



· 论 著 ·

血清来源外泌体携带miR-143-3p靶向调控BPTF影响胃癌恶性进展的作用机制

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[摘要] 背景和目的: miR-143-3p可抑制胃癌细胞的增殖、侵袭、迁移、细胞周期和上皮-间充质转化。BPTF作为一种致癌基因, 在胃癌中高表达并与患者的不良预后有关。外泌体作为由各种细胞释放的一种具有脂质双层结构的外囊泡, 携带miRNA可在胃癌的发生、发展中发挥抑癌作用。但血清来源外泌体携带miR-143-3p是否可通过靶向调控BPTF影响胃癌恶性进展目前尚不清楚。本研究旨在探究血清来源外泌体携带miR-143-3p靶向调控BPTF影响胃癌恶性进展的作用机制。方法: 以实时荧光定量反转录聚合酶链反应 (quantitative reverse transcription polymerase chain reaction, qRT-PCR) 检测胃癌组织及人胃癌细胞系MKN-45、HGC-27、MGC-803中miR-143-3p及BPTF的表达。提取并鉴定胃癌患者和健康志愿者血清中外泌体, 并进行MGC-803细胞摄取实验。以蛋白质印迹法 (Western blot) 和双荧光素酶报告基因实验验证血清来源外泌体携带miR-143-3p对BPTF的靶向调控作用。将MGC-803细胞随机分为正常组、Exo-miR-143-3p组、siRNA-NC组、si-BPTF组、Exo-miR-143-3p+pcDNA组和Exo-miR-143-3p+pcDNA-BPTF组, 采用血清来源外泌体携带miR-143-3p、BPTF siRNA阴性对照、BPTF siRNA空载质粒及BPTF过表达质粒分组干预后进行qRT-PCR和Western blot实验检测BPTF表达。采用流式细胞术、transwell及细胞计数试剂盒-8 (cell counting kit-8, CCK-8) 实验检测细胞凋亡、侵袭、迁移及增殖程度。通过皮下接种MGC-803细胞构建BALB/c裸鼠移植瘤模型, 采用相同分组和干预方法后检测裸鼠皮下肿瘤重量和体积。结果: 与配对癌旁组织相比, 胃癌组织中miR-143-3p表达显著降低 ($P<0.05$), BPTF mRNA表达显著升高 ($P<0.05$)。与GES-1细胞相比, MKN-45、HGC-27和MGC-803细胞中的miR-143-3p表达显著降低 ($P<0.05$), BPTF mRNA表达显著升高 ($P<0.05$)。本研究成功提取血清来源外泌体。血清来源外泌体可被MGC-803细胞成功摄取, 且健康志愿者血清来源外泌体可上调MGC-803细胞中miR-143-3p的表达。血清来源外泌体携带miR-143-3p可靶向调控BPTF。与正常组相比, Exo-miR-143-3p组、si-BPTF组及Exo-miR-143-3p+pcDNA组BPTF的相对表达、侵袭数、迁移数、细胞活性、裸鼠皮下肿瘤重量和体积降低 ($P<0.05$), 凋亡率升高 ($P<0.05$)。与Exo-miR-143-3p组相比, Exo-miR-143-3p+pcDNA-BPTF组BPTF的相对表达、侵袭数、迁移数、细胞活性、裸鼠皮下肿瘤重量和体积升高 ($P<0.05$), 凋亡率降低 ($P<0.05$)。结论: 血清来源外泌体携带miR-143-3p可靶向下调BPTF抑制胃癌恶性进展。

[关键词] 血清来源外泌体; miR-143-3p; 靶向调控; BPTF; 胃癌; 恶性进展

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Mechanism by which miR-143-3p-carrying serum-derived exosomes affect malignant progression of gastric cancer via targeted regulation of BPTF QUAN Zhenhao, LIN Lin, XU Feipeng, HUANG Renwei, CHEN Rihong, SUN Kaiyu (Department of Gastroenterology, Affiliated Hospital of Guangdong Medical University, Zhanjiang 524001, Guangdong Province, China)

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[**Abstract**] **Background and purpose:** miR-143-3p can inhibit the proliferation, invasion, migration, cell cycle and epithelial-mesenchymal transition of gastric cancer cells. *BPTF*, as an oncogene, is highly expressed in gastric cancer and is associated with poor prognosis in patients. Exosomes, as a type of lipid bilayer structure released by various cells, carrying miRNA, can play an anti-cancer role in the occurrence and development of gastric cancer. However, it is currently unclear whether serum-derived exosomes carrying miR-143-3p can affect the malignant progression of gastric cancer through targeted regulation of *BPTF*, and further clarification is needed. The aim of this study was to investigate the mechanism by which serum-derived exosomes carrying miR-143-3p regulate the *BPTF* and affect the malignant progression of gastric cancer. **Methods:** Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was used to detect the expressions of miR-143-3p and *BPTF* in gastric cancer tissues and human gastric cancer cell lines MKN-45, HGC-27 and MGC-803. Exosomes extracted from the serum of gastric cancer and healthy volunteers, were identified, and then subjected to MGC-803 cell uptake experiments. Western blot and dual luciferase reporter gene experiments were used to verify the targeted regulatory effect of miR-143-3p-carrying serum-derived exosomes on *BPTF*. MGC-803 cells were randomly divided into normal group, Exo-miR-143-3p group, siRNA-NC group, si-*BPTF* group, Exo-miR-143-3p+pcDNA group and Exo-miR-143-3p+pcDNA-*BPTF* group. After using serum-derived exosomes carrying miR-143-3p, *BPTF* siRNA negative control, *BPTF* siRNA empty vector and *BPTF* overexpression plasmid intervention in grouping, qRT-PCR and Western blot experiments were used to detect *BPTF* expression. Flow cytometry, transwell and cell counting kit-8 (CCK-8) experiments were used to measure cell apoptosis, invasion, migration and proliferation. Xenograft gastric cancer model was constructed by subcutaneous inoculation of MGC-803 cells in BALB/c nude mice. After the same grouping and intervention, the subcutaneous tumor weight and volume of nude mice were detected. **Results:** Compared with paired adjacent cancer tissues, the expression of miR-143-3p in gastric cancer tissues was significantly reduced ($P < 0.05$), while the expression of *BPTF* mRNA was significantly increased ($P < 0.05$). Compared with GES-1 cells, the expression of miR-143-3p in MKN-45, HGC-27 and MGC-803 cells was significantly reduced ($P < 0.05$), while the expression of *BPTF* mRNA was significantly increased ($P < 0.05$). This study successfully extracted serum-derived exosomes. Serum-derived exosomes could be successfully taken up by MGC-803 cells, and healthy volunteers' serum-derived exosomes could upregulate the miR-143-3p expression in MGC-803 cells. The miR-143-3p-carrying serum-derived exosomes could target and regulate *BPTF*. Compared with the normal group, the Exo-miR-143-3p group, si-*BPTF* group and Exo-miR-143-3p+pcDNA group showed a decrease in *BPTF* relative expression, invasion number, migration number, cell activity, subcutaneous tumor weight and volume in nude mice ($P < 0.05$), and an increase in apoptotic rate ($P < 0.05$). Compared with the Exo-miR-143-3p group, the Exo-miR-143-3p+pcDNA-*BPTF* group showed an increase in *BPTF* relative expression, invasion number, migration number, cell activity, subcutaneous tumor weight and volume in nude mice ($P < 0.05$), and a decrease in apoptotic rate ($P < 0.05$). **Conclusion:** The miR-143-3p-carrying serum-derived exosomes can downregulate *BPTF* and inhibit malignant progression of gastric cancer.

[**Key words**] Serum-derived exosomes; miR-143-3p; Targeted regulation; *BPTF*; Gastric cancer; Malignant progression

胃癌一般起源于胃黏膜细胞, 幽门螺杆菌感染被认为是其主要致病因素, 是全球第5大常见癌症及引发癌症相关死亡的第3大原因, 目前疗效依然不能令人满意, 截至目前仍是重大的全球健康问题^[1-3]。外泌体是由各种细胞释放的一种具有脂质双层结构的外囊泡, 其内包含脂质、蛋白质、微小RNA、生长因子、细胞因子等分子物质, 在恶性肿瘤的治疗中发挥关键作用^[4]。有研究^[3]显示, 外泌体携带miR-502-5p可在胃癌的发生、发展中发挥抑制作用。miR-143-3p在大多数恶性肿瘤中下调, 并可通过调控靶基因抑制肿瘤的发生、发展^[5], 海绵化miR-143-3p可促进包括胃癌在内的各种肿瘤细胞的增殖、侵袭、

迁移、细胞周期和上皮-间充质转化^[6], miR-143-3p模拟物可通过抑制细胞增殖、促进细胞凋亡, 从而对肝细胞癌发挥抗肿瘤作用^[7]。*BPTF*作为一种致癌基因, 在幽门螺杆菌诱导的胃癌中高表达并与患者的不良预后有关^[8], 敲低*BPTF*可抑制胶质瘤细胞的恶性行为和进展^[9], 下调*BPTF*可抑制肺腺癌细胞增殖和肿瘤生长并加速其凋亡^[10], 由此可知, miR-143-3p和*BPTF*可作为胃癌的潜在治疗靶标。通过生物信息学分析可知, miR-143-3p可能通过与*BPTF*的结合位点结合来调控其表达, 而miR-143-3p可被外泌体携带, 因而推测, 血清来源外泌体携带miR-143-3p可能通过靶向调控*BPTF*影响胃癌恶性进展。本

研究对此进行探讨,旨在为胃癌的靶向治疗提供新的理论依据。

1 材料和方法

1.1 材料

1.1.1 研究对象

收集2024年5月—2025年4月在广东医科大学附属医院进行手术切除的30例胃癌患者(均签署知情同意书)的胃癌组织和距离癌组织3~5 cm的配对癌旁组织,储存在液氮中备用。收集以上30例胃癌患者和30名健康志愿者的血液,4℃下以1 000×g离心10 min收集血清,储存在-80℃备用。5周龄BALB/c雄性裸鼠[无特定病原体(specific pathogen free, SPF)级、18~21 g]由上海西普尔-必凯实验动物有限公司提供,质量合格证号为2008001661519,生产许可证号为SCXK(沪)2020-0016,使用许可证号为SYXK(粤)2022-0286,在恒温22~24℃、12 h/12 h明暗交替照明、45%~60%恒湿的SPF级环境中饲养,提供充足饲料和纯净水使裸鼠自由进食和饮水。本研究已通过广东医科大学附属医院临床科研伦理委员会批准(批件号:YJKT2023-073-02)。

人胃黏膜上皮细胞GES-1、人胃癌细胞系MKN-45(货号IM-H088)、HGC-27(货号IM-H085)、MGC-803(货号IM-H086)均购自厦门逸漠生物科技有限公司。

1.1.2 试剂和材料

血清来源外泌体分离试剂(批次号4478360)、总外泌体RNA和蛋白质分离试剂盒(批次号4478545)、一步法实时荧光定量反转录聚合酶链反应(quantitative reverse transcription polymerase chain reaction, qRT-PCR)试剂盒(批次号11732020)、辣根过氧化物酶(horseradish peroxidase, HRP)-羊抗兔二抗(批次号A16110)均购自美国Thermo Fisher Scientific公司,外泌体绿色荧光标记试剂盒(PKH67)(批次号IME-M001)购自厦门逸漠生物科技有限公司,细胞计数试剂盒-8(cell counting kit-8, CCK-8)(批次号HY-K0301)、双荧光素酶试剂盒(批次号HY-K1013)、结晶紫(批次号HY-B0324A)均购自美国MCE公司,由生工生物工程(上海)股份有限公司合成构建miR-143-3p mimics、miR-143-3p阴性对照(miR-143-3p-NC)、空载质粒(pc-NC)、BPTF过表达质粒(pc-BPTF),Annexin V-异硫氰酸荧光素

(fluorescein isothiocyanate, FITC)凋亡检测试剂盒(批次号ab14085)、兔抗人BPTF、CD63、CD9、肿瘤易感基因101(tumor susceptibility gene 101, TSG101)、GM130及 β -actin一抗(批次号ab288159、ab134045、ab236630、ab125011、ab52649、ab5694)均购自英国Abcam公司。

1.1.3 主要仪器

MX3005P荧光定量分析仪购自美国Agilent公司, Talos F200i透射电镜、PowerEase™ Touch 350W型蛋白电泳套装、EVOS™ M7000显微成像系统均购自美国Thermo Fisher Scientific公司, FV1000共聚焦激光扫描生物显微镜购自日本Olympus公司, MACSQuant® Tyto流式细胞仪购自德国Miltenyi Biotec公司, Biotek Synergy H1酶标仪购自美国Bio-Tek公司。

1.2 方法

1.2.1 采用qRT-PCR检测人胃癌组织及胃癌细胞系中miR-143-3p及BPTF的表达

细胞培养:将GES-1、MKN-45、HGC-27和MGC-803细胞快速解冻、清洗后用细胞培养液重悬,接种在T25培养瓶中, GES-1、MKN-45和MGC-803细胞培养液为89%RPMI-1640+10%胎牛血清+1%双抗, HGC-27细胞培养液为89%DMEM+10%胎牛血清+1%双抗,细胞均置于37℃、CO₂体积分数为5%的恒温培养箱内培养,融合度达到80%时以1:2的比例进行传代,后续实验均使用传代2次的对数期细胞进行。

取胃癌组织与配对癌旁组织,剪碎置于TRIzol试剂内研磨提取总RNA,取对数生长期GES-1、MKN-45、HGC-27和MGC-803细胞置于TRIzol试剂内裂解提取总RNA,用一步法qRT-PCR试剂盒并依据其说明书中指导配制反应体系,行qRT-PCR来检测miR-143-3p与BPTF基因表达,实验数据用 $2^{-\Delta\Delta Ct}$ 算法分析,用内参基因U6做对照量化miR-143-3p的相对表达,用内参基因 β -actin做对照量化BPTF的相对表达,基因引物序列见表1。

1.2.2 外泌体提取、鉴定以及外泌体中miR-143-3p表达检测

取胃癌患者和健康志愿者血清,将0.2 mL血清来源外泌体分离试剂加到1 mL血清中,4℃下温育30 min,按其说明指导进行离心分离,获得血清中外泌体,以2%多聚甲醛溶液固定外泌体,滴加在400目铜网上(涂有碳支持膜)吸附20 min,滴加1%戊二醛再固定5 min,滴加

pH=7.0的尿素-草酸溶液(染色5 min)、pH=4.0的2%甲基纤维素及4%乙酸尿素混合液(染色10 min)进行双染,铜网风干后于透射电子显微镜下观察、拍照外泌体。

表1 基因引物序列

Tab. 1 Gene primer sequence

Primer name	Sequence (5'-3')
<i>BPTF</i>	Forward: CTGCTGCTTCCCAGAAGAGG; Reverse: GCCTCACTTTGCAAGATGCC
β -actin	Forward: TGGATCAGCAAGCAGGAGTA; Reverse: TCGCCACATTGTGAACCTT
miR-143-3p	Forward: CGCGTGAGATGAAGCACTG; Reverse: AGTGCAGGTCCGAGGTATT
<i>U6</i>	Forward: GCTTCGGCAGCACATATACTAAAAT; Reverse: CGCTTACGAATTTGCGTGTCA

取总外泌体RNA和蛋白质分离试剂盒,按其制造商说明指导提取外泌体中总RNA和蛋白质,测定总蛋白浓度后进行蛋白质变性,通过电泳、湿转将蛋白质分离后进行转印,上样量为20 μ g/孔,滴加CD63、CD9、TSG101和GM130兔抗人一抗,4 $^{\circ}$ C下温育过夜,充分洗膜后滴加HRP-大鼠抗兔二抗,37 $^{\circ}$ C下温育2 h,抗体均用5%牛血清白蛋白稀释:CD63(1:1 000)、CD9(1:1 500)、TSG101(1:1 000)、GM130(1:1 000)、二抗(1:2 000),再次洗膜后进行蛋白显色,拍摄外泌体蛋白条带图像。

取外泌体总RNA,按“1.2.1”中方法行qRT-PCR来检测miR-143-3p的相对表达。

1.2.3 胃癌细胞对外泌体的摄取检测

取胃癌患者和健康志愿者血清来源外泌体各10 μ g,添加100 μ mol/L的PKH67外泌体绿色荧光标记染料工作液50 μ L,温育10 min,滴入10 mL磷酸缓冲盐溶液(phosphate-buffered saline, PBS)混匀后以相同方法再次提取外泌体,以PBS重悬,采用10 μ g外泌体温育 1×10^5 个MGC-803细胞,分别设为胃癌患者组和健康志愿者组,将未温育外泌体正常培养的细胞设为正常组,24 h后固定细胞,以4',6-二脒基-2-苯基吲哚(4',6-diamidino-2-phenylindole, DAPI)复染核,于激光共聚焦显微镜下观察MGC-803细胞对外泌体的摄取情况。

miR-143-3p对BPTF调控作用的检测:取胃癌患者未温育外泌体、分别温育胃癌患者和健康志愿者血清来源外泌体的MGC-803细胞,按“1.2.1”中方法行qRT-PCR来检测miR-143-3p表达。

1.2.4 血清来源外泌体携带miR-143-3p对BPTF靶向调控作用检测

miR-143-3p对BPTF调控作用的检测:取胃癌患者未温育外泌体、分别温育胃癌患者和健康志愿者血清来源外泌体的MGC-803细胞,按“1.2.1”中方法行qRT-PCR来检测BPTF基因表达,按“1.2.2”中方法行蛋白质印迹法(Western blot)实验,以 β -actin作为内参蛋白来检测BPTF蛋白的相对表达。

miR-143-3p对BPTF靶向作用的检测:将MGC-803细胞接种在24孔板上,生长至对数期时用miR-143-3p mimics、miR-143-3p阴性对照(NC-miR-143-3p)分别与野生型、突变型BPTF 3'-非翻译区(untranslated region, UTR)报告质粒两两联合转染,24 h后取双荧光素酶试剂盒,按其制造商说明指导检测各联合转染组细胞双荧光素酶的相对活性。

1.2.5 MGC-803细胞分组干预

MGC-803细胞接种在24孔板上,生长至对数期时用外泌体及质粒进行分组干预:以健康志愿者血清来源外泌体干预的细胞设为携带miR-143-3p外泌体(Exo-miR-143-3p)组,以10 μ g外泌体温育 1×10^5 个细胞^[11];转染BPTF siRNA阴性对照、BPTF siRNA的细胞分别设为siRNA-NC组、si-BPTF组,均以50 nmol/L的终浓度转染;以健康志愿者血清来源外泌体干预的同时转染空载质粒、BPTF过表达质粒的细胞分别设为Exo-miR-143-3p+pcDNA组和Exo-miR-143-3p+pcDNA-BPTF组,均以1 μ g的量转染,正常培养不做干预及转染的细胞设为正常组,细胞均干预24 h。

1.2.6 各组MGC-803细胞BPTF表达及凋亡检测

取分组干预后的各组MGC-803细胞,按“1.2.2”中方法行Western blot实验,以 β -actin作为内参蛋白来检测BPTF蛋白的相对表达。

取分组干预后的各组MGC-803细胞,用胰酶消化、PBS清洗,计数后每组取 5×10^5 个细胞重悬在PBS内,滴加5 μ L Annexin V-FITC及2.5 μ L碘化丙啶(propidium iodide, PI)双染,充分清洗后流式上机,利用流式细胞仪检测各组MGC-803细胞凋亡率。

1.2.7 各组MGC-803细胞侵袭、迁移及增殖检测

侵袭、迁移检测:取分组干预后的各组MGC-803细胞,用胰酶消化、PBS清洗,计数后每组取 5×10^5 个细胞重悬在无血清RPMI-1640培养基内,接种在transwell小室上层,小室下层添加含20%血清的RPMI-1640培养基,培养24 h后

擦除上层细胞，清洗、固定下层细胞后采用结晶紫染色，清洗后显微成像，进行细胞计数后得到各组细胞侵袭或迁移数。进行侵袭检测时transwell小室于细胞接种前用基质胶包被处理，而进行迁移检测时不用基质胶包被处理。

增殖检测：将MGC-803细胞接种在96孔板上，生长至对数期时用“1.2.5”中方法分组干预，同时设置不接种细胞的空白对照组，然后温育CCK-8试剂，10 μL/孔，2 h后测定各组吸光度(D)值，按CCK-8说明指导测定各组细胞活性。细胞活性的计算公式为：细胞活性(%) = $(D_{\text{实验组}} - D_{\text{空白对照组}}) / (D_{\text{正常组}} - D_{\text{空白对照组}}) \times 100\%$ 。

1.2.8 MGC-803裸鼠移植瘤模型构建、分组干预及皮下肿瘤体积检测

取BALB/c裸鼠，将 2×10^6 个MGC-803细胞(重悬在0.2 mL PBS内)皮下接种在右腋部，饲养7 d后裸鼠皮下长出黄豆大小硬块，表明MGC-803裸鼠移植瘤模型构建成功^[12]，共36只裸鼠，成功率为100%，采用随机数表法将其随机分为正常组、Exo-miR-143-3p组、siRNA-NC组、si-BPTF组、Exo-miR-143-3p+pcDNA组和Exo-miR-143-3p+pcDNA-BPTF组，每组6只裸鼠。

Exo-miR-143-3p组裸鼠于皮下肿瘤中心注射10 μg健康志愿者血清来源外泌体(重悬在20 μL PBS内)^[11]；siRNA-NC组、si-BPTF组裸鼠于皮下肿瘤中心分别注射BPTF siRNA阴性对照、BPTF siRNA，注射量均为10 μg(以20 μL PBS溶解)；Exo-miR-143-3p+pcDNA组、Exo-miR-143-3p+pcDNA-BPTF组裸鼠于皮下肿瘤中心注射10 μg健康志愿者血清来源外泌体，同时于皮下肿瘤中心分别注射空载质粒、BPTF过表达质粒，注射量均为100 μg，外泌体核质粒均重悬或溶解在20 μL PBS内，裸鼠均每周注射2次，注射3周后对裸鼠进行安乐死，取出皮下肿瘤拍照，测定其重量后测量其最长径和最短径，计算各组皮下肿瘤体积。皮下肿瘤体积的计算公式为：皮下肿瘤体积(mm³) = 最短径² × 最长径/2。

1.3 统计学处理

本研究利用GraphPad Prism 8.0软件对数据进行统计分析，实验数据符合正态分布，以 $\bar{x} \pm s$ 表示。对数据进行方差齐性检验，若方差齐，两组间差异进行t检验，多组间差异比较行单因素方差分析，两两之间进一步差异比较行SNK-q检验；若方差不齐，采用Kruskal-Wallis检验分析多组间差异，若差异显著则进一步采用Nemenyi

检验进行事后两两比较。检验水准为单侧检验， $P < 0.05$ 为差异有统计学意义。

2 结果

2.1 人胃癌组织及胃癌细胞系中miR-143-3p及BPTF表达

与配对癌旁组织相比，胃癌组织miR-143-3p表达显著降低($P < 0.05$)，BPTF mRNA表达显著升高($P < 0.05$)；与GES-1细胞相比，MKN-45、HGC-27和MGC-803细胞miR-143-3p表达显著降低($P < 0.05$)，BPTF mRNA表达显著升高($P < 0.05$ ，表2)。

表2 人胃癌组织及胃癌细胞系中miR-143-3p及BPTF mRNA表达相对表达

Tab. 2 Relative expression of miR-143-3p and BPTF mRNA in human gastric cancer tissues and gastric cancer cell lines		
($\bar{x} \pm s$)		
Group	miR-143-3p	BPTF mRNA
Paired adjacent cancer tissues (n=30)	1.00 ± 0.06	1.00 ± 0.11
Gastric cancer tissue (n=30)	0.34 ± 0.04 ^a	2.09 ± 0.13 ^a
t value	50.131	-35.058
P value	<0.001	<0.001
GES-1 cells (n=6)	1.00 ± 0.07	1.00 ± 0.12
MKN-45 cells (n=6)	0.39 ± 0.05 ^b	1.97 ± 0.14 ^b
HGC-27 cells (n=6)	0.41 ± 0.04 ^b	2.02 ± 0.10 ^b
MGC-803 cells (n=6)	0.23 ± 0.03 ^b	2.24 ± 0.15 ^b
F value	284.130	120.372
P value	<0.001	<0.001

^a: $P < 0.001$, compared with paired adjacent cancer tissues; ^b: $P < 0.001$, compared with GES-1 cells.

2.2 外泌体鉴定及其miR-143-3p表达

用透射电镜观察到血清来源外泌体呈典型的“杯托”形态并呈现双层膜结构，且粒径在30~100 nm之间，符合外泌体粒径特征(图1)。Western blot检测结果显示，血清来源外泌体表达外泌体表征蛋白CD63、CD9、TSG101，不表达GM130蛋白(图2)，最终说明本研究成功提取血清来源外泌体。与健康志愿者(1.01 ± 0.08)相比，胃癌患者血清来源外泌体中的miR-143-3p表达(0.22 ± 0.05)显著降低($t = 45.866$, $P < 0.001$ ，表3)。

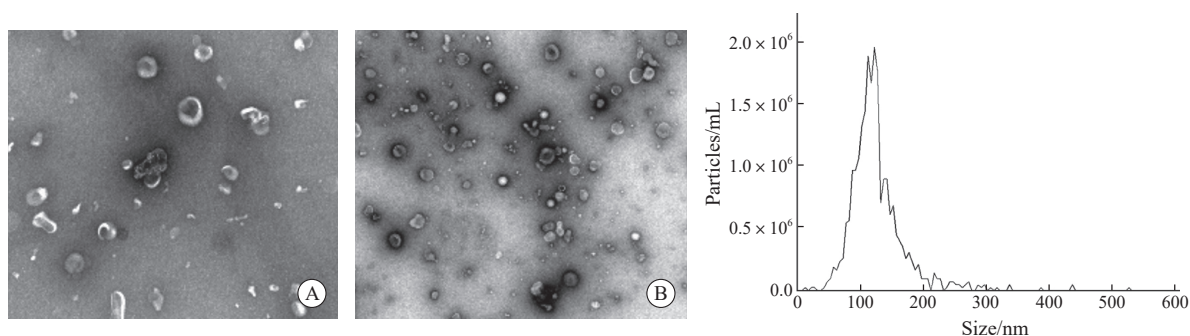


图1 透射电镜观察血清来源外泌体形态及粒径 ($\times 5\ 000$)

Fig. 1 Observation of morphology and particle size of serum-derived exosomes using transmission electron microscopy ($\times 5\ 000$)

A: Gastric cancer patients' serum-derived exosomes; B: Healthy volunteers' serum-derived exosomes.

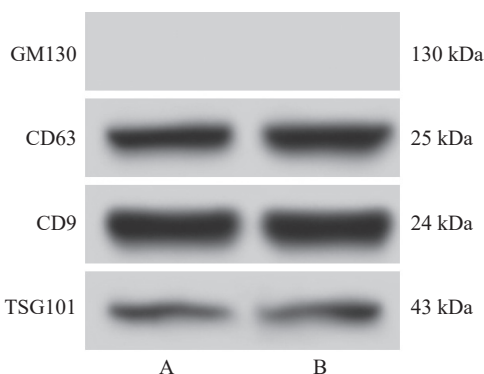


图2 Western blot检测血清来源外泌体表征蛋白表达

Fig. 2 Detection of characterization protein expression of serum-derived exosomes by Western blot

A: Gastric cancer patients' serum-derived exosomes; B: Healthy volunteers' serum-derived exosomes.

表3 血清来源外泌体中miR-143-3p相对表达

Tab. 3 Relative expression of miR-143-3p in serum-derived exosomes

Group	miR-143-3p
Healthy volunteer group	1.01 \pm 0.08
Gastric cancer patient group	0.22 \pm 0.05 ^a
<i>t</i> value	20.512
<i>P</i> value	<0.001

^a: $P < 0.001$, compared with the healthy volunteer group.

2.3 MGC-803细胞对外泌体的摄取验证及血清来源外泌体对细胞miR-143-3p表达的影响

用激光共聚焦显微镜观察到胃癌患者组和健康志愿者组外泌体感染的MGC-803细胞中均有绿色荧光, 正常组MGC-803细胞中没有绿色荧光(图3)。与正常组相比, 胃癌患者组MGC-803细胞miR-143-3p表达稍有升高, 但差异无统计学意义($P > 0.05$); 健康志愿者组MGC-803细胞miR-143-3p表达显著升高($P < 0.05$, 表4)。上述结果说明MGC-803细胞可有效地摄取血清来源外泌体, 且外泌体摄取可导致MGC-803细胞中miR-143-3p水平升高。

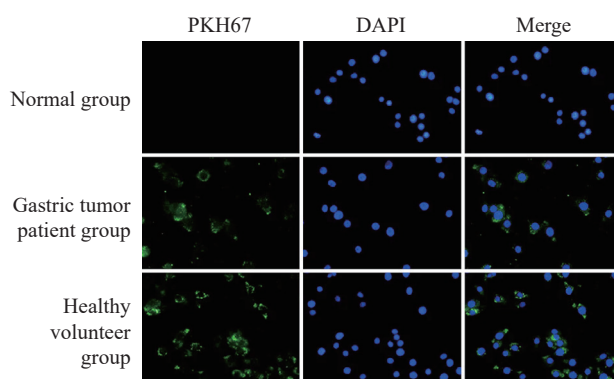


图3 激光共聚焦显微镜下观察MGC-803细胞对外泌体的摄取情况 ($\times 200$)

Fig. 3 Observation of the uptake of extracellular vesicles by MGC-803 cells under laser confocal microscopy ($\times 200$)

表4 血清来源外泌体感染的MGC-803细胞中miR-143-3p相对表达

Tab. 4 Relative expression of miR-143-3p in serum-derived exosomes cultured MGC-803 cells

Group	miR-143-3p
Normal group	1.00 \pm 0.09
Gastric cancer patient group	1.13 \pm 0.14
Healthy volunteer group	2.70 \pm 0.13 ^a
<i>F</i> value	361.332
<i>P</i> value	<0.001

^a: $P < 0.001$, compared with the normal group.

2.4 血清来源外泌体携带miR-143-3p对BPTF的靶向调控作用验证

与正常组相比, 胃癌患者组MGC-803细胞BPTF表达稍有降低, 但差异无统计学意义($P > 0.05$); 健康志愿者组MGC-803细胞BPTF表达显著降低($P < 0.05$, 图4, 表5)。

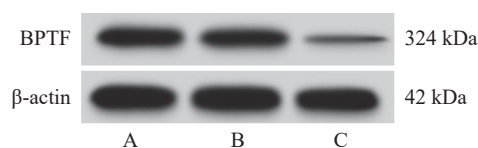


图4 Western blot检测血清来源外泌体温育MGC-803细胞中BPTF蛋白表达

Fig. 4 Detection of BPTF protein expression of serum-derived exosomes cultured MGC-803 cells by Western blot

A: Normal group, B: Gastric cancer patient group, C: Healthy volunteer group.

表5 血清来源外泌体温育的MGC-803细胞中BPTF相对表达
Tab. 5 Relative expression of BPTF in serum-derived exosomes cultured MGC-803 cells

Group	BPTF mRNA	BPTF protein
Normal group	1.00±0.09	0.70±0.07
Gastric cancer patient group	0.92±0.10	0.63±0.08
Healthy volunteer group	0.21±0.05 ^a	0.18±0.04 ^a
<i>F</i> value	165.233	111.116
<i>P</i> value	<0.001	<0.001

^a: *P*<0.001, compared with the normal group.

与NC-miR-143-3p相比, miR-143-3p mimics与野生型BPTF 3'-UTR报告质粒联合转染组细胞相对萤光素酶活性值降低 (*P*<0.05); 与NC-miR-143-3p相比, miR-143-3p mimics与突变型BPTF 3'-UTR报告质粒联合转染组细胞相对萤光素酶活性值无明显变化 (*P*>0.05, 图5, 表6)。

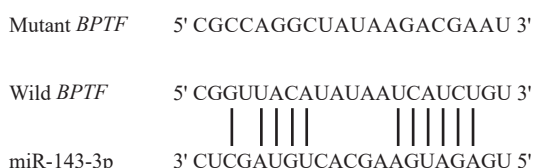


图5 miR-143-3p与BPTF间结合位点及突变位点设计示意图

Fig. 5 Schematic diagram of binding site and mutation site design between miR-143-3p and BPTF

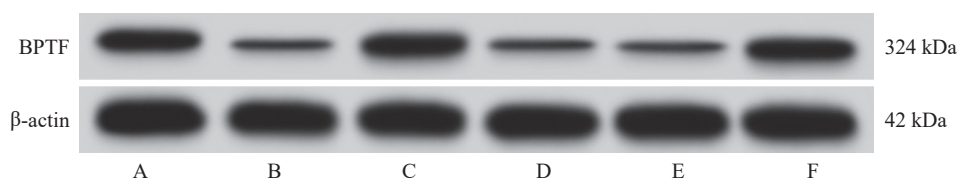


图6 Western blot检测各组MGC-803细胞中BPTF蛋白表达

Fig. 6 Detection of BPTF protein expression in MGC-803 cells from each group by Western blot

A: Normal group; B: Exo-miR-143-3p group; C: siRNA-NC group; D: si-BPTF group; E: Exo-miR-143-3p+pcDNA group; F: Exo-miR-143-3p+pcDNA-BPTF group.

表6 各联合转染组细胞相对萤光素酶活性值

Tab. 6 Relative luciferase activity values of cells in each co-transfection group

Group	Mutant BPTF 3'-UTR reporter plasmid	Wild BPTF 3'-UTR reporter plasmid
NC-miR-143-3p	1.02±0.13	1.01±0.08
miR-143-3p mimics	1.00±0.14	0.23±0.05 ^a
<i>t</i> value	0.256	20.252
<i>P</i> value	0.803	<0.001

^a: *P*<0.001, compared with NC-miR-143-3p.

2.5 各组MGC-803细胞miR-143-3p、BPTF表达及凋亡检测结果

与正常组相比, Exo-miR-143-3p组、si-BPTF组、Exo-miR-143-3p+pcDNA组BPTF相对表达降低 (*P*<0.05), 凋亡率升高 (*P*<0.05); siRNA-NC组BPTF相对表达及凋亡率无明显变化 (*P*>0.05)。与Exo-miR-143-3p组相比, Exo-miR-143-3p+pcDNA-BPTF组BPTF相对表达升高 (*P*<0.05), 凋亡率降低 (*P*<0.05); Exo-miR-143-3p+pcDNA组BPTF相对表达及凋亡率无明显变化 (*P*>0.05, 图6、7, 表7)。

2.6 各组MGC-803细胞侵袭、迁移及MGC-803裸鼠皮下肿瘤体积检测结果

与正常组相比, Exo-miR-143-3p组、si-BPTF组、Exo-miR-143-3p+pcDNA组侵袭数、迁移数、细胞活性及裸鼠皮下肿瘤体积降低 (*P*<0.05), siRNA-NC组侵袭数、迁移数、细胞活性及裸鼠皮下肿瘤体积无明显变化 (*P*>0.05)。与Exo-miR-143-3p组相比, Exo-miR-143-3p+pcDNA-BPTF组侵袭数、迁移数、细胞活性及裸鼠皮下肿瘤体积升高 (*P*<0.05), Exo-miR-143-3p+pcDNA组侵袭数、迁移数、细胞活性及裸鼠皮下肿瘤体积无明显变化 (*P*>0.05, 图8、9, 表8)。

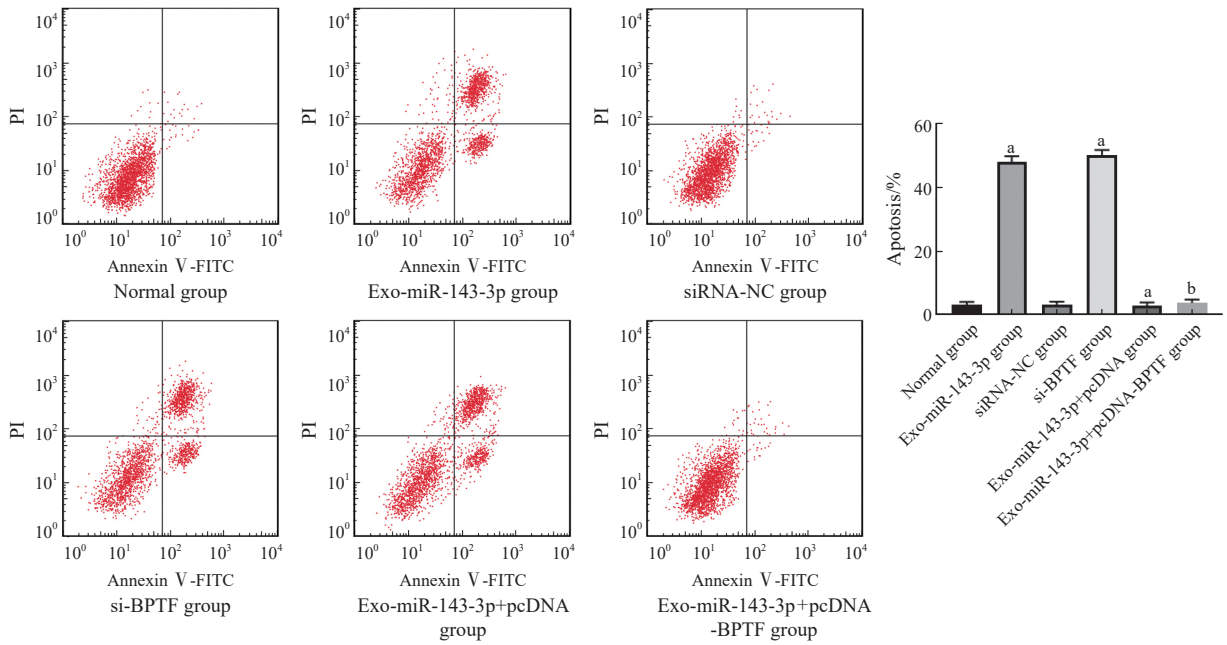


图7 流式细胞实验检测各组MGC-803细胞凋亡率

Fig. 7 Detection of apoptosis rate of MGC-803 cells in each group by flow cytometry experiment

^a: $P < 0.001$, compared with the normal group; ^b: $P < 0.001$, compared with the Exo-miR-143-3p group.

表7 各组MGC-803细胞BPTF相对表达以及凋亡率

Tab. 7 Relative expression of BPTF and apoptosis rate of MGC-803 cells in each group

Group	BPTF mRNA	BPTF protein	Apoptosis rate/%
Normal group	1.00 ± 0.06	0.66 ± 0.05	3.41 ± 0.92
Exo-miR-143-3p group	0.24 ± 0.03 ^a	0.20 ± 0.03 ^a	48.16 ± 1.75 ^a
siRNA-NC group	1.01 ± 0.07	0.67 ± 0.07	3.32 ± 1.04
si-BPTF group	0.23 ± 0.04 ^a	0.19 ± 0.04 ^a	50.25 ± 1.63 ^a
Exo-miR-143-3p+pcDNA group	0.25 ± 0.05 ^a	0.22 ± 0.05 ^a	47.19 ± 1.57 ^a
Exo-miR-143-3p+pcDNA-BPTF group	0.95 ± 0.08 ^b	0.64 ± 0.08 ^b	3.96 ± 1.13 ^b
<i>F</i> value	303.389	118.417	1 920.924
<i>P</i> value	<0.001	<0.001	<0.001

($\bar{x} \pm s, n=6$)

^a: $P < 0.001$, compared with the normal group; ^b: $P < 0.001$, compared with the Exo-miR-143-3p group.

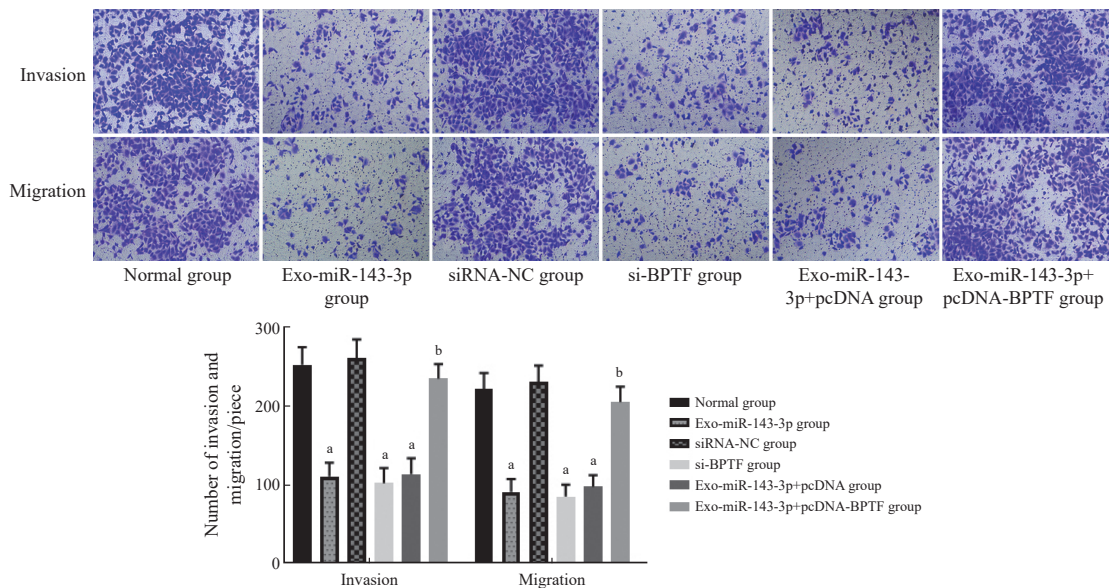


图8 Transwell实验检测各组MGC-803细胞侵袭与迁移 (×200)

Fig. 8 Detection of invasion and migration of MGC-803 cells in each group by Transwell experiment (×200)

^a: $P < 0.001$, compared with the normal group; ^b: $P < 0.001$, compared with the Exo-miR-143-3p group.

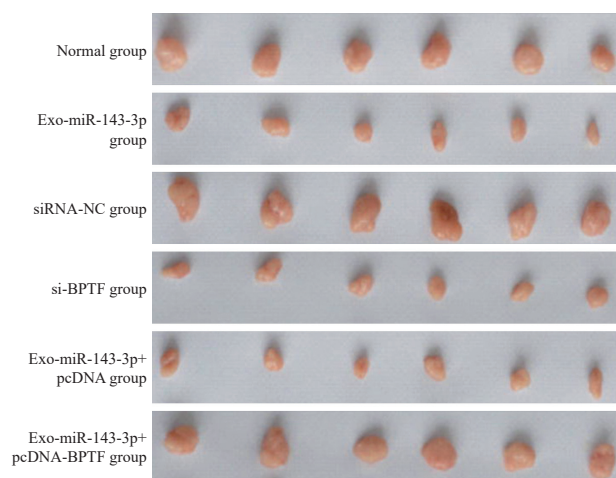


图9 各组MGC-803裸鼠皮下肿瘤图片

Fig. 9 Subcutaneous tumor images of MGC-803 nude mice in each group

表8 各组MGC-803细胞侵袭数、迁移数、细胞活性以及裸鼠皮下肿瘤体积

Tab. 8 Invasion and migration numbers, cell activity of MGC-803 cells and subcutaneous tumor volume of nude mice in each group

Group	Number of invasions/piece	Number of migrations/piece	Cell activity/%	Subcutaneous tumor weight/g	Subcutaneous tumor volume/mm ³
Normal group	251.50 ± 22.34	220.50 ± 20.55	100.00 ± 0.00	1.15 ± 0.13	1103.45 ± 42.76
Exo-miR-143-3p group	110.50 ± 17.53 ^a	91.00 ± 16.32 ^a	51.04 ± 15.36 ^a	0.39 ± 0.08 ^a	348.26 ± 33.54 ^a
siRNA-NC group	260.00 ± 23.70	229.50 ± 21.04	103.52 ± 16.14	1.24 ± 0.12	1139.34 ± 31.45
si-BPTF group	102.00 ± 19.25 ^a	85.00 ± 15.28 ^a	47.80 ± 14.23 ^a	0.37 ± 0.09 ^a	325.17 ± 36.20 ^a
Exo-miR-143-3p+pcDNA group	113.50 ± 20.13 ^a	97.50 ± 14.65 ^a	52.75 ± 17.63 ^a	0.41 ± 0.07 ^a	359.42 ± 30.98 ^a
Exo-miR-143-3p+pcDNA-BPTF group	234.00 ± 18.45 ^b	204.50 ± 19.60 ^b	94.15 ± 13.85 ^b	1.13 ± 0.14 ^b	1083.51 ± 41.16 ^b
F value	86.420	90.174	21.656	95.053	800.301
P value	<0.001	<0.001	<0.001	<0.001	<0.001

^a: P<0.001, compared with the normal group; ^b: P<0.001, compared with the Exo-miR-143-3p group.

泌体作为一种由脂质双层组成的外囊泡，可携带微小RNA、蛋白质等物质，能被内吞到细胞内，进而通过将包括miRNA在内的肿瘤抑制因子带入癌细胞内来发挥抗癌作用^[3, 17-18]。本研究发现，miR-143-3p在胃癌组织及人胃癌细胞系中表达明显降低，胃癌患者血清来源外泌体中miR-143-3p表达水平相比健康志愿者明显降低，表明miR-143-3p与胃癌的发生有关。外泌体摄取实验结果显示，胃癌MGC-803细胞可成功摄取血清来源外泌体，健康志愿者血清来源外泌体可携带miR-143-3p，并可上调MGC-803细胞miR-143-3p表达。本研究功能实验结果显示，以携带miR-143-3p的血清来源外泌体干预MGC-803细胞，可明显降低其细胞侵袭数、迁移数、细胞活性，升高细胞凋亡率，表明血清来源外泌体携带miR-143-3p可在体外抑制胃癌细胞增殖、侵袭及迁移，促进其凋亡；于MGC-803裸鼠移植瘤皮下肿

3 讨 论

胃癌的最主要治疗方法是进行胃切除术，早期患者治愈率较高，但胃癌早期通常没有症状，多数患者进行治疗时胃癌已进展至晚期，且经常发生远端器官转移，此时患者还需辅助进行化疗、放疗、免疫治疗等其他治疗手段，但患者预后很差，还需探寻更有效的治疗方式来提升胃癌患者疗效^[2, 13-14]。miR-143-3p作为一种肿瘤抑制因子，在胃癌中表达显著下调，可作为胃癌诊断标志物，并通过调控靶基因表达介导胃癌的恶性进展^[15]，升高miR-143-3p表达水平可抑制宫颈癌裸鼠体内肿瘤细胞增殖、侵袭，并诱导肿瘤细胞凋亡，最终抑制宫颈癌的肿瘤进展^[16]。外

瘤中心注射携带miR-143-3p的血清来源外泌体，可降低裸鼠皮下肿瘤体积，表明血清来源外泌体携带miR-143-3p可抑制胃癌裸鼠体内肿瘤生长，提示血清来源外泌体携带miR-143-3p可明显抑制胃癌恶性进展。

BPTF是一种重要的表观遗传因子，已被证实发生在癌症的发生、发展中发挥重要作用，可对黑色素瘤起到明显的促肿瘤作用^[19]，BPTF抑制剂可抑制其表观遗传功能，联合厄洛替尼可在体内和体外协同抑制胃癌肿瘤生长，并提升胃癌细胞对厄洛替尼的敏感性^[20]。本研究发现，BPTF在胃癌组织及人胃癌细胞系中表达明显升高，且Western blot和双荧光素酶报告基因实验结果显示，血清来源外泌体携带miR-143-3p可靶向下调BPTF表达，表明BPTF参与介导胃癌的发生过程。本研究结果显示，以携带miR-143-3p的血清来源外泌体干预的同时用质粒过表达BPTF，可

减弱血清来源外泌体携带miR-143-3p对MGC-803细胞增殖、侵袭及迁移的抑制作用, 消除其对MGC-803细胞凋亡的促进作用, 拮抗其对MGC-803裸鼠移植瘤皮下肿瘤生长的抑制作用, 提示血清来源外泌体携带miR-143-3p可通过靶向下调BPTF来抑制胃癌的恶性进展。

综上所述, 血清来源外泌体携带miR-143-3p可明显降低胃癌细胞增殖、侵袭及迁移活性, 促进其凋亡, 抑制体内胃癌肿瘤生长, 最终明显阻碍胃癌恶性进展, 靶向下调BPTF是对胃癌细胞发挥以上抗肿瘤作用的分子机制。本研究为胃癌的诊断治疗提供了新的诊断生物标志物和治疗靶向标志物, 并为胃癌的临床治疗提供了新思路和新方向。但本研究只是使用了一种胃癌细胞系进行研究, 且只在皮下注射的裸鼠移植瘤模型中进行了体内验证, 存在一定局限性, 后续可通过多种胃癌细胞系及原位接种动物模型进行进一步验证, 为临床转化提供更坚实的理论基础。

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