doi:10.3969/j.issn.1003-6350.2017.09.001

·论　　著·

转染反义MIF对人胃癌细胞的影响

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【摘要】 **目的**　探讨反义巨噬细胞移动抑制因子(MIF)对人胃癌MGC-803细胞的影响。**方法**　将胃癌MGC-803细胞分为pcDNA3.1-Anti MIF组与空白对照组。pcDNA3.1-Anti MIF组采用转染技术将MIF反义RNA真核表达质粒(pcDNA3.1-AntiMIF)转入MGC-803细胞;空白对照组转染pcDNA3.1-sh-MIF质粒。qRT-PCR与蛋白质印迹法检测转染效率;采用MTT法、侵袭实验、AnnexinV- FITC和PI染色法分别检测反义MIF对MGC-803细胞增殖、侵袭、凋亡的影响。**结果**　qRT-PCR结果显示,pcDNA3.1-Anti MIF组MIF mRNA表达量(2.086±0.248)较空白对照组(6.992±0.342)明显下调;Western blot显示,pcDNA3.1vAntiMIF组MIF蛋白表达水平量(0.361±0.043)较空白对照组(1.171±0.091)明显下调;MTT实验结果显示,pcDNA3.1-AntiMIF组MGC-803细胞的OD值(0.436±0.017)较空白对照组(0.563±0.019)明显下降;侵袭实验结果显示,pcDNA3.1-AntiMIF组穿过基质胶的MGC-803细胞数(73.67±8.54)较空白对照组(137.30±11.91)明显减少;AnnexinV-FITC和PI染色法结果显示,pcDNA3.1-AntiMIF组MGC-803细胞的凋亡率(21.61±4.62)%较空白对照组(7.67±0.63)%明显增加。以上各项指标比较差异均具有显著统计学意义(P<0.01)。**结论**　反义MIF能抑制人胃癌MGC-803细胞的增殖与侵袭,并能诱导凋亡。

【关键词】 巨噬细胞移动抑制因子;胃癌;增殖;侵袭;凋亡
【中图分类号】 R735.2　【文献标识码】 A　【文章编号】 1003—6350(2017)09—1377—04

Effects of antisense macrophage migration inhibitory factor in human gastric cancer cells. *ZHANG Xue-mei [1], SUN Yan-liang [2], YANG Yang [1], LAN Hui [3], WANG Xi-mei [1]. 1. Hunan University of Medicine, Huaihua 418000, Hunan, CHINA; 2. First Affiliated Hospital of Hunan University of Medicine, Huaihua 418000, Hunan, CHINA; 3. First People's Hospital of Huaihua City, Huaihua 418000, Hunan, CHINA*

【**Abstract**】 **Objective**　To explore the effect of antisense macrophage migration inhibitory factor (MIF) on human gastric cancer MGC-803 cells. **Methods**　Human gastric cancer MGC-803 cells were assigned into two groups to receive transfection of antisense MIF plasmid pcDNA3.1-Anti MIF (pcDNA3.1-Anti MIF group) and pcDNA3.1-sh-MIF (control group). The qRT-PCR and Western blot analysis were employed for detecting transfection efficiency. The ability of proliferation and invasion, as well as apoptosis rate of MGC-803 cells regulated by antisense MIF were evaluated by MTT, Transwell invasion assays, and Annexin V/propidium iodide staining. **Results**　qRT-PCR results showed that the expression of MIF mRNA in pcDNA3.1-Anti MIF group was significantly lower than that in control group, (2.086±0.248) *vs* (6.992±0.342). Western blot analysis results showed that the expression of MIF protein in pcDNA3.1-Anti MIF group was significantly lower than that in control group, (0.361±0.043) *vs* (1.171±0.091). MTT assay showed that the OD value of MGC-803 cells in the pcDNA3.1-Anti MIF group was significantly lower than that in control group, (0.436±0.017) *vs* (0.563±0.019). Transwell invasion assays results showed that the number of cells through the matris glue in pcDNA3.1-Anti MIF group was significantly lower than that in the control group, (73.67±8.54) *vs* (137.30±11.91). Annexin V/propidium iodide staining showed that the apoptosis rate of MGC-803 cells in the pcDNA3.1-Anti MIF group was significantly higher than that in control group, (21.61±4.62)% *vs* (7.67±0.63)%. The differences were all statistically significant (P<0.01). **Conclusion**　Antisense MIF can inhibit the proliferation and invasion of human gastric cancer MGC-803 cells, as well as induce apoptosis.

【**Key words**】 Macrophage migration inhibitory factor; Gastric cancer; Proliferation; Invasion; Apoptosis

胃癌的发病率与死亡率居消化道恶性肿瘤之首[1],严重危害人类的生命与健康,其对人体的危害不仅在于肿瘤细胞生长失控而引起的克隆性异常增生,同时,肿瘤的侵袭和转移更是导致胃癌患者死亡的重要原因。因此,寻求一种有效抑制胃癌细胞生长,防止胃癌转移和复发的新方法显得极为紧迫。目前,基因治疗作为一种新的治疗手段已逐渐成为肿瘤治疗的一种重要策略[2]。

基金项目:湖南省教育厅科学研究项目(编号:13C741)
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巨噬细胞移动抑制因子(macrophage migration inhibitory factors,MIF)是一种多功能细胞因子,广泛表达于人体多种细胞和组织,与多种炎症性疾病、自体免疫性疾病及肿瘤等密切相关。研究显示,MIF可从多个层次促进肿瘤的发生发展,主要通过促进细胞增殖、抑制细胞凋亡、促进血管生成等共同促进肿瘤的发生发展和侵袭转移[3-5]。抑制MIF分泌及活性可抑制恶性肿瘤的生长和转移。本研究利用基因重组技术构建MIF反义RNA真核表达质粒pcDNA3.1-AntiMIF,将其转染胃癌细胞株MGC-803细胞,观察MIF对胃癌细胞的作用,从而为以MIF为靶点抗胃癌的基因治疗提供新思路。

1 材料与方法

1.1 细胞系 人胃癌MGC-803由南华大学肿瘤研究惠赠,细胞用含10%胎牛血清的RPMI-1640培养,置于37℃、5% CO_2的恒温箱中培养。

1.2 主要材料 Lipofectamine 2000购自美国Invitrogen公司,pcDNA3.1-Anti-MIF与pcDNA3.1-sh-MIF(空载体)由Invitrogen公司设计构建,RNA抽提试剂盒购于Applied Biosystems公司,MIF和β-actin抗体购于美国Santa Cruz公司,MTT粉购自美国Sigma公司,Transwell小室购于美国BD公司。

1.3 pcDNA3.1-Anti MIF转染及细胞分组 培养人胃癌MGC-803细胞,将MGC-803细胞分为pcDNA3.1-Anti MIF组与空白对照组。pcDNA3.1-Anti MIF组转染pcDNA3.1-Anti MIF质粒,空白对照组转染pcDNA3.1-sh-MIF质粒。于6孔板内按2 mL/孔铺加MGC-803细胞悬液,放置于37℃、5% CO_2的细胞培养箱中培养至细胞汇合度达30%~50%。在无菌EP管中配好pcDNA3.1-Anti MIF质粒、pcDNA3.1-sh-MIF质粒以及Lipofectamin2000;室温中放置20 min,使脂质体与质粒DNA形成复合体。用无血清培养液轻洗待转细胞后加入无血清RPMI-1640 (1 mL),再将孵育好的pcDNA3.1-Anti MIF质粒与pcDNA3.1-sh-MIF质粒脂质体混合液加至6孔板中,将细胞置于37℃、5% CO_2培养箱中继续培养6 h后换成有RPMI -1640完全细胞培养基,再继续培养48 h。

1.4 qRT-PCR 用RNA抽提试剂盒抽提上述两组细胞中总RNA,逆转录合成cDNA于-80℃冰箱保存。PCR扩增反应体系为20 μL,其中包括PCR primers (5 mmol/L) 0.4 μL,正向引物:5'-AGTGGTGTCCGAGAAGTCAG-3';反向引物:5'-TTAGGCGAAGGTGGAGTTGT-3', RT product 2.0 μL, *Taq* DNA polymerase (5 U/μL) 0.2 μL, 2×SYBR Mix 10 μL, 灭菌蒸馏水7.4 μL。混匀,反应条件如下:50℃ 2 min, 95℃ 10 min后95℃ 15 s, 60℃ 60 s, 40个循环,溶解曲线条件:95℃ 15 s, 60℃ 60 s, 95℃ 15 s。以U6 snRNA为内参,所测定的MIF的相对表达量采用$2^{-\Delta\Delta CT}$法分析。

1.5 Western blot检测 收集上述两组细胞,提取细胞总蛋白,采用BCA法测定蛋白浓度。每组取等量蛋白样本进行SDS-PAGE凝胶电泳,再将蛋白转至PVDF膜上,采用5%脱脂牛奶进行封闭2 h,再加MIF抗体或β-actin抗体,于4℃下继续过夜。TBST洗膜30 min,加入二抗室温孵育1 h,TBST洗膜30 min,然后加ECL发光剂,X片曝光、显影、定影。

1.6 MTT法检测细胞增殖 消化上述两组细胞,取200 μL即5 000个细胞接种于96孔板中,设置6个复孔,培养48 h后取出,每孔加20 μL MTT液,继续培养4 h后取出,每孔中加150 μL DMSO,低速振荡10 min,选择波长为570 nm,在酶标仪上测定各孔吸光值,实验重复3次。

1.7 Transwell侵袭实验 在Transwell小室中铺加适量基质胶稀释液,过夜并成膜。上述两组细胞中分别取100 μL即含1×10^5个细胞/mL的细胞稀释液接种至Transwell小室的上室,取含10%胎牛血清的RPMI-1640培养液500 μL加入下室,置于37℃、5% CO_2的细胞培养箱中培养36 h后取出,用棉签擦弃小室中上层未穿过基质胶的细胞,磷酸盐缓冲液(PBS)轻洗,再将Transwell小室放置于4%多聚甲醛中,以固定小室背面穿出的细胞,结晶紫染色,PBS液洗,倒置,晾干。光学显微镜下观察并摄相,随机选取4个高倍视野进行细胞计数,取平均值,实验重复3次。

1.8 细胞凋亡实验 采用AnnexinV-FITC/PI染色法。将上述两组组培养至80%汇合度,用PBS液洗涤细胞2次并离心收集,取1×10^6个细胞,加入100 μL的结合缓冲液悬浮细胞,加Annexin V-FITC 5 μL混匀,再加PI 1 μL混匀。室温下避光反应15~30 min,上机,流式细胞仪检测。

1.9 统计学方法 应用SPSS13.0软件进行统计学分析,计量资料以均数±标准差($\bar{x}\pm s$)表示,两组比较采用t检验,以$P<0.05$为差异有统计学意义。

2 结 果

2.1 反义MIF抑制人胃癌MGC-803细胞的增殖与侵袭 qRT-PCR与Western blot显示结果显示,pcDNA3.1-AntiMIF组MIF mRNA与蛋白表达水平较空白对照组均明显下调,差异有显著统计学意义($P<0.01$),提示转染成功;MTT实验结果显示,pcDNA3.1-AntiMIF组MGC-803细胞的OD值较空白对照组明显下降,差异有显著统计学意义($P<0.01$);侵袭实验结果显示,pcDNA3.1-AntiMIF组穿过基质胶的MGC-803细胞数较空白对照组明显减少,差异有显著统计学意义($P<0.01$)。见表1。

2.2 反义MIF诱导人胃癌细胞凋亡 凋亡实验结果显示,pcDNA3.1-AntiMIF组MGC-803细胞的凋亡率为(21.61±4.62)%,与空白对照组的(7.67±0.63)%比较,差异均具有显著统计学意义($t=8.020$, $P<0.01$)。

表1 两组细胞mRNA、蛋白灰度值、OD值和穿过基质胶的细胞数比较($\bar{x}\pm s$)

组别	mRNA	蛋白灰度值	OD值	穿过基质胶的细胞数
pcDNA3.1-Anti MIF组	2.086±0.248	0.361±0.043	0.436±0.017	73.67±8.54
空白对照组	6.992±0.342	1.171±0.091	0.563±0.019	137.30±11.91
t值	28.300	9.637	4.961	8.593
P值	<0.01	<0. 01	<0. 01	<0. 01

3 讨 论

自分泌MIF信号刺激巨噬细胞产生肿瘤坏死因子、白介素-1、一氧化氮、过氧化氢以及其他炎症因子[6]。在大鼠模型胃炎的发病机制中研究发现，MIF是重要的调节因子，尤其在幽门螺杆菌(*Helicobacter pylori*，HP)感染的胃炎中MIF的表达量明显上调[7-8]。研究显示，沉默MIF后能预防HP诱导的胃炎发生[9]。此外，大量的研究亦显示，MIF在多种肿瘤中高表达，如肺癌[10]、肝癌[11]、乳腺癌[12]、胃癌[13]、结肠癌[14]以及前列腺癌[15]等。且MIF高表达通过旁分泌途径能促进肿瘤细胞增殖与抑制凋亡[16-17]。这些结果表明，MIF在慢性炎症与癌症性疾病中可能扮演着重要角色。

研究显示，MIF在正常胃黏膜、慢性胃窦炎、肠上皮化生以及胃癌组织黏膜中的表分别为12.19%、52.12%、66.11%、95.51%，且其在胃癌中的表达水平与胃癌患者的临床分期、淋巴结转移以及预后明显相关[13]。还有研究显示，MIF在胃癌患者血清中的表达浓度亦明显增高[18]。研究表明MIF可能是肿瘤治疗的重要分子靶点。目前，已有以MIF为靶点，使用MIF特异性抑制剂、阻断MIF受体、制备MIF单抗、靶向MIF的基因治疗等方法来降低MIF分泌和活性，进而抑制恶性肿瘤生长和转移的相关研究报道[19-20]。研究显示，在结肠癌细胞中转染MIF反义质粒下调MIF后，癌细胞的增殖能力明显减弱[14]。在前列腺癌DU-145细胞中，抑制MIF与其受体CD74能明显减弱癌细胞的增殖与侵袭能力[15]。然而，沉默MIF是否影响胃癌的发展以及MIF在胃癌的治疗中是否是一个潜在的治疗靶分子，有待验证。

本研究首先采用转染技术将MIF反义RNA真核表达质粒转于人胃癌MGC-803细胞，以沉默该细胞中MIF的表达。qRT-PCR与蛋白质印迹法验证转染效率，结果显示，转染组MIF的蛋白与mRNA水平较对照均明显下调，提示转染成功。接下来进一步采用MTT法、侵袭以及凋亡实验明确其对胃癌细胞生物活行为的影响。MTT与侵袭实验结果显示，在胃癌细胞中沉默MIF后，细胞的增殖与侵袭明显下降。而凋亡实验结果显示，在胃癌细胞中沉默MIF后，细胞的凋亡诱导作用明显增强。

综上所述，在胃癌细胞中沉默MIF能抑制癌细胞的增殖与侵袭，并能诱导癌细胞凋亡，接下来的研究重点将放在构建胃癌体内模型上，以进一步研究在体内沉默MIF的表达对胃癌细胞生物学行为的影响，且进一步探讨其具体机制，为胃癌的临床靶向治疗提供重要的理论基础和实验依据。

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doi:10.3969/j.issn.1003-6350.2017.09.002

·论 著·

人肝癌细胞HepG2接种于BALB/C裸小鼠移植瘤的性别差异

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【摘要】 **目的** 研究性别对人肝癌细胞HepG2接种于BALB/C裸小鼠成瘤及移植瘤生长趋势的影响。**方法** 18只4~6周龄BALB/C裸小鼠雌雄各半,按照性别分成雌雄两组,每组9只,将全部裸小鼠肩胛部接种处于对数生长期且浓度为1.0×10^7/mL的HepG2细胞0.2 mL,10 d后观察两组的成瘤情况并测量肿瘤体积。**结果** 雌性组成瘤模型4只,成瘤率为44.44%;雄性组成瘤模型9只,成瘤率为100.00%,差异有统计学意义(P=0.029);造模成功的移植瘤体积走势图显示移植瘤生长速度雄性组大于雌性组。**结论** 人肝癌细胞HepG2接种于BALB/C裸小鼠成瘤率及移植瘤生长速度有明显的性别差异。

【关键词】 HepG2;成瘤;性别;裸小鼠
【中图分类号】 R-332 【文献标识码】 A 【文章编号】 1003—6350(2017)09—1380—03

Gender difference of human hepatocellular carcinoma HepG2 cell xenografts in BALB/C nude mice.

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【**Abstract**】 **Objective** To study the effect of gender on the tumorigenicity and growth trend after human hepatocellular carcinoma HepG2 cells were inoculated into BALB/C nude mice. **Methods** Eighteen 4-6 weeks' old BALB/C nude mice were divided into two groups (male group and female group) according to the gender, with 9 in each group. All the nude mice scapular were inoculated with 0.2 mL HepG2 cells in logarithmic growth phase and at the concentration of 1×10^7/mL. After 10 days, the tumor formation of the two groups was observed, and the tumor volume was measured. **Results** The number of tumorigenicity model in female group was 4, and tumorigenicity rate was 44.44%. The number of tumorigenicity model in male group was 9, and tumorigenicity rate was 100.00%, with statistically significant difference (P=0.029). The transplanted tumor volume trend figure of the successful model showed that the tumor growth in male group was higher than that in the female group. **Conclusion** Tumorigenicity rate and growth rate of transplanted tumor have obvious gender difference after human hepatocellular carcinoma HepG2 cells were inoculated into BALB/C nude mice.

【**Key words**】 HepG2; Tumorigenicity; Gender; Nude mice

基金项目:国家自然科学基金(编号:81274135)
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(收稿日期:2017-01-10)