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粉防己碱通过miR-149-5p/*HMGA2*轴调节食管癌细胞的增殖、凋亡、迁移和侵袭

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摘要 该文旨在探讨粉防己碱(Tet)通过miR-149-5p/高迁移率族蛋白A2(*HMGA2*)轴对食管癌细胞增殖、凋亡、迁移和侵袭的影响。qRT-PCR检测食管癌细胞系(EC9706、Eca109、TE-13、TE-1)以及正常食管上皮细胞(HEEC)中miR-149-5p、*HMGA2* mRNA表达水平, 筛选最佳细胞系进行后续实验; 分别用不同浓度(5、10、20 μmol/L) Tet处理筛选得到的最佳细胞系EC9706 48 h, 利用CCK-8法检测细胞的存活率获得合适的Tet浓度; 取对数生长期EC9706细胞, 随机分为对照组、Tet组、Tet+抑制剂阴性对照(inhibitor NC)组、Tet+miR-149-5p抑制剂(miR-149-5p inhibitor)组, qRT-PCR检测各细胞中miR-149-5p、*HMGA2* mRNA表达水平; 流式细胞术检测各组细胞凋亡; Western blot检测间充质标志物N-钙黏蛋白(N-cadherin)、B淋巴细胞瘤-2相关蛋白(Bax)、HMGA2、E-钙黏蛋白(E-cadherin)、细胞周期素D1(CyclinD1)表达水平; Transwell测定细胞迁移与侵袭情况; CCK-8法检测细胞增殖情况; 双荧光素酶实验检验miR-149-5p、*HMGA2*靶向关系。10 μmol/L Tet为该研究最佳浓度。*HMGA2*为miR-149-5p的靶向结合位点。与HEEC细胞相比, 食管癌细胞系中miR-149-5p表达水平显著降低, *HMGA2* mRNA表达水平升高(P<0.05), 其中EC9706细胞中两者水平变化最为显著, 因此, EC9706细胞作为后续实验的最佳实验细胞。与对照组相比, Tet组EC9706细胞24 h、48 h的D_{450}值, 迁移与侵袭细胞数, CyclinD1、N-cadherin、*HMGA2*表达水平显著下降, 凋亡率及Bax、E-cadherin表达水平显著上升(P<0.05); 与Tet+inhibitor NC组相比, Tet+miR-149-5p inhibitor组EC9706细胞24 h、48 h的D_{450}值, 迁移与侵袭细胞数, CyclinD1、N-cadherin、*HMGA2*表达水平显著上升, 凋亡率及Bax、E-cadherin表达水平显著下降(P<0.05)。Tet可能通过上调miR-149-5p, 抑制*HMGA2*表达, 进而诱导食管癌细胞凋亡, 抑制其增殖、迁移和侵袭。

关键词 粉防己碱; miR-149-5p/*HMGA2*轴; 食管癌细胞; 迁移; 侵袭; 增殖; 凋亡

Tetrandrine Regulates the Proliferation, Apoptosis, Migration and Invasion of Esophageal Cancer Cells Via the miR-149-5p/*HMGA2* Axis

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Abstract This study aims to investigate the effects of Tet (tetrandrine) on the proliferation, apoptosis, migration and invasion of esophageal cancer cells through the miR-149-5p/*HMGA2* (high mobility group protein A2)

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axis. The expression levels of miR-149-5p and *HMGA2* mRNA in esophageal cancer cell lines (EC9706, Eca109, TE-13, TE-1) and normal esophageal epithelial cells HEECs were detected by qRT-PCR, and the best cell lines were screened for subsequent experiments; the optimal cell line EC9706 obtained through screening was treated with different concentrations (5, 10, and 20 μmol/L) of Tet for 48 h respectively. CCK-8 assay was used to detect cell viability to obtain appropriate Tet concentration. EC9706 cells in logarithmic growth phase were randomly divided into the Control group, Tet group, Tet+inhibitor negative control (inhibitor NC) group, and Tet+miR-149-5p inhibitor (miR-149-5p inhibitor) group. qRT-PCR was used to detect the expression levels of miR-149-5p and *HMGA2* mRNA in each cell; flow cytometry was used to detect apoptosis in each group; Western blot was used to detect the expression levels of cellular epithelial marker proteins E-cadherin, CyclinD1, mesenchymal marker N-cadherin, Bax (B-lymphoma-2-associated protein), and HMGA2; Transwell was used to measure cell migration and invasion; CCK-8 assay was used to detect cell proliferation; and dual-luciferase experiment was used to examine the targeting relationship between miR-149-5p and *HMGA2*. 10 μmol/L Tet was the best concentration in this study. *HMGA2* was a targeted binding site for miR-149-5p. Compared with HEEC cells, the expression of miR-149-5p in esophageal cancer cell lines was obviously decreased, and the expression of *HMGA2* mRNA was increased (P<0.05), among which EC9706 cells had the most obvious changes and they were used as the best experimental cells for subsequent experiments. Compared with the Control group, the D_{450} value, the numbers of migrating and invasive EC9706 cells, the expression of CyclinD1, N-cadherin and *HMGA2* in the Tet group at 24 h and 48 h were obviously decreased, and the apoptosis rate and the expression of Bax and E-cadherin were obviously increased (P<0.05); compared with Tet+inhibitor NC group, the D_{450} value, the numbers of migrating and invasive EC9706 cells, the expression of CyclinD1, N-cadherin and *HMGA2* in the Tet+miR-149-5p inhibitor group at 24 h and 48 h were obviously increased, and the apoptosis rate and the expression of Bax and E-cadherin were obviously decreased (P<0.05). Tet may up-regulate miR-149-5p and inhibit the expression of *HMGA2*, thereby inducing apoptosis of esophageal cancer cells and inhibiting their proliferation, migration and invasion.

Keywords tetrandrine; miR-149-5p/*HMGA2* axis; esophageal cancer cells; migration; invasion; proliferation; apoptosis

食管癌是消化道常见的恶性肿瘤, 其发病率及死亡率分别全球排名第七和第六, 且呈逐年增长趋势[1-2]。食管癌被发现时大多数处于中晚期, 总体5年生存率约为10%[3]。微小RNA(microRNA, miRNA)是小的内源性非编码RNA, 它们在多种复杂的生理过程中如细胞增殖和凋亡、免疫防御等方面发挥重要作用, miRNA的异常表达与许多人类疾病如癌症等有关[4]。miR-149-5p是miRNA的家族成员, 已被证明在胃癌中下调, 与癌症包括食管癌的恶性行为密切相关[5]。高迁移率族蛋白A2(high mobility group protein A2, *HMGA2*)与DNA中富含AT的区域结合, 改变染色质结构以促进基因转录, 研究表明*HMGA2*在大多数恶性肿瘤如食管癌中高度表达[6]。Starbase数据库(https://starbase.sysu.edu.cn/)显示, miR-149-5p与*HMGA2*具有结合位点, 但miR-149-5p/*HMGA2*轴在食管癌中的作用鲜有报道。粉防己碱(tetrandrine, Tet)是一种双苄基异喹啉, 具有多种药理活性, 已显示出作为抗癌剂的有前途药物[7]。据报道, Tet可以抑制多种癌如结直肠癌细胞的增殖和诱导细胞凋亡, 但在食管癌中鲜有报道[8]。本文旨在探讨Tet对食管癌细胞恶性行为的影响及机制。

1 材料与方法

1.1 细胞来源

由中国科学院典型培养物保藏中心细胞库提供食管癌细胞系(EC9706、Eca109、TE-13、TE-1)以及正常食管上皮细胞(HEEC)。所有细胞均保存在RPMI-1640培养基中, 并进行常规培养。

1.2 主要试剂

Annexin V-FITC/PI凋亡试剂盒、CCK-8购自百奥创新科技有限公司; Tet购自青岛捷世康生物科技有限公司; TRIzol试剂购自Invitrogen公司; ECL试剂

购自翌圣生物科技股份有限公司; 逆转录试剂盒购自Fermentas公司; Transwell小室购自Corning公司; miR-149-5p抑制剂(miR-149-5p inhibitor)及抑制剂阴性对照(inhibitor NC)、miR-149-5p模拟物(miR-149-5p mimics)及模拟物阴性对照(mimics NC)购自上海基因制药有限公司; 上皮标志蛋白E-钙黏蛋白(E-cadherin)、细胞周期素D1(CyclinD1)、间充质标志物N-钙黏蛋白(N-cadherin)、B淋巴细胞瘤-2相关蛋白(B-lymphoma-2-associated protein, Bax)、HMGA2一抗购自Abcam公司; 荧光定量PCR试剂盒购自TaKaRa公司。

1.3 方法

1.3.1 qRT-PCR检测细胞中miR-149-5p、*HMGA2* mRNA表达情况 使用TRIzol试剂从食管癌细胞系、正常食管上皮细胞中分离总RNA, 将总RNA(包含miRNA)与茎环引物混合, 逆转录酶和dNTPs进行miRNA逆转录; 加入引物、逆转录酶和dNTPs进行mRNA逆转录, 并使用SYBR PrimeScript RT PCR试剂盒进行qRT-PCR分析, PCR设置热循环条件: 95 °C初始变性10 min; 95 °C变性15 s, 55 °C退火40 s, 72 °C延伸34 s, 37个循环。miR-149-5p、*HMGA2*分别以*U6*、*β-actin*为标准化的内部对照, 通过$2^{-\Delta\Delta Ct}$方法分析实验数据。引物序列见表1。

1.3.2 不同浓度的Tet对细胞存活率的影响 将对数生长期EC9706细胞接种于96孔板中, 根据前期预实验, 分别于培养基中加入5、10、20 μmol/L Tet处理EC9706细胞, 记为5 μmol/L Tet组、10 μmol/L Tet组、20 μmol/L Tet组, 以未经任何处理的细胞记为对照组。处理48 h后, 每孔加入CCK-8试剂(10 μL), 37 °C培养细胞2 h, 使用酶标仪测量波长为450 nm的*D*值, 分析存活率。

1.3.3 细胞分组与处理 取对数生长期EC9706细胞, 设置对照(Control)组、Tet组、Tet+inhibitor NC组、Tet+miR-149-5p inhibitor组; 其中Tet组为10 μmol/L Tet处理细胞, Tet+inhibitor NC组、Tet+miR-149-5p inhibitor组分别采用转染inhibitor NC、miR-149-5p inhibitor至EC9706细胞, 48 h后, 随后以Tet(10 μmol/L)处理细胞, 48 h后收集各组细胞, 并进行相关结果分析。

1.3.4 CCK-8法 在规定时间向每孔添加10 μL CCK-8 , 37 °C培养细胞2 h。按照制造商提供的说明书, 使用酶标仪在450 nm的波长下测量*D*值。

1.3.5 流式细胞术 将200 μL Annexin V-FITC和10 μL PI添加到各组细胞(10^6个/孔)悬浮液中, 在37 °C下在黑暗中保持30 min。使用流式细胞仪测定细胞凋亡率。

1.3.6 Transwell实验 在无血清RPMI-1640培养基中培养EC9706细胞(2×10^4个细胞)并将其接种到Transwell上室, 下室加入含有10% FBS的RPMI-1640培养基。在37 °C、5% CO_2中孵育48 h后, 用棉签仔细擦去未迁移的细胞, 迁移的细胞室温下经4%多聚甲醛固定15 min, 0.1%结晶紫室温下染色20 min。镜下观察细胞迁移能力。其中侵袭测定中Transwell上室需提前用Matrigel预涂30 min, 其余操作同上述迁移操作步骤。

1.3.7 Western blot实验 采用裂解缓冲液从各组细胞中获得蛋白质, 通过SDS-PAGE分离等量蛋白质前, 进行蛋白质浓度测定, 随后转膜、室温封闭1 h后, 将膜与一抗(1:1 000)在4 °C下孵育过夜, 洗膜后, 并与相应的二抗(1:2 000)在室温下孵育1 h。添加化学发光底物检测条带, ImageJ软件进行半定量分析, 其中一抗为Bax、HMGA2、E-cadherin、β-actin、

表1 qRT-PCR引物序列

Table 1 qRT-PCR primer sequence

基因名称 Gene name	方向 Direction	序列(5′→3′) Sequence (5′→3′)
miR-149-5p	F	CCC TCA TTC TGT GCC ACA CTC CAG CTG GG
	R	TGG TGT CGT GGA GTCG
HMGA2	F	AGT CCC TCT AAA GCA GCT CA
	R	GTC CTC TTC GGC AGA CTC TT
β-actin	F	ATC CAC GAA ACT ACC TTC AAC TC
	R	GAG GAG CAA TGA TCT TGA TCT TC
U6	F	GCT TCG GCA GCA CAT ATA CTA AAA T
	R	CGC TTC ACG AAT TTG CGT GTC AT

CyclinD1、N-cadherin(稀释比例为1:1 000), 二抗为山羊抗兔IgG(稀释比例为1: 2 000)。

1.3.8 qRT-PCR检测细胞中miR-149-5p、*HMGA2* mRNA表达 参照上述1.3.1的操作步骤检测各组细胞中miR-149-5p、*HMGA2* mRNA表达水平。

1.3.9 双荧光素酶实验检验miR-149-5p、*HMGA2*关系 采用Lipofectamine™ 2000转染试剂盒将*HMGA2*突变(mutation type, MUT)、野生(wild type, WT) 3′UTR区与miR-149-5p mimics或mimics NC共转染, 48 h后测定荧光素酶活性。

1.4 统计分析

SPSS 22.0软件用于统计分析, $P<0.05$表示差异具有统计学意义。数据以($\bar{x}\pm s$)表示, 多组间用单因素方差分析, 两组间用SNK-q检验。

2 结果

2.1 食管癌细胞系、正常食管上皮细胞中miR-149-5p、*HMGA2* mRNA表达水平

与HEEC细胞相比, EC9706、Eca109、TE-13、TE-1细胞中miR-149-5p表达水平显著下降, *HMGA2* mRNA表达水平显著升高($P<0.05$), 其中EC9706细胞中两者水平变化最为显著, 并作为后续实验的最佳实验细胞。见表2。

2.2 不同浓度Tet对细胞存活率的影响

不同浓度Tet组存活率较对照组下降($P<0.05$), 其中10 μmol/L Tet组细胞存活率接近50%, 故选用10 μmol/L Tet进行后续实验研究。见表3。

2.3 Tet对miR-149-5p、*HMGA2* mRNA表达水平的影响

Tet组较对照组miR-149-5p水平升高, *HMGA2* mRNA表达水平下降($P<0.05$); Tet+miR-149-5p inhibitor组较Tet+inhibitor NC组miR-149-5p表达水平显著下降, *HMGA2* mRNA表达水平显著升高($P<0.05$)。见表4。

2.4 Tet对细胞增殖的影响

24 h、48 h D_{450}比较: Tet组较对照组下降($P<0.05$); Tet+miR-149-5p inhibitor组较Tet+inhibitor NC组增加($P<0.05$), 见表5。

表2 比较各细胞中miR-149-5p、*HMGA2* mRNA表达水平

Table 2 Comparison of mRNA expression levels of miR-149-5p and *HMGA2* in different cells

组别 Groups	miR-149-5p	*HMGA2* mRNA
HEEC	0.82±0.09	0.19±0.02
Eca109	0.42±0.05*	0.49±0.05*
TE-13	0.53±0.05*	0.55±0.05*
TE-1	0.59±0.06*	0.58±0.05*
EC9706	0.34±0.04*	0.78±0.08*
F	55.492	95.392
P	0.000	0.000

*$P<0.05$, 与HEEC细胞相比。$\bar{x}\pm s$, $n=6$。

*$P<0.05$ compared with HEEC cells. $\bar{x}\pm s$, $n=6$.

表3 不同浓度Tet对细胞存活率的影响

Table 3 Effects of different concentrations of Tet on cell survival rate

组别 Groups	细胞存活率/% Cell survival rate /%
Control	100.00±0.00
5 μmol/L Tet	75.08±7.51*
10 μmol/L Tet	48.11±4.82*
20 μmol/L Tet	34.59±3.46*
F	221.424
P	0.000

*$P<0.05$, 与HEEC细胞相比。$\bar{x}\pm s$, $n=6$。

*$P<0.05$ compared with HEEC cells. $\bar{x}\pm s$, $n=6$.

表4 比较各组中miR-149-5p、*HMGA2* mRNA表达水平

Table 4 Comparison of mRNA expression levels of miR-149-5p and *HMGA2* in each group

组别 Groups	miR-149-5p	*HMGA2* mRNA
Control	0.35±0.04	0.79±0.08
Tet	0.76±0.08*	0.28±0.03*
Tet+inhibitor NC	0.73±0.08	0.29±0.03
Tet+miR-149-5p inhibitor	0.45±0.05#	0.53±0.06#
F	58.923	118.288
P	0.000	0.000

*P<0.05, 与对照组比较; #P<0.05, 与Tet+inhibitor NC组比较。$\bar{x}\pm s$, n=6.

*P<0.05 compared with Control group; #P<0.05 compared with Tet+inhibitor NC group. $\bar{x}\pm s$, n=6.

表5 比较各组中D_{450}值的变化

Table 5 Comparison of D_{450} values in each group

组别 Groups	D_{450}		
	0 h	24 h	48 h
Control	0.22±0.03	0.58±0.06	0.87±0.09
Tet	0.26±0.03	0.31±0.04*	0.57±0.06*
Tet+inhibitor NC	0.27±0.03	0.33±0.04	0.55±0.06
Tet+miR-149-5p inhibitor	0.25±0.03	0.55±0.06#	0.82±0.09#
F	3.111	46.673	28.265
P	0.049	0.000	0.000

*P<0.05, 与对照组比较; #P<0.05, 与Tet+inhibitor NC组比较。$\bar{x}\pm s$, n=6.

*P<0.05 compared with Control group; #P<0.05 compared with Tet+inhibitor NC group. $\bar{x}\pm s$, n=6.

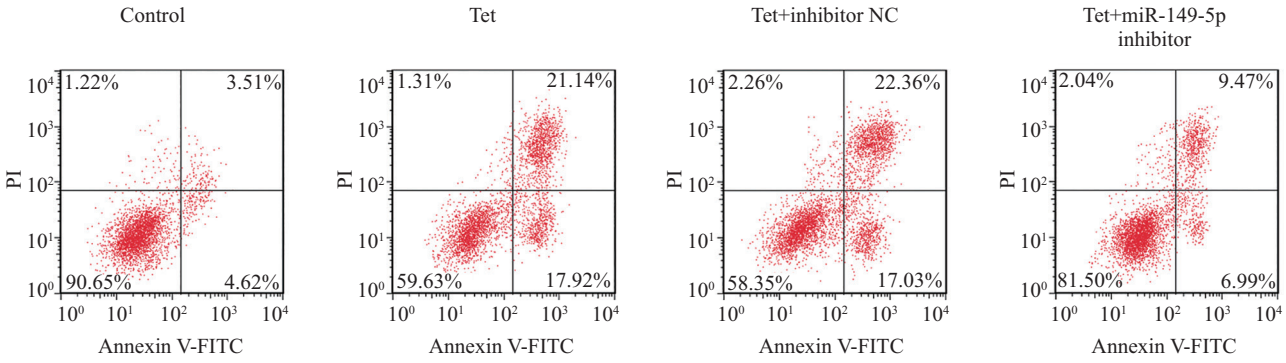


图1 各组细胞凋亡状况

Fig.1 Cell apoptosis in each group

2.5 Tet对凋亡率的影响

凋亡率: Tet组较对照组升高(P<0.05); Tet+miR-149-5p inhibitor组较Tet+inhibitor NC组下降(P<0.05), 见图1和表6。

2.6 Tet对细胞侵袭、迁移的影响

侵袭、迁移比较: Tet组较对照组下降(P<0.05); Tet+miR-149-5p inhibitor组较Tet+inhibitor NC组增加(P<0.05), 见图2和表7。

2.7 Tet对相关蛋白表达水平的影响

Tet组较对照组E-cadherin、Bax水平增加, CyclinD1、N-cadherin、HMGA2表达水平降低(P<0.05); Tet+miR-149-5p inhibitor组较Tet+inhibitor NC组E-cadherin、Bax水平降低, CyclinD1、N-cadherin、HMGA2水平增加(P<0.05), 见图3和表8。

2.8 miR-149-5p、*HMGA2*靶向关系

Starbase数据库显示miR-149-5p、*HMGA2*结

表6 Tet对各组细胞凋亡的影响

Table 6 Effects of Tet on apoptosis in each group

组别 Groups	细胞凋亡率/% Apoptosis rate /%
Control	8.07±0.81
Tet	38.98±3.91*
Tet+inhibitor NC	39.11±3.92
Tet+miR-149-5p inhibitor	16.37±1.64#
F	177.419
P	0.000

*P<0.05, 与对照组比较; #P<0.05, 与Tet+inhibitor NC组比较。$\bar{x}\pm s$, n=6.

*P<0.05 compared with Control group; #P<0.05 compared with Tet+inhibitor NC group. $\bar{x}\pm s$, n=6.

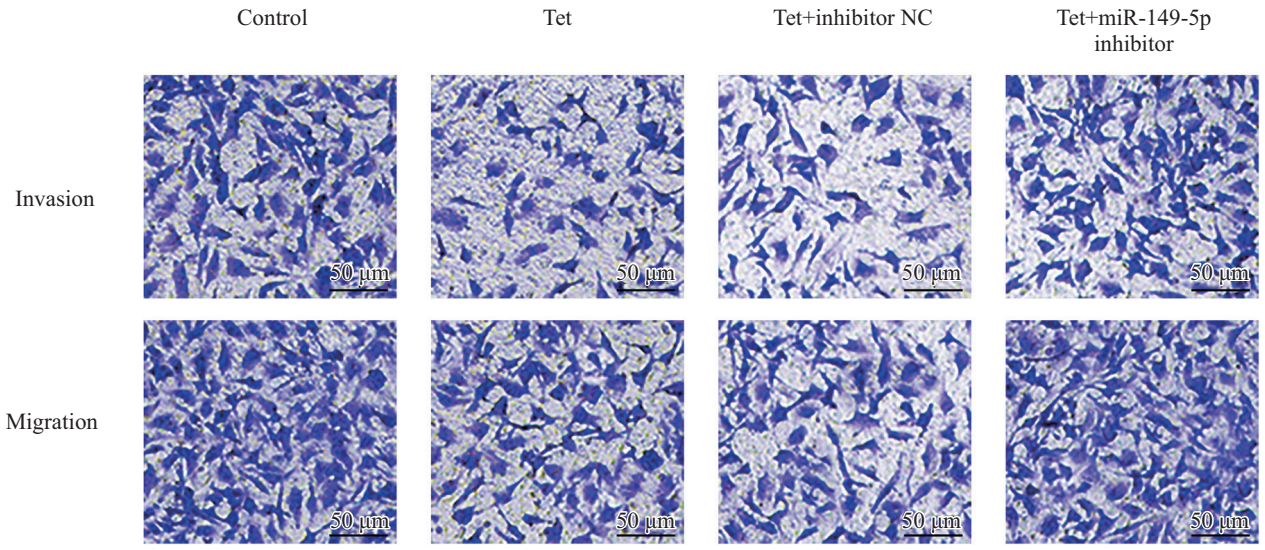


图2 各组细胞侵袭、迁移状况

Fig.2 Cell invasion and migration in each group

表7 Tet对各组细胞侵袭、迁移的影响

Table 7 Effects of Tet on cell invasion and migration in each group

组别 Groups	细胞侵袭数 Number of cell invasions	细胞迁移数 Number of cell migration
Control	127.34±12.74	293.15±29.32
Tet	61.20±6.13*	111.02±11.11*
Tet+inhibitor NC	61.34±6.14	115.34±11.54
Tet+miR-149-5p inhibitor	121.11±12.12#	272.09±27.21#
F	82.871	124.702
P	0.000	0.000

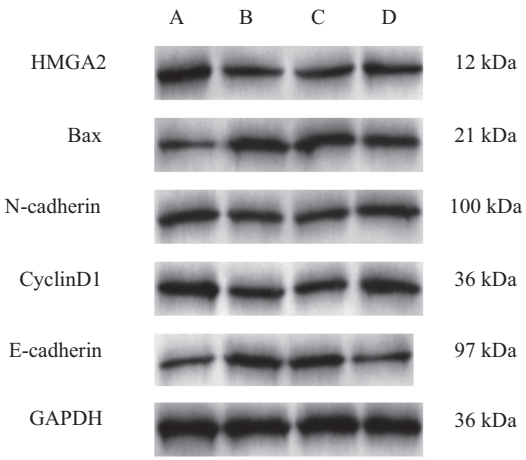
*P<0.05, 与对照组比较; #P<0.05, 与Tet+inhibitor NC组比较。$\bar{x}\pm s$, n=6.

*P<0.05 compared with Control group; #P<0.05 compared with Tet+inhibitor NC group. $\bar{x}\pm s$, n=6.

合位点见图4。与mimics NC+*HMGA2* WT组相比, miR-149-5p mimics+*HMGA2*-WT组荧光素酶活性显著降低(P<0.05), 见表9。

3 讨论

目前食管癌的治疗主要采用放疗、化疗、手术切除、免疫治疗等方法, 但晚期EC患者的预后仍然



A: 对照组; B: Tet组; C: Tet+inhibitor NC组; D: Tet+miR-149-5p inhibitor组。

A: Control group; B: Tet group; C: Tet+inhibitor NC group; D: Tet+miR-149-5p inhibitor group.

图3 各组细胞中蛋白表达情况

Fig.3 Protein expression in cells of each group

表8 Tet对各组细胞相关蛋白表达的影响

Table 8 Effects of Tet on expression of related proteins in cells of each group

组别 Groups	E-cadherin/β-actin	CyclinD1/β-actin	N-cadherin/β-actin	Bax/β-actin	HMGA2/β-actin
Control	0.28±0.03	0.94±0.10	0.75±0.08	0.34±0.04	0.76±0.08
Tet	0.64±0.07*	0.46±0.05*	0.45±0.05*	0.84±0.09*	0.31±0.04*
Tet+inhibitor NC	0.62±0.07	0.48±0.05	0.43±0.05	0.82±0.09	0.34±0.04
Tet+miR-149-5p inhibitor	0.34±0.04#	0.83±0.09#	0.68±0.07#	0.51±0.06#	0.67±0.07#
F	67.902	61.809	38.417	66.794	86.400
P	0.000	0.000	0.000	0.000	0.000

*P<0.05, 与对照组比较; #P<0.05, 与Tet+inhibitor NC组比较。$\bar{x}\pm s$, n=6.

*P<0.05 compared with Control group; #P<0.05 compared with Tet+inhibitor NC group. $\bar{x}\pm s$, n=6.

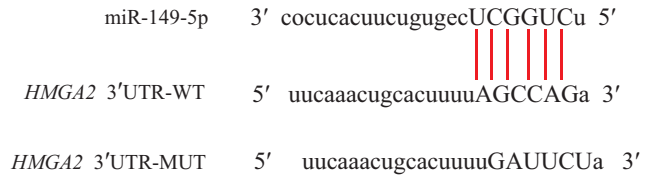


图4 miR-149-5p、*HMGA2*的结合位点

Fig.4 Binding sites of miR-149-5p and *HMGA2*

不理想, 5年生存率较低[9], 因此开发有效的治疗方法成为目前治疗食管癌的研究重点。

天然化合物及其衍生物的是抗癌药物的主要来源之一。中医认为虚、热、瘀、毒是食管癌的影响因素, 因此需清热解毒、补气活血、健脾补肾等[10]。Tet是一种从传统中药植物中提取的生物碱, 除具有免疫抑制、抗病毒等多种生物活性外, 还表现出显著的抗肿瘤活性[11]。如在宫颈癌细胞研究中[12], Tet可以联合顺铂抑制癌细胞生长。但目前Tet对食管癌的作用尚无报道。本研究发现5 μmol/L Tet、10 μmol/L Tet、20 μmol/L Tet处理EC9706细胞后, 细胞存活率均得到不同程度的降低, 表明Tet可以抑制EC9706细胞增殖, 实验选取10 μmol/L Tet为后续研究浓度, 结果发现经Tet处理后, EC9706细胞增殖、侵袭、迁移细胞数均得到抑制, 同时Tet促进EC9706细胞凋亡, 表明Tet可以抑制EC9706细胞的恶性行为。细

表9 靶向验证miR-149-5p、*HMGA2*的关系

Table 9 Targeted validation of the relationship between miR-149-5p and *HMGA2*

组别 Groups	荧光素酶相对活性 Relative activity of luciferase
mimics NC+*HMGA2*-WT	1.04±0.11
miR-149-5p mimics+*HMGA2*-WT	0.45±0.05*
mimics NC+*HMGA2*-MUT	1.03±0.11
miR-149-5p mimics+*HMGA2*-MUT	1.06±0.11
F	58.536
P	0.000

*P<0.05, 与mimics NC+*HMGA2*-WT组比较。

*P<0.05 compared with mimics NC+*HMGA2*-WT group.

胞周期蛋白如CyclinD1控制癌细胞的快速周期性生长；凋亡Bax蛋白属于BCL-2家族，Bax表达量增加是凋亡细胞的特征[13]。在癌症进展过程中，上皮–间质转化过程改变上皮癌细胞的黏附情况、细胞外基质水平，使其变为可移动的间充质细胞，从而促进癌细胞的迁移、侵袭[14]，E-cadherin、N-cadherin是E-cadherin、N-cadherin过程的标志蛋白，Tet处理可下调N-cadherin、CyclinD1表达，上调E-cadherin、Bax表达，进一步表明Tet可能具有抗食管癌的作用，但具体作用机制仍需探究。

miRNA失调与癌症发展有关，已有研究表明miRNA在食管癌的发病机制中发挥着关键作用[15]。miR-149-5p作为miRNA的家族成员，在食管癌研究中鲜有报道，但FENG等[16]研究发现miR-149-5p在结直肠癌细胞和组织中表达水平显著降低，上调其表达可以抑制结直肠癌细胞的恶性行为。miR-149-5p在肝癌细胞中的表达水平降低[17]，上调其表达可以抑制细胞的活力、增殖和迁移能力。本研究发现EC9706细胞中miR-149-5p表达水平降低，结合先前报道显示miR-149-5p在食管鳞状细胞癌组织下调[18]，提示miR-149-5p可能参与食管癌发展。另外，miR-149-5p、*HMGA2*存在结合位点，且双荧光素酶实验检验*HMGA2*为miR-149-5p的直接靶点。WU等[19]研究发现*HMGA2*在食管鳞状细胞癌组织及细胞中表达，这可为食管癌治疗提供一种有效策略。本研究发现HMGA2 mRNA及蛋白在EC9706细胞中表达水平显著升高，与CHE等[20]研究结果相吻合。miR-149-5p参与调控骨肉瘤细胞迁移，其机制与*HMGA2*有关[21]，本研究推测miR-149-5p/*HMGA2*可能参与食管癌的发展。经Tet处理后，EC9706细胞的恶性行为被抑制，但miR-149-5p表达水平升高，HMGA2 mRNA及蛋白表达水平降低，表明Tet发挥抗癌作用，可能通过上调miR-149-5p表达、下调*HMGA2*表达实现。为进一步验证该结论，文章引入miR-149-5p inhibitor进行反面回复性实验，结果发现抑制miR-149-5p表达逆转了Tet对EC9706细胞的抗癌作用，若采用miR-149-5p mimics联合Tet处理细胞，推测可能加强Tet对EC9706细胞的抗癌作用，反差效果远不如miR-149-5p inhibitor显著，表明Tet可以诱导EC9706细胞凋亡，抑制其增殖、侵袭及迁移，可能与上调miR-149-5p、抑制*HMGA2*有关。

综上所述，Tet通过上调miR-149-5p、抑制*HMGA2*表达诱导EC9706细胞凋亡、抑制其恶性生物学行为，为临床治疗食管癌提供潜在药物，但由于疾病发病机制及药物作用机制复杂，仍需进一步验证。

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