四川大学学报（医学版） 2024，55（4）：893－901
J Sichuan Univ（Med Sci） doi: 10.12182/20240760205

在线全文

人母系蛋白3参与胃癌细胞恶性行为且与患者不良预后相关*

李 靖，喻大军，陈少华，谢 波△，汪 虎
蚌埠医科大学第一附属医院 肿瘤外科（蚌埠 233000）

【摘要】 **目的** 探索胃癌患者人母系蛋白3（MATN3）表达的临床意义及其致病机制。**方法** 收集2022年1月－2022年12月蚌埠医科大学第一附属医院肿瘤外科收治的胃癌患者100例，采用免疫组化法检测胃癌组织和癌旁组织的MATN3表达情况，比较各病理特征、化疗耐药与胃癌组织MATN3表达水平的关系。利用Kaplan-Meier生存曲线分析MATN3与胃癌患者无复发生存期（recurrence-free survival, RFS）和总生存期（overall survival, OS）的关系，Cox等比例风险回归分析影响胃癌患者预后的因素。转染*MATN3*至人胃癌细胞MGC803，将细胞分为高表达组（LV-*MATN3*组）及其对照（LV-NC组），低表达组（sh-*MATN3*组）及其对照（sh-NC组），CCK8法检测细胞增殖，Transwell实验检测细胞的迁移和侵袭，RT-qPCR检测*MATN3* mRNA表达水平。将转染*MATN3*的MGC-803细胞注射到裸鼠皮下，构建移植瘤模型，RT-qPCR检测肿瘤组织中*MATN3* mRNA表达水平。**结果** 免疫组化结果显示胃癌组织MATN3高表达率〔64.00%（64/100）〕高于癌旁组织〔31.00%（31/100）〕（*P*<0.05）。MATN3高表达与年龄≥60岁、肿瘤部位胃体（主体胃）、肿瘤直径≥5 cm、淋巴结转移（N1～N3）、组织学分化（中高分化）、肿瘤浸润深度（T3～T4）、TNM分期（Ⅲ～Ⅳ）、远处器官有转移、有复发和死亡因素有关（*P*<0.05）。化疗耐药组MATN3高表达率为79.49%（31/39），高于化疗敏感组的54.10%（33/61）（*P*<0.05）。随访11～22个月，100例胃癌患者中有3例失访，随访率97.00%。生存曲线分析显示，MATN3高表达患者的RFS和OS低于MATN3低表达患者（*P*均<0.001）。Cox等比例风险回归多因素分析显示，MATN3高表达〔风险比（HR）=2.291，95%置信区间（CI）：1.268～5.392〕、肿瘤部位主体胃（HR=2.057，95%CI：1.441～5.666）、淋巴结转移N1-N3（HR=2.011，95%CI：1.010～2.274）、肿瘤浸润深度T3～T4（HR=2.977，95%CI：1.032～7.853）、TNM分期Ⅲ～Ⅳ（HR=2.008，95%CI：1.049～3.902）和远处器官有转移（HR=2.505，95%CI：1.529～5.000）均为影响胃癌患者RFS和OS的独立危险因素（*P*<0.05）。细胞和动物实验结果显示，与LV-NC组比较，LV-*MATN3*组细胞增殖、迁移和侵袭能力明显升高（*P*<0.05），肿瘤体积以及肿瘤组织中*MATN3* mRNA表达水平升高（*P*<0.05）；与sh-NC组比较，sh-*MATN3*组细胞增殖、迁移和侵袭能力降低（*P*<0.05），肿瘤体积缩小（*P*<0.05），肿瘤组织中*MATN3* mRNA表达水平降低（*P*<0.05）。**结论** MATN3参与胃癌细胞恶性行为，在胃癌组织高表达，且与病理特征、耐药性和患者不良预后相关。

【关键词】 免疫组化法 MATN3 胃癌 病理特征 耐药 预后 关系

Relationship Between the Expression of Human Matricellular Protein 3 and the Pathological Features, Drug Resistance, and Prognosis of Gastric Cancer Based on Immunohistochemical Method

LI Jing, YU Dajun, CHEN Shaohua, XIE Bo△*, WANG Hu. Department of Surgical Oncology, The First Affiliated Hospital of Bengbu Medical University*, Bengbu 233000, China
△ Corresponding author, E-mail: xb19821127@163.com

【**Abstract**】 **Objective** To observe the relationship between the expression of human matricellular protein 3 (MATN3) and the pathological features, drug resistance, and prognosis of gastric cancer based on immunohistochemical method. **Methods** A total of 100 gastric cancer patients treated at the First Affiliated Hospital of Bengbu Medical College from January 2022 to December 2022 were included. MATN3 expression in gastric cancer tissues and paracancerous tissues was assessed by immunohistochemistry. The expression of MATN3 was compared across pathological features. Patients were divided into sensitive and resistant groups based on chemotherapy resistance, and MATN3 expression was compared between these groups. The relationship between MATN3 and recurrence-free survival (RFS) and overall survival (OS) of gastric cancer patients was analyzed using Kaplan-Meier survival curves. Univariate and multifactorial Cox regression analyses were used to analyze the factors affecting the prognosis of gastric cancer patients. Human gastric cancer cells MGC803 were transfected with *MATN3*. The cells were divided into a high expression group (LV-*MATN3* group) and its control group (LV-NC group) and a low expression group (sh-*MATN3* group) and its control group (sh-NC group). Cell proliferation was assessed using the CCK8 assay, cell migration and invasion were assessed using the Transwell assay, and *MATN3* mRNA expression levels were measured using RT-qPCR. A nude mouse xenograft model was constructed by hypodermic injection of MGC-803 cells transfected with *MATN3*, and *MATN3* mRNA expression levels in tumor tissues were measured using RT-qPCR. **Results** Immunohistochemical results showed a significantly

* 安徽省高校自然科学研究重点项目（No. KJ2021A0729）和蚌埠医科大学自然科学重点项目（No. 2022byzd029）资助
△ 通信作者，E-mail: xb19821127@163.com
出版日期：2024-07-20

higher rate of high MATN3 expression in gastric cancer tissues (64.00%, 64/100) compared to adjacent non-cancerous tissues (31.00%, 31/100) (P<0.05). High MATN3 expression was associated with age ⩾60 years old, tumor location in the gastric body, tumor size ⩾5 cm, lymph node metastasis (N1-N3), histological differentiation (moderate to high), tumor invasion depth (T3-T4), TNM stage (Ⅲ-Ⅳ), distant organ metastasis, recurrence, and mortality (P<0.05). Among patients with chemotherapy resistance, the high MATN3 expression rate was 79.49% (31/39) in the resistant group compared to 54.10% (33/61) in the sensitive group (P<0.05). Follow-up duration ranged from 11 to 22 months, with a 97.00% follow-up rate and 3 cases lost to follow-up. Kaplan-Meier survival curve analysis showed that patients with high MATN3 expression had significantly lower RFS and OS compared to those with low MATN3 expression (RFS: log-rank=17.291, P<0.001; OS: log-rank=21.719, P<0.001). Multivariate Cox analysis identified high MATN3 expression (hazard ratio [HR]=2.291, 95% confidence interval [CI]: 1.268-5.392), tumor location in the gastric body (HR=2.057, 95% CI: 1.441-5.666), lymph node metastasis (N1-N3) (HR=2.011, 95% CI: 1.010-2.274), tumor invasion depth (T3-T4) (H=2.977, 95% CI: 1.032-7.853), TNM stage Ⅲ-Ⅳ (HR=2.008, 95% CI: 1.049-3.902), and distant organ metastasis (HR=2.505, 95% CI: 1.529-5.000) as independent risk factors affecting RFS and OS (P<0.05). Cell and animal experiments demonstrated that compared to the LV-NC group, the LV-*MATN3* group exhibited significantly higher cell proliferation, migration, and invasion (P<0.05), as well as increased tumor volume and *MATN3* mRNA expression in tumor tissues (P<0.05). Conversely, the sh-*MATN3* group showed significantly reduced cell proliferation, migration, and invasion, along with decreased tumor volume and *MATN3* mRNA levels compared to the sh-NC group (P<0.05). **Conclusion** MATN3 is highly expressed in gastric cancer tissues and is associated with various pathological features, drug resistance and poor prognosis. MATN3 holds potential as a diagnostic marker for poor prognosis and may play a role in the malignant behaviors of gastric cancer cells, including proliferation, migration, and invasion.

【Key words】 Immunohistochemistry MATN3 Gastric cancer Pathological features Drug resistance Prognosis Relationship

胃癌是临床发病率较高的消化道恶性肿瘤，占全身恶性肿瘤的10%～15%[1]。胃癌早期治疗接受根治性手术或术后辅助化疗，术后5年生存率在90%以上[2-3]。化学疗法是目前中晚期胃癌患者的主要治疗方式，但化疗耐药仍是中晚期胃癌患者实现最佳预后的主要临床障碍[4]。因此寻找一种可评价胃癌病理特征及预后的生物标志物，有助于指导治疗方案，改善患者预后。人母系蛋白3基因（martrilin-3，*MATN3*）是一种蛋白质编码基因，主要存在于原代软骨细胞，可编码含血管假性血友病因子A结构域成员的蛋白质家族，参与了细胞外基质的丝状网络的形成，还可参与细胞增殖及分化过程[5]，*MATN3*突变与常见的骨病和罕见的软骨发育不良有关[6]。此外，一些研究报告了几种肿瘤中MATN3的明显失调，例如胰腺导管腺癌和骨肉瘤[7]。既往已有研究发现，MATN3在胃癌中的表达具有临床预后意义[8-9]。但关于MATN3对胃癌患者的临床病理特征分析并不全面，且暂无研究探讨其与化疗耐药的关系。本研究通过探讨MATN3与胃癌病理特征、化疗耐药及预后的关系，并进一步行体外、体内实验，旨在为胃癌的诊断、治疗和预后评估提供新的思路。

1 资料与方法

1.1 实验设计

本实验首先回顾性分析患者临床病理资料，采用免疫组化法检测胃癌组织和癌旁组织的MATN3表达情况，分析MATN3与胃癌病理特征、化疗耐药及预后的关系。然后构建*MATN3*基因高表达/低表达胃癌细胞，CCK8法检测细胞增殖，Transwell实验检测细胞的迁移和侵袭，RT-qPCR检测*MATN3* mRNA表达水平；用转染*MATN3*的胃癌细胞构建鼠移植瘤模型，RT-qPCR检测肿瘤组织中*MATN3* mRNA表达水平。

1.2 患者资料分析

回顾性分析临床资料，纳入2022年1月–2022年12月蚌埠医科大学第一附属医院肿瘤外科收治的胃癌患者100例，纳入标准：①确诊时无远处转移的原发性胃癌，诊断标准参考《胃癌诊疗规范（2011年版）》[10]，经病理学检查再次确诊；②均于我院完成胃癌根治术并接受奥沙利铂+替吉奥联合化疗方案；③临床资料完整无流失；④均为首次诊断，既往未行相关治疗；⑤患者年龄在18～85岁之间。排除标准：①合并其他恶性肿瘤；②为哺乳期或妊娠期妇女；③合并重要器官严重功能障碍；④Fish检测确诊Her-2阳性的患者。本研究操作均经医院伦理会批准同意，批准号伦科批字2023第073号。收集胃癌患者临床病理资料，分析各病理特征与MATN3表达的关系。

1.2.1 免疫组化染色

取胃癌患者的胃癌组织和癌旁组织（距离胃癌肿块>

5 cm, 经确定为无癌组织), 制备石蜡切片, 采用免疫组化法检测MATN3表达情况。其中MATN3抗体购自abcam公司。将石蜡切片脱蜡至水, 经修复液和双氧水溶液干预后, 血清封闭30 min。取出石蜡切片, 加入一抗MATN3(1∶200)4 ℃孵育过夜, 加入与一抗相应种属的二抗(1∶10000)室温孵育50 min, 经DAB显色, 复染细胞核后脱水封片。MATN3阳性主要位于细胞质和细胞外基质中, 呈黄色或棕黄色, 阅片时采用免疫反应评分(immune response score, IRS)进行判断, IRS评分范围0 ~ 12分, 其中IRS≤4分为MATN3低表达, IRS>4分为MATN3高表达。

1.2.2 耐药敏感患者分组

术后化疗方案: 采用奥沙利铂+替吉奥联合化疗6周期, 21 d/周期。参考文献[11-12]并根据实体瘤疗效评价标准和有无复发情况, 将患者分为2组: 停止化疗后>6个月复发或不复发的患者纳入敏感组; 术后化疗期间出现复发或停止化疗后<6个月复发的患者纳入耐药组。基于免疫组化染色结果比较两组MATN3表达情况。

1.2.3 随访

每月采用电话或挂号等方式进行随访, 随访11 ~ 22个月, 截至日期2023年10月, 中位时间16个月, 在此期间收集到97.00%(97/100)的患者完整信息, 失访的3例做删失值处理, 不纳入后续分析。基于免疫组化染色结果, 比较MATN3高表达和MATN3低表达患者无复发生存期(recurrence-free survival, RFS)和总生存期(overall survival, OS)。

1.3 细胞实验

1.3.1 构建*MATN3*基因高表达和低表达胃癌细胞

将人胃癌细胞MGC803(南京科佰生物科技有限公司)接种于6孔板中, 放入37 ℃、体积分数5%CO_2培养箱中培养至细胞密度达到80%以上。将已培养好的MGC803细胞用无血清培养基洗涤后, 加入1 mL无血清培养基, 使用Lipofectamine 3000试剂(美国Thermo Fisher)转染过表达*MATN3*载体(LV-*MATN3*)和对照空载体(LV-NC)、干扰*MATN3*载体(sh-*MATN3*)和对照空载体(sh-NC)(上海汉恒生物科技有限公司), 采用RT-qPCR验证转染效率, 将转染成功的细胞使用嘌呤霉素筛选稳定表达的细胞株。将细胞分为高表达组(LV-*MATN3*组)及其对照(LV-NC组), 低表达组(sh-*MATN3*组)及其对照(sh-NC组)。

1.3.2 CCK8检测

取对数生长期、转染后的MGC803细胞, 以每孔1×10^4细胞接种至96孔细胞培养板中, 置于培养箱中培养0、24、48、72 h, 另设置无细胞空白(调零孔)组。培养板中每孔加入10 μL CCK8溶液(美国MCE), 室温避光孵育2 h。采用酶标仪于450 nm处光密度(OD)值, 确定细胞增殖活性。

1.3.3 Transwell迁移和侵袭实验

将已进行血清饥饿的MGC803细胞进行消化、重悬和计数, 调整细胞悬液浓度至1×10^5 mL^{-1}。迁移实验是将MGC803细胞悬液接种到Transwell小室内, 而侵袭实验是将MGC803细胞悬液接种至已被matrigel胶包被的Transwell小室内, 置于37 ℃培养箱中培养48 h, 加入多聚甲醛溶液, 室温固定细胞, 加入结晶紫溶液, 染色清洗晾干。揭膜, 封片, 置于200× Qimaging Micropublisher 5.0 RTV显微镜(日本Olympus)下观察, 计数每个视野中的细胞数。

1.3.4 RT-qPCR检测

各组细胞采用Trizol(美国Thermo Fisher)提取总RNA, 测定RNA浓度和纯度后, 使用cDNA第一链合成试剂盒(上海碧云天生物试剂有限公司)合成RNA。使用南京诺唯赞生物科技股份有限公司的AceQ Universal SYBR qPCR Master Mix试剂盒进行RT-qPCR实验。在CFX96实时荧光定量PCR仪器(美国Bio-rad)上进行PCR扩增。引物序列由上海生工生物工程股份有限公司设计和提供, *MATN3*上游引物5′-GCCTTTGGACTTGGTGTTCAT-3′, 下游引物5′-TCTGGAATTCACCAAGGTGAA-3′; *GAPDH*上游引物5′-CATGTTGCAACCGGGAAGGA-3′, 下游引物5′-GCGCCCAATACGACCAAATCAGA-3′。以*GAPDH*为内参, 使用$2^{-\Delta\Delta Ct}$计算各组*MATN3* mRNA表达水平。

1.4 裸鼠移植瘤模型构建和检测

将6周龄、体质量18 ~ 20 g的BALB/c-nude裸鼠(江苏集萃药康生物科技股份有限公司)饲养于无特定病原菌动物级环境, 随机分为LV-NC组、LV-*MATN3*组、sh-NC组和sh-*MATN3*组, 每组6只, 共24只, 每组体质量纳入范围为18 ~ 20 g, 各组体质量相当(P>0.05)。各组裸鼠均在右侧腋下皮下注射0.1 mL对应组别细胞悬液(浓度为1×10^7 mL^{-1}), 于接种后第3天、第7天、第10天、第14天、第17天和第21天观察并记录肿瘤体积。于接种后第21天处死裸鼠, 剥离肿瘤, 并拍照记录。采用RT-qPCR检测肿瘤组织中*MATN3* mRNA表达水平, 方法同1.3.4小节。本动物实验方案已通过蚌埠医学院动物伦理委员会批准, 批准号伦动科批字[2022]第283号。

1.5 统计学方法

本实验数据采用SPSS20.0软件分析, 计数资料以

$\bar{x}\pm s$为主，组间比较采用两独立样本t检验，不满足正态分布采用中位数(四分位间距)表示，采取Kruskal-Wallis H秩和检验。计数资料组间比较采用χ^2检验。绘制Kaplan-Meier生存曲线分析MATN3与胃癌患者RFS和OS的关系，log-rank检验比较存活率。将与MATN3有关的指标通过Cox等比例风险回归分析确定影响胃癌患者RFS和OS的因素。多重共线性检验：对于容差>0.1及方差膨胀因子(variance inflation factor, VIF)<10可认为不具有多重共线性。P<0.05为差异有统计学意义。

2 结果

2.1 胃癌各组织MATN3表达情况比较

胃癌组织、癌旁组织MATN3高表达率分别为64.00%(64/100)、31.00%(31/100)，而胃癌组织、癌旁组织MATN3低表达率分别为36.00%(36/100)、69.00%(69/100)，其中胃癌组织MATN3高表达率高于癌旁组织(χ^2=21.835, P<0.05)。见图1。

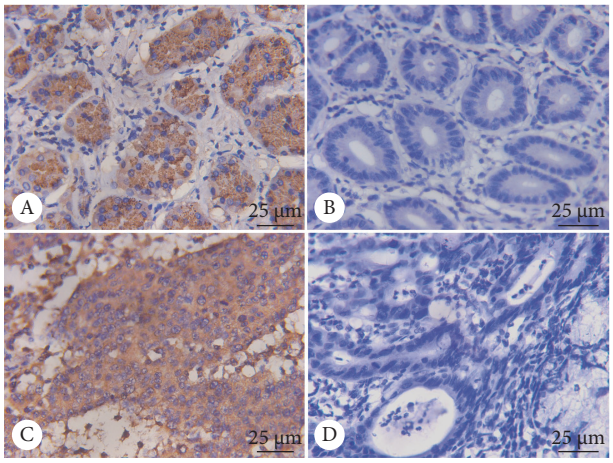

图 1 免疫组化法观察胃癌各组织MATN3表达情况比较

Fig 1 Comparison of MATN3 expression in various tissues of gastric cancer observed by Immunohistochemistry

A, MATN3 is highly expressed in paracancerous tissues; B, MATN3 is lowly expressed in paracancerous tissues; C, MATN3 is highly expressed in cancer tissues; D, MATN3 is lowly expressed in cancer tissues.

2.2 MATN3表达情况与胃癌病理特征参数关系分析

见表1～表2。年龄≥60岁、肿瘤部位胃体(主体胃)、肿瘤直径≥5 cm、淋巴结转移越多(N1～N3)、组织学分化越差(趋向于中高分化)、肿瘤浸润深度越深(T3～T4)、TNM分期越高、远处器官有转移、有复发、死亡，MATN3表达明显呈高表达(P<0.05)，且上述指标之间不存在共线性(容差均>0.1, VIF均<10)，可进一步纳入回归分析。而MATN3表达与性别、脉管癌栓、切胃方式、根治性术式和病理类型无关(P>0.05)。胃癌出现远处器官转移40例，其中肝转移19例，肺转移12例，骨转移2例，肾转移6例，脑转移1例，主要以肝、肺转移为主。

2.3 不同MATN3表达胃癌患者耐药分析

61例敏感组MATN3高表达率和低表达率分别为54.10%(33/61)和45.90%(28/61)，39例耐药组MATN3高表达率和低表达率分别为79.49%(31/39)和20.51%(8/39)，耐药组MATN3高表达率高于敏感组，差异有统计学意义(χ^2=6.656, P<0.05)。

2.4 MATN3表达在胃癌患者中的预后价值

随访100例胃癌患者，3例失访，随访率97.00%。Kaplan-Meier生存分析结果显示，MATN3高表达患者RFS和OS分别为46.88%(30/64)和78.13%(50/64)，MATN3低表达患者RFS和OS分别为77.78%(28/36)和94.44%(34/36)。MATN3高表达患者的RFS和OS低于MATN3低表达患者(RFS: log-rank=17.291, P<0.001; OS: log-rank=21.719, P<0.001)。见图2。提示MATN3阳性表达会引起胃癌患者的不良预后。Cox单因素回归分析结果显示MATN3高表达、年龄≥60岁、肿瘤部位胃体(主体胃)、肿瘤直径≥5 cm、淋巴结转移(N1～N3)、组织学分化(中高分化)、肿瘤浸润深度(T3～T4)、TNM分期(Ⅲ～Ⅳ)和远处器官有转移均为影响胃癌患者RFS和OS的危险因素(P<0.05)。Cox多因素回归分析结果显示，MATN3高表达、肿瘤部位胃体(主体胃)、淋巴结转移(N1～N3)、肿瘤浸润深度(T3～T4)、TNM分期(Ⅲ～Ⅳ)和远处器官有转移均为胃癌患者RFS和OS的独立危险因素(P<0.05)。见表3～表4。

2.5 *MATN3*表达对胃癌细胞增殖、迁移和侵袭的影响

RT-qPCR结果显示，过表达*MATN3*基因的LV-*MATN3*和干扰*MATN3*基因的sh-*MATN3*已成功转染至胃癌细胞。CCK-8结果显示，与LV-NC组比较，LV-*MATN3*组细胞增殖能力升高(P<0.05)；与sh-NC组比较，sh-*MATN3*组细胞增殖能力降低(P<0.05)。迁移和侵袭实验显示，与LV-NC组比较，LV-*MATN3*组细胞迁移和侵袭能力升高(P<0.05)；与sh-NC组比较，sh-*MATN3*组细胞迁移和侵袭能力降低(P<0.05)。见图3。

2.6 *MATN3*表达对裸鼠胃癌成瘤能力的影响

RT-qPCR结果显示，与LV-NC组比较，LV-*MATN3*组肿瘤组织中*MATN3* mRNA表达水平升高(P<0.05)；与sh-NC组比较，sh-*MATN3*组肿瘤组织中*MATN3* mRNA表达水平降低(P<0.05)。裸鼠皮下成瘤实验显示，与LV-NC组比较，LV-*MATN3*组肿瘤体积增大(P<0.05)；与sh-NC组比较，sh-*MATN3*组肿瘤体积缩小(P<0.05)。见图4。

表 1　MATN3表达情况与胃癌病理特征参数关系分析

Table 1　Analysis of the relationship between MATN3 expression status and parameters of pathological features of gastric cancer

Factor	MATN3/case (%)		χ^2	P
	High expression (n=64)	Low expression (n=36)		
Age			10.982	<0.001
<60 yr.	25 (25.00)	21 (58.33)		
⩾60 yr.	48 (75.00)	15 (41.67)		
Sex			0.992	0.319
Male	42 (65.63)	20 (55.56)		
Female	22 (34.88)	16 (44.44)		
Tumor location			16.039	<0.001
Cardia (upper stomach)	15 (23.44)	18 (50.00)		
Gastric body (main stomach)	37 (57.81)	6 (16.67)		
Gastric antrum pylorus (lower stomach)	12 (18.75)	12 (33.33)		
Tumor diameter			11.111	0.001
<5 cm	24 (37.50)	26 (72.22)		
⩾5 cm	40 (62.50)	10 (27.78)		
Histological subdivision			36.043	<0.001
Low polarization	11 (17.19)	25 (69.44)		
Middle polarization	20 (31.25)	11 (30.56)		
High polarization	33 (51.56)	0 (0.00)		
Lymphatic node transfer			58.924	<0.001
N0	3 (4.69)	26 (72.22)		
N1	21 (32.81)	10 (27.78)		
N2	25 (39.06)	0 (0.00)		
N3	15 (23.44)	0 (0.00)		
Tumor infiltration depth			48.214	<0.001
T1	4 (6.25)	21 (58.33)		
T2	20 (31.25)	15 (41.67)		
T3	23 (35.94)	0 (0.00)		
T4	17 (26.56)	0 (0.00)		
Vascular thrombosis			0.188	0.665
No	30 (46.88)	19 (52.78)		
Yes	34 (53.13)	17 (47.22)		
Stomach cutting method			2.068	0.150
Partial excision	26 (40.64)	20 (55.56)		
Total gastrectomy	38 (59.38)	16 (44.44)		
Radical operation			3.242	0.198
D1	10 (15.63)	9 (25.00)		
D2	42 (65.63)	17 (47.22)		
D3	12 (18.74)	10 (27.78)		
TNM stage			55.415	<0.001
Ⅰ	2 (3.13)	23 (63.89)		
Ⅱ	24 (37.50)	13 (36.11)		
Ⅲ	20 (31.25)	0 (0.00)		
Ⅳ	18 (28.13)	0 (0.00)		
Pathological type			1.487	0.685
Indocyte carcinoma	11 (17.19)	9 (25.00)		
Adenocarcinoma	39 (60.94)	22 (61.11)		
Squamous carcinoma	6 (9.38)	2 (5.56)		
Other types	8 (12.50)	3 (8.33)		
Distant organ metastasis			12.760	<0.001
No	30 (46.88)	30 (83.33)		
Yes	34 (53.13)	6 (16.67)		
Relapse			9.032	0.003
No	30 (46.88)	28 (77.78)		
Yes	34 (53.12)	8 (22.22)		
Death			6.157	0.010
No	50 (78.13)	34 (94.44)		
Yes	14 (21.88)	2 (5.56)		

表 2 MATN3高表达与其他因素的多重共线性检验

Table 2 Multicollinearity test of MATN3 high expression and other factors

Factor	Tolerance	VIF
Age≥60 yr.	0.595	2.766
Tumor site in the gastric body (main body stomach)	0.792	1.321
Tumor diameter≥5 cm	0.659	2.669
Histological subdivision (middle and high polarization)	0.317	2.668
Lymphatic node transfer (N1-N3)	0.465	2.468
Tumor infiltration depth (T3-T4)	0.421	2.942
TNM stage (Ⅲ-Ⅳ)	0.714	1.850
Distant organ metastasis (yes)	0.621	1.529
Relapse (yes)	0.704	1.971
Death (yes)	0.422	2.060

VIF: variance inflation factor.

3 讨论

MATN3是细胞外基质蛋白家族成员，对晚期胃癌患者具有诊断和预后价值[13]。JIA等[14]研究显示，MATN3可预测胃癌的总生存期、无病生存期和药物敏感性。本研究结果发现MATN3在胃癌组织高表达，提示MATN3可作为新的诊断、预后生物标记和治疗靶点。此外，本研究还发现MATN3表达与年龄、肿瘤部位、肿瘤直径、肿瘤浸润深度、TNM分期、淋巴结转移、组织学分化、远处器官有转移、有复发和死亡因素有关，为MATN3临床应用提供更多依据。

临床关于患者是否耐药主要是通过停用化疗药物的时间和肿瘤复发时间来确定，无法提前筛查出耐药患者[15]，因此发现能预测化疗耐药的靶点是目前研究的热点。本研究发现化疗耐药患者的MATN3高表达明显高

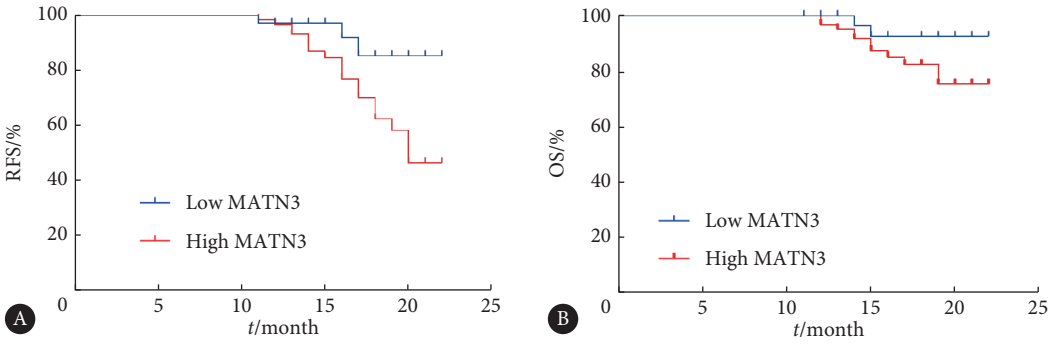

图 2 MATN3不同表达下胃癌患者的RFS（A）和OS（B）

Fig 2 RFS (A) and OS (B) of gastric cancer patients with different expressions of MATN3

RFS: recurrence-free survival; OS: overall survival; High expression (n=64) and low expression (n=36) at the initiate month.

表 3 Cox等比例风险回归分析影响胃癌患者RFS的危险因素

Table 3 Cox proportional hazards regression analysis of risk factors affecting RFS in gastric cancer patients

Factor	One-way			Multifactorial		
	HR	95% CI	P	HR	95% CI	P
MATN3 expression (high vs. low)	11.251	0.665-17.683	<0.001	2.291	1.268-5.392	<0.001
Age (≥60 yr. vs. <60 yr.)	7.014	0.600-14.694	0.004	–	–	–
Tumor site in the gastric body (main body stomach vs. other)	9.118	0.370-18.080	<0.001	2.057	1.441-5.666	0.029
Tumor diameter (≥5 cm vs. <5 cm)	6.606	0.845-8.052	<0.001	–	–	–
Lymphatic node transfer (N1-N3 vs. N0)	15.693	3.233-23.364	<0.001	2.011	1.010-2.274	0.035
Histological subdivision (middle and high polarization vs. low polarization)	37.071	1.179-42.377	<0.001	–	–	–
Tumor infiltration depth (T3-T4 vs. T1-T2)	21.773	9.084-30.001	<0.001	2.008	1.049-3.902	0.002
TNM stage (Ⅲ-Ⅳ vs. Ⅰ-Ⅱ)	24.020	11.202-29.994	0.008	2.977	1.032-7.853	0.007
Distant organ metastasis (yes vs. no)	16.053	9.138-25.770	<0.001	2.505	1.529-5.000	<0.001

HR: hazards ratio; CI: confidence interval.

表 4　Cox等比例风险回归分析影响胃癌患者OS的危险因素

Table 4　Cox proportional hazards regression analysis of risk factors affecting OS in gastric cancer patients

Factor	One-way			Multifactorial		
	HR	95% CI	*P*	HR	95% CI	*P*
MATN3 expression (high vs. low)	6.009	3.582-9.690	0.014	3.181	1.325-7.605	0.010
Age (≥60 yr. vs. <60 yr.)	3.111	1.007-10.185	0.030	–	–	–
Tumor site in the gastric body (main body stomach vs. other)	18.198	15.486-29.328	<0.001	1.219	1.041-1.428	0.014
Tumor diameter (≥5 cm vs. <5 cm)	2.866	0.830-4.209	0.039	2.376	1.223-4.617	0.051
Lymphatic node transfer (N1-N3 vs. N0)	16.596	10.204-23.623	0.004	4.933	1.397-7.427	0.028
Histological subdivision (middle and high polarization vs. low polarization)	30.747	19.333-34.816	0.018	–	–	–
Tumor infiltration depth (T3-T4 vs. T1-T2)	24.493	11.205-27.470	<0.001	2.098	1.744-3.345	0.001
TNM stage (Ⅲ-Ⅳ vs. Ⅰ-Ⅱ)	26.628	14.120-30.586	<0.001	3.841	3.135-6.119	0.001
Distant organ metastasis (yes vs. no)	13.110	7.483-22.038	0.012	2.014	1.005-1.022	0.002

HR: hazards ratio; CI: confidence interval.

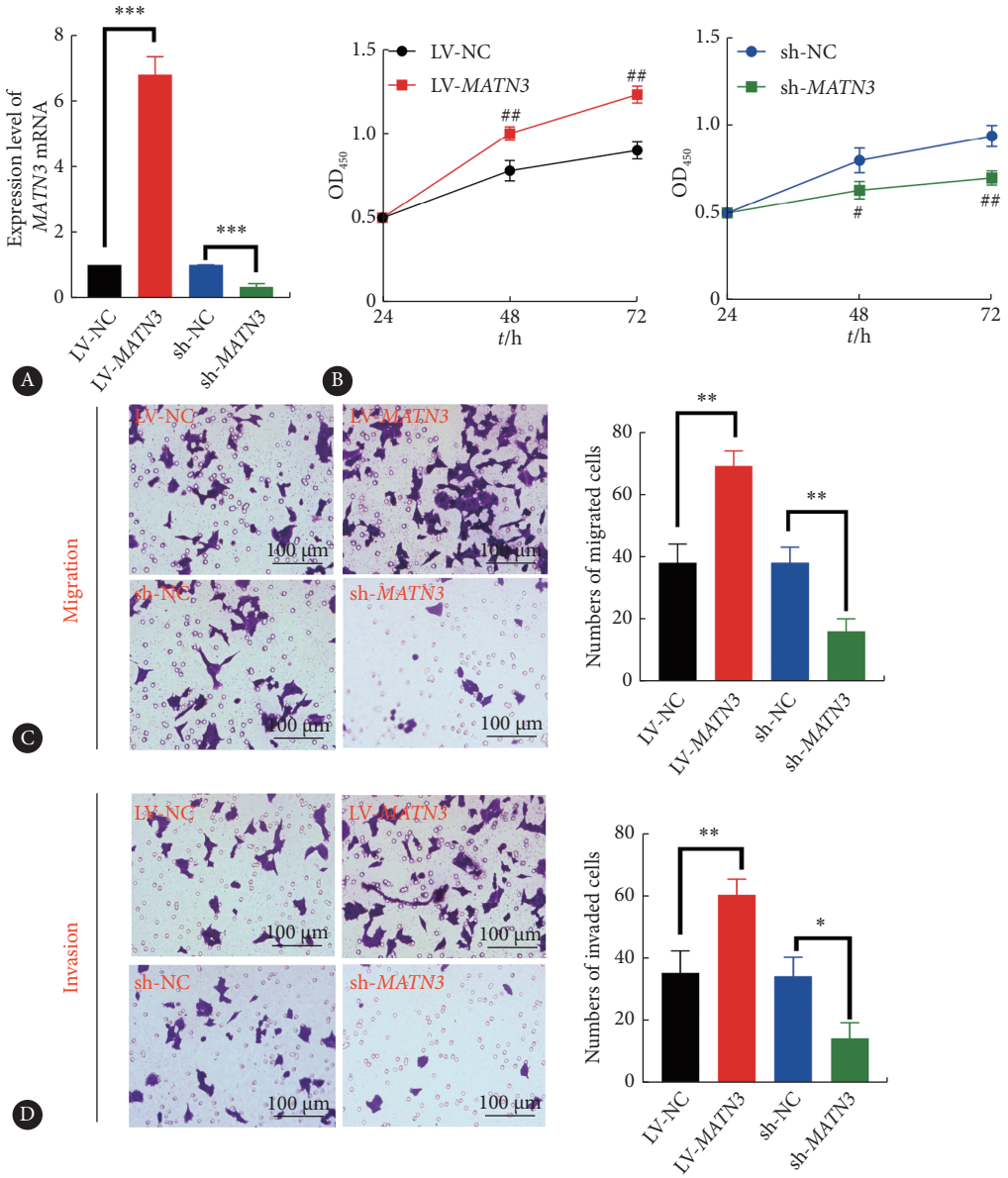

图 3　干扰或过表达*MATN3*基因对胃癌细胞增殖、迁移和侵袭的影响

Fig 3　Effects of *MATN3* gene interference or overexpression on proliferation, migration and invasion of gastric cancer cells

A, RT-qPCR to verify the expression level of *MATN3* mRNA in gastric cancer cells; B, CCK-8 to assess the proliferation ability of gastric cancer cells; C, gastric cancer cells in Transwell migration test; D, gastric cancer cells in Transwell invasion test. * P<0.05, ** P<0.01, *** P<0.001. $^{\#}$ P<0.05, $^{\#\#}$ P<0.01, vs. LV-NC or sh-NC. n=3.

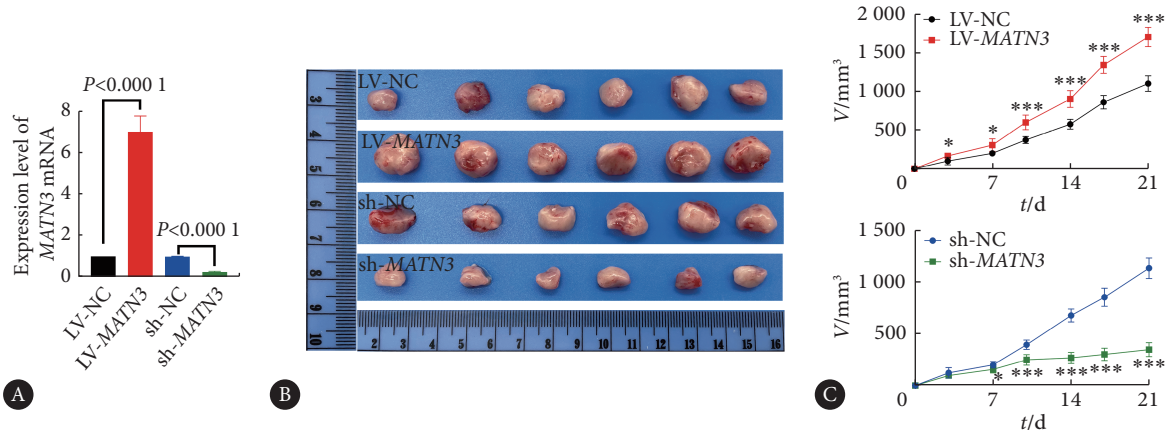

图 4 干扰或过表达*MATN3*基因对裸鼠胃癌成瘤能力的影响

Fig 4 Effect of *MATN3* gene disruption or overexpression on tumorigenicity of gastric cancer in nude mice

A, RT-qPCR to deterrmine the expression level of *MATN3* mRNA in tumor tissues; B, the appearance of tumor tissues; C, tumor volume curves in the nude mice of each group. * $P<0.05$, *** $P<0.001$, vs. LV-NC or sh-NC. $n=6$.

于化疗敏感患者，推测MATN3可能参与胃癌耐药的机制，意味着MATN3可能作为一种生物标志物，用于预测胃癌患者的化疗疗效。但具体作用机制暂不清楚，还待后续进行细胞耐药实验详细探讨。本研究通过分析MATN3与胃癌预后的关系，结果显示，MATN3高表达患者的RFS和OS明显低于MATN3低表达患者，且MATN3高表达、肿瘤部位主体胃、淋巴结转移N1～N3、肿瘤浸润深度T3～T4、TNM分期Ⅲ～Ⅳ期和远处器官有转移均为影响胃癌患者不良预后的独立危险因素。提示MATN3可能作为评估胃癌的预后指标。与LI等[16]研究结果相似，但LI等研究暂未发现肿瘤部位对不良预后的影响，本研究进行探讨发现胃体癌导致预后差，肿瘤部位、浸润深度和TNM分期是影响器官转移、复发或死亡的主要因素。本研究结果发现胃癌的远处器官转移以肝、肺为主，存在少量的骨、肾和脑转移。早期检测MATN3表达情况有助于预测患者预后情况，帮助制定针对性治疗方案，从而改善患者预后，另外，对MATN3的深入调查也可能阐述胃癌的发生、发展机制，对制定胃癌治疗策略具有一定价值。

本研究进一步进行体内外实验结果显示，过表达*MATN3*基因表达可显著升高胃癌细胞的增殖、迁移和侵袭等恶性行为，并促进胃癌肿瘤组织生长，即肿瘤细胞的恶性生物学行为参与癌症的发生发展，影响其预后；而干扰*MATN3*基因可降低胃癌细胞的增殖、迁移和侵袭能力，从而抑制肿瘤的生长。这给出了MATN3高表达在临床上加重胃癌疾病的致病机制的一个线索。

综上所述，胃癌组织MATN3呈异常高表达状态，且MATN3表达情况与胃癌病理特征、耐药、预后等存在一定联系，并可能参与胃癌细胞增殖、迁移和侵袭等恶性行为，检测MATN3可帮助指导治疗方案及评价预后。但由于研究对象来源于单中心，尚需多中心样本进行验证。本研究创新之处在于通过分析MATN3与胃癌病理特征、耐药及预后的关系，有助于判断患者的病情严重程度和预后，为临床治疗提供依据。研究结果提示通过抑制MATN3的表达或功能，可能有助于提高胃癌患者的化疗敏感性，改善患者的预后。并且抑制MATN3的表达可降低胃癌的增殖、迁移和侵袭能力。本研究还为今后深入研究MATN3在胃癌中的作用机制奠定基础，有望为胃癌的防治提供新的策略。

* * *

作者贡献声明 李靖负责论文构思、数据审编、正式分析、经费获取、提供资源、初稿写作和审读与编辑写作，喻大军负责经费获取、提供资源、监督指导和验证，陈少华负责研究方法，谢波负责研究项目管理和提供资源，汪虎负责调查研究和提供资源。所有作者已经同意将文章提交给本刊，且对将要发表的版本进行最终定稿，并同意对工作的所有方面负责。

Author Contribution LI Jing is responsible for conceptualization, data curation, formal analysis, funding acquisition, resources, writing--original draft, and writing--review and editing. YU Dajun is responsible for funding acquisition, resources, supervision, and validation. CHEN Shaohua is responsible for methodology. XIE Bo is responsible for project administration and resources. WANG Hu is responsible for investigation and resources. All authors consented to the submission of the article to the Journal. All authors approved the final version to be published and agreed to take responsibility for all aspects of the work.

利益冲突 所有作者均声明不存在利益冲突

Declaration of Conflicting Interests All authors declare no competing interests.

参 考 文 献

[1] 张露, 张薇, 尚高峰. 血清TK1、CEA、CA19-9检测在胃癌诊断中的应用及与胃癌病理特征的相关性. 检验医学与临床, 2022, 19(9): 1237–1240. doi: 10.3969/j.issn.1672-9455.2022.09.022.
ZHANG L, ZHANG W, SHANG G F. Application of serum TK1, CEA and CA19-9 detection in the diagnosis of gastric cancer and their correlation with pathological features of gastric cancer. Lab Med Clin, 2022, 19(9): 1237–1240. doi: 10.3969/j.issn.1672-9455.2022.09.022.

[2] 宋圆圆, 李晓丽. 磷酸化STAT3蛋白与胃癌患者预后及临床病理特征关系的Meta分析. 胃肠病学和肝病学杂志, 2020, 29(2): 148–154. doi: 10.3969/j.issn.1006-5709.2020.02.006.
SONG Y Y, LI X L. Correlation of the expression of p-STAT3 protein with prognosis and clinicopatho-logical characteristics in gastric cancer patients: a meta-analysis. Chin J Gastroenterol Hepatol, 2020, 29(2): 148–154. doi: 10.3969/j.issn.1006-5709.2020.02.006.

[3] 刘庆伟, 李勇, 檀碧波, 等. 早期胃癌患者临床病理特征与淋巴结转移因素分析. 中华普通外科杂志, 2022, 37(4): 255–259. doi: 10.3760/cma.j.cn113855-20210831-00525.
LIU Q W, LI Y, TAN B B, *et al*. Clinicopathological characteristics and lymph node metastasis in patients with early gastric cancer. Chin J Gen Surg, 2022, 37(4): 255–259. doi: 10.3760/cma.j.cn113855-20210831-00525.

[4] HE W, LIANG B, WANG C, *et al*. MSC-regulated lncRNA MACC1-AS1 promotes stemness and chemoresistance through fatty acid oxidation in gastric cancer. Oncogene, 2019, 38(23): 4637–4654. doi: 10.1038/s41388-019-0747-0.

[5] WEI C, LI M, LIN S, *et al*. Characterization of tumor mutation burden-based gene signature and molecular subtypes to assist precision treatment in gastric cancer. Biomed Res Int, 2022, 2022: 4006507. doi: 10.1155/2022/4006507.

[6] DASA V, EASTWOOD J R B, PODGORSKI M, *et al*. Exome sequencing reveals a novel COL2A1 mutation implicated in multiple epiphyseal dysplasia. Am J Med Genet A, 2019, 179(4): 534–541. doi: 10.1002/ajmg.a.61049.

[7] THRIFT A P, El-SERAG H B. Burden of gastric cancer. Clin Gastroenterol Hepatol, 2020, 18(3): 534–542. doi: 10.1016/j.cgh.2019.07.045.

[8] 罗安, 朱欣彦, 胡晔东, 等. 基于肿瘤基质评分的胃癌预后基因分析. 同济大学学报(医学版), 2020, 41(4): 418–425. doi: 10.16118/j.1008-0392.2020.04.003.
LUO A, ZHU X Y, HU Y D, *et al*. Analysis of prognosis-related genes of gastric cancer based on tumor stromal score. J Tongji Univ (Med Sci), 2020, 41(4): 418–425. doi: 10.16118/j.1008-0392.2020.04.003.

[9] 吴平利. MATN3在胃癌中的表达及其临床预后意义. 合肥: 安徽医科大学, 2018. doi: 10.7666/d.D01503271.
WU P L. Expression level of MATN3 in gastric cancer and its clinical prognostic significance. Hefei: Anhui Medical University, 2018. doi: 10.7666/d.D01503271.

[10] 中华人民共和国卫生部医政司. 胃癌诊疗规范(2011年版). 中国医学前沿杂志(电子版), 2012, 4(5): 62–71. doi: 10.3969/j.issn.1674-7372.2012.05.015.
Department of Medical Administration, Ministry of Health, People's Republic of China. Gastric cancer diagnosis and treatment guidelines (2011 edition). Chin J Front Med Sci (E Vers), 2012, 4(5): 62–71. doi: 10.3969/j.issn.1674-7372.2012.05.015.

[11] LAI M Y, KANG S Y, SUN Y T, *et al*. Comparison of response evaluation criteria in solid tumors and tumor regression grade in evaluating the effect of preoperative systemic therapy of gastric cancer. BMC Cancer, 2022, 22(1): 1031. doi: 10.1186/s12885-022-10125-1.

[12] 钟兰, 黄涛, 谢贤和, 等. XRCC1基因Arg194Trp位点多态性与胃癌铂类药物化疗敏感性的关系. 山东医药, 2017, 57(8): 5–8. doi: 10.3969/j.issn.1002-266X.2017.08.002.
ZHONG L, HUANG T, XIE X H, *et al*. Correlation between *XRCC1* gene Arg194Trp polymorphism and platinum-based chemotherapy in patients with gastric cancer. Shandong Med J, 2017, 57(8): 5–8. doi: 10.3969/j.issn.1002-266X.2017.08.002.

[13] WANG P, XIAO W S, LI Y H, *et al*. Identification of MATN3 as a novel prognostic biomarker for gastric cancer through comprehensive TCGA and GEO data mining. Dis Markers, 2021, 2021: 1769635. doi: 10.1155/2021/1769635.

[14] JIA X, CHEN B, LI Z, *et al*. Identification of a four-gene-based SERM signature for prognostic and drug sensitivity prediction in gastric cancer. Front Oncol, 2022, 11: 799223. doi: 10.3389/fonc.2021.799223.

[15] YUAN L, XU Z Y, RUAN S M, *et al*. Long non-coding RNAs towards precision medicine in gastric cancer: early diagnosis, treatment, and drug resistance. Mol Cancer, 2020, 19(1): 96. doi: 10.1186/s12943-020-01219-0.

[16] LI D, XU J, DONG X, *et al*. Diagnostic and prognostic value of MATN3 expression in gastric carcinoma: TCGA database mining. J Gastrointest Oncol, 2021, 12(4): 1374–1383. doi: 10.21037/jgo-21-267.

（2024 – 05 – 27收稿, 2024 – 06 – 27修回）
编辑　吕　熙