一临床医学院;b. 神经外科,山西 太原 030001;2. 山西省人民医院 介入科,山西 太原 030012;3. 大同市第五人民医院 神经外科,山西 大同 037000)

[摘 要] **目的:**分析微小染色体维持蛋白3(minichromosome maintenance protein 3,MCM3)在脑胶质瘤中的表达情况、临床意义和可能参与的生物学过程,并探究其与胶质瘤免疫的关系。**方法:**在线检索GEPIA和Oncomine数据库获得MCM3在胶质瘤组织中的表达情况,利用CGGA数据库在线分析MCM3表达和胶质瘤临床病理特征的关系。同时收集2019年1月到2020年3月在山西省人民医院神经外科接受手术治疗的24例胶质瘤患者的肿瘤标本和8例非肿瘤对照标本,采用免疫组化SP法检测MCM3的表达,对生物信息学分析结果进行验证。在TCGA和CGGA数据库中利用Kaplan-Meier生存曲线评价MCM3对胶质瘤预后的作用。通过Linkedomic数据库、STRING数据库和Cytoscape软件获得与MCM3表达显著相关的基因。使用DAVID数据库对MCM3及其显著相关基因进行GO和KEGG分析,探究基因功能。最后在TIMER数据库中探究MCM3表达和胶质瘤免疫浸润的关系。**结果:**综合生物信息学与临床数据分析显示,MCM3在胶质瘤组织中相对正常组织呈高表达(P=0.024),其表达量随着病理级别逐渐升高(P=0.001)。生存分析显示,MCM3高表达与胶质瘤不良预后有关(P<0.05)。GO和KEGG分析显示,MCM3及其显著相关基因主要富集于细胞周期、DNA复制和调节DNA损伤修复等方面。TIMER数据库分析结果显示,在胶质瘤队列中,MCM3与多种免疫浸润细胞具有相关性(P<0.05)。**结论:**MCM3在胶质瘤中高表达且与不良预后有关,其可能与胶质瘤细胞的细胞周期、DNA复制、调节DNA损伤修复和免疫微环境有关。MCM3能促进胶质瘤的进展,可作为胶质瘤患者预后判断指标和潜在的治疗靶点。

[关键词] 胶质瘤;微小染色体维持蛋白3;基因表达;预后;肿瘤免疫;生物信息学
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The expression of minichromosome maintenance protein 3 in brain glioma and its clinical significances

YAN Runzhi[1a], MU Wei[1a,2], LI Jinhu[1b], WANG Jianfang[3], LIU Xiaodong[1b], FAN Yimin[1b](1. a. The First Clinical Medical College; b. Department of Neurosurgery, Shanxi Medical University, Taiyuan 030001, Shanxi, China; 2. Department of Interventional Radiology, Shanxi Provincal People's Hospital, Taiyuan 030012, Shanxi, China; 3. Department of Neurosurgery, the Fifth People's Hospital of Datong City, Datong 037000, Shanxi, China)

[Abstract] Objective: Analyze the expression, clinical significance and possible biological processes of minichromosome maintenance protein 3 (MCM3) in brain gliomas, and explore its relationship with glioma immunity. **Methods:** The databases GEPIA and Oncomine were searched online to obtain the expression of MCM3 in glioma tissues. The CGGA database was used to analyze the relationship between MCM3 expression and clinicopathological characteristics of gliomas online.At the same time, 24 tumor specimens of patients with glioma who underwent surgical treatment in the Department of Neurosurgery of Shanxi Provincial People's Hospital from January 2019 to March 2020 and 8 non-tumor control specimens were collected, and used immunohistochemical SP method to detect the expression of MCM3 to verify the bioinformatics analysis results. Kaplan-Meier survival curve was used to evaluate the effect of

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[作者简介] 闫润芝(1994-),男,硕士生,主要从事胶质瘤免疫治疗的机制研究,E-mail:2317415676@qq.com
[通信作者] 范益民(FAN Yimin,corresponding author),博士,主任医师,教授,博士生导师,主要从事颅脑肿瘤相关的基础和临床研究,E-mail:fanyimin5678@126.com

MCM3 on the prognosis of glioma in the TCGA and CGGA databases. The significant related genes of MCM3 were obtained through Linkedomic database, STRING database and Cytoscape software. Use the DAVID database to perform GO and KEGG analysis of MCM3 and its significant related genes to explore the biological processes they may participate in. Finally, explore the relationship between MCM3 expression and glioma immune infiltrating cells in the TIMER database. **Results:** Comprehensive bioinformatics analysis and clinical data verification showed that MCM3 was highly expressed in glioma tissues relative to normal tissues (P=0.024), and its expression level gradually increased with the pathological grade (P=0.001). Survival analysis showed that high expression of MCM3 was related to the poor prognosis of glioma (P<0.05). GO and KEGG analysis of MCM3 and its significant related genes showed that these genes are mainly enriched in cell cycle, DNA replication and regulation of DNA damage repair. TIMER database showed that in the glioma cohort, MCM3 was correlated with a variety of immune infiltrating cells (P<0.05). **Conclusions:** MCM3 is highly expressed in gliomas and is related to poor prognosis, which may be related to the cell cycle, DNA replication, regulation of DNA damage repair and immune microenvironment of glioma cells. MCM3 can promote the progression of glioma, and can be used as a prognostic indicator and potential therapeutic target for patients with glioma.

[Key words] glioma; minichromosome maintenance protein 3 (MCM3); gene expression; prognosis; tumor immunity; bioinformatics

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肿瘤生物标志物的鉴定有助于选择常规疗法、预测生存预后和开发分子靶向治疗方法。对于胶质瘤患者来说，一些潜在的差异表达基因有望成为新的生物标志物，可以帮助临床医生改善肿瘤的诊断和分级，更加精准地制定治疗策略，避免过度或过少治疗。微小染色体维持蛋白（minichromosome maintenance protein，MCM）家族在真核生物中高度保守，至少包含6种不同的核蛋白（MCM2~7）[1]。MCM蛋白形成的六聚体蛋白复合物是DNA复制许可复合体的关键组成部分[2]，其可能参与DNA复制叉的形成以及其他DNA复制相关蛋白质的募集[3]。MCM3是MCM蛋白家族的重要成员之一，作为该复合体的一个亚基，由808个氨基酸残基组成，相对分子质量为105 000[4-5]。MCM3在多种肿瘤中过度表达，如肾细胞癌[6]、子宫内膜癌[7]、乳腺癌[8]等，但其在胶质瘤中的表达情况和作用机制国内外文献研究较少。因此，本研究运用生物信息学分析结合免疫组化验证探究MCM3在胶质瘤中的差异表达和临床意义。以评估其作为胶质瘤潜在诊断和预后生物标记物的可能。

1　资料和方法

1.1　MCM3在多数据库胶质瘤队列中的表达

GEPIA（http://gepia.cancer-pku.cn/index.html）数据库是一个可分析癌症和正常基因表达谱的公共数据库。其整合了来自TCGA数据库的肿瘤数据和来自GTEx数据库的正常组织数据，可用于分析癌症亚型、驱动基因和致癌因素，从而挖掘新型癌症靶点和标记物[9]。Oncomine（https://www.oncomine.org）数据库是目前世界上最大的癌基因芯片数据库和整合数据挖掘平台。迄今为止该数据库已经收集了729个基因表达数据集，90 000多个癌症组织和正常组织的样本数据，可用于癌基因差异分析和相关临床数据分析。检索GEPIA和Oncomine数据库中MCM3在人类肿瘤组织和正常组织的相对表达情况。CGGA（http://www.cgga.org.cn）数据库收集了来自中国队列的2 000多个样本的脑肿瘤数据集，包括全外显子测序、DNA甲基化和mRNA测序等数据以及相应的临床资料。在CGGA数据库mRNAseq_325数据集中检索MCM3，获得其表达情况与胶质瘤临床病理特征的关系。临床病理特征分组：将胶质瘤按不同的病理级别分为Ⅱ、Ⅲ、Ⅳ级；根据IDH状态分为IDH突变型和IDH野生型；根据患者年龄分为高龄组（≥42岁）和低龄组（＜42岁）。

1.2　临床资料收集验证

选取2019年1月到2020年3月在山西省人民医院神经外科接受手术治疗并且经过病理证实的胶质瘤组织24例，其中男性13例、女性11例；年龄6~74岁，中位年龄44岁，平均年龄（41.79 ± 21.33）岁。肿瘤分级为I级5例、II级6例、III级7例和成胶质细胞瘤（glioblastoma，GBM）6例。同时收集8例非肿瘤脑组织（取自癫痫患者的病灶组织）作为对照组。

使用免疫组化SP法检测MCM3在胶质瘤和非肿瘤脑组织中的表达情况。实验步骤按照说明书进行，MCM3抗体原液按1∶600稀释，阴性对照组用PBS代替一抗，阳性对照为试剂公司提供的照片。组织经包埋、切片、脱蜡、EDTA加热抗原修复、DAB显色、苏木精复染，然后将每张切片置于400倍光学显微镜下观察，随机选择5个视野，计数每个视野内的阳性细胞。在无背景染色情况下以细胞核染成黄色至棕褐色细颗粒状为阳性反应。根据阳性细胞的百分比评分：阳性细胞≤5%为0分；>5%且≤25%为1分；>25%且≤50%为2分；>50%且≤75%为3分；>75%为4分。根据阳性细胞着色强度计分：不着色为0分，浅黄色为1分，棕黄色为2分，棕褐色为3分。最终表达分级用阳性细胞百分比得分×着色强度得分，

按计算结果分级，≤4分为低表达，＞5分为高表达。同时收集胶质瘤患者临床病理特征，探究MCM3表达与年龄、性别、胶质瘤级别和IDH状态的相关性。

1.3 MCM3表达与胶质瘤患者生存预后的关系

CGGA和TCGA（https://portal.gdc.cancer.gov/）数据库提供在线生存分析，根据MCM3表达水平对胶质瘤患者进行分组，MCM3表达量≥中位数者为高表达组，反之为低表达组。计算95%置信区间（CI）的对数秩*P*值和危险比（*HR*），并在图上显示，以展示MCM3在胶质瘤中的表达情况对生存预后的影响。

1.4 MCM3的显著相关基因筛选和功能分析

Linkedomic（http://www. linkedomics. org/login. php）是一个用于分析32个TCGA癌症相关多维数据集的网络平台[10]。利用该平台的LinkFinder模块探索TCGA胶质瘤队列中与MCM3相关的共表达基因。STRING（https://string-db.org）可以研究蛋白相互作用（protein-protein interaction，PPI）网络，其包含多个物种的大量蛋白功能互作信息。通过该数据库构建MCM3及其相关共表达基因的PPI网络。Cytoscape是一个用于生物分子相互作用网络集成模型研究的图形可视化软件。通过该软件挖掘MCM3在该PPI网络中的显著相关节点，获得MCM3的显著相关基因。功能分析包括基因本体（gene ontology，GO）分析和京都基因与基因组百科全书（Kyoto encyclopedia of genes and genomes，KEGG）信号通路分析。DAVID（https://david-d.ncifcrf.gov）是一个生物信息数据库，为大规模的基因或蛋白列表提供系统综合的生物功能注释信息[11-12]。利用该数据库对MCM3及其显著相关基因进行GO和KEGG信号通路注释分析。

1.5 分析MCM3与胶质瘤免疫浸润的关系

TIMER（https://cistrome.shinyapps.io/timer/）数据库是用于对各种类型癌症的免疫浸润进行系统分析的综合资源[13-15]。通过该数据库Gene模块展示在低级别胶质瘤（low-grade glioma，LGG）和GBM数据集中MCM3表达与6种免疫细胞（B细胞、$CD4^+$T细胞、$CD8^+$T细胞、嗜中性粒细胞、巨噬细胞和树突状细胞）的关系。

1.6 统计学处理

采用单因素方差分析对GEPIA和Oncomine数据库中MCM3在胶质瘤中的表达进行分析，表达数据经过$\log_2$处理，$|\log_2FC|>1$则认为是差异基因。CGGA数据库基因芯片样本中数据的两组间比较采用*t*检验，多组间比较采用单因素方差分析。绘制Kaplan-Meier生存曲线，选用Log Rank法检验组间差异性。免疫组化检测结果使用SPSS 20.0软件进行分析，使用卡方检验进行组间比较。以上各分析结果均以$P<0.05$或$P<0.01$表示差异具有统计学意义。对于MCM3及其相关的共表达基因的表达水平，采用Spearman检验评估其相关性。利用STRING数据库构建PPI网络，评估组合分数大于0.4则定义为显著的交互关系。

2 结 果

2.1 MCM3在胶质瘤组织中高表达

GEPIA和Oncomine数据库的分析结果（图1）显示，在胶质瘤中MCM3的表达水平显著高于正常组织。免疫组化SP法检测结果（图2，表1）也显示，胶质瘤组织中MCM3的表达水平显著高于非瘤脑组织（$P<0.05$）；MCM3集中表达在细胞核中，胞质有少量表达，镜下观察到阳性表达处有浅黄色至棕黄色沉淀。

2.2 MCM3表达与胶质瘤临床病理特征相关

CGGA数据库分析结果（图3）显示，MCM3表达与胶质瘤的级别（$P=2.9\times10^{-22}$）、IDH状态（$P=2.5\times10^{-5}$）、年龄（$P=0.014$）有关，与胶质瘤患者性别、原复发无关。临床资料（表2）显示，MCM3表达与胶质瘤级别有关（$P=0.001$），与年龄（$P=0.113$）、性别（$P=0.916$）和IDH状态（$P=0.831$）无关。

2.3 MCM3高表达提示胶质瘤不良预后

CGGA数据库生存分析结果（4A、4B）显示，MCM3表达与胶质瘤预后显著相关，MCM3高表达提示不良预后（原发性胶质瘤$P<0.000\ 1$，继发性胶质瘤$P=0.001\ 2$）。TCGA数据库生存分析结果（图4C）显示，MCM3表达也与胶质瘤预后显著相关，MCM3高表达提示不良预后（$P<0.01$）。

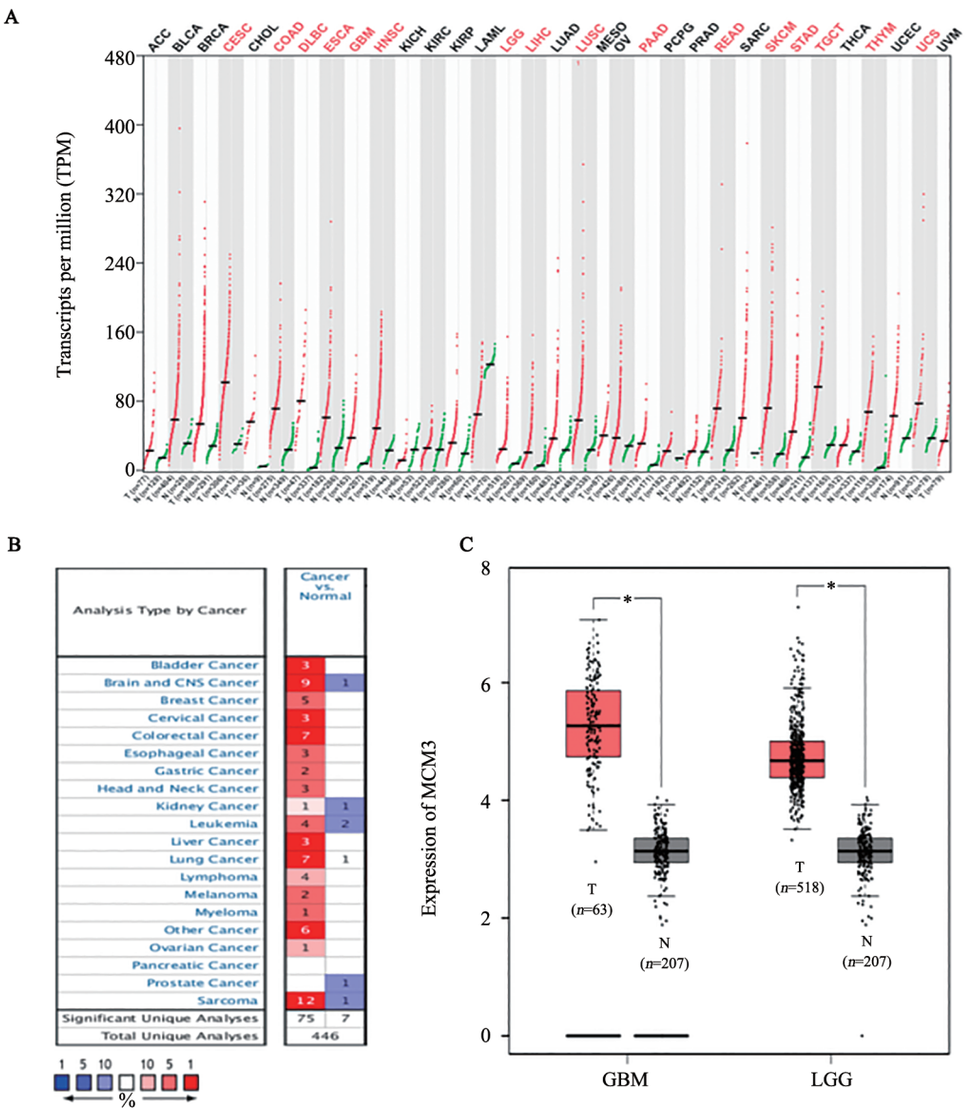
2.4 MCM3显著相关基因的功能分析

在Linkedomic数据库中得到MCM3正负相关共表达基因热图（图5），根据*P*值排序选择前50个基因，输入STRING数据库后得到PPI网络，其中节点数为51、边数为227、平均节点度为8.9、平均局部聚类系数为0.563（图6A、6B）。将该PPI网络导入Cytoscape软件中，以MCM3作为中心节点，得到MCM3的显著相关节点23个，即MCM3的显著相关基因为ARPP21、TIMELESS、KIF2C、MYBL2、CDCA8、RAD51、MCM2、CDK2、AURKB、PCNA、RNASEH2A、RAD51AP1、GINS2、ASF1B、TPX2、GINS4、ORC1、MCM5、NDC80、TCF19、KIFC1、TACC3、RAD54L（图6C）。利用DAVID数据库对MCM3及其相关的23个基因进行GO分析，结果（表3）显示：（1）在生物学过程（biological process，BP），富集于DNA复制、细胞分裂、G1/S有丝分裂细胞周期的转变等方面；（2）在细胞组成（cell component，CC），

富集于核质、微管细胞骨架、MCM复合体、端粒区域等方面；(3)在分子功能(molecular function，MF)，富集于ATP结合、蛋白质结合、DNA解旋酶活性、3'-5' DNA解旋酶活性、微管运动等方面。KEGG分析显示，这些基因的功能主要富集于细胞周期、DNA复制、同源重组等方面。

2.5　MCM3表达与胶质瘤免疫浸润有关

TIMER数据库中显示，在LGG队列中MCM3表达与不同的免疫浸润细胞具有相关性，包括B细胞（P=9.85×10^{-19}，相关系数cor=0.389）、CD8^{+}T细胞（P=2.12×10^{-8}，cor=0.253）、CD4^{+}T细胞（P=4.59×10^{-15}，cor=0.349）、树突状细胞（P=6.11×10^{-22}，cor=0.422）、中性粒细胞（P=1.37×10^{-14}，cor=0.343）和巨噬细胞（P=1.54×10^{-17}，cor=0.378）（图7A）。在GBM队列中MCM3表达与树突状细胞相关（P=9.59×10^{-4}，cor=0.161）（图7B）。



*P<0.05

A: The expression of MCM3 in various tumor tissues in the GEPIA database; B: The expression of MCM3 in various tumor tissues in the Oncomine database; C: The expression of MCM3 in glioma and normal tissues in the GEPIA database; T: Tumor group; N: Normal group

图1 MCM3在胶质瘤组织中的表达

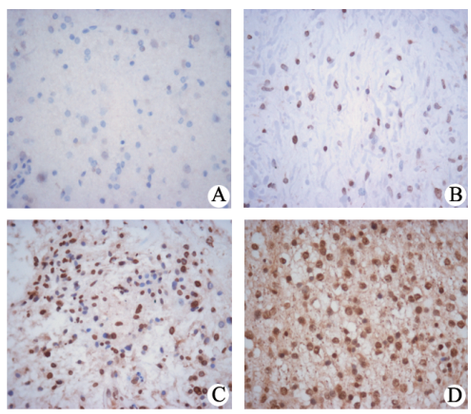
Fig.1 Expression of MCM3 in glioma tissues

3　讨　论

胶质瘤是中枢系统最常见的原发恶性肿瘤，是起源于中枢神经系统胶质细胞的低级和高级脑肿瘤[16]，占原发性脑肿瘤的80%以上[17]。根据世界卫生组织(WHO)制定的组织学标准，从肿瘤恶性程度上

划分为I~IV级。I级胶质瘤通常是良性和可手术的，治疗效果较好；II级胶质瘤经手术结合放化疗后有一定疗效但易复发，也可进展为III级间变性胶质瘤；III级胶质瘤一般很难治愈，中位生存期为3~10年[18]。GBM是最高级别的星形细胞瘤，侵袭性强、极易复发，患者中位生存期不足15个月[19]。尽管目前恶性胶质瘤的治疗已取得广泛的进展，但患者预后仍然很差。相关研究[20]表明，胶质瘤更倾向于为一种受多基因影响、多步骤发展的遗传相关疾病。因此，寻找高效、高敏感度的生物标志物对改善早期诊断、肿瘤分类以及量身定制治疗策略具有重要意义。

MCM蛋白复合物与DNA复制的起始和延伸有关[21]。MCM2~7形成的六聚体复合物与DNA复制起始点结合，细胞周期蛋白依赖性激酶如Cdc6、Cdt1和bf4/Cdc7等可以激活该复合物，导致DNA合成的启动[22]。同时该复合物也具有解链酶的活性，在DNA链延伸过程中发挥作用。研究表明，在消化系统肿瘤[23]、泌尿生殖系统肿瘤[24-26]、肺癌[27]等肿瘤中，MCM具有成为生物标志物的可能性，且MCM水平的升高不仅可以识别恶性细胞，还可以识别癌前细胞和肿瘤复发[28-30]。MCM3在成髓细胞瘤和恶性黑色素瘤的研究中已被证实可作为独立预后的指标[31-32]。HUA等[33]发现，MCM3的表达在胶质瘤中上调，并与III级肿瘤患者的总生存率相关。



A, B：MCM3 showed no or low expression in the non-tumor control group; C, D：MCM3 is moderately or highly expressed in glioma tissues

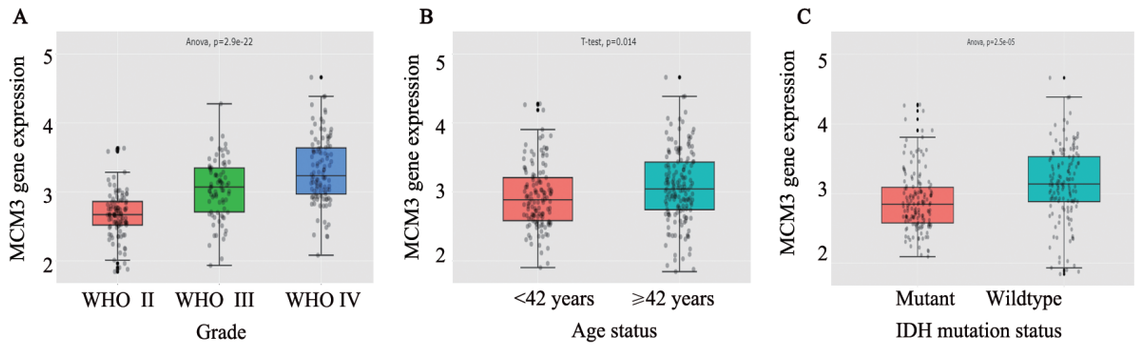
图2 MCM3在胶质瘤组织和非肿瘤组织中的表达情况（×400）

Fig.2 MCM3 expression in glioma tissue and non-tumor tissue（×400）

表1 MCM3在胶质瘤组织和非肿瘤对照组中的表达情况

Tab.1 The expression of MCM3 in glioma tissue and non-tumor control group

Group	Case	MCM3 expression		Positive rate（%）	χ^2	*P*
		High	Low			
Glioma tissue	24	15	9	62.5	5.100	0.024
Non-tumor tissue	8	2	6	25.0		



A: The higher the expression of MCM3, the higher the grade of glioma; B: High expression of MCM3 is more likely to occur in patients with high age groups (≥42 years old); C: High expression of MCM3 is more likely to occur in patients with IDH wild-type glioma

图3 CGGA数据库中MCM3表达与胶质瘤临床病理特征的关系

Fig.3 Relationship between MCM3 expression in CGGA database and clinicopathological characteristics of glioma

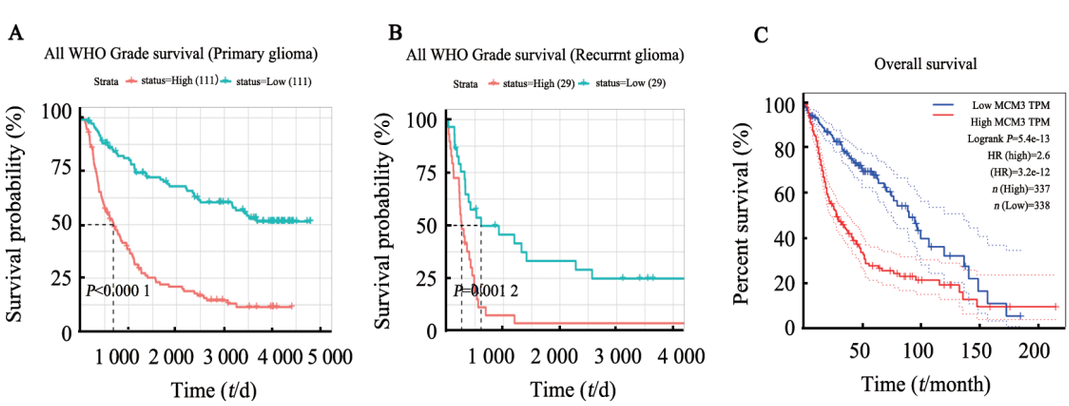
本研究联合多个在线数据库中的胶质瘤队列数据对胶质瘤中MCM3的表达和临床意义进行分析发现，与非肿瘤组织相比，MCM3在胶质瘤组织中呈高表达。CGGA数据库分析显示，MCM3表达与胶质瘤的级别有关，MCM3表达越高，胶质瘤级别越高，且MCM3高表达更易发生在IDH野生型和高年龄的胶质瘤患者中。但临床标本验证结果显示，MCM3表达仅与胶质瘤病理级别相关，究

其原因，可能是样本量较小导致的偏差，后续期待更多实验验证。生存分析显示，MCM3 高表达提示不良预后，该结论可在 CGGA 和 TCGA 数据库中得到验证。通过 MCM3 与其 50 个共表达基因构建 PPI 网络，并以 MCM3 为中心节点筛选出其显著相关基因 23 个，其中 15 个基因在胶质瘤中异常表达且都与胶质瘤预后有关，这些基因集中在细胞周期、DNA 复制和损伤调节等方面。相关文献证明，细胞周期的调控和 DNA 修复是胶质瘤发生进展的重要原因。比如 miRNA 对恶性神经胶质瘤细胞周期的控制已得到公认。miRNA 的失调有助于肿瘤增殖[34]。PEDERSEN 等[35]发现，在 GBM 中，GBM 癌症干细胞样细胞（GBM cancer stem-like cell，GSC）显示出优异的 DNA 修复能力，从而保护其免受电离辐射或替莫唑胺化疗等治疗方法的影响。MCM3 及其相关分子也可能基于对细胞周期和 DNA 损伤修复的调控影响胶质瘤的进展。SÖLING 等[36]发现，MCM3 在人星形细胞肿瘤中过度表达，并且其在一部分脑肿瘤和脑转移瘤患者中引起癌症限制性体液免疫反应，可能是胶质瘤的相关抗原。本研究发现，在胶质瘤中 MCM3 表达与多种免疫细胞浸润相关。提示 MCM3 可能通过影响免疫浸润细胞参与胶质瘤微环境的免疫应答。相关结论有待进一步验证。

表 2　MCM3 表达水平与胶质瘤临床病理特征的相关性

Tab.2　Correlation between MCM3 expression level and clinicopathological characteristics of glioma

Clinicopathological characteristics	Case	Expression of MCM3		χ^2	*P*
		High	Low		
Gender					
Male	11	7	4	0.011	0.916
Female	13	8	5		
Age (*t*/a)					
＜42	11	5	6	2.517	0.113
≥42	13	10	3		
Pathological grade					
Ⅰ/Ⅱ	11	3	8	10.752	0.001
Ⅲ/Ⅳ	13	12	1		
IDH status					
Wild	10	6	4	0.046	0.831
Mutant	14	9	5		

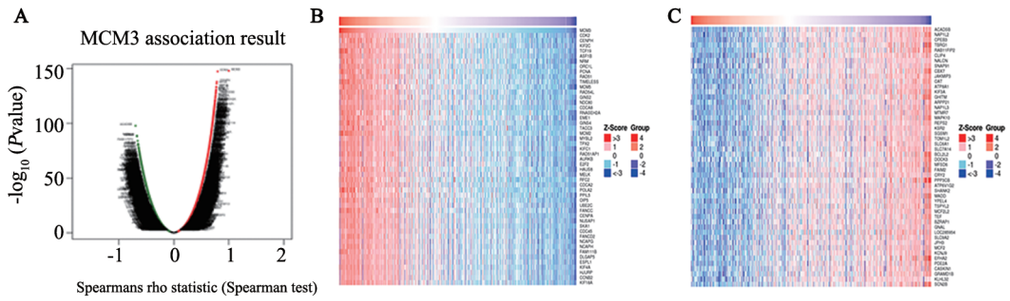


A, B: The higher the expression of MCM3 in the CGGA database, the worse the survival rate of glioma patients;
C: The higher the expression of MCM3 in the TCGA database, the worse the survival rate of glioma patients

图 4　CGGA 和 TCGA 数据库中 MCM3 表达与胶质瘤生存预后关系

Fig.4　The relationship between the expression of MCM3 in CGGA and TCGA databases and the prognosis of glioma

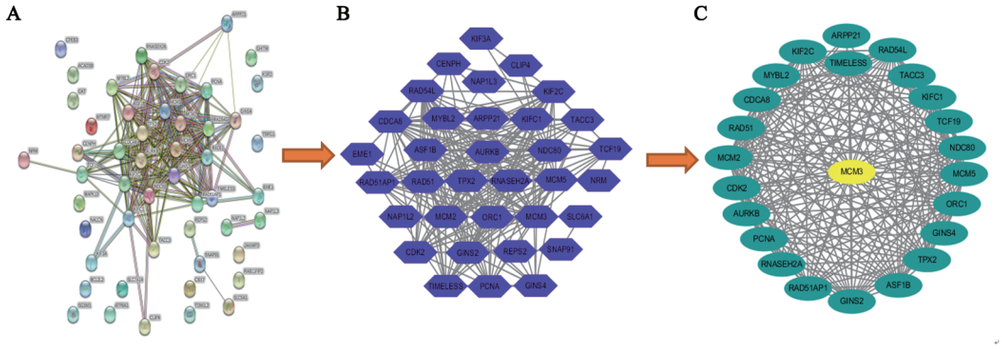
综上所述，MCM3 在胶质瘤中高表达且与临床病理特征和不良预后相关，显示 MCM3 可能成为临床上有用的胶质瘤生物标志物。



A: Spearman test analysis of the correlation between MCM3 and other genes in the TCGA glioma cohort; B: Genes positively related to MCM3 expression; C: Genes negatively related to MCM3 expression

图5 Linkedomic数据库中与MCM3正负共表达的相关基因

Fig.5 Related genes co-expressed with MCM3 positive and negative in the Linkedomic database



A, B: PPI network of MCM3 and its first 50 related genes; C: 23 significant MCM3 related genes screened out with MCM3 as the central node in the PPI network

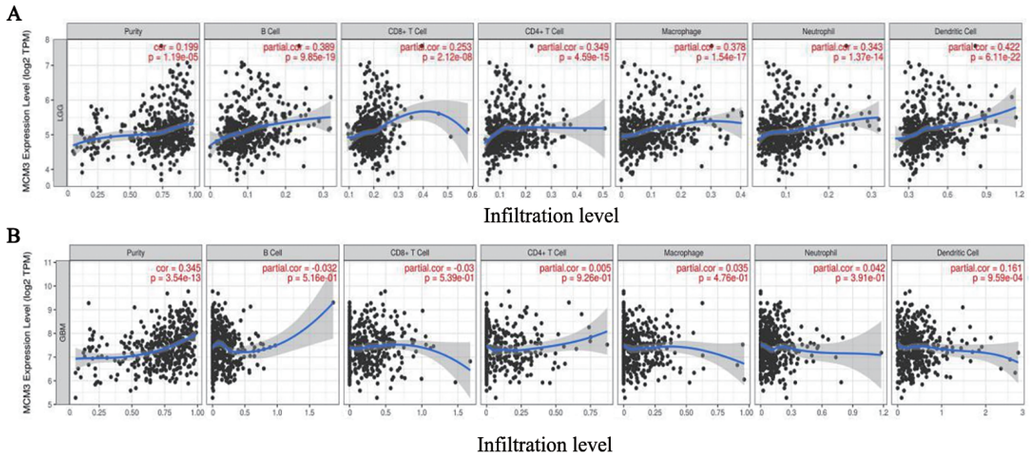
图6 MCM3及其相关基因的PPI网络

Fig.6 PPI network of MCM3 and related genes

表3 MCM3及其显著相关基因的GO和KEGG分析

Tab.3 GO and KEGG analysis of MCM3 and its significant related genes

Category	Term	Count	Percentage of genes (%)	*P*
GOTERM_BP_DIRECT	GO:0006260~DNA replication	10	20	<0.01
GOTERM_BP_DIRECT	GO:0051301~cell division	9	18	<0.01
GOTERM_BP_DIRECT	GO:0006270~DNA replication initiation	4	8	<0.01
GOTERM_BP_DIRECT	GO:0000082~G1/S transition of mitotic cell cycle	5	10	<0.01
GOTERM_BP_DIRECT	GO:0007062~sister chromatid cohesion	5	10	<0.01
GOTERM_CC_DIRECT	GO:0005654~nucleoplasm	22	44	<0.01
GOTERM_CC_DIRECT	GO:0015630~microtubule cytoskeleton	6	12	<0.01
GOTERM_CC_DIRECT	GO:0000790~nuclear chromatin	6	12	<0.01
GOTERM_CC_DIRECT	GO:0042555~MCM complex	3	6	<0.01
GOTERM_CC_DIRECT	GO:0000784~nuclear chromosome, telomeric region	5	10	<0.01
GOTERM_MF_DIRECT	GO:0005524~ATP binding	14	28	<0.01
GOTERM_MF_DIRECT	GO:0005515~protein binding	38	76	<0.01
GOTERM_MF_DIRECT	GO:0003678~DNA helicase activity	3	6	<0.01
GOTERM_MF_DIRECT	GO:0043138~3'-5' DNA helicase activity	2	4	<0.05
GOTERM_MF_DIRECT	GO:0003777~microtubule motor activity	3	6	<0.05
KEGG_PATHWAY	hsa03030:DNA replication	5	10	<0.01
KEGG_PATHWAY	hsa04110:Cell cycle	5	10	<0.01
KEGG_PATHWAY	hsa03440:Homologous recombination	3	6	<0.01



A: MCM3 expression in the LGG cohort is related to B cells, $CD8^+$ T cells, $CD4^+$ T cells, dendritic cells, neutrophils and macrophages; B: MCM3 expression in the GBM cohort is related to dendritic cells

图7 TIMER数据库胶质瘤队列中MCM3表达与不同免疫浸润细胞的关系

Fig.7 Relationship between MCM3 expression and different immune infiltrating cells in the TIMER database glioma cohort

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