

利多卡因调节RhoA/ROCK轴 对结直肠癌细胞生物学行为的影响

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摘要 该文主要探讨利多卡因(lidocaine, Lido)通过调节Ras同源基因家族成员A(RhoA)/Rho相关的卷曲螺旋激酶(ROCK)轴对结直肠癌(CRC)细胞生物学行为的影响。该研究使用0~1 250 $\mu\text{mol/L}$ 的利多卡因处理人结直肠癌细胞LS513, CCK-8法检测细胞活力筛选适宜药物浓度。将细胞分为对照组(Control组)、利多卡因低浓度组(Lido-L组, 500 $\mu\text{mol/L}$ Lido)、利多卡因中浓度组(Lido-M组, 750 $\mu\text{mol/L}$ Lido)、利多卡因高浓度组(Lido-H组, 1 000 $\mu\text{mol/L}$ Lido)和利多卡因高浓度+ROCK信号通路激活剂LPA组(Lido-H+LPA组, 1 000 $\mu\text{mol/L}$ Lido+10 $\mu\text{mol/L}$ LPA)。Edu检测细胞增殖; 划痕愈合实验和Transwell小室实验分别检测细胞迁移和侵袭能力; 流式细胞仪检测细胞凋亡情况; Western blot检测PCNA、Bax、Bcl-2、RhoA、ROCK 1、E-cadherin和N-cadherin蛋白表达情况。该研究得出与0 $\mu\text{mol/L}$ 利多卡因相比, 500 $\mu\text{mol/L}$ 、750 $\mu\text{mol/L}$ 、1 000 $\mu\text{mol/L}$ 和1 250 $\mu\text{mol/L}$ 利多卡因处理的LS513细胞活力显著降低($P<0.05$), 选择500 $\mu\text{mol/L}$ 、750 $\mu\text{mol/L}$ 和1 000 $\mu\text{mol/L}$ 的利多卡因进行后续实验。与Control组相比, Lido-L组、Lido-M组和Lido-H组LS513细胞Edu阳性率, 划痕愈合率, 细胞侵袭数及PCNA、N-cadherin、Bcl-2、RhoA和ROCK 1蛋白表达水平降低($P<0.05$), 细胞凋亡率以及E-cadherin和Bax蛋白表达增加($P<0.05$); 与Lido-H组相比, Lido-H+LPA组LS513细胞Edu阳性率, 划痕愈合率, 细胞侵袭数及PCNA、N-cadherin、Bcl-2、RhoA和ROCK 1蛋白表达水平显著增加($P<0.05$), 细胞凋亡率、E-cadherin和Bax蛋白表达水平显著降低($P<0.05$)。利多卡因可能通过抑制RhoA/ROCK信号通路抑制结直肠癌细胞恶性生物学行为。

关键词 利多卡因; Ras同源基因家族成员A/Rho相关的卷曲螺旋激酶信号通路; 结直肠癌; 恶性生物学行为

Effect of Lidocaine Regulating RhoA/ROCK Axis on Biological Behavior of Colorectal Cancer Cells

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Abstract This study focused on the effects of Lido (lidocaine) on the biological behavior of CRC (colorectal cancer) cells by regulating the RhoA (Ras homologous gene family member A)/ROCK (Rho associated coiled-

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coil forming protein kinase) axis. In this study, human colorectal cancer cells LS513 were treated with 0-1 250 $\mu\text{mol/L}$ lidocaine, and cell viability was detected by CCK-8 method to screen suitable drug concentration. The cells were grouped into Control group, lidocaine low concentration group (Lido-L group, 500 $\mu\text{mol/L}$ Lido), lidocaine medium concentration group (Lido-M group, 750 $\mu\text{mol/L}$ Lido), lidocaine high concentration group (Lido-H group, 1 000 $\mu\text{mol/L}$ Lido) and lidocaine high concentration+ROCK signal pathway activator LPA group (Lido-H+LPA group, 1 000 $\mu\text{mol/L}$ Lido+10 $\mu\text{mol/L}$ LPA group). Edu was applied to detect cell proliferation; scratch healing test and transwell chamber test were applied to detect cell migration and invasion ability, respectively; flow cytometry was applied to detect cell apoptosis; Western blot was applied to detect the expression of PCNA, Bax, Bcl-2, RhoA, ROCK 1, E-cadherin, and N-cadherin proteins. In this study, compared with 0 $\mu\text{mol/L}$ Lido, the activity of LS513 cells treated with 500 $\mu\text{mol/L}$, 750 $\mu\text{mol/L}$, 1 000 $\mu\text{mol/L}$ and 1 250 $\mu\text{mol/L}$ Lido obviously decreased ($P<0.05$), lidocaine of 500 $\mu\text{mol/L}$, 750 $\mu\text{mol/L}$ and 1 000 $\mu\text{mol/L}$ was selected for subsequent experiments. Compared with the Control group, the Edu positive rate, scratch healing rate, cell invasion number, and PCNA, N-cadherin, Bcl-2, RhoA, and ROCK 1 protein expression of LS513 cells in the Lido-L group, Lido-M group, and Lido-H group decreased sequentially ($P<0.05$), the apoptosis rate, E-cadherin and Bax protein expression increased sequentially ($P<0.05$); compared with the Lido-H group, the Edu positive rate, scratch healing rate, cell invasion number, and PCNA, N-cadherin, Bcl-2, RhoA, and ROCK 1 protein expression of LS513 cells in the Lido-H+LPA group obviously increased ($P<0.05$), the apoptosis rate, E-cadherin, and Bax protein expression were obviously reduced ($P<0.05$). Lidocaine may inhibit the malignant biological behavior of colorectal cancer cells by inhibiting RhoA/ROCK signaling pathway.

Keywords lidocaine; Ras homolog gene family member A/Rho associated coiled-coil forming protein kinase signal pathway; colorectal cancer; malignant biological behavior

结直肠癌 (colorectal cancer, CRC) 是一种预后不良的致命疾病, 是癌症死亡的第四大原因, 肥胖、缺乏体育锻炼和吸烟等不利风险因素都与结直肠癌发病率有关^[1]。局部麻醉剂可能具有抗癌作用, 利多卡因 (lidocaine, Lido) 是一种熟悉的局部麻醉剂, 具有镇痛和抗炎作用^[2-3], 在细胞水平上涉及的作用机制包括抑制增殖、侵袭和迁移^[4]。研究表明, 利多卡因可以通过上调 miR-520a-3p 来抑制 EGFR 的表达, 诱导结直肠癌细胞凋亡和抑制增殖^[5]。据报道 RhoA/ROCK 在控制细胞运动方面非常重要, RhoA 是小鸟苷三磷酸酶 (GTPases) Ras 超家族的成员, 因其在细胞骨架重塑中的作用而备受关注, RhoA 的作用是由 Rho 相关的含有卷曲螺旋的蛋白激酶 (Rho associated coiled-coil forming protein kinase, ROCK) 介导的, 最终导致细胞迁移、黏附、增殖、收缩和凋亡等变化^[6]。研究表明 MYO1B 能通过 RhoA/ROCK/FAK 信号转导促进 F-肌动蛋白重排和局灶性黏附组装来增强结直肠癌转移水平^[7]。麻木素能抑制 RhoA 激活, 干扰 ROCK 表达, 进而抑制细胞增殖和迁移^[8]。本文探讨利多卡因通过 RhoA/ROCK 信号通路对结直肠癌细胞恶性生物学行为的影响机制, 为结直肠癌的

治疗提供参考。

1 材料与方法

1.1 材料

人结直肠癌细胞 LS513 (CL-0640) 购自武汉普诺赛生命科技有限公司; 利多卡因 (PHR1257)、溶血磷脂酸 (lysophosphatidic acid, LPA) (L7260) 购自德国 Merck 公司; CCK-8 检测试剂盒 (E-CK-A362)、Edu 检测试剂盒 (E-CK-A376) 购自武汉伊莱瑞特生物科技股份有限公司; Attune NxT 流式细胞仪 (A28993) 购自美国 ThermoFisher 公司; 抗体 PCNA (ab18197)、Bax (ab32503)、Bcl-2 (ab182858)、RhoA (ab187027)、ROCK 1 (ab134181)、E-cadherin (ab40772)、N-cadherin (ab76011)、GAPDH (ab8245)、兔抗小鼠 IgG (ab6728) 均购自英国 Abcam 公司。

1.2 方法

1.2.1 细胞活力检测 人结直肠癌细胞 LS513 在培养箱 (37 $^{\circ}\text{C}$ 、5% CO_2) 中于补充有 10% 胎牛血清 (fetal bovine serum, FBS)、5% 青霉素/链霉素的 DMEM 培养基中生长, 细胞达到 80% 以上时进行实验。

使用 CCK-8 法检测细胞活力。将对数生长

期LS513细胞(1×10^5 个)接种于96孔板中孵育(37 °C、5% CO₂)培养,然后将细胞与0 μmol/L、250 μmol/L、500 μmol/L、750 μmol/L、1 000 μmol/L、1 250 μmol/L的利多卡因,分别孵育(37 °C、5% CO₂) 24 h、48 h和72 h,随后,去除培养基,向每个孔中添加100 μL基础培养基和10 μL CCK-8试剂,并将细胞再培养4 h。在波长为450 nm处,酶标仪检测吸光度(D)值。

1.2.2 细胞分组与处理 将对数生长期的细胞分为对照组(Control组)、利多卡因低浓度组(Lido-L组)、利多卡因中浓度组(Lido-M组)、利多卡因高浓度组(Lido-H组)和利多卡因高浓度+ROCK信号通路激活剂LPA组(Lido-H+LPA组)。Lido-L组、Lido-M组、Lido-H组分别使用500、750和1 000 μmol/L的利多卡因处理细胞24 h, Lido-H+LPA组使用1 000 μmol/L的利多卡因和10 μmol/L的ROCK信号通路激活剂LPA^[9]处理细胞24 h, Control组给予等量的生理盐水。

1.2.3 细胞增殖检测 取各组LS513细胞接种到96孔板进行培养,约 5×10^4 个/孔。与10 μmol/L Edu于37 °C培养2 h,并在4%甲醛中室温固定15 min。用Apollo反应混合物和DAPI(鉴定细胞核)染色30 min,在荧光显微镜下对增殖阳性细胞进行拍照并在显微镜下计数。

1.2.4 细胞迁移和侵袭能力检测 将LS513细胞(2×10^5 个/孔)接种在6孔板中。在达到90%~100%融合后,使用无菌移液管尖端将细胞单层划成直线,然后仔细清洗以去除分离的细胞。随后,细胞在培养箱(37 °C、5% CO₂)中培养24 h。在0 h和24 h处拍摄记录划痕的照片。计算细胞划痕愈合率。划痕愈合率(%)= $[(W_{0h}-W_{24h})/W_{0h}] \times 100\%$ 。

Transwell检测细胞侵袭。上层用无血清培养基填充细胞。下层补充血清培养基。37 °C下孵育24 h,室温下甲醛固定20 min。然后,用0.5%结晶紫对细胞染色10 min。最后,使用光学显微镜拍摄图像,随机选择5个视野,计算每个视野侵袭细胞数量。

1.2.5 细胞凋亡检测 各组细胞处理24 h后,将细胞用胰蛋白酶室温消化2 min并在PBS中洗涤3次,然后在结合缓冲液中重新悬浮。在黑暗中用5 μL膜联蛋白V-FITC和10 μL PI溶液双重染色10 min后,采用流式细胞仪检测细胞凋亡情况。

1.2.6 相关蛋白表达检测 使用RIPA溶液裂解各组细胞培养液提取细胞总蛋白,BCA测定蛋白浓度,10% SDS-PAGE分离蛋白,然后转移至PVDF膜

上,封闭缓冲液室温封闭1 h,后与一抗Bax(1:2000)、Bcl-2(1:2 000)、RhoA(1:5 000)、ROCK 1(1:1 000)、E-cadherin(1:1 000)、N-cadherin(1:1 000)和GAPDH(1:5 000)于4 °C孵育过夜,与二抗兔抗小鼠IgG(1:2 000)室温孵育1 h。EDL显影,使用ImageJ软件测量蛋白质条带的灰度值。

1.3 数据分析

所有实验数据采用SPSS 27进行统计分析,实验数据符合正态分布以平均值±标准差表示,多组间比较采用单因素方差分析,组间两两比较采用LSD-*t*检验。 $P < 0.05$ 为差异具有统计学意义。

2 结果

2.1 利多卡因对LS513细胞活力的影响

培养24 h、48 h和72 h后,与0 μmol/L利多卡因相比,500 μmol/L、750 μmol/L、1 000 μmol/L和1 250 μmol/L利多卡因处理的LS513细胞活力显著降低($P < 0.05$),250 μmol/L的利多卡因处理后细胞活力虽有降低但差异不具有统计学意义($P > 0.05$)。1 250 μmol/L的利多卡因处理后细胞活力较低,因此选择500 μmol/L、750 μmol/L和1 000 μmol/L的利多卡因进行后续实验(图1和表1)。

2.2 利多卡因对LS513细胞增殖的影响

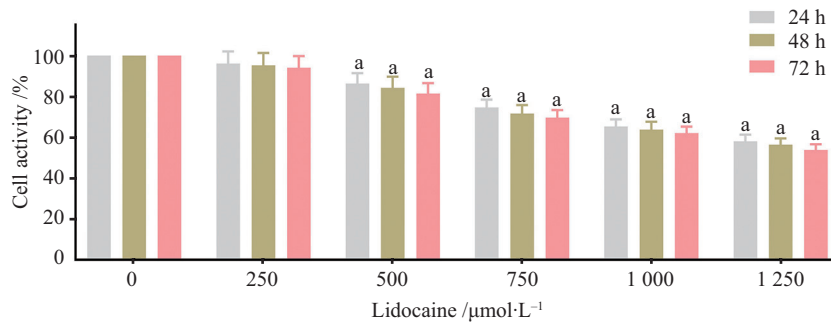
与Control组相比,Lido-L组、Lido-M组和Lido-H组LS513细胞Edu阳性率、PCNA蛋白表达水平呈浓度依赖性降低($P < 0.05$);与Lido-H组相比,Lido-H+LPA组LS513细胞Edu阳性率、PCNA蛋白表达水平显著增加($P < 0.05$)(图2、图3、图4和表2)。

2.3 利多卡因对LS513细胞迁移和侵袭能力的影响

与Control组相比,Lido-L组、Lido-M组和Lido-H组LS513细胞划痕愈合率和细胞侵袭数呈浓度依赖性降低($P < 0.05$);与Lido-H组相比,Lido-H+LPA组LS513细胞划痕愈合率和细胞侵袭数显著增加($P < 0.05$)(图5、图6、图7和表3)。

2.4 利多卡因对LS513细胞凋亡情况和凋亡相关蛋白的影响

与Control组相比,Lido-L组、Lido-M组和Lido-H组LS513细胞凋亡率和Bax蛋白表达水平显著增加($P < 0.05$),Bcl-2蛋白表达水平显著降低($P < 0.05$),且呈浓度依赖性;与Lido-H组相比,Lido-H+LPA组LS513细胞凋亡率和Bax蛋白表达水平显著降低($P < 0.05$),Bcl-2蛋白表达水平显著增加($P < 0.05$)(图



^a*P*<0.05与0 μmol/L组比较。n=6。

^a*P*<0.05 vs 0 μmol/L group. n=6.

图1 利多卡因对LS513细胞活力的影响

Fig.1 Effects of lidocaine on the viability of LS513 cells

表1 利多卡因对LS513细胞活力的影响

Table 1 Effects of lidocaine on the viability of LS513 cells

分组 Groups	细胞活力/% Cell activity /%		
	24 h	48 h	72 h
0 μmol/L	100.00±0	100.00±0	100.00±0
250 μmol/L	96.07±5.94	95.24±6.03	94.05±5.64
500 μmol/L	86.26±5.15 ^a	84.19±5.47 ^a	81.44±5.02 ^a
750 μmol/L	74.58±3.86 ^a	71.62±4.13 ^a	69.58±3.77 ^a
1 000 μmol/L	65.31±3.41 ^a	63.74±3.85 ^a	62.03±3.15 ^a
1 250 μmol/L	58.06±3.19 ^a	56.38±3.04 ^a	53.82±2.76 ^a
<i>F</i>	104.076	102.523	137.158
<i>P</i>	<0.001	<0.001	<0.001

^a*P*<0.05与0 μmol/L组比较。n=6。

^a*P*<0.05 vs 0 μmol/L group. n=6.

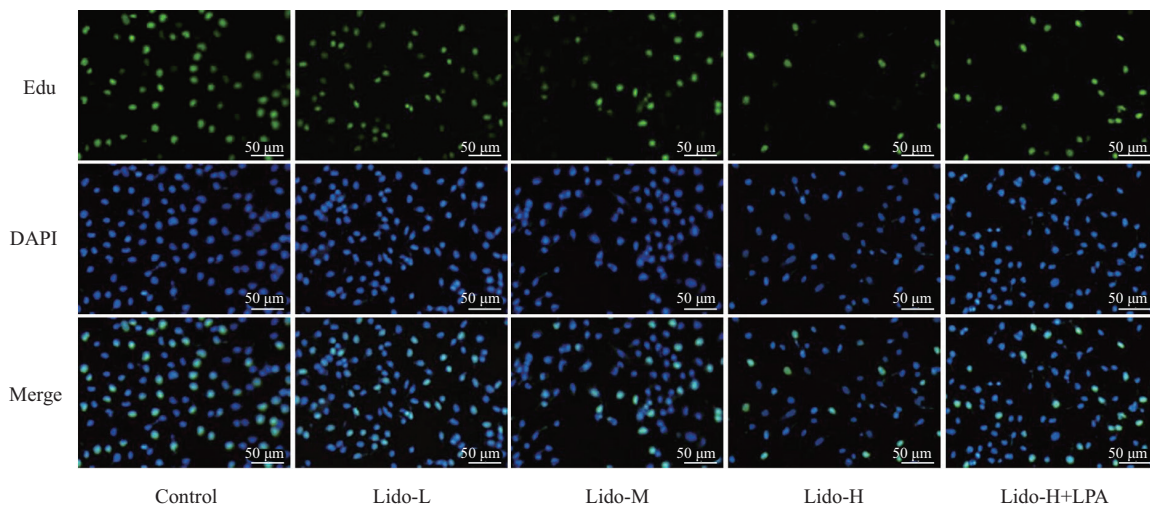


图2 利多卡因对LS513细胞增殖的影响

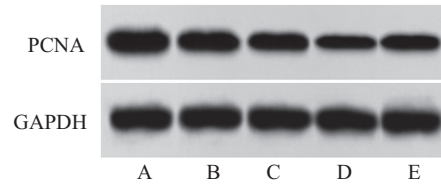
Fig.2 Effect of lidocaine on proliferation of LS513 cells

8、图9、图10和表4)。

2.5 利多卡因对LS513细胞E-cadherin和N-cadherin蛋白表达的影响

与Control组相比, Lido-L组、Lido-M组和

Lido-H组LS513细胞E-cadherin蛋白表达水平显著增加(*P*<0.05), N-cadherin蛋白表达水平显著降低(*P*<0.05), 且呈浓度依赖性; 与Lido-H组相比, Lido-H+LPA组LS513细胞E-cadherin蛋白表达水平显著

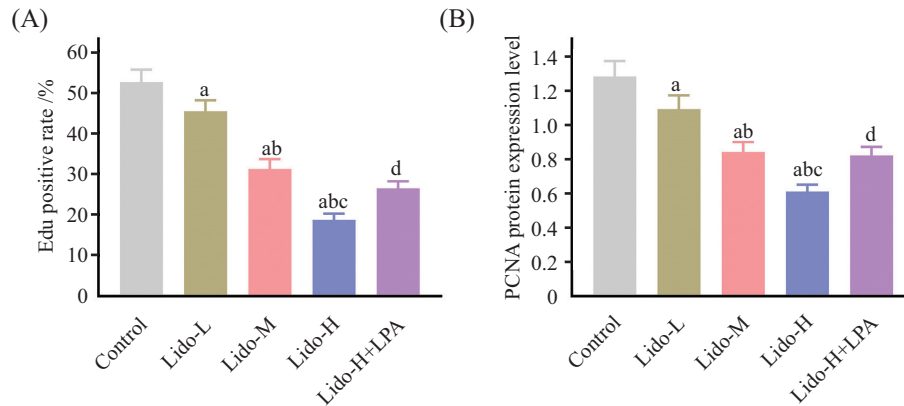


A: Control组; B: Lido-L组; C: Lido-M组; D: Lido-H组; E: Lido-H+LPA组。

A: Control group; B: Lido-L group; C: Lido-M group; D: Lido-H group; E: Lido-H+LPA group.

图3 利多卡因对增殖相关蛋白水平的影响

Fig.3 Effects of lidocaine on proliferation-related protein levels



A: Edu阳性率; B: PCNA蛋白表达。^a $P < 0.05$ vs Control组; ^b $P < 0.05$ vs Lido-L组; ^c $P < 0.05$ vs Lido-M组; ^d $P < 0.05$ vs Lido-H组。 $n = 6$ 。

A: Edu positive rate; B: PCNA protein expression. ^a $P < 0.05$ vs Control group; ^b $P < 0.05$ vs Lido-L group; ^c $P < 0.05$ vs Lido-M group; ^d $P < 0.05$ vs Lido-H group. $n = 6$.

图4 利多卡因对LS513细胞增殖的影响

Fig.4 Effect of lidocaine on proliferation of LS513 cells

表2 利多卡因对LS513细胞增殖的影响

Table 2 Effects of lidocaine on proliferation of LS513 cells

分组 Groups	Edu阳性率/% Edu positive rate /%	PCNA蛋白表达量 PCNA protein expression level
Control	52.43±3.01	1.28±0.09
Lido-L	45.28±2.68 ^a	1.09±0.08
Lido-M	31.15±2.42 ^{ab}	0.84±0.06
Lido-H	18.69±1.53 ^{abc}	0.61±0.04
Lido-H+LPA	26.42±1.74 ^d	0.82±0.05
<i>F</i>	207.671	94.423
<i>P</i>	<0.001	<0.001

^a $P < 0.05$ vs Control组; ^b $P < 0.05$ vs Lido-L组; ^c $P < 0.05$ vs Lido-M组; ^d $P < 0.05$ vs Lido-H组。 $n = 6$ 。

^a $P < 0.05$ vs Control group; ^b $P < 0.05$ vs Lido-L group; ^c $P < 0.05$ vs Lido-M group; ^d $P < 0.05$ vs Lido-H group. $n = 6$.

降低 ($P < 0.05$), N-cadherin蛋白表达水平显著增加 ($P < 0.05$)(图11、图12和表5)。

2.6 利多卡因对LS513细胞通路相关蛋白表达的影响

与Control组相比, Lido-L组、Lido-M组和Lido-H组LS513细胞RhoA和ROCK 1蛋白表达呈浓度依赖性降低($P < 0.05$); 与Lido-H组相比, Lido-H+LPA组

LS513细胞RhoA和ROCK 1蛋白表达水平显著增加 ($P < 0.05$)(图13、图14和表6)。

3 讨论

CRC是最常见的癌症之一,也是全球主要的健康负担,在全球发病率和死亡率方面均排名第三,其病因非常复杂,涉及遗传和环境因素^[10]。因此,迫切

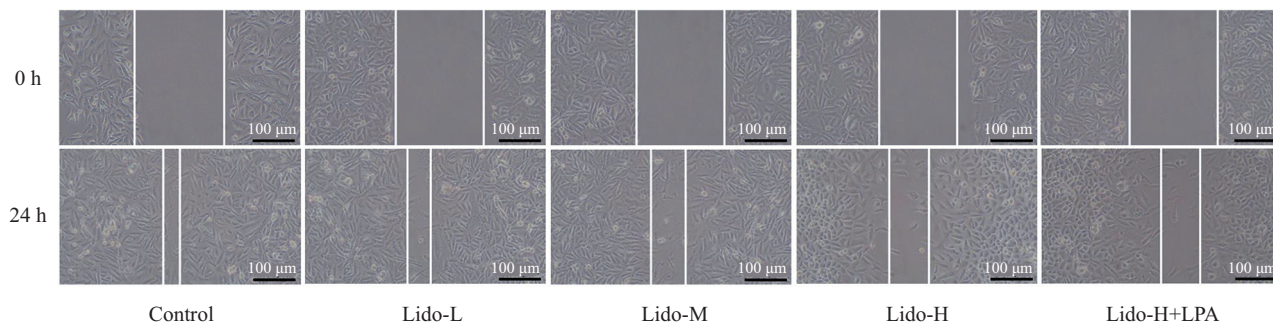


图5 划痕实验检测LS513细胞迁移能力

Fig.5 Migration ability of LS513 cells detected by scratch test

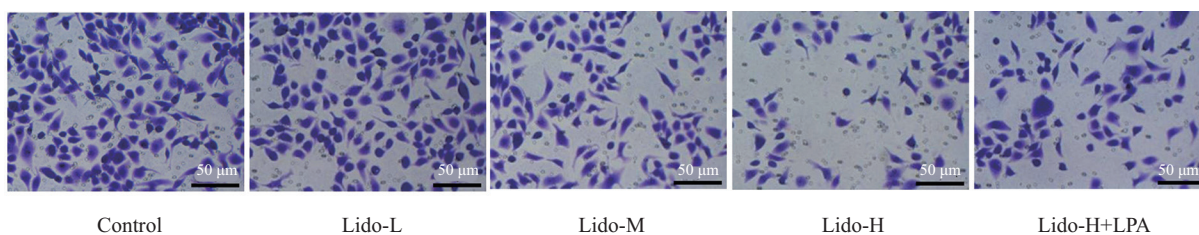
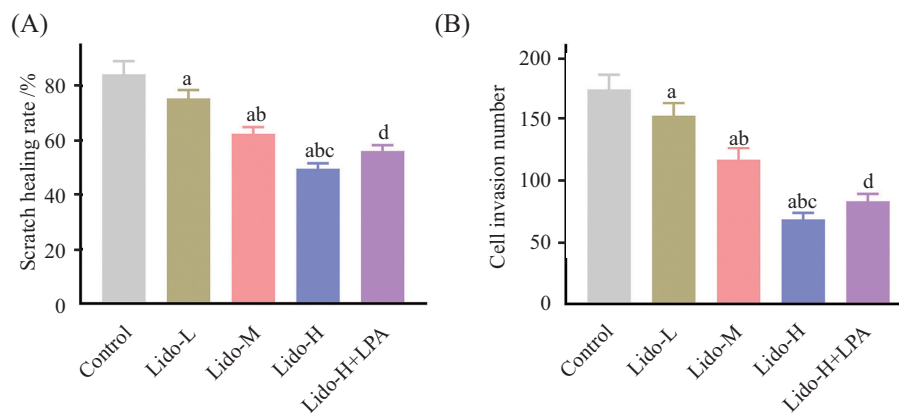


图6 Transwell小室检测LS513细胞侵袭能力

Fig.6 Invasion ability of LS513 cells detected by Transwell chamber



A: 细胞划痕愈合率; B: 细胞侵袭数。^a $P < 0.05$ vs Control组; ^b $P < 0.05$ vs Lido-L组; ^c $P < 0.05$ vs Lido-M组; ^d $P < 0.05$ vs Lido-H组。 $n = 6$ 。

A: cell scratch healing rate; B: cell invasion number. ^a $P < 0.05$ vs Control group; ^b $P < 0.05$ vs Lido-L group; ^c $P < 0.05$ vs Lido-M group; ^d $P < 0.05$ vs Lido-H group. $n = 6$ 。

图7 利多卡因对LS513细胞迁移和侵袭能力的影响

Fig.7 Effects of lidocaine on migration and invasion abilities of LS513 cells

需要开发新的CRC治疗药物。研究表明, 麻醉药物对免疫系统和癌症细胞功能有影响, 其中一些药物已显示对癌症细胞具有直接的抗增殖和促进凋亡作用, 对癌症复发和转移有影响^[11]。利多卡因是一种常用的局部麻醉剂, 发挥着多种作用, 可能对多种肿瘤细胞具有抗肿瘤作用, 对癌细胞具有抗增殖或细胞毒性作用^[12]。WANG等^[13]研究表明利多卡因能通过circITFG2/miR-1204/SOCS2轴抑制CRC细胞的增殖、转移并促进细胞凋亡, 阻碍CRC进展。本研究

中利多卡因干预后, LS513细胞增殖活力、迁移和侵袭能力降低, 表明利多卡因能抑制结直肠癌细胞的增殖、迁移和侵袭, 与报道类似。

促凋亡和抗凋亡Bcl-2家族蛋白与细胞凋亡有关, 细胞凋亡通常由细胞内半胱氨酸蛋白酶(半胱天冬酶)介导, 其启动和执行细胞凋亡过程^[14]。Bax接收到凋亡信号, 发生低聚并转移到线粒体膜, 随后导致细胞色素c和其他凋亡因子释放到细胞质中, 引发半胱氨酸天冬氨酸级联反应, 最终导致细胞死亡^[15]。

表3 利多卡因对LS513细胞迁移和侵袭能力的影响

Table 3 Effects of lidocaine on migration and invasion abilities of LS513 cells

分组 Groups	划痕愈合率/% Scratch healing rate /%	细胞侵袭数 Cell invasion number
Control	84.27±4.83	174.33±12.04
Lido-L	75.39±3.12 ^a	153.07±10.36 ^a
Lido-M	62.43±2.47 ^{ab}	117.40±9.35 ^{ab}
Lido-H	49.57±2.02 ^{abc}	68.93±5.14 ^{abc}
Lido-H+LPA	56.08±2.17 ^d	83.50±6.04 ^d
<i>F</i>	125.440	148.858
<i>P</i>	<0.001	<0.001

^a*P*<0.05 vs Control组; ^b*P*<0.05 vs Lido-L组; ^c*P*<0.05 vs Lido-M组; ^d*P*<0.05 vs Lido-H组。n=6。

^a*P*<0.05 vs Control group; ^b*P*<0.05 vs Lido-L group; ^c*P*<0.05 vs Lido-M group; ^d*P*<0.05 vs Lido-H group。n=6。

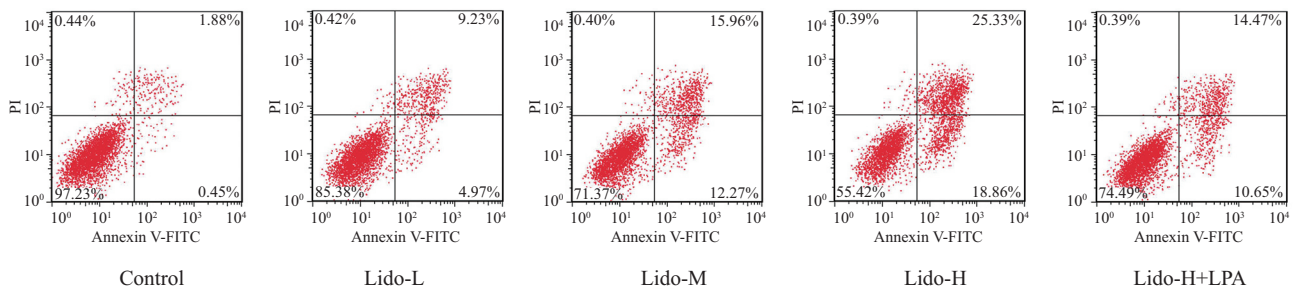
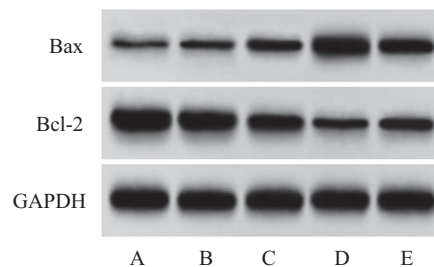


图8 流式细胞仪检测利多卡因对LS513细胞凋亡情况的影响

Fig.8 The effect of lidocaine on apoptosis of LS513 cells was detected by flow cytometry



A: Control组; B: Lido-L组; C: Lido-M组; D: Lido-H组; E: Lido-H+LPA组。

A: Control group; B: Lido-L group; C: Lido-M group; D: Lido-H group; E: Lido-H+LPA group。

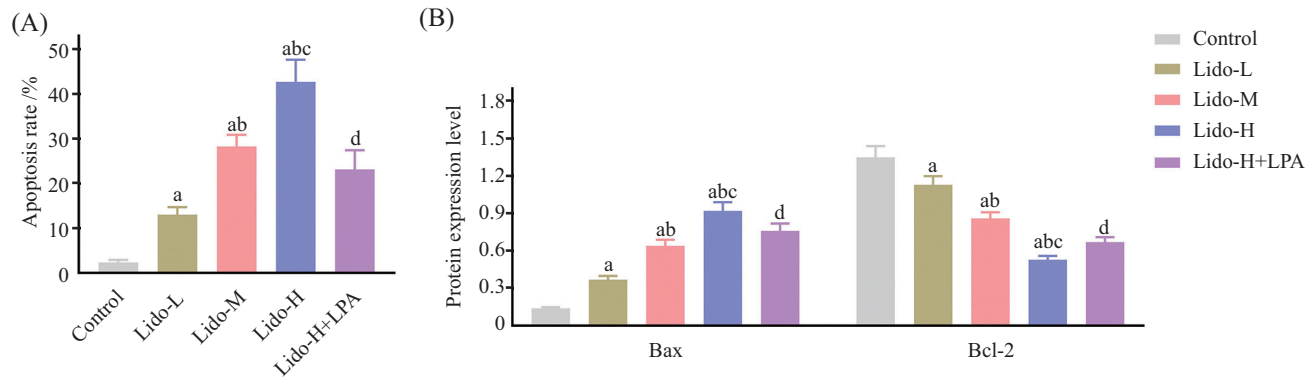
图9 利多卡因对凋亡相关蛋白水平的影响

Fig.9 Effect of lidocaine on apoptosis-related protein levels

上皮-间充质转化(epithelial-mesenchymal transition, EMT)参与癌症细胞的生长、侵袭和迁移,上皮相关标记蛋白的下调和间充质标记蛋白的上调诱导细胞黏附到间质,增强肿瘤细胞的侵袭性^[16]。E-钙黏蛋白(E-cadherin)是一种跨膜分子,维持细胞-细胞结构的完整性并影响细胞黏附,在肿瘤转移中起重要作用^[17]。N-钙黏蛋白(N-cadherin)是EMT中的间充质标志物,介导同源和异型细胞黏附,其高表达与肿瘤的侵袭性有关^[18]。LU等^[19]研究表明豆蔻素对CRC细胞MMP-2、MMP-9和N-钙黏蛋白的表达有抑制

作用,对E-钙黏蛋白表达有促进作用,豆蔻素通过下调ADRB2的表达抑制结肠癌细胞的迁移、侵袭、EMT和肺转移。PCNA是增殖相关蛋白,与细胞增殖密切相关^[20],本研究中利多卡因处理后,LS513细胞凋亡率以及Bax和E-cadherin蛋白表达水平增加,细胞Edu阳性率及PCNA、Bcl-2和N-cadherin蛋白表达水平降低,上皮-间充质转化过程受到抑制。表明利多卡因能通过调节相关蛋白表达抑制细胞增殖、侵袭和迁移,促进细胞凋亡。

研究表明,RhoA/ROCK信号通路可能参与调



A: 细胞凋亡率; B: 凋亡相关蛋白表达。^a $P < 0.05$ vs Control组; ^b $P < 0.05$ vs Lido-L组; ^c $P < 0.05$ vs Lido-M组; ^d $P < 0.05$ vs Lido-H组。 $n = 6$ 。

A: cell apoptosis rate; B: expression of apoptosis-related proteins. ^a $P < 0.05$ vs Control group; ^b $P < 0.05$ vs Lido-L group; ^c $P < 0.05$ vs Lido-M group; ^d $P < 0.05$ vs Lido-H group. $n = 6$ 。

图10 利多卡因对LS513细胞凋亡率和凋亡相关蛋白的影响

Fig.10 Effects of lidocaine on apoptosis rate and apoptosis-related proteins in LS513 cells

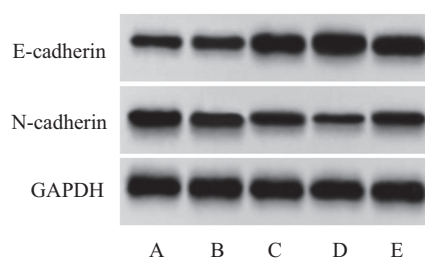
表4 利多卡因对LS513细胞凋亡率和凋亡相关蛋白的影响

Table 4 Effects of lidocaine on apoptosis rate and apoptosis-related proteins in LS513 cells

分组 Groups	细胞凋亡率/% Apoptosis rate /%	Bax蛋白表达量 Bax protein expression level	Bcl-2蛋白表达量 Bcl-2 protein expression level
Control	2.37±0.56	0.14±0.01	1.35±0.09
Lido-L	13.05±1.67 ^a	0.37±0.03 ^a	1.12±0.07 ^a
Lido-M	28.28±2.61 ^{ab}	0.64±0.05 ^{ab}	0.86±0.05 ^{ab}
Lido-H	42.74±4.93 ^{abc}	0.92±0.07 ^{abc}	0.53±0.03 ^{abc}
Lido-H+LPA	23.18±4.26 ^d	0.76±0.06 ^d	0.67±0.04 ^d
<i>F</i>	134.109	242.700	184.217
<i>P</i>	<0.001	<0.001	<0.001

^a $P < 0.05$ vs Control组; ^b $P < 0.05$ vs Lido-L组; ^c $P < 0.05$ vs Lido-M组; ^d $P < 0.05$ vs Lido-H组。 $n = 6$ 。

^a $P < 0.05$ vs Control group; ^b $P < 0.05$ vs Lido-L group; ^c $P < 0.05$ vs Lido-M group; ^d $P < 0.05$ vs Lido-H group. $n = 6$ 。



A: Control组; B: Lido-L组; C: Lido-M组; D: Lido-H组; E: Lido-H+LPA组。

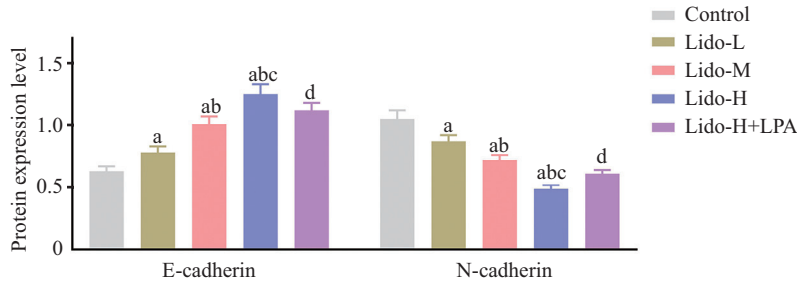
A: Control group; B: Lido-L group; C: Lido-M group; D: Lido-H group; E: Lido-H+LPA group.

图11 利多卡因对LS513细胞E-cadherin和N-cadherin蛋白表达的影响

Fig.11 Effect of lidocaine on expression of E-cadherin and N-cadherin in LS513 cells

节细胞迁移和转移^[21]。RhoA是小GTP酶Rho家族中研究最多的成员之一,它以GTP激活的形式激活下游效应分子,从而影响细胞骨架、细胞黏附、细胞迁移和EMT过程,RhoA的异常表达在CRC等多种肿瘤中很常见,并且RhoA激活与肿瘤转移有关^[22]。ROCK是Rho下游的一种关键丝氨酸/苏氨酸激酶,通过下

游靶标的磷酸化介导RhoA诱导的应激纤维和局灶性黏连的形成,在促进各种类型癌症的侵袭和迁移方面发挥着作用^[23]。DING等^[24]研究表明lncRNA BANCR通过激活RhoA/ROCK途径促进M2巨噬细胞极化、CRC细胞增殖和侵袭、结直肠癌转移。YU等^[25]研究表明血清素通过激活RhoA/ROCK 1/2



^a $P < 0.05$ vs Control组; ^b $P < 0.05$ vs Lido-L组; ^c $P < 0.05$ vs Lido-M组; ^d $P < 0.05$ vs Lido-H组。 $n = 6$ 。

^a $P < 0.05$ vs Control group; ^b $P < 0.05$ vs Lido-L group; ^c $P < 0.05$ vs Lido-M group; ^d $P < 0.05$ vs Lido-H group。 $n = 6$ 。

图12 利多卡因对LS513细胞E-cadherin和N-cadherin蛋白表达的影响

Fig.12 Effects of lidocaine on expression of E-cadherin and N-cadherin proteins in LS513 cells

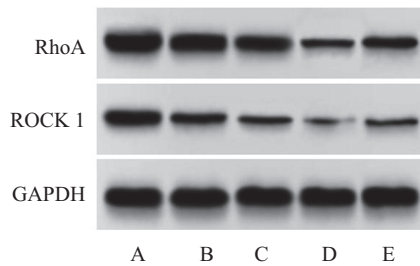
表5 利多卡因对LS513细胞E-cadherin和N-cadherin蛋白表达的影响

Table 5 Effects of lidocaine on expression of E-cadherin and N-cadherin proteins in LS513 cells

分组	E-cadherin蛋白表达量	N-cadherin蛋白表达量
Groups	E-cadherin protein expression level	N-cadherin protein expression level
Control	0.63±0.04	1.05±0.07
Lido-L	0.78±0.05 ^a	0.87±0.05 ^a
Lido-M	1.01±0.06 ^{ab}	0.72±0.04 ^{ab}
Lido-H	1.25±0.08 ^{abc}	0.49±0.03 ^{abc}
Lido-H+LPA	1.12±0.06 ^d	0.61±0.03 ^d
<i>F</i>	107.407	133.667
<i>P</i>	<0.001	<0.001

^a $P < 0.05$ vs Control组; ^b $P < 0.05$ vs Lido-L组; ^c $P < 0.05$ vs Lido-M组; ^d $P < 0.05$ vs Lido-H组。 $n = 6$ 。

^a $P < 0.05$ vs Control group; ^b $P < 0.05$ vs Lido-L group; ^c $P < 0.05$ vs Lido-M group; ^d $P < 0.05$ vs Lido-H group。 $n = 6$ 。

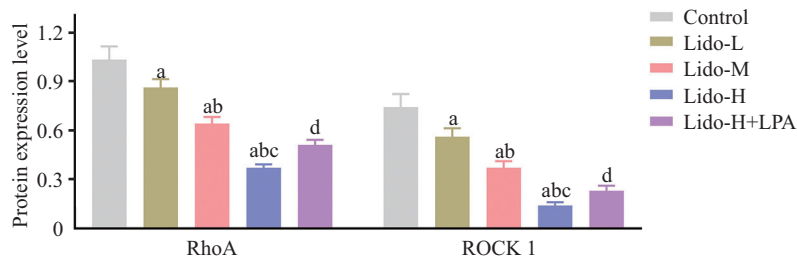


A: Control组; B: Lido-L组; C: Lido-M组; D: Lido-H组; E: Lido-H+LPA组。

A: Control group; B: Lido-L group; C: Lido-M group; D: Lido-H group; E: Lido-H+LPA group。

图13 利多卡因对LS513细胞通路相关蛋白表达的影响

Fig.13 Effect of lidocaine on pathway-related proteins expression in LS513 cells



^a $P < 0.05$ vs Control组; ^b $P < 0.05$ vs Lido-L组; ^c $P < 0.05$ vs Lido-M组; ^d $P < 0.05$ vs Lido-H组。 $n = 6$ 。

^a $P < 0.05$ vs Control group; ^b $P < 0.05$ vs Lido-L group; ^c $P < 0.05$ vs Lido-M group; ^d $P < 0.05$ vs Lido-H group。 $n = 6$ 。

图14 利多卡因对LS513细胞通路相关蛋白表达的影响

Fig.14 Effects of lidocaine on pathway-related proteins expression in LS513 cells

表6 利多卡因对LS513细胞通路相关蛋白表达的影响

Table 6 Effects of lidocaine on the expression of pathway-related proteins in LS513 cells

分组 Groups	RhoA蛋白表达量 RhoA protein expression level	ROCK 1蛋白表达量 ROCK 1 protein expression level
Control	1.03±0.08	0.74±0.08
Lido-L	0.86±0.05 ^a	0.56±0.05 ^a
Lido-M	0.64±0.04 ^{ab}	0.37±0.04 ^{ab}
Lido-H	0.37±0.02 ^{abc}	0.14±0.02 ^{abc}
Lido-H+LPA	0.51±0.03 ^d	0.23±0.03 ^d
<i>F</i>	178.907	151.449
<i>P</i>	<0.001	<0.001

^a*P*<0.05 vs Control组; ^b*P*<0.05 vs Lido-L组; ^c*P*<0.05 vs Lido-M组; ^d*P*<0.05 vs Lido-H组。n=6。

^a*P*<0.05 vs Control group; ^b*P*<0.05 vs Lido-L group; ^c*P*<0.05 vs Lido-M group; ^d*P*<0.05 vs Lido-H group。n=6。

信号转导,上调YAP表达,促进体外和体内结肠癌细胞的生长。本研究中利多卡因处理LS513细胞后,RhoA和ROCK 1蛋白表达水平下调,通路激活剂LPA逆转了利多卡因下调RhoA和ROCK 1表达的作用。这表明利多卡因可能通过抑制RhoA/ROCK信号通路抑制结直肠癌细胞增殖、迁移、侵袭及EMT进程。

综上所述,利多卡因可能通过抑制RhoA/ROCK信号通路,抑制结直肠癌细胞增殖、迁移和侵袭及EMT进程,并促进细胞凋亡。本研究仅在体外细胞实验中初步探究了利多卡因对结直肠癌细胞恶性生物学的影响,今后还需开展体内实验验证利多卡因对结直肠癌的影响。

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