·基础研究·

S100钙结合蛋白A16在胃癌转移中的作用和机制

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[摘 要] **目的**:研究S100钙结合蛋白A16(S100 calcium binding protein A16,S100A16)对胃癌细胞迁移的影响及其分子机制。**方法**:慢病毒感染构建稳定过表达S100A16的胃癌细胞株SGC-7901和MGC-803;划痕实验和Transwell实验检测胃癌细胞的迁移能力;SGC-7901细胞通过脂质体转染S100A16 siRNA,Western blot检测E-钙黏蛋白和波形蛋白;免疫共沉淀检测S100A16和闭合小环蛋白2(zonula occludens 2,ZO-2)的结合情况;免疫荧光观察S100A16和ZO-2在胃癌细胞中的定位。**结果**:S100A16在胃癌细胞中表达高于胃上皮细胞;稳定过表达S100A16的胃癌细胞SGC-7901和MGC-803构建成功;过表达S100A16促进胃癌细胞SGC-7901和MGC-803迁移;转染S100A16 siRNA抑制SGC-7901细胞中S100A16表达,E-钙黏蛋白表达升高,波形蛋白下调;S100A16和ZO-2存在相互作用,并在空间上存在共定位。**结论**:S100A16过表达促进胃癌细胞迁移,其作用机制可能是通过和ZO-2相互作用而实现。

[关键词] 胃癌;S100钙结合蛋白A16;紧密连接蛋白ZO-2;迁移

[中图分类号] R735.2 [文献标志码] A [文章编号] 1007-4368(2019)09-1285-07

doi:10.7655/NYDXBNS20190905

S100 calcium binding protein A16 promotes metastasis of gastric cancer

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[**Abstract**] **Objective**: This study aims to investigate the effect and mechanism of S100 calcium binding protein A16 (S100A16) on migration of gastric cancer cells. **Methods**: Gastric cancer cell lines SGC-7901 and MGC-803 with stable overexpression of S100A16 were constructed by lentivirus infection . Wound healing assay and Transwell assay were used to analyze the migration of gastric cancer cells. Liposome transfection method was used to transfect the S100A16 siRNA into SGC-7901, and expressons of E-Cadherin and Vimentin were detected by Western blot. The interaction between S100A16 and zonula occludens 2 (ZO-2) was detected by co-immunoprecipitation. The location of S100A16 and ZO-2 was detected by immunofluorescence. **Results**: S100A16 expression was higher in gastric cancer cells than in normal gastric epithelial cells. Cell lines with S100A16 overexpression were stably constructed. Overexpression of S100A16 promoted the migration of gastric cancer cells. E-Cadherin protein increased, S100A16 and Vimentin proteins decreased in SGC-7901 cells by transfection of S100A16 siRNA. S100A16 interacted with ZO-2 in SGC-7901 cells. **Conclusion**: Overexpression of S100A16 promotes the migration of gastric cancer cells. Its mechanism may be through the interaction of ZO-2.

[**Key words**] gastric cancer; S100 calcium binding protein A16; zonula occludens 2; migration

[J Nanjing Med Univ, 2019, 39(09): 1285-1291]

胃癌是我国是常见的消化道恶性肿瘤之一,其发病率在男性中位列第2,在女性中位列第3。约90%的胃癌属于胃腺癌,根据Lauren分型,胃癌可以分为肠型胃癌和弥漫性胃癌[1-2]。早期胃癌患者可以通过根治性手术治疗,术后5年生存率高达90%。但是早期胃癌缺乏特异性征象,检出率低,大多数

[基金项目] 国家自然科学基金面上项目(81570779)

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胃癌患者确诊时已发展为中晚期，错过手术最佳时期，有些甚至失去手术机会[3]。因此研究胃癌发生发展，阐明胃癌发病的分子机制具有重要意义。

S100钙结合蛋白A16（S100 calcuium binding protein A16，S100A16）是S100家族新成员，属于酸性钙结合蛋白。其特异性在于只有C端可以和钙离子结合，N端因缺乏谷氨酸残基而不具备和钙离子结合的功能[4-5]。S100A16和肿瘤的侵袭和转移过程密切相关[6-7]，本课题组既往研究发现S100A16参与胃癌的发生发展[8]，但S100A16调控胃癌生物学功能的分子机制尚未明确。本研究旨在探讨S100A16对胃癌细胞转移的影响及其分子机制，为后续对胃癌的研究提供初步理论依据和实验基础。

1　材料和方法

1.1　材料

RPMI-1640培养基（Gibco公司，美国）；兔抗S100A16、兔抗闭合小环蛋白（zonula occludens 2，ZO-2）、鼠抗波形蛋白（Vimentin）及GAPDH（武汉三鹰Proteintech Group公司）；兔抗E-钙黏蛋白（E-cadherin）（Bioworld公司，美国），鼠抗ZO-2（Invitrogen公司，美国）；脂质体核酸转染试剂（上海翊圣生物公司），DAPI（上海碧云天公司）；Transwell小室（Millipore公司，美国）；Alexa Fluor 488 donkey anti-rabbit（Life Technologies公司，美国），Alexa Fluor 594 donkey anti-mouse（Invitrogen公司，美国）；S100A16过表达慢病毒及其阴性对照慢病毒由上海吉凯基因公司合成，S100A16 siRNA及其阴性对照序列由上海吉玛公司设计合成。

1.2　方法

1.2.1　细胞培养

人胃黏膜正常上皮细胞GES-1、人胃癌细胞SGC-7901和MGC-803置于含10%胎牛血清RPMI-1640培养基中，37 ℃、5%CO_2恒温培养箱中培养。

1.2.2　S100A16过表达稳定细胞株的构建

取生长状态良好的SGC-7901和MGC-803细胞，使用10%胎牛血清RPMI-1640培养基制备3×10^4~5×10^4个/mL细胞悬液，铺板于6孔板中，37 ℃培养16~24 h。待细胞汇合度约30%，加入慢病毒感染。37 ℃培养12~16 h后更换完全培养基继续培养，48~72 h观察荧光表达情况和细胞状态，加入含2 μg/mL嘌呤霉素的RPMI-1640培养基，每3~4 d更换1次含嘌呤霉素的培养基进行加压筛选。提取细胞总蛋白，Western blot方法检测S100A16蛋白表达情况，将筛选好的细胞扩增培养，冻存保种。

1.2.3　蛋白质免疫印迹实验（Western blot）

使用含PMSF的RIPA裂解液提取细胞总蛋白，BCA法测定蛋白浓度。蛋白变性后取等量蛋白上样，进行SDS-PAGE凝胶电泳，湿转法转膜至PVDF膜上；5%脱脂奶粉溶液室温封闭2 h，4 ℃一抗孵育过夜；次日TBST洗膜3次，每次10 min；孵育相应二抗，室温孵育2 h；TBST洗膜3次，每次10 min；用ECL发光液显影曝光，保存图像。

1.2.4　划痕实验

取对数生长期细胞胰酶消化后，于6孔板中加入约4×10^5个/孔细胞；待细胞汇合80%~90%，取10 μL无菌枪头沿直尺划“十”字；PBS小心洗涤2~3次，更换为无血清培养基；放入37 ℃ 5%CO_2恒温培养箱培养；分别于0、24 h使用倒置显微镜观察并拍照保存图像。

1.2.5　Transwell实验

取对数生长期细胞，胰酶消化后用无血清培养基制成细胞悬液；取200 μL细胞悬液（约2×10^5个细胞）铺板于24孔板8 μm孔径Transwell上层小室中，下室中加入600 μL含20%胎牛血清的培养基；置于37 ℃ 5% CO_2恒温培养箱中培养12~24 h。从24孔板中小心取出小室，4%多聚甲醛固定30 min后结晶紫染色；吸走上室中液体，用棉签小心擦去上室中未迁移细胞；清水漂洗后静置晾干，置于显微镜下观察拍照并且保存图像。

1.2.6　siRNA转染实验

取对数生长期细胞，按4×10^5个/孔细胞铺板于6孔板中，置于37 ℃ 5% CO_2恒温培养箱培养。待细胞密度70%~80%时按照转染试剂说明书进行转染，4~6 h后更换为正常含血清培养基，转染48 h后提取细胞总蛋白，Western blot检测蛋白表达情况。

1.2.7　免疫共沉淀实验

提取细胞蛋白，BCA法测蛋白浓度，定量后分别加入兔IgG和兔抗S100A16抗体置于4 ℃、70 r/min摇床摇动过夜。次日在蛋白-抗体混合液中加入预清洗的Protein A agrose，缓慢摇动4~6 h，预冷的温和裂解液洗涤离心后加入2×蛋白上样缓冲液，95 ℃煮样10 min，取上清进行Western blot检测结果。

1.2.8　免疫荧光实验

细胞铺板后去除旧培养基，PBS清洗后加入4%多聚甲醛固定30 min，5%BSA室温封闭1 h，一抗4 ℃孵育过夜。次日使用PBS洗涤3次，每次5 min；37 ℃孵育第1种荧光二抗30 min，PBS洗涤后，相同

方法孵育第2种荧光二抗；DAPI染核，PBS洗涤后，抗荧光淬灭封片剂封片，于激光共聚焦显微镜下拍照，保存图像。

1.3　统计学方法

使用GraphPadInStat 3软件进行相关统计学分析，实验数据采用均数±标准差（$\bar{x} \pm s$）表示，两组间比较采用t检验，$P \leqslant 0.05$为差异有统计学意义。

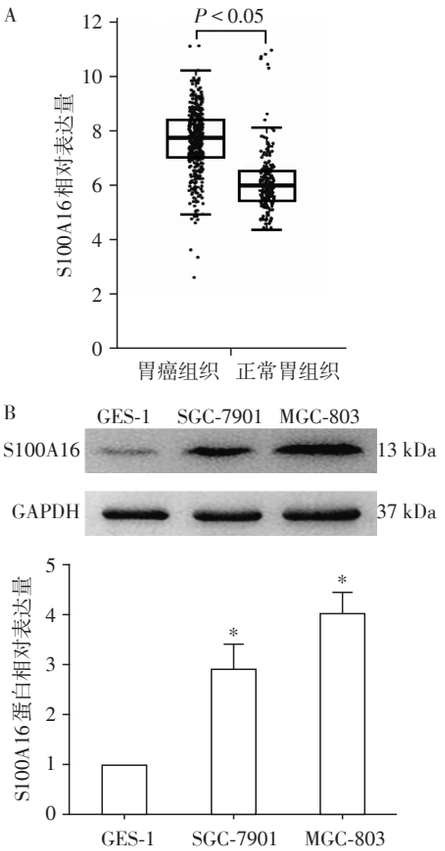
2　结　果

2.1　S100A16在胃癌及胃癌细胞中高表达

GEPIA数据库（http://gepia.cancer-pku.cn）中包含TCGA和GTEx数据，分析正常胃组织和胃癌组织中S100A16表达情况。结果显示S100A16在胃癌组织中表达显著高于正常胃组织（图1A）。Western blot方法检测S100A16在正常胃黏膜细胞GES-1和胃癌细胞SGC-7901、MGC-803中表达情况。结果显示S100A16在胃癌细胞中表达显著高于正常胃黏膜细胞（图1B），两者结论一致。

2.2　S100A16过表达稳转胃癌细胞的建立和鉴定

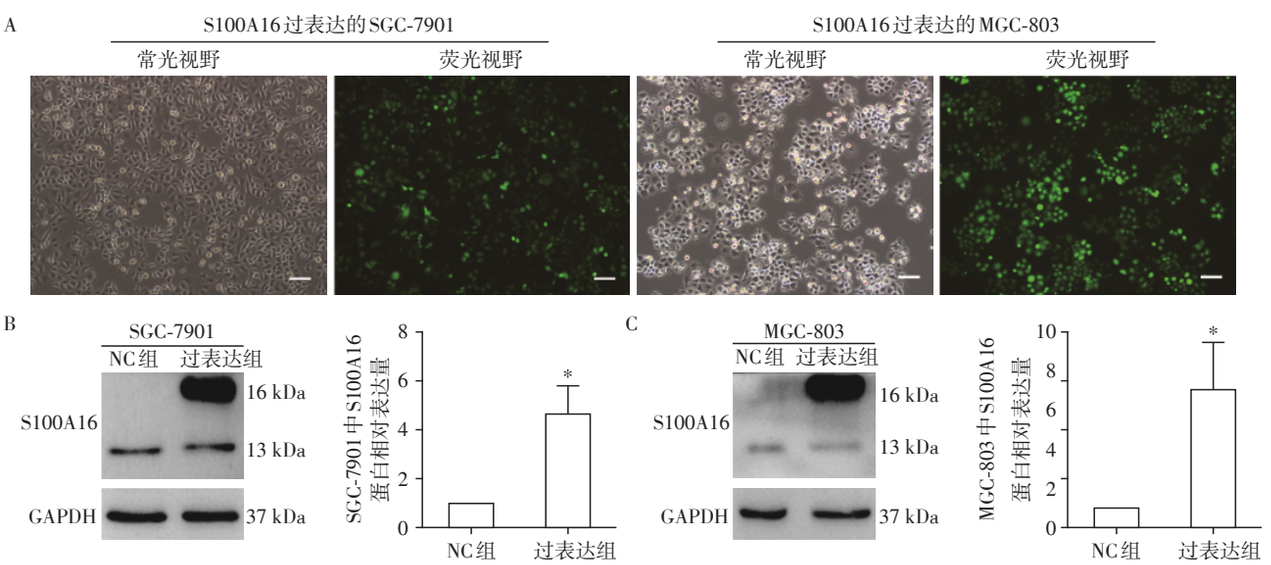
为了研究S100A16对胃癌细胞生物学功能影响，在胃癌细胞SGC-7901和MGC-803中加入阴性对照和S100A16过表达慢病毒进行感染，观察荧光效果，Western blot检测S100A16表达情况（内源性S100A16蛋白的分子量为13 kDa，外源性S100A16蛋白分子量为16 kDa）。结果显示（图2），和对照组相比，慢病毒感染组S100A16表达水平显



A：GEPIA数据库分析胃癌组织和正常胃组织S100A16表达情况；B：Western blot检测S100A16蛋白水平，与正常胃黏膜细胞GES-1比较，*$P < 0.05$。

图1　胃癌和胃癌细胞中S100A16表达水平

Figure 1　Expression of S100A16 in gastric cancer tissues and cells



A：荧光观察S100A16慢病毒感染情况（×100）；B、C：Western blot检测S100A16蛋白在胃癌细胞SGC-7901（B）和MGC-803（C）中的表达水平，NC：阴性对照，与NC组比较，*$P < 0.05$。

图2　S100A16稳定过表达胃癌细胞成功建立

Figure 2　Establishment of gastric cancer cells with stable overexpression of S100A16

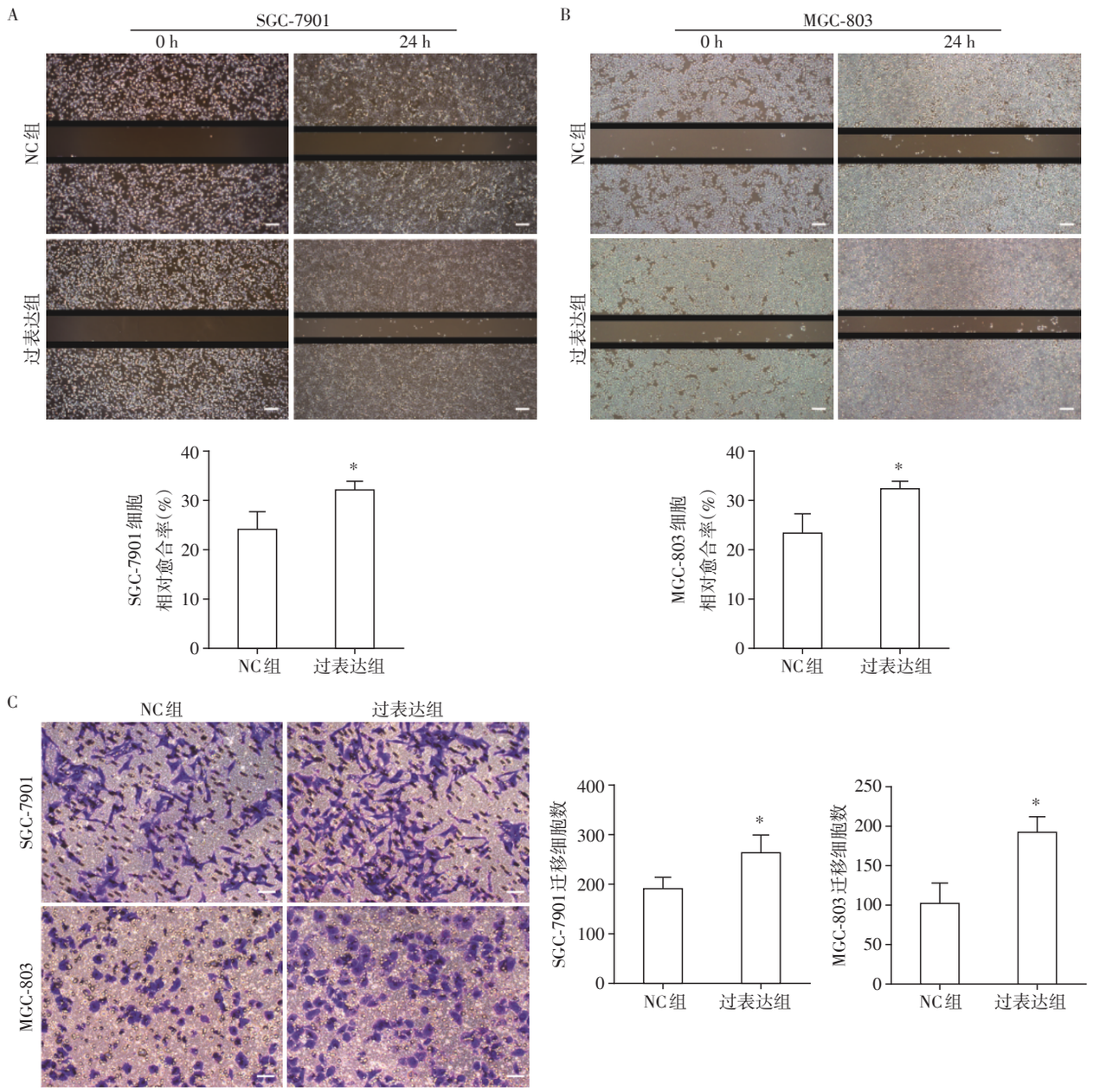
著增高。S100A16过表达的胃癌细胞SGC-7901和MGC-803成功建立，可用于后续进一步实验。

2.3　S100A16促进胃癌细胞迁移

使用过表达S100A16胃癌细胞SGC-7901和MGC-803及其相应对照细胞，划痕实验结果显示，随着时间增加，细胞向划痕处移动，SGC-7901和MGC-803过表达组间距较对照组明显变窄（图3A、B）。Transwell实验结果显示，SGC-7901和MGC-803过表达组穿过小室的细胞数量较对照组明显增多（图3C）。以上结果均提示S100A16过表达促进胃癌细胞迁移。

2.4　下调S100A16可抑制胃癌细胞上皮间质转化

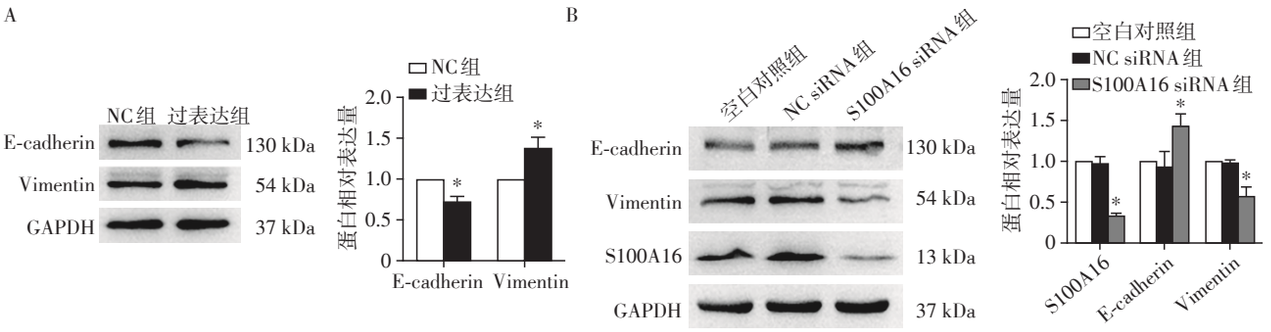
SGC-7901细胞中S100A16过表达后E-钙黏蛋白表达降低，波形蛋白表达增加（图4A）。在SGC-7901中转染对照siRNA和S100A16 siRNA，S100A16蛋白表达显著降低，说明成功敲低S100A16。S100A16敲低后，E-钙黏蛋白表达升高，波形蛋白表达降低（图4B），说明干扰下调S100A16可以抑制胃



A、B：划痕实验检测胃癌细胞SGC-7901（A）、MGC-803（B）的迁移能力（×40）；C：Transwell实验检测SGC-7901、MGC-803细胞的迁移能力（×200），与NC组比较，*$P<0.05$。

图3　S100A16过表达促进胃癌细胞SGC-7901、MGC-803的迁移能力

Figure 3　Overexpression of S100A16 promoted the migration of SGC-7901 and MGC-803



A：Western blot检测E-钙黏蛋白和波形蛋白表达，与NC组比较，$^*P<0.05$；B：Western blot检测S100A16 siRNA干扰效率及E-钙黏蛋白和波形蛋白表达，与空白对照组比较，$^*P<0.05$。

图4　S100A16对胃癌细胞SGC-7901中E-钙黏蛋白和波形蛋白表达的影响

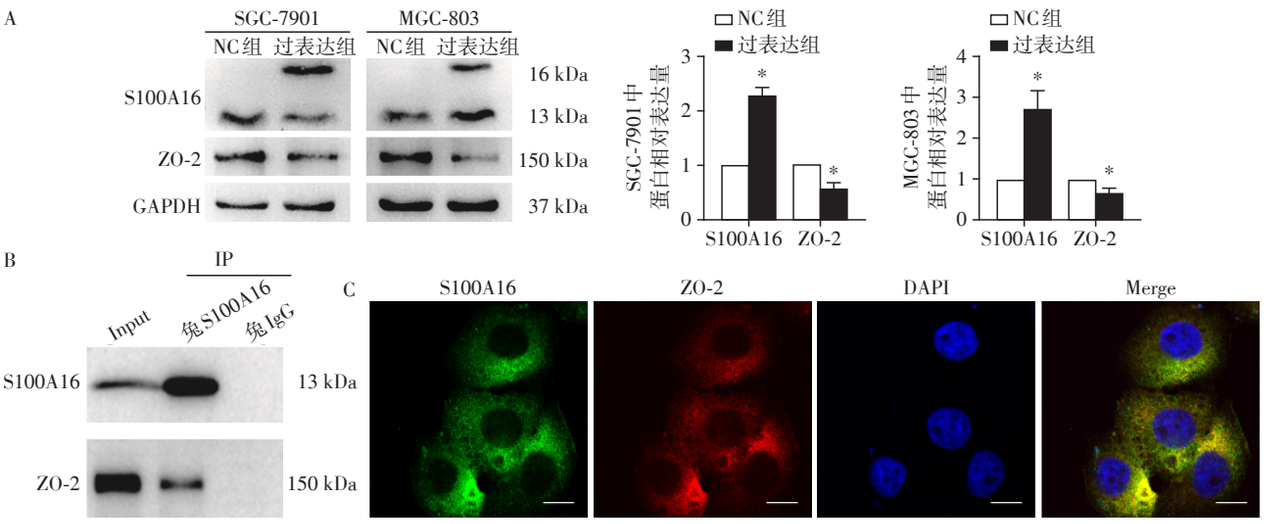
Figure 4　Effect of S100A16 on the expression of E-cadherin and Vimentin proteins in SGC-7901

癌细胞上皮间质转化。

为深入研究S100A16促进胃癌细胞迁移的分子机制，将对照组和过表达S100A16的胃癌细胞组采用免疫共沉淀后进行质谱分析鉴定，筛选出可能相互作用的蛋白。根据质谱分析结果筛选出紧密连接蛋白ZO-2，其丰度高且表达变化显著。ZO-2，也称作TJP2（tight junction protein 2），由TJP2（Gene ID：9414）基因编码，是膜结合鸟苷酸激酶家族（membrane-associated guanylate kinase，MAGUK）成员之一，属于紧密连接中的膜支架蛋白成分，在形成和维持紧密连接的通透性屏障功能、保持细胞极性等方面均发挥着重要作用[9]。为了验证质谱结果，Western blot结果显示，和对照组相比，S100A16过表达组ZO-2表达下降（图5A）；免疫共沉淀结果显示S100A16和ZO-2存在相互作用（图5B）；免疫荧光结果显示S100A16和ZO-2两者在空间上存在共定位（图5C），以上结果提示S100A16和ZO-2存在相互作用。

3　讨　论

S100蛋白家族和肿瘤的发生密切相关，包括肿瘤细胞的增殖、转移、血管生成和免疫逃避等[10-12]。本研究中S100A16在胃癌中表达上调，促进胃癌细胞迁移。上皮间质转化在肿瘤转移过程中发挥重要作用，也是胃癌侵袭和转移的重要机制之一[13]。S100家族成员可以通过上皮间质转化促进肿瘤细胞转移，Tian等[14]发现S100A7通过上皮间质转化促进宫颈癌细胞的转移，Hua等[15]发现S100A4通过上



A：Western blot检测SGC-7901、MGC-803细胞中S100A16和ZO-2表达情况，与NC组比较，$^*P<0.05$；B：免疫共沉淀检测SGC-7901中S100A16和ZO-2相互作用；C：免疫荧光检测SGC-7901中S100A16和ZO-2的共定位（×600）。

图5　S100A16和ZO-2蛋白在胃癌细胞中的关系

Figure 5　Relationship between S100A16 and ZO-2 proteins in gastric cancer cells

皮间质转化促进子宫内膜癌发展，Zhou等[16]发现S100A16可以通过Notch1途径促进乳腺癌上皮间质转化。

在上皮间质转化过程中，破坏上皮细胞间的紧密连接是第一步，也是关键步骤，是肿瘤细胞增加迁移能力的起始步骤[17]。ZO-2下调使细胞间紧密连接防护功能下降[18]。ZO-2能够和多种蛋白质存在相互作用，包括细胞-细胞黏附蛋白、细胞骨架成分和核因子[9]。研究表明ZO-2在肿瘤中表达下降[19-20]，Luczka等[20]发现ZO-2在肺癌中表达降低，抑制ZO-2可以增加肿瘤细胞的迁移和侵袭能力，提示ZO-2可能起到类似肿瘤抑制蛋白的功能。Wnt信号通路和上皮间质转化密切相关[21]，GSK-3β是Wnt信号通路核心参与者，Dominguez-Calderon等[22]发现敲低ZO-2，GSK-3β ser9位点磷酸化水平增加，促进β-catenin转录活性；而Tapia等[9,23]发现ZO-2过表达，GSK-3β ser9位点磷酸化水平降低，抑制β-catenin转录活性。提示Wnt信号通路可能参与其中，但仍需实验进一步证实。本实验室前期研究提示S100A16影响胃癌生物学功能[8]。敲低S100A16可以抑制胃癌细胞上皮间质转化，S100A16和ZO-2两者存在相互作用，且两者存在空间上共定位。Orre等[24]发现S100A4和p53结合并且促进p53降解，Tomiyama等[25]发现S100A16在人宫颈癌细胞中通过蛋白酶体途径促进p53的降解，因此推测S100A16可能促进ZO-2的降解，但是其机制仍有待进一步研究证实。希望能够以此为线索为胃癌转移的研究提供初步理论依据，为胃癌转移的治疗提供新思路。

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[收稿日期] 2019-02-01

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[收稿日期] 2019-01-17