

# 熊果酸通过调控FKBP38/mTOR/SREBPs信号通路 对宫颈癌细胞增殖、迁移和侵袭的作用

倪欣<sup>1</sup> 李艳春<sup>2</sup> 张琼慧<sup>3</sup> 徐晓锋<sup>4\*</sup>

(<sup>1</sup>上海健康医学院附属嘉定区中心医院麻醉科, 上海 201800; <sup>2</sup>上海市瑞金医院南翔分院妇产科, 上海 201802;

<sup>3</sup>上海市嘉定区徐行镇社区卫生服务中心妇女保健科, 上海 201808;

<sup>4</sup>上海健康医学院附属嘉定区中心医院妇产科, 上海 201800)

**摘要** 该研究旨在探究熊果酸通过FK506结合蛋白38(FKBP38)/哺乳动物雷帕霉素靶蛋白(mTOR)/固醇调节元件结合蛋白(SREBPs)信号通路对宫颈癌细胞增殖、侵袭和迁移的作用。将HeLa细胞分为CK组(正常培养细胞)、L-熊果酸组(5  $\mu\text{mol/L}$ )、M-熊果酸组(10  $\mu\text{mol/L}$ )、H-熊果酸组(20  $\mu\text{mol/L}$ )和FKBP38抑制剂FKBP51-Hsp90-IN-1(0.1  $\mu\text{mol/L}$ )+H-熊果酸(20  $\mu\text{mol/L}$ )组。CCK-8法检测细胞活性, 流式细胞术检测细胞凋亡情况, 划痕实验检测细胞迁移情况, Transwell法检测细胞侵袭情况, Western blot检测Bax、Bcl-2、cleaved-Caspase-3、FKBP38、mTOR、SREBPs蛋白表达情况。L-熊果酸组、M-熊果酸组、H-熊果酸组较CK组细胞D值、划痕愈合率、侵袭细胞数以及Bcl-2、p-mTOR/mTOR、SREBPs蛋白表达水平降低, 细胞凋亡率以及Bax、cleaved-Caspase-3、FKBP38蛋白表达水平升高( $P<0.05$ ); FKBP51-Hsp90-IN-1+H-熊果酸组较H-熊果酸组D值、划痕愈合率、侵袭细胞数以及Bcl-2、p-mTOR/mTOR、SREBPs蛋白水平显著增加, 细胞凋亡率以及Bax、cleaved-Caspase-3、FKBP38蛋白水平显著降低( $P<0.05$ )。熊果酸可能通过调控FKBP38/mTOR/SREBPs信号通路, 进而抑制HeLa细胞的增殖、迁移及侵袭等。

**关键词** 熊果酸; FKBP38/mTOR/SREBPs通路; 宫颈癌; 迁移; 侵袭

## The Effects of Ursolic Acid on Proliferation, Migration, and Invasion of Cervical Cancer Cells through Regulating the FKBP38/mTOR/SREBPs Signaling Pathway

NI Xin<sup>1</sup>, LI Yanchun<sup>2</sup>, ZHANG Qionghui<sup>3</sup>, XU Xiaofeng<sup>4\*</sup>

(<sup>1</sup>Department of Anesthesiology, Jiading District Central Hospital Affiliated Shanghai University of Medicine & Health Sciences, Shanghai 201800, China; <sup>2</sup>Department of Obstetrics and Gynecology, Nanxiang Branch of Ruijin Hospital, Shanghai 201802, China;

<sup>3</sup>Department of Women's Health, Community Health Service Center of Xuhang Town, Jiading District, Shanghai 201808, China;

<sup>4</sup>Department of Obstetrics and Gynecology, Jiading District Central Hospital Affiliated Shanghai University of Medicine & Health Sciences, Shanghai 201800, China)

**Abstract** This study aims to investigate the mechanism of action of ursolic acid in regulating the proliferation, invasion and migration of cervical cancer cells through the FKBP38 (FK506-binding protein 38)/mTOR (mammalian target of rapamycin)/SREBPs (sterol regulatory element binding proteins) signalling pathway. HeLa cells were assigned into CK group (normal cultured cells), L-ursolic acid group (5  $\mu\text{mol/L}$ ), M-ursolic acid group (10  $\mu\text{mol/L}$ ), H-ursolic acid group (20  $\mu\text{mol/L}$ ), and FKBP38 inhibitor FKBP51-Hsp90-IN-1 (0.1  $\mu\text{mol/L}$ )+H-ursolic acid (20  $\mu\text{mol/L}$ ) group. The

收稿日期: 2024-11-11

接受日期: 2024-12-24

上海市嘉定区卫生健康委员会科研项目(批准号: 2022-KY-11)资助的课题

\*通信作者。Tel: 18930861912, E-mail: 61256233@qq.com

Received: November 11, 2024

Accepted: December 24, 2024

This work was supported by the Scientific Research Project of Jiading District Health Commission, Shanghai (Grant No.2022-KY-11)

\*Corresponding author. Tel: +86-18930861912, E-mail: 61256233@qq.com

CCK-8 assay was used to detect cell viability. Flow cytometry was used to detect cell apoptosis. The scratch assay was used to detect cell migration. The Transwell assay was used to detect cell invasion, and Western blot was used to detect the protein expression levels of Bax, Bcl-2, cleaved-Caspase-3, FKBP38, mTOR and SREBPs. The *D* value, scratch healing rate, number of invasive cells, the protein expression of Bcl-2, p-mTOR/mTOR, and SREBPs in the L-ursolic acid group, M-ursolic acid group, and H-ursolic acid group were lower than those in the CK group, and the apoptosis rate, the protein expression of Bax, cleaved-Caspase-3, and FKBP38 were higher ( $P<0.05$ ). The *D* value, scratch healing rate, number of invasive cells, the protein expression of Bcl-2, p-mTOR/mTOR, and SREBPs in the FKBP51-Hsp90-IN-1+H-ursolic acid group were higher than those in the H-ursolic acid group, and the apoptosis rate, the protein expression of Bax, cleaved-Caspase-3, and FKBP38 were lower ( $P<0.05$ ). Ursolic acid may inhibit the proliferation, migration, and invasion of HeLa cells by modulating the FKBP38/mTOR/SREBPs signaling pathway.

**Keywords** ursolic acid; FKBP38/mTOR/SREBPs pathway; cervical cancer; migration; invasion

宫颈癌是常见女性恶性肿瘤的一种,具有发病率高以及死亡率高的特点,严重影响患者以及整个家庭的生活<sup>[1]</sup>。随着生活方式的改变,宫颈癌的发病年龄逐渐降低,严重威胁女性健康<sup>[2]</sup>。因此,对宫颈癌的具体发病机制进行深入研究,对于提高患者的生存率至关重要<sup>[3]</sup>。熊果酸是从山茱萸、泽兰等中草药中提取得到的一种三萜类化合物,具有多种生物学活性,如抗炎、抗菌、抗氧化以及抗肿瘤等<sup>[4-6]</sup>。已有相关研究发现,熊果酸对乳腺癌、结直肠癌等细胞的生长具有抑制作用<sup>[7-8]</sup>。但其对宫颈癌的作用并不十分清楚。FK506结合蛋白38(FK506 binding protein 38, FKBP38)存在于卵巢、肝脏等多种组织中,且较多定位于线粒体<sup>[9-10]</sup>。已有相关研究发现,FKBP38在乳腺癌中表达水平降低<sup>[11]</sup>。已有报道显示,FKBP38过表达可以抑制哺乳动物雷帕霉素靶蛋白(mammalian target of rapamycin, mTOR)磷酸化的发生,进而抑制固醇调节元件结合蛋白(sterol regulatory element binding proteins, SREBPs)的激活<sup>[12]</sup>。YAN等<sup>[13]</sup>研究发现,FKBP38可以通过抑制mTOR通路,进而抑制子宫内膜癌的进展。但熊果酸是否通过调控FKBP38/mTOR/SREBPs通路在宫颈癌中发挥作用尚不清楚。因此,本研究进行体外实验探究熊果酸对宫颈癌增殖、迁移及侵袭的影响,并分析其机制是否与FKBP38/mTOR/SREBPs通路有关,从而为熊果酸在宫颈癌治疗中的应用提供理论依据。

## 1 材料与方法

### 1.1 材料

宫颈癌HeLa细胞(货号:YT800317)、BCA试剂盒(货号:YT8173)购自北京伊塔生物科技有限公司;

DMEM培养基(货号:A-CSH795-500mL)购自武汉佰瑞得生物技术有限公司;CCK-8试剂盒(货号:BY-Q50356)购自武汉艾美捷科技有限公司;熊果酸(货号:A3221)购自北京康瑞纳生物科技有限公司;FKBP38抑制剂(FKBP51-Hsp90-IN-1)(货号:HY-158130)购自美国MedChemExpress公司;Annexin V-FITC/PI试剂盒(货号:S0185XL)购自上海雅吉生物科技有限公司;FKBP38(货号:ab129113)、mTOR(货号:ab134903)、p-mTOR(货号:ab109268)、SREBPs(货号:ab313881)、Bax(货号:ab270742)、Bcl-2(货号:ab117115)、cleaved-Caspase-3(货号:ab32042)、GAPDH(货号:ab199553)抗体以及山羊抗兔二抗(货号:ab205718)购自英国Abcam公司;酶标仪(型号:Multiskan SkyHigh)、凝胶成像仪(型号:iBright™ CL750)、光学显微镜(型号:EVOS M5000)购自美国ThermoFisher Scientific公司;CO<sub>2</sub>细胞培养箱(型号:CellXpert C170i)购自德国Eppendorf公司;流式细胞仪(型号:NL-CLC 1L-3L)购自美国Cyttek Biosciences公司。

### 1.2 方法

**1.2.1 细胞培养与分组** 将宫颈癌HeLa细胞置于DMEM培养基(10%胎牛血清+100 U/mL青霉素和100 μg/mL链霉素)中培养(37 °C、5% CO<sub>2</sub>)。熊果酸和FKBP38抑制剂(FKBP51-Hsp90-IN-1)分别加入PBS缓冲液,配制到合适的浓度。其中CK组为正常培养细胞;L-熊果酸组、M-熊果酸组、H-熊果酸组分别加入含不同浓度(5、10、20 μmol/L)熊果酸<sup>[14]</sup>的培养基;FKBP51-Hsp90-IN-1+H-熊果酸组的培养基中加入0.1 μmol/L FKBP51-Hsp90-IN-1<sup>[15]</sup>和20 μmol/L熊果酸,24 h后进行后续实验。

**1.2.2 CCK-8试剂盒检测细胞活性** 当细胞生长

至对数生长期时,收集各组细胞,接种于96孔板( $5 \times 10^3$ 个/孔)中,置于37 °C、5% CO<sub>2</sub>细胞培养箱中培养,48 h后,在每孔中加入10  $\mu$ L CCK-8试剂,接着在37 °C、5% CO<sub>2</sub>细胞培养箱中培养4 h。酶标仪检测450 nm波长下的吸光度(*D*)值。

**1.2.3 流式细胞术检测细胞凋亡情况** 各组细胞培养48 h后,用胰蛋白酶消化(37 °C、5 min),收集100  $\mu$ L细胞悬液( $1 \times 10^5$ 个),PBS洗涤2~3次(每次3~5 min)后室温3 000 r/min离心6 min,保留沉淀,并加入缓冲液重悬细胞,之后分别加入5  $\mu$ L Annexin V-FITC和PI室温孵育10 min。流式细胞仪测定细胞的凋亡率。

**1.2.4 划痕实验检测细胞迁移情况** 将各组HeLa细胞以 $2 \times 10^5$ 个/孔的密度接种于6孔板中,37 °C、5% CO<sub>2</sub>细胞培养箱中培养24 h后,使用100  $\mu$ L移液器枪头的尖端轻轻划动6孔板,进行拍照,之后继续在37 °C、5% CO<sub>2</sub>细胞培养箱中培养24 h,光学显微镜观察细胞迁移情况。计算划痕愈合率,划痕愈合率=[(0 h划痕宽度-24 h划痕宽度)/0 h划痕宽度] $\times 100\%$ 。

**1.2.5 Transwell法检测细胞侵袭情况** 收集各组细胞并重悬至 $4 \times 10^4$ 个/mL,取100  $\mu$ L HeLa细胞加入含有Matrigel的Transwell上室,而在Transwell下室加入600  $\mu$ L DMEM培养基(含有血清),置于37 °C、5% CO<sub>2</sub>细胞培养箱培养,在室温下用4 %多聚甲醛固定20 min,并在室温下用结晶紫染色30 min。显微镜下随机观察统计5个视野细胞数量。

**1.2.6 Western blot检测FKBP38/mTOR/SREBPs通路及凋亡相关蛋白表达情况** 收集每组HeLa细胞置于RIPA裂解物中分离总蛋白,测定蛋白浓度后,在10% SDS-PAGE电泳孔槽中加入蛋白,电泳分离等量的蛋白样品、转膜、室温封闭2 h。随后将膜分别与一抗FKBP38(1:1 000)、mTOR(1:1 000)、p-mTOR(1:1 000)、SREBPs(1:1 000)、Bax(1:1 000)、

Bcl-2(1:1 000)、cleaved-Caspase-3(1:1 000)和内参GAPDH(1:1 000)于4 °C孵育24 h,洗膜,随后用二抗(1:5 000)于37 °C孵育2 h。使用Image Lab™定量蛋白条带。目标蛋白相对表达量=目标蛋白灰度值/内参蛋白灰度值。

### 1.3 统计学处理

用SPSS 25.0软件分析数据。数据表示为( $\bar{x} \pm s$ ),多组比较用单因素方差分析,两两比较用SNK-*q*检验。*P*<0.05表示有统计学差异。

## 2 结果

### 2.1 熊果酸降低HeLa细胞活性

L-熊果酸组、M-熊果酸组、H-熊果酸组较CK组细胞*D*值呈剂量依赖性降低(*P*<0.05);FKBP51-Hsp90-IN-1+H-熊果酸组较H-熊果酸组*D*值显著升高(*P*<0.05,表1)。这表明熊果酸可降低HeLa细胞活性。

### 2.2 熊果酸促进HeLa细胞凋亡

L-熊果酸组、M-熊果酸组、H-熊果酸组较CK组细胞凋亡率呈剂量依赖性升高(*P*<0.05);FKBP51-Hsp90-IN-1+H-熊果酸组较H-熊果酸组细胞凋亡率显著降低(*P*<0.05,图1和表2)。这表明熊果酸可促进HeLa细胞凋亡。

### 2.3 熊果酸抑制HeLa细胞迁移

L-熊果酸组、M-熊果酸组、H-熊果酸组较CK组划痕愈合率呈剂量依赖性降低(*P*<0.05);FKBP51-Hsp90-IN-1+H-熊果酸组较H-熊果酸组划痕愈合率显著升高(*P*<0.05,图2和表3)。这表明熊果酸可抑制HeLa细胞迁移。

### 2.4 熊果酸抑制HeLa细胞侵袭

L-熊果酸组、M-熊果酸组、H-熊果酸组较CK组侵袭细胞数呈剂量依赖性减少(*P*<0.05);FKBP51-Hsp90-IN-1+H-熊果酸组较H-熊果酸组侵袭细胞数

表1 熊果酸影响HeLa细胞活性

Table 1 Effects of ursolic acid on the viability of HeLa cells

| 组别<br>Group                            | <i>D</i> 值<br><i>D</i> value |
|----------------------------------------|------------------------------|
| CK group                               | 1.57 $\pm$ 0.16              |
| L-ursolic acid group                   | 1.21 $\pm$ 0.13*             |
| M-ursolic acid group                   | 0.81 $\pm$ 0.09**            |
| H-ursolic acid group                   | 0.53 $\pm$ 0.06***           |
| FKBP51-Hsp90-IN-1+H-ursolic acid group | 1.25 $\pm$ 0.13@             |

\**P*<0.05, 与CK组相比; \*\**P*<0.05, 与L-熊果酸组相比; \*\*\**P*<0.05, 与M-熊果酸组相比; @*P*<0.05, 与H-熊果酸组相比。

\**P*<0.05 compared with CK group; \*\**P*<0.05 compared with L-ursolic acid group; \*\*\**P*<0.05 compared with M-ursolic acid group; @*P*<0.05 compared with H-ursolic acid group.

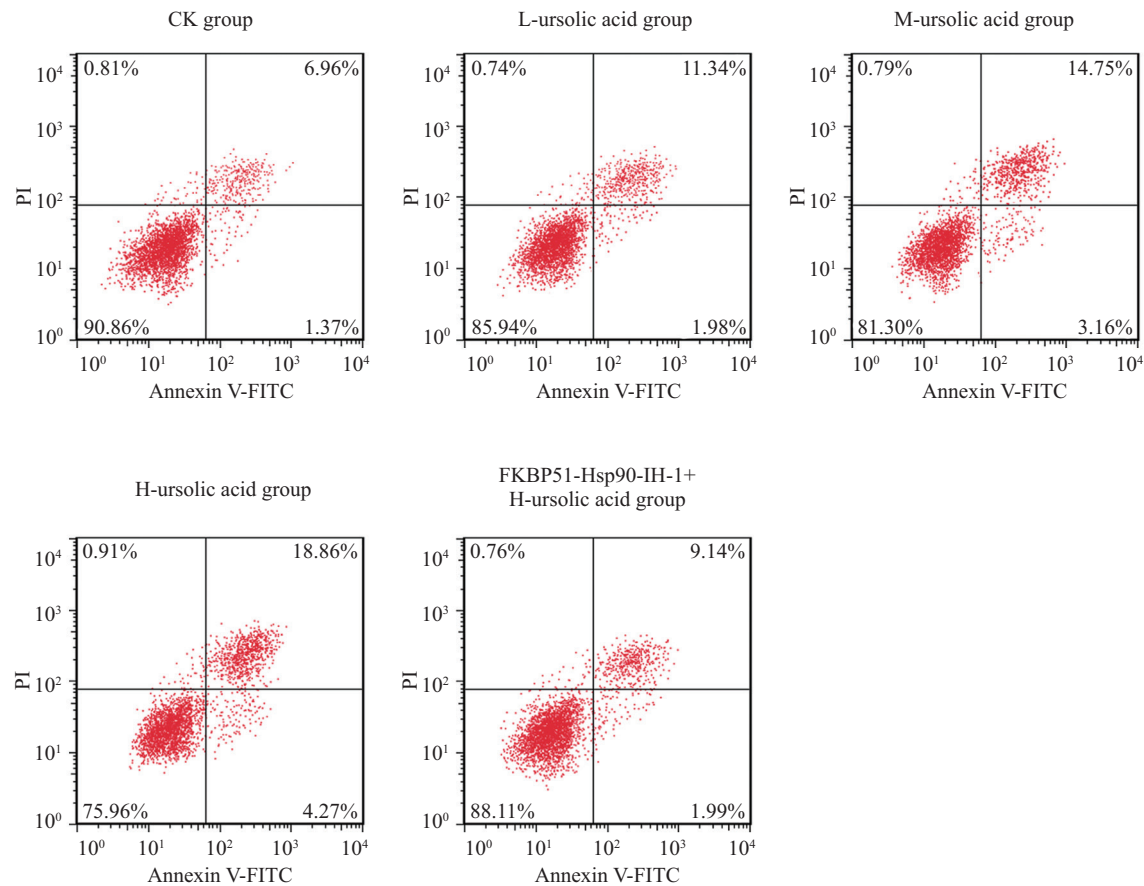


图1 流式细胞术检测细胞凋亡情况  
Fig.1 Detection of cell apoptosis by flow cytometry

表2 熊果酸影响HeLa细胞凋亡  
Table 2 Effects of ursolic acid on apoptosis of HeLa cells

| 组别<br>Group                            | 凋亡率/%<br>Apoptosis rate /% |
|----------------------------------------|----------------------------|
| CK group                               | 8.13±0.82                  |
| L-ursolic acid group                   | 13.02±1.39*                |
| M-ursolic acid group                   | 17.59±1.82*#               |
| H-ursolic acid group                   | 23.07±2.37*##&             |
| FKBP51-Hsp90-IN-1+H-ursolic acid group | 11.06±1.15@                |

\* $P<0.05$ , 与CK组相比; # $P<0.05$ , 与L-熊果酸组相比; & $P<0.05$ , 与M-熊果酸组相比; @ $P<0.05$ , 与H-熊果酸组相比。  
\* $P<0.05$  compared with CK group; # $P<0.05$  compared with L-ursolic acid group; & $P<0.05$  compared with M-ursolic acid group; @ $P<0.05$  compared with H-ursolic acid group.

显著增加 ( $P<0.05$ , 图3和表4)。这表明熊果酸可抑制HeLa细胞侵袭。

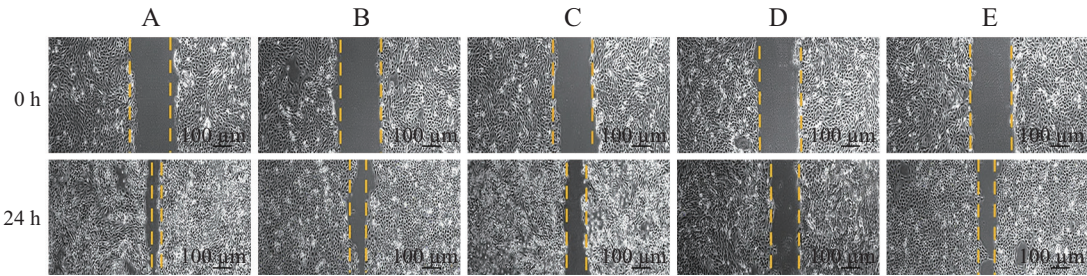
2.5 熊果酸对HeLa细胞中凋亡相关蛋白表达的影响

L-熊果酸组、M-熊果酸组、H-熊果酸组较CK组 Bax、cleaved-Caspase-3蛋白水平升高, Bcl-2蛋白水平降低 ( $P<0.05$ ), 且随着熊果酸浓度的增加, Bax、cleaved-Caspase-3蛋白表达量逐渐增加, Bcl-2蛋白表达

量逐渐减少 ( $P<0.05$ ); FKBP51-Hsp90-IN-1+H-熊果酸组较H-熊果酸组 Bax、cleaved-Caspase-3蛋白水平显著降低, Bcl-2蛋白水平显著升高 ( $P<0.05$ , 图4、图5和表5)。这表明熊果酸可影响HeLa细胞中凋亡相关蛋白表达。

2.6 熊果酸对HeLa细胞中FKBP38/mTOR/SREBPs信号通路相关蛋白表达的影响

L-熊果酸组、M-熊果酸组、H-熊果酸组较CK组 FKBP38蛋白水平升高, p-mTOR/mTOR、SREBPs蛋白



A: CK组; B: L-熊果酸组; C: M-熊果酸组; D: H-熊果酸组; E: FKBP51-Hsp90-IN-1+H-熊果酸组。  
A: CK group; B: L-ursolic acid group; C: M-ursolic acid group; D: H-ursolic acid group; E: FKBP51-Hsp90-IN-1+H-ursolic acid group.

图2 各组HeLa细胞的迁移能力  
Fig.2 Migration ability of HeLa cells in each group

表3 熊果酸影响HeLa细胞迁移  
Table 3 Effects of ursolic acid on the migration of HeLa cells

| 组别<br>Group                            | 划痕愈合率/%<br>Scratch healing rate /% |
|----------------------------------------|------------------------------------|
| CK group                               | 78.26±7.95                         |
| L-ursolic acid group                   | 61.37±6.23*                        |
| M-ursolic acid group                   | 49.51±5.07*#                       |
| H-ursolic acid group                   | 35.69±3.61*#&                      |
| FKBP51-Hsp90-IN-1+H-ursolic acid group | 62.55±6.39@                        |

\* $P<0.05$ , 与CK组相比; # $P<0.05$ , 与L-熊果酸组相比; & $P<0.05$ , 与M-熊果酸组相比; @ $P<0.05$ , 与H-熊果酸组相比。  
\* $P<0.05$  compared with CK group; # $P<0.05$  compared with L-ursolic acid group; & $P<0.05$  compared with M-ursolic acid group; @ $P<0.05$  compared with H-ursolic acid group.

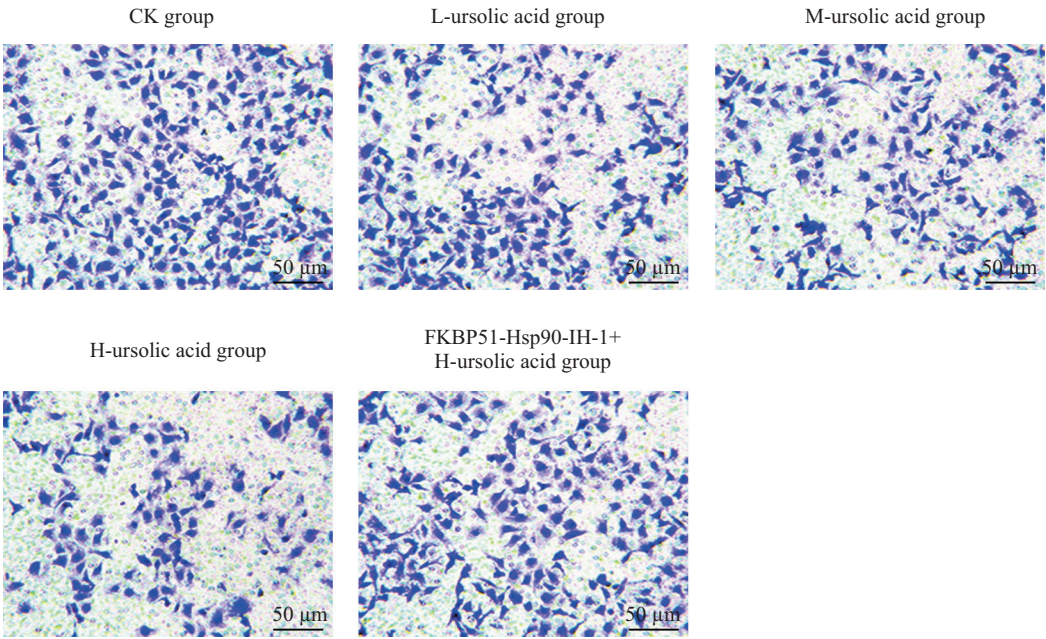


图3 各组HeLa细胞的侵袭能力  
Fig.3 Invasive ability of HeLa cells in each group

水平降低( $P<0.05$ ), 且随着熊果酸浓度的增加, FKBP38蛋白表达量逐渐增加, p-mTOR/mTOR、SREBPs蛋白表达量逐渐减少( $P<0.05$ ); FKBP51-Hsp90-IN-1+H-熊

果酸组较H-熊果酸组FKBP38蛋白水平显著降低, p-mTOR/mTOR、SREBPs蛋白水平显著升高( $P<0.05$ , 图6、图7和表6)。这表明熊果酸可影响HeLa细胞中

表4 熊果酸影响HeLa细胞侵袭  
Table 4 Effects of ursolic acid on the invasion of HeLa cells

| 组别<br>Group                            | 侵袭细胞数<br>Number of invasive cells |
|----------------------------------------|-----------------------------------|
| CK group                               | 178.26±18.71                      |
| L-ursolic acid group                   | 141.39±14.36*                     |
| M-ursolic acid group                   | 120.56±12.54*#                    |
| H-ursolic acid group                   | 81.57±8.36*#&                     |
| FKBP51-Hsp90-IN-1+H-ursolic acid group | 146.29±14.91@                     |

\**P*<0.05, 与CK组相比; #*P*<0.05, 与L-熊果酸组相比; &*P*<0.05, 与M-熊果酸组相比; @*P*<0.05, 与H-熊果酸组相比。  
\**P*<0.05 compared with CK group; #*P*<0.05 compared with L-ursolic acid group; &*P*<0.05 compared with M-ursolic acid group; @*P*<0.05 compared with H-ursolic acid group.

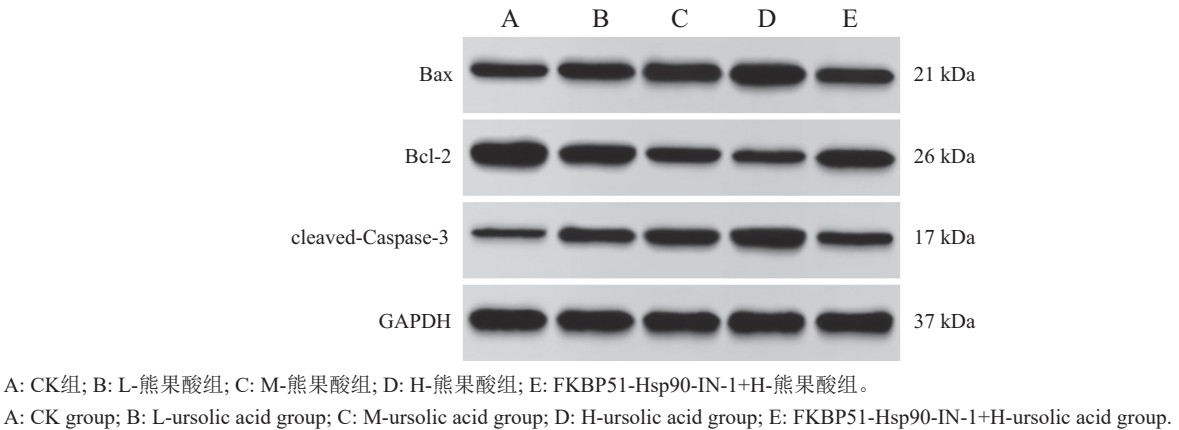


图4 Western blot检测Bax、Bcl-2、cleaved-Caspase-3蛋白表达情况  
Fig.4 Detection of the expression levels of Bax, Bcl-2, and cleaved-Caspase-3 proteins by Western blot

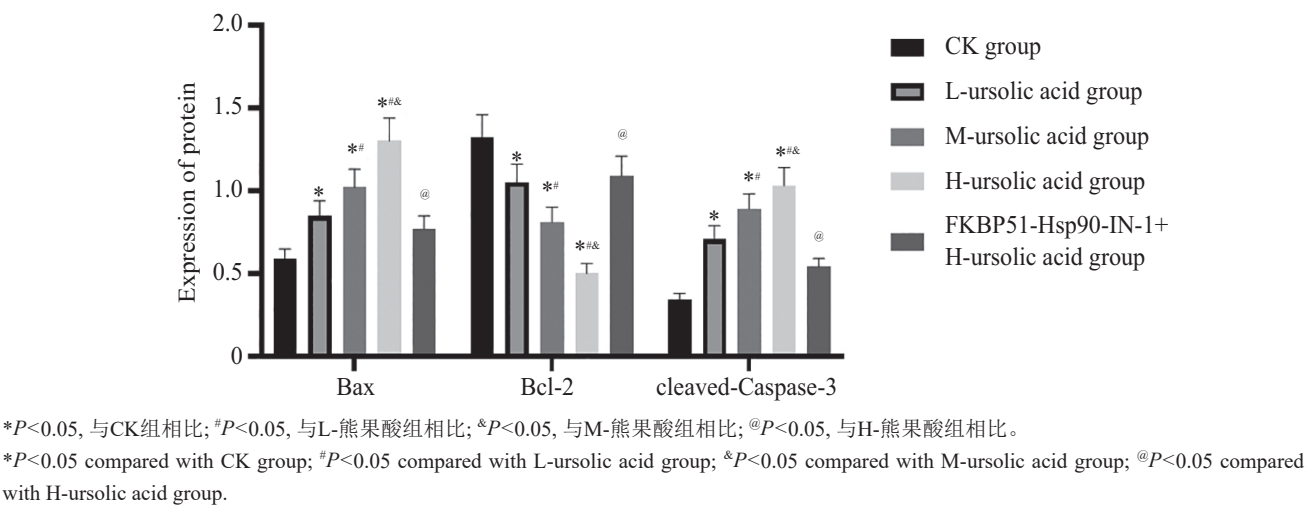


图5 Bax、Bcl-2、cleaved-Caspase-3蛋白表达情况  
Fig.5 Expression levels of Bax, Bcl-2, and cleaved-Caspase-3 proteins

FKBP38/mTOR/SREBPs相关蛋白表达。

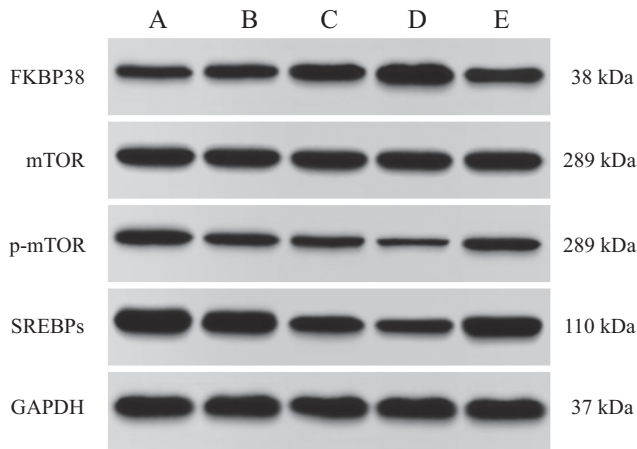
3 讨论

宫颈癌作为一种常见妇科恶性肿瘤,是造成女性癌症死亡相关的主要原因,严重威胁女性健康<sup>[16]</sup>。据相关数据显示,每年新增宫颈癌确诊病例可达50万之多,并预计至2024年,美国新增病例达13 820例,死亡病例达4 360例<sup>[17-18]</sup>。目前针对宫颈癌的常用治疗方

表5 各组HeLa细胞中Bax、Bcl-2、cleaved-Caspase-3蛋白水平  
Table 5 Protein levels of Bax, Bcl-2, and cleaved-Caspase-3 in HeLa cells of each group

| 组别<br>Group                            | Bax/GAPDH                    | Bcl-2/GAPDH                  | cleaved-Caspase-3/GAPDH      |
|----------------------------------------|------------------------------|------------------------------|------------------------------|
| CK group                               | 0.59±0.06                    | 1.32±0.14                    | 0.34±0.04                    |
| L-ursolic acid group                   | 0.85±0.09*                   | 1.05±0.11*                   | 0.71±0.08*                   |
| M-ursolic acid group                   | 1.02±0.11 <sup>#</sup>       | 0.81±0.09 <sup>#</sup>       | 0.89±0.09 <sup>#</sup>       |
| H-ursolic acid group                   | 1.30±0.14 <sup>*#&amp;</sup> | 0.50±0.06 <sup>*#&amp;</sup> | 1.03±0.11 <sup>*#&amp;</sup> |
| FKBP51-Hsp90-IN-1+H-ursolic acid group | 0.77±0.08 <sup>@</sup>       | 1.09±0.12 <sup>@</sup>       | 0.54±0.05 <sup>@</sup>       |

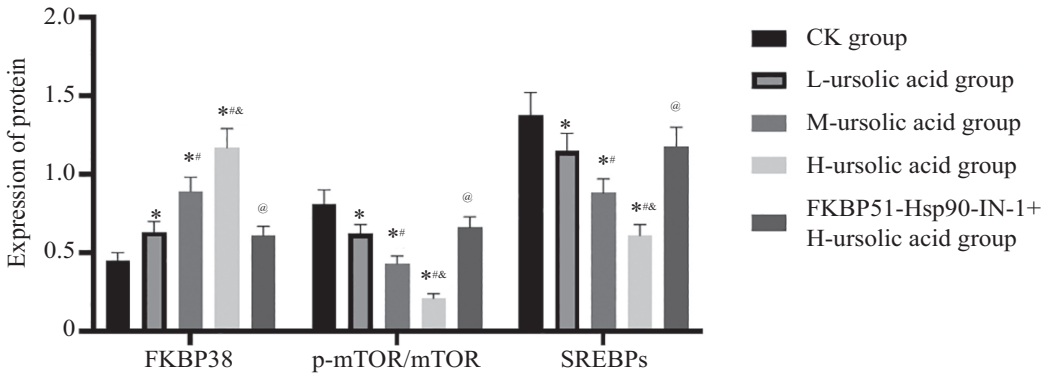
\**P*<0.05, 与CK组相比; <sup>#</sup>*P*<0.05, 与L-熊果酸组相比; <sup>&</sup>*P*<0.05, 与M-熊果酸组相比; <sup>@</sup>*P*<0.05, 与H-熊果酸组相比。  
\**P*<0.05 compared with CK group; <sup>#</sup>*P*<0.05 compared with L-ursolic acid group; <sup>&</sup>*P*<0.05 compared with M-ursolic acid group; <sup>@</sup>*P*<0.05 compared with H-ursolic acid group.



A: CK组; B: L-熊果酸组; C: M-熊果酸组; D: H-熊果酸组; E: FKBP51-Hsp90-IN-1+H-熊果酸组。  
A: CK group; B: L-ursolic acid group; C: M-ursolic acid group; D: H-ursolic acid group; E: FKBP51-Hsp90-IN-1+H-ursolic acid group.

图6 Western blot检测FKBP38/mTOR/SREBPs信号通路相关蛋白表达情况

Fig.6 Detection of the expression of proteins related to the FKBP38/mTOR/SREBPs signaling pathway by Western blot



\**P*<0.05, 与CK组相比; <sup>#</sup>*P*<0.05, 与L-熊果酸组相比; <sup>&</sup>*P*<0.05, 与M-熊果酸组相比; <sup>@</sup>*P*<0.05, 与H-熊果酸组相比。  
\**P*<0.05 compared with CK group; <sup>#</sup>*P*<0.05 compared with L-ursolic acid group; <sup>&</sup>*P*<0.05 compared with M-ursolic acid group; <sup>@</sup>*P*<0.05 compared with H-ursolic acid group.

图7 FKBP38/mTOR/SREBPs信号通路相关蛋白表达情况

Fig.7 Expression of proteins related to the FKBP38/mTOR/SREBPs signaling pathway

法为化疗等药物治疗与手术切除相结合,但是预后效果仍未达到理想状态<sup>[19]</sup>。宫颈癌的发病机制十分复杂,因此,深入探究其发病机制可以为宫颈癌的治疗以及寻找新的靶点提供重要理论依据。

目前关于抗肿瘤药物的使用,与化学合成药物相比,天然植物提取物具有生物活性多,且副作用更小的优点<sup>[20]</sup>。熊果酸是从中草药中提取到的天然植物提取物,具有抗肿瘤的药理作用<sup>[21]</sup>。已有相关

表6 各组HeLa细胞中FKBP38、p-mTOR/mTOR、SREBPs蛋白水平  
Table 6 Protein levels of FKBP38, p-mTOR/mTOR, and SREBPs in HeLa cells of each group

| 组别<br>Group                            | FKBP38/GAPDH | p-mTOR/mTOR  | SREBPs/GAPDH |
|----------------------------------------|--------------|--------------|--------------|
| CK group                               | 0.45±0.05    | 0.81±0.09    | 1.38±0.14    |
| L-ursolic acid group                   | 0.63±0.07*   | 0.62±0.06*   | 1.15±0.11*   |
| M-ursolic acid group                   | 0.89±0.09*#  | 0.43±0.05*#  | 0.88±0.09*#  |
| H-ursolic acid group                   | 1.17±0.12*#& | 0.21±0.03*#& | 0.61±0.07*#& |
| FKBP51-Hsp90-IN-1+H-ursolic acid group | 0.61±0.06@   | 0.66±0.07@   | 1.18±0.12@   |

\* $P<0.05$ , 与CK组相比; # $P<0.05$ , 与L-熊果酸组相比; & $P<0.05$ , 与M-熊果酸组相比; @ $P<0.05$ , 与H-熊果酸组相比。

\* $P<0.05$  compared with CK group; # $P<0.05$  compared with L-ursolic acid group; & $P<0.05$  compared with M-ursolic acid group; @ $P<0.05$  compared with H-ursolic acid group.

研究显示,熊果酸的抗肿瘤作用可能与下调肿瘤生长、迁移等相关基因以及上调凋亡相关基因的表达有关,从而进一步抑制肿瘤的发展<sup>[22]</sup>。据已有研究显示,Bax可以促进细胞凋亡,Bcl-2在抑制细胞凋亡的同时还抑制Bax的表达,而cleaved-Caspase-3是一种可以反映细胞凋亡情况的指标<sup>[23-24]</sup>。在本研究中,经过不同剂量的熊果酸处理后,CCK-8法检测细胞活性,发现其活性降低;流式细胞术检测细胞凋亡情况,发现其凋亡率增加;划痕实验检测细胞迁移能力,发现其迁移能力降低;Transwell法检测细胞侵袭能力,发现其侵袭能力降低;Western blot检测Bcl-2、Bax、cleaved-Caspase-3蛋白表达情况,发现其中Bcl-2蛋白表达水平降低,Bax、cleaved-Caspase-3蛋白表达水平升高,这表明熊果酸可以抑制细胞的增殖、迁移、侵袭,并通过影响Bax、cleaved-Caspase-3、Bcl-2蛋白的表达,进而促进细胞的凋亡。

研究发现,在子宫内膜癌细胞中,过表达FKBP38蛋白,可以对细胞的增殖以及侵袭进行抑制<sup>[25]</sup>。已有相关研究发现,FKBP38通过对mTOR进行抑制,从而抑制子宫内膜癌细胞的增殖以及转移<sup>[15]</sup>。CHEN等<sup>[26]</sup>研究发现,在结直肠癌中,mTOR/SREBP-1/SCD1信号通路参与细胞反应。本研究结果显示,与CK组相比,熊果酸处理组的FKBP38蛋白表达水平显著提高,mTOR蛋白磷酸化以及SREBPs蛋白表达水平降低,且HeLa细胞的生物学行为受到影响,表明熊果酸可能通过调控FKBP38/mTOR/SREBPs信号通路,进而对其生物学行为进行影响。为了进一步验证这一猜想,本研究利用FKBP38抑制剂设计并进行回复实验,结果发现,与H-熊果酸组比较,FKBP51-Hsp90-IN-1+H-熊果酸组细胞活性、迁移能力、侵袭能力增

加,Bcl-2、p-mTOR/mTOR、SREBPs蛋白水平增加,细胞凋亡率降低以及Bax、cleaved-Caspase-3、FKBP38蛋白水平显著降低,因此推测熊果酸可能通过调控FKBP38/mTOR/SREBPs信号通路,进而影响HeLa细胞的增殖、迁移、侵袭和凋亡。

综上所述,本研究表明熊果酸可能通过对FKBP38/mTOR/SREBPs信号通路进行调控,从而抑制HeLa细胞增殖、迁移、侵袭,以及促进HeLa细胞的凋亡。但是本研究仍然存在一定的不足之处,如只是在细胞上进行了体外验证,并未进行动物体内验证,针对这一不足,后续会进一步开展相关实验。

## 参考文献 (References)

- [1] 李颖,王凯,胡莉. KDR调节PI3K/AKT/mTOR信号通路对宫颈癌增殖、迁移和侵袭的影响[J]. 中国优生与遗传杂志(LI Y, WANG K, HU L. The influence of KDR regulating the PI3K/AKT/mTOR signaling pathway on the proliferation, migration and invasion of cervical cancer [J]. Chinese Journal of Birth Health & Heredity), 2022, 30(12): 2113-20.
- [2] 范佳杨,蔡鑫,熊蓉,等. 埃索美拉唑诱导宫颈癌细胞增殖、迁移、侵袭和凋亡作用及机制[J]. 中国药理学与毒理学杂志(FAN J Y, CAI X, XIONG R, et al. Effects and mechanisms of esomeprazole on the proliferation, migration, invasion and apoptosis of cervical cancer cells [J]. Chinese Journal of Pharmacology and Toxicology), 2023, 9(6): 418-25.
- [3] VOLKOVA L V, PASHOV A I, OMELCHUK N N. Cervical carcinoma: oncobiology and biomarkers [J]. Int J Mol Sci, 2021, 22(22): 12571-6.
- [4] 陈燕,何沙,陈春林. 熊果酸药理作用研究进展[J]. 宜春学院学报(CHEN X, HE S, CHEN C L. Research progress on the pharmacological effects of ursolic acid [J]. Journal of Yichun University), 2023, 45(9): 21-6.
- [5] NAVABHARATH M, SRIVASTAVA V, GUPTA S, et al. Ursolic acid and solasodine as potent anti-mycobacterial agents for combating paratuberculosis: an anti-inflammatory and in silico analysis [J]. Molecules, 2022, 28(1): 274-81.
- [6] LIU K M, HUANG Y, WAN P P, et al. Ursolic acid protects neurons in temporal lobe epilepsy and cognitive impairment by re-

- pressing inflammation and oxidation [J]. *Front Pharmacol*, 2022, 13(1): 877898-903.
- [7] ZHANG M, XIANG F, SUN Y, et al. Ursolic acid inhibits the metastasis of colon cancer by downregulating ARL4C expression [J]. *Oncol Rep*, 2024, 51(2): 27-35.
- [8] ALAM M, ALI S, AHMED S, et al. Therapeutic potential of ursolic acid in cancer and diabetic neuropathy diseases [J]. *Int J Mol Sci*, 2021, 22(22): 12162-8.
- [9] 周玉霞, 赵茴茴, 帅领, 等. Fkbp38基因缺失导致小鼠早发性卵巢功能不全: 基于激活 mTOR通路并诱导细胞凋亡[J]. *南方医科大学学报*(ZHOU Y X, ZHAO H H, SHUAI L, et al. Fkbp38 gene deletion leads to premature ovarian insufficiency in mice: based on activation of the mTOR pathway and induction of apoptosis [J]. *Journal of Southern Medical University*), 2022, 42(11): 1611-7.
- [10] AGUILERA M O, ROBLEDO E, MELANI M, et al. FKBP8 is a novel molecule that participates in the regulation of the autophagic pathway [J]. *Biochim Biophys Acta Mol Cell Res*, 2022, 1869(5): 119212-8.
- [11] 闫云静, 赖婉梅, 吴晓丽, 等. FKBP38蛋白在乳腺癌中的表达研究[J]. *现代生物医学进展*(YAN Y J, LAI Y M, WU X L, et al. Research on the expression of FKBP38 protein in breast cancer [J]. *Progress in Modern Biomedicine*), 2024, 24(1): 12-7.
- [12] XIAO P T, XIE Z S, KUANG Y J, et al. Discovery of a potent FKBP38 agonist that ameliorates HFD-induced hyperlipidemia via mTOR/P70S6K/SREBPs pathway [J]. *Acta Pharm Sin B*, 2021, 11(11): 3542-52.
- [13] YAN Y, WANG S, ZHANG Z, et al. FKBP38 suppresses endometrial cancer cell proliferation and metastasis by inhibiting the mTOR pathway [J]. *Arch Biochem Biophys*, 2024, 752(1): 109891-7.
- [14] 卢文, 杨艳梅, 马敬全, 等. 自噬在熊果酸抑制人宫颈癌HeLa细胞增殖中的作用[J]. *中国肿瘤*(LU W, YANG Y M, MA J Q, et al. The role of autophagy in the inhibitory effect of ursolic acid on the proliferation of human cervical cancer HeLa cells [J]. *China Cancer*), 2016, 25(7): 553-8.
- [15] WANG L, KUMAR R, WINBLAD B, et al. Structure-based discovery of small molecule inhibitors of FKBP51-Hsp90 protein-protein interaction [J]. *Eur J Med Chem*, 2024, 270(1): 116356-62.
- [16] SUNG H, FERLAY J, SIEGEL R L, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries [J]. *CA Cancer J Clin*, 2021, 71(3): 209-49.
- [17] PERKINS R B, WENTZENSEN N, GUIDO R S, et al. Cervical cancer screening: a review [J]. *JAMA*, 2023, 330(6): 547-58.
- [18] SIEGEL R L, GIAQUINTO A N, JEMAL A. Cancer statistics, 2024 [J]. *CA Cancer J Clin*, 2024, 74(1): 12-49.
- [19] JOHNSON C A, JAMES D, MARZAN A, et al. Cervical cancer: an overview of pathophysiology and management [J]. *Semin Oncol Nurs*, 2019, 35(2): 166-74.
- [20] NEWMAN D J, CRAGG G M. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019 [J]. *J Nat Prod*, 2020, 83(3): 770-803.
- [21] LIAN M Q, CHNG W H, LIANG J, et al. Plant-derived extracellular vesicles: recent advancements and current challenges on their use for biomedical applications [J]. *J Extracell Vesicles*, 2022, 11(12): e12283.
- [22] BOKHTIA R M, PHAM A M, BIHARI GUPTA K, et al. Ursolic acid conjugates: a new frontier in anticancer drug development [J]. *Chembiochem*, 2024, 29(1): e202400376.
- [23] CAMPBELL K J, TAIT S W G. Targeting BCL-2 regulated apoptosis in cancer [J]. *Open Biol*, 2018, 8(5): 180002-11.
- [24] 秦安敏, 司应明, 付盈盈, 等. 二氢杨梅素诱导人卵巢癌HO-8910细胞凋亡及机制研究[J]. *蚌埠医学院学报*(QIN A M, SI Y M, FU Y Y, et al. Research on the apoptosis induction of human ovarian cancer HO-8910 cells by dihydromyricetin and its mechanism [J]. *Journal of Bengbu Medical College*), 2021, 46(10): 1340-5.
- [25] 闫云静, 王帅, 穆云萍, 等. FKBP38蛋白对人源子宫内膜癌细胞增殖及侵袭的调控[J]. *中国药理学通报*(YAN Y J, WANG S, MU Y P, et al. Regulation of FKBP38 protein on the proliferation and invasion of human endometrial carcinoma cells [J]. *Chinese Pharmacological Bulletin*), 2020, 36(8): 1140-5.
- [26] CHEN H, QI Q, WU N, et al. Aspirin promotes RSL3-induced ferroptosis by suppressing mTOR/SREBP-1/SCD1-mediated lipogenesis in PIK3CA-mutant colorectal cancer [J]. *Redox Biol*, 2022, 55(1): 102426-32.