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扶正软坚抗癌方调控Akt/MDM2/P53信号通路对肝癌HepG2细胞恶性生物学行为的影响

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［摘 要］ **目的：** 探讨扶正软坚抗癌方调控蛋白激酶B（Akt）/鼠双微体2（MDM2）/P53信号通路对肝癌HepG2细胞恶性生物学行为的影响。**方法：** 采用0、0.05、0.10、0.20、0.40、0.80、1.60、3.20和6.40 g·mL^{-1}扶正软坚抗癌方分别处理HepG2细胞48 h，CCK-8法检测HepG2细胞存活率，筛选扶正软坚抗癌方浓度用于后续实验。将HepG2细胞分为对照组、低剂量扶正软坚抗癌方组（0.2 g·mL^{-1}）、中剂量扶正软坚抗癌方组（0.4 g·mL^{-1}）、高剂量扶正软坚抗癌方组（0.8 g·mL^{-1}）、SC79组（8 mg·L^{-1} SC79）和高剂量扶正软坚抗癌方＋SC79组（0.8 g·mL^{-1}扶正软坚抗癌方＋8 mg·L^{-1} SC79）。CCK-8法检测各组HepG2细胞增殖活性，克隆形成实验检测各组HepG2细胞克隆形成率，流式细胞术检测各组HepG2细胞凋亡率，Transwell小室实验检测各组HepG2细胞迁移和侵袭细胞数，Western blotting法检测各组HepG2细胞中增殖细胞核抗原（PCNA）、含半胱氨酸的天冬氨酸蛋白酶3（Caspase-3）、基质金属蛋白酶（MMP）-2、MMP-9、磷酸化Akt（p-Akt）、磷酸化MDM2（p-MDM2）和P53蛋白表达水平。**结果：** 随着扶正软坚抗癌方浓度（0、0.05、0.10、0.20、0.40、0.80、1.60、3.20和6.40 g·mL^{-1}）的升高，HepG2细胞存活率逐渐降低（$P<0.05$），选取0.2、0.4和0.8 g·mL^{-1}扶正软坚抗癌方用于后续实验。CCK-8法检测，与对照组比较，低、中和高剂量扶正软坚抗癌方组HepG2细胞增殖活性均明显降低（$P<0.05$），并呈剂量依赖性，SC79组HepG2细胞增殖活性明显升高（$P<0.05$）；与高剂量扶正软坚抗癌方组比较，高剂量扶正软坚抗癌方＋SC79组HepG2细胞增殖活性明显升高（$P<0.05$）。克隆形成实验检测，与对照组比较，低、中和高剂量扶正软坚抗癌方组HepG2细胞克隆形成率均明显降低（$P<0.05$），并呈剂量依赖性，SC79组HepG2细胞克隆形成率明显升高（$P<0.05$）；与高剂量扶正软坚抗癌方组比较，高剂量扶正软坚抗癌方＋SC79组HepG2细胞克隆形成率明显升高（$P<0.05$）。流式细胞术检测，与对照组比较，低、中和高剂量扶正软坚抗癌方组HepG2细胞凋亡率均明显降低（$P<0.05$），并呈剂量依赖性，SC79组HepG2细胞凋亡率明显升高（$P<0.05$）；与高剂量扶正软坚抗癌方组比较，高剂量扶正软坚抗癌方＋SC79组HepG2细胞凋亡率明显升高（$P<0.05$）。Transwell小室实验检测，与对照组比较，低、中和高剂量扶正软坚抗癌方组HepG2细胞迁移和侵袭细胞数均明显降低（$P<0.05$），并呈剂量依赖性，SC79组HepG2细胞迁移和侵袭细胞数均明显升高（$P<0.05$）；与高剂量扶正软坚抗癌方组比较，高剂量扶正软坚抗癌方＋SC79组HepG2细胞迁移和侵袭细胞数均明显升高（$P<0.05$）。Western blotting法检测，与对照组比较，低、中和高剂量扶正软坚抗癌方组HepG2细胞中PCNA、MMP-2、MMP-9、p-Akt和p-MDM2蛋白表达水平均明显降低（$P<0.05$），并呈剂量依赖性；Caspase-3和P53蛋白表达水平均明显升高（$P<0.05$），并呈剂量依赖性；SC79组HepG2细胞中PCNA、MMP-2、MMP-9、p-Akt和p-MDM2蛋白表达水平均明显升高（$P<0.05$），Caspase-3和P53蛋白表达水平均明显降低（$P<$

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0.05)；与高剂量扶正软坚抗癌方组比较，高剂量扶正软坚抗癌方＋SC79组HepG2细胞中PCNA、MMP-2、MMP-9、p-Akt和p-MDM2蛋白表达水平均明显升高（$P<0.05$），Caspase-3和P53蛋白表达水平均明显降低（$P<0.05$）。**结论：** 扶正软坚抗癌方抑制HepG2细胞增殖、迁移和侵袭，促进细胞凋亡，其作用机制与抑制Akt/MDM2信号通路、上调P53蛋白表达有关。

［**关键词**］ 扶正软坚抗癌方；蛋白激酶B；鼠双微体2；P53；肝肿瘤；细胞增殖；细胞凋亡

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Effect of Fuzheng Ruanjian Anticancer Formula on malignant biological behaviors of hepatocellulars carcinoma HepG2 cells by regulating Akt/MDM2/P53 signaling pathway

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ABSTRACT **Objective:** To discuss the effect of Fuzheng Ruanjian Anticancer Formula on the malignant biological behaviors of the hepatocellular carcinoma HepG2 cells by requlating protein kinase B (Akt)/murine double minute 2 (MDM2)/P53 signaling pathway. **Methods:** The HepG2 cells were treated with 0, 0.05, 0.10, 0.20, 0.40, 0.80, 1.60, 3.20, and 6.40 $g \cdot mL^{-1}$ Fuzheng Ruanjian Anticancer Formula for 48 h. CCK-8 method was used to detect the survival rates of the HepG2 cells in various groups, and the concentrations of Fuzheng Ruanjian Anticancer Formula for the subsequent experiments were screened. The HepG2 cells were divided into control group, low dose of Fuzheng Ruanjian Anticancer Formula group (0.2 $g \cdot mL^{-1}$), medium dose of Fuzheng Ruanjian Anticancer Formula group (0.4 $g \cdot mL^{-1}$), high dose of Fuzheng Ruanjian Anticancer Formula group (0.8 $g \cdot mL^{-1}$), SC79 group (8 $mg \cdot L^{-1}$ SC79), and high dose of Fuzheng Ruanjian Anticancer Formula+SC79 group (0.8 $g \cdot mL^{-1}$ Fuzheng Ruijian Anticancer Formula+8 $mg \cdot L^{-1}$ SC79). CCK-8 method was used to detect the proliferation activities of the HepG2 cells in various groups; clone formation assay was used to detect the clone formation rates of the HepG2 cells in various groups; flow cytometry was used to detect the apoptotic rates of the HepG2 cells in various groups; Transwell chamber assay was used to detect the numbers of migration and invasion HepG2 cells in various groups; Western blotting method was used to detect the expression levels of proliferating cell nuclear antigen (PCNA), cysteine aspartate specific proteinase (Caspase-3), matrix metalloproteinase (MMP)-2, MMP-9, phosphorylated Akt (p-Akt), phosphorylated MDM2 (p-MDM2), and P53 proteins in the HepG2 cells in various groups. **Results:** As the increasing of concentrations of Fuzheng Ruanjian Anticancer Formula (0, 0.05, 0.10, 0.20, 0.40, 0.80, 1.60, 3.20, and 6.40 $g \cdot mL^{-1}$), the surival rates of the HepG2 cells were gradually decreased ($P<0.05$), and 0.2, 0.4, and 0.8 $g \cdot mL^{-1}$ Fuzheng Ruanjian Anticancer Formula were selected for the subsequent experiments. The CCK-8 assay results showed that compared with control group, the proliferation activities of the HepG2 cells in low, medium, and high doses of Fuzheng Ruanjian Anticancer Formula groups were significantly decreased ($P<0.05$), in a dose-dependent manner, while the proliferation activity of the cells in SC79 group was significantly increased ($P<0.05$). Compared with high dose of Fuzheng Ruanjian Anticancer Formula group, the proliferation activity of the HepG2 cells in high dose of Fuzheng Ruanjian Anticancer Formula+SC79 group was significantly increased ($P<0.05$). The clone formation assay results showed that compared with

control group, the clone formation rates of the HepG2 cells in low, medium, and high doses of Fuzheng Ruanjian Anticancer Formula groups were significantly decreased ($P<0.05$) in a dose-dependent manner, while the clone formation rate of the cells in SC79 group was significantly increased ($P<0.05$); compared with high dose of Fuzheng Ruanjian Anticancer Formula group, the clone formation rate of the cells in high dose of Fuzheng Ruanjian Anticancer Formula+SC79 group was significantly increased ($P<0.05$). The flow cytometry results showed that compared with control group, the apoptotic rates of the HepG2 cells in low, medium, and high doses of Fuzheng Ruijian Anticancer Formula groups were significantly increased ($P<0.05$) in a dose-dependent manner, while the apoptotic rate of the cells in SC79 group was significantly decreased ($P<0.05$); compared with high dose of Fuzheng Ruanjian Anticancer Formula group, the apoptotic rate of the cells in high dose of Fuzheng Ruanjian Anticancer Formula+SC79 group was significantly decreased($P<0.05$). The Transwell chamber assay results showed that compared with control group, the numbers of migration and invasion HepG2 cells in low, medium, and high doses of Fuzheng Ruanjian Anticancer Formula groups were significantly decreased ($P<0.05$) in a dose-dependent manner, while the numbers of migration and invasion cells in SC79 group were significantly increased ($P<0.05$); compared with high dose of Fuzheng Ruanjian Anticancer Formula group, the numbers of migration and invasion HepG2 cells in high dose of Fuzheng Ruanjian Anticancer Formula+SC79 group were significantly increased ($P<0.05$). The Western blotting results showed that compared with control group, the expression levels of PCNA, MMP-2, MMP-9, p-Akt, and p-MDM2 proteins in the cells in low, medium, and high doses of Fuzheng Ruanjian Anticancer Formula groups were significantly decreased ($P<0.05$) in a dose-dependent manner, while the expression levels of Caspase-3 and P53 proteins were significantly increased ($P<0.05$) in a dose-dependent manner, while the expression levels of PCNA, MMP-2, MMP-9, p-Akt, and p-MDM2 proteins in the cells in SC79 group were significantly increased ($P<0.05$), and the expression levels of Caspase-3 and P53 proteins were significantly decreased ($P<0.05$); compared with high dose of Fuzheng Ruanjian Anticancer Formula group, the expression levels of PCNA, MMP-2, MMP-9, p-Akt, and p-MDM2 proteins in the cells in high dose of Fuzheng Ruanjian Anticancer Formula+SC79 group were significantly increased ($P<0.05$), while the expression levels of Caspase-3 and P53 proteins were significantly decreased ($P<0.05$). **Conclusion**: Fuzheng Ruanjian Anticancer Formula may inhibit the proliferation, migration, and invasion of the HepG2 cells and promote the apoptosis, and its mechanism may be related to suppressing the Akt/MDM2 signaling pathway and upregulating the P53 proteim expression.

KEYWORDS Fuzheng Ruanjian Anticancer Formula; Protein kinase B; Mouse double minute 2; P53; Liver neoplasm; Cell proliferation; Apoptosis

肝细胞癌作为原发性肝癌的主要组织学类型，严重危害人们的健康[1]。大部分住院患者在诊断时已达到中晚期或不符合手术条件，因此手术切除是20%～30%患者的首选治疗方法[2]。此外，肝癌发病机制复杂，对临床常用疗法不敏感。即使是一线化疗药物联合放疗也会产生多种不良反应，包括正常组织的异常代谢和患者生活质量降低。迄今为止，尚无广泛接受的肝癌治疗方法。因此，迫切需要探索与肝癌相关的机制，并开发更有效的治疗措施。扶正软坚抗癌方由人参、黄芪、当归、黄精、莪术、白花蛇舌草和山慈菇7味中药组成。其中单味药即可抑制肝癌进展，如黄芪能抑制磷脂酰肌醇3激酶（phosphatidyqinositol-3-kinase，PI3K）/蛋白激酶B（protein kinase B，Akt）通路发挥对肝癌细胞的抑制作用，白花蛇舌草提取物可抑制肝癌细胞增殖，促进细胞凋亡[3-4]。研究[5]显示：抑制Akt/鼠双微体2（murine double minute 2，MDM2）通路且上调P53蛋白表达可以抑制肝癌细胞增殖，诱导细胞凋亡。因此，本研究探讨扶正软坚抗癌方通过调控Akt/MDM2/P53信号通路对肝癌细胞恶性生物学行为的影响，并阐明其作用机制。

1 材料与方法

1.1 细胞、药物、主要试剂和仪器 人肝癌HepG2

细胞购自北京伊塔生物科技有限公司。扶正软坚抗癌方（人参10 g、黄芪15 g、当归10 g、黄精10 g、莪术10 g、白花蛇舌草20 g和山慈菇10 g）购自四川新绿色药业科技发展股份有限公司，所有药材加6倍体积水浸泡1 h，加热煎煮2 h收集煎液，将剩余药渣继续加6倍体积水煎煮收集煎液，合并2次煎液后低温减压浓缩为干浸膏，取0.2 g干浸膏溶解于1 mL 0.1%二甲基亚砜（dimethyl sulfoxide，DMSO）中，采用0、0.05、0.10、0.20、0.40、0.80、1.60、3.20和6.40 $g\cdot mL^{-1}$ DMEM培养液稀释药物。Akt激活剂SC79购自美国MCE公司，CCK-8试剂盒购自无锡菩禾生物医药技术有限公司，Annexin Ⅴ-FITC细胞凋亡检测试剂盒购自上海富雨生物科技有限公司，兔源一抗增殖细胞核抗原（proliferating cell nuclear antigen，PCNA）、含半胱氨酸的天冬氨酸蛋白酶3（cysteinyl aspartate specific proteinase-3，Caspase-3）、基质金属蛋白酶（matrix metalloproteinase，MMP）-2、MMP-9、磷酸化Akt（phosphorylated Akt，p-Akt）、Akt、磷酸化MDM2（phosphorylated MDM2，p-MDM2）、MDM2、P53、β-actin及辣根过氧化物酶（horseradish peroxidase，HRP）标记的羊抗兔二抗均购自英国Abcam公司。SAF-680T型酶标仪购自上海旦鼎国际贸易有限公司，CytoFLEX型流式细胞仪购自上海贝克曼库尔特商贸有限公司，DM6M LIBS型光学显微镜购自德国徕卡公司，DYCZ-25D型蛋白电泳仪购自北京六一仪器厂。

1.2　细胞培养和扶正软坚抗癌方浓度筛选　将HepG2细胞置于含有10%胎牛血清的DMEM培养基中培养。取对数生长期的HepG2细胞，以每孔1×10^4个细胞的密度接种于96孔细胞培养板中，采用0、0.05、0.10、0.20、0.40、0.80、1.60、3.20和6.40 $g\cdot mL^{-1}$扶正软坚抗癌方分别处理HepG2细胞48 h，各孔中加入10 μL CCK-8试剂，孵育2 h后，采用酶标仪检测波长450 nm处吸光度（A）值，计算细胞存活率。细胞存活率=（实验孔A值−空白孔A值)/(对照孔A值−空白孔A值）×100%。根据细胞存活率筛选合适的扶正软坚抗癌方浓度用于后续实验。

1.3　细胞分组　取对数生长期HepG2细胞，分为对照组、低剂量扶正软坚抗癌方组、中剂量扶正软坚抗癌方组、高剂量扶正软坚抗癌方组、SC79组和高剂量扶正软坚抗癌方+SC79组。低、中和高剂量扶正软坚抗癌方组HepG2细胞分别采用0.2、0.4和0.8 $g\cdot mL^{-1}$扶正软坚抗癌方处理48 h，SC79组HepG2细胞采用8 $mg\cdot L^{-1}$ SC79处理48 h[6]，高剂量扶正软坚抗癌方+SC79组HepG2细胞采用0.8 $g\cdot mL^{-1}$扶正软坚抗癌方和8 $mg\cdot L^{-1}$ SC79同时处理48 h，对照组HepG2细胞为正常培养的HepG2细胞，不作任何处理。

1.4　CCK-8法检测各组HepG2细胞增殖活性　取HepG2细胞，以每孔1×10^4个细胞的密度接种于96孔细胞培养板中，按照“1.3”中细胞分组进行对应处理后，每孔中加入10 μL CCK-8溶液，37 ℃孵育2 h，采用酶标仪检测波长450 nm处A值，并计算细胞增殖活性。细胞增殖活性=（实验孔A值−空白孔A值）/（对照孔A值−空白孔A值）×100%。

1.5　克隆形成实验检测各组HepG2细胞克隆形成率　取HepG2细胞，以每孔500个细胞的密度接种于6孔细胞培养板中，置入细胞培养箱中培养2周后，甲醇固定，0.1%结晶紫染色，观察细胞克隆形成情况，计算各组HepG2细胞克隆形成率。克隆形成率=形成克隆细胞数/接种总细胞数×100%。

1.6　流式细胞术检测各组HepG2细胞凋亡率　将“1.3”中培养的各组细胞用磷酸盐缓冲液（phosphate buffered saline，PBS）洗涤，再用Annexin Ⅴ-FITC/PI染色。37 ℃下避光孵育25 min后，采用流式细胞仪检测细胞凋亡情况，计算细胞凋亡率。细胞凋亡率=凋亡细胞数/总细胞数×100%。

1.7　Transwell小室实验检测各组HepG2细胞迁移和侵袭细胞数　细胞迁移实验：采用不含胎牛血清的DMEM培养液调整各组HepG2细胞密度为5×10^4 mL^{-1}，取200 μL细胞悬液加入Transwell上室中，再向Transwell下室中加入600 μL含10%胎牛血清的DMEM培养液。37 ℃孵育24 h，甲醛固定，0.5%结晶紫溶液染色，光学显微镜下观察各组HepG2细胞迁移情况，采用Image J软件计算迁移细胞数。细胞侵袭实验：预先将Matrigel基质胶涂覆于Transwell上室，待其自然干燥后，取上述200 μL细胞悬液加入到Transwell上室中，剩余步骤与细胞迁移实验一致，计算侵袭细胞数。

1.8　Western blotting法检测各组HepG2细胞中PCNA、Caspase-3、MMP-2、MMP-9、p-Akt、p-MDM2和P53蛋白表达水平　采用预冷的RIPA裂解缓冲液提取HepG2细胞总蛋白质，将蛋白质进行定量、电泳、转膜和封闭后，加入一抗β-actin、PCNA、Caspase-3、MMP-2、MMP-9、p-Akt、Akt、p-MDM2、MDM2和P53均为1∶1 000。4 ℃下孵育过夜。次日，加入二抗，于室温下孵育1 h。ECL试剂显色，采用Image J软件分析蛋白条带灰度值，以β-actin为内参，计算目的蛋白表达水平。目的蛋白表达水平＝目的蛋白条带灰度值/β-actin蛋白条带灰度值。

1.9　统计学分析　采用SPSS 25.0统计软件进行统计学分析。各组HepG2细胞增殖活性、克隆形成率、细胞凋亡率、迁移细胞数、侵袭细胞数和细胞中PCNA、Caspase-3、MMP-2及MMP-9蛋白和Akt/MDM2/P53信号通路相关蛋白表达水平均符合正态分布，以$\bar{x}\pm s$表示，多组间样本均数比较采用单因素方差分析，组间样本均数两两比较采用SNK-q检验。以$P<0.05$为差异有统计学意义。

2　结　果

2.1　不同浓度扶正软坚抗癌方处理后HepG2细胞存活率　随着扶正软坚抗癌方浓度（0、0.05、0.10、0.20、0.40、0.80、1.60、3.20和6.40 g·mL^{-1}）的升高，HepG2细胞存活率（99.93%±0.07%、96.88%±0.11%、86.88%±0.25%、79.83%±0.36%、71.26%±2.18%、62.59%±2.56%、43.15%±2.05%、39.26%±1.52%和28.87%±1.16%）逐渐降低（$P<0.05$）。0.20、0.40和0.80 g·mL^{-1}扶正软坚抗癌方处理下，HepG2细胞存活率＞50%，因此选择0.20、0.40和0.80 g·mL^{-1}扶正软坚抗癌方作为低、中和高剂量扶正软坚抗癌方组。用于后续实验。

2.2　各组HepG2细胞增殖活性　与对照组比较，低、中和高剂量扶正软坚抗癌方组HepG2细胞增殖活性均明显降低（$P<0.05$），并呈剂量依赖性，SC79组HepG2细胞增殖活性明显升高（$P<0.05$）。与高剂量扶正软坚抗癌方组比较，高剂量扶正软坚抗癌方＋SC79组HepG2细胞增殖活性明显升高（$P<0.05$）。见表1。

表1　各组HepG2细胞增殖活性

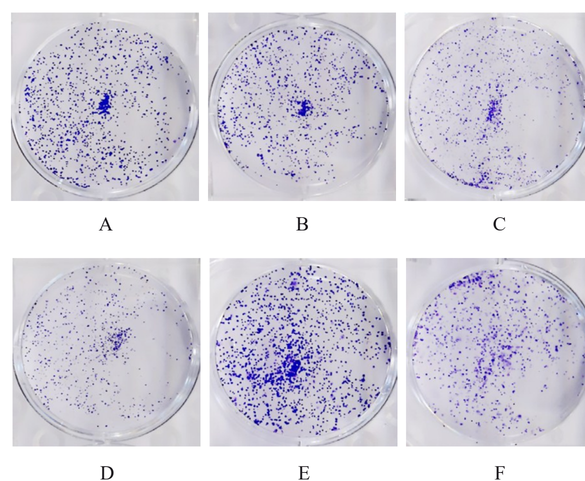
Tab. 1　Proliferation activities of HepG2 cells in various groups

（$n=6$, $\bar{x}\pm s$, η/%）

Group	Proliferation activity
Control	0.81±0.06
Fuzheng Ruanjian Anticancer Formula	
Low dose	0.73±0.06*
Medium dose	0.65±0.05$^{*\triangle}$
High dose	0.43±0.03$^{*\triangle\#}$
SC79	0.96±0.07*
High dose of Fuzheng Ruanjian Anticancer Formula＋SC79	0.69±0.05$^{\bigcirc}$

$^{*}P<0.05$ compared with control group; $^{\triangle}P<0.05$ compared with low dose of Fuzheng Ruanjian Anticancer Formula group; $^{\#}P<0.05$ compared with medium dose of Fuzheng Ruanjian Anticancer Formula group; $^{\bigcirc}P<0.05$ compared with high dose of Fuzheng Ruanjian Anticancer Formula group.

2.3　各组HepG2细胞克隆形成率　与对照组比较，低、中和高剂量扶正软坚抗癌方组HepG2细胞克隆形成率均明显降低（$P<0.05$），并呈剂量依赖性，SC79组HepG2细胞克隆形成率明显升高（$P<0.05$）。与高剂量扶正软坚抗癌方组比较，高剂量扶正软坚抗癌方＋SC79组HepG2细胞克隆形成率明显升高（$P<0.05$）。见图1和表2。



A: Control group; B: Low dose of Fuzheng Ruanjian Anticancer Formula group; C: Medium dose of Fuzheng Ruanjian Anticancer Formula group; D: High dose of Fuzheng Ruanjian Anticancer Formula group; E: SC79 group; F: High dose of Fuzheng Ruanjian Anticancer Formula＋SC79 group.

图1　克隆形成实验检测各组HepG2细胞克隆形成情况（结晶紫）

Fig. 1　Clone formation of HepG2 cells in various groups detected by clone formation experiment (Crystal violet)

2.4　各组HepG2细胞凋亡率　与对照组比较，

表2　各组HepG2细胞克隆形成率

Tab. 2　Clone formation rates of HepG2 cells in various groups

($n=6$, $\bar{x}\pm s$, η/%)

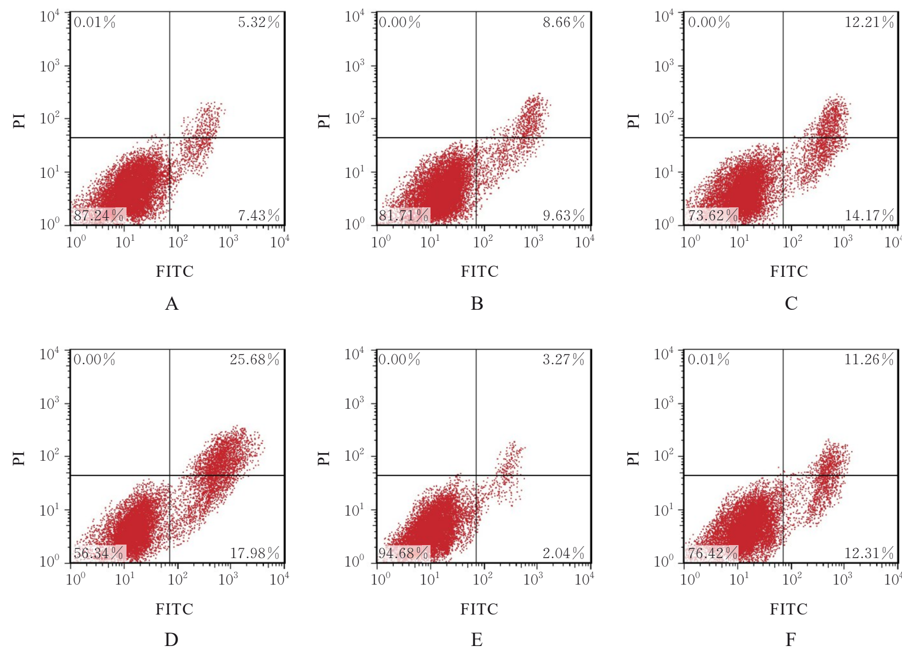
Group	Clone formation rate
Control	54.45±2.18
Fuzheng Ruanjian Anticancer Formula	
Low dose	48.82±2.09*
Medium dose	41.13±2.11*△
High dose	26.68±1.26*△#
SC79	68.83±3.15*
High dose of Fuzheng Ruanjian Anticancer Formula＋SC79	43.38±2.13○

*$P<0.05$ compared with control group; △$P<0.05$ compared with low dose of Fuzheng Ruanjian Anticancer Formula group; #$P<0.05$ compared with medium dose of Fuzheng Ruanjian Anticancer Formula group; ○$P<0.05$ compared with high dose of Fuzheng Ruanjian Anticancer Formula group.

低、中和高剂量扶正软坚抗癌方组HepG2细胞凋亡率均明显降低（$P<0.05$），并呈剂量依赖性，SC79组HepG2细胞凋亡率明显升高（$P<0.05$）。与高剂量扶正软坚抗癌方组比较，高剂量扶正软坚抗癌方＋SC79组HepG2细胞凋亡率明显升高（$P<0.05$）。见图2和表3。

2.5　各组HepG2细胞迁移和侵袭细胞数　与对照组比较，低、中和高剂量扶正软坚抗癌方组HepG2细胞迁移和侵袭细胞数均明显降低（$P<0.05$），并呈剂量依赖性，SC79组HepG2细胞迁移和侵袭细胞数均明显升高（$P<0.05$）。与高剂量扶正软坚抗癌方组比较，高剂量扶正软坚抗癌方＋SC79组HepG2细胞迁移和侵袭细胞数均明显升高（$P<0.05$）。见图3和4及表4。

2.6　各组HepG2细胞中PCNA、Caspase-3、MMP-2、MMP-9、p-Akt、p-MDM2和P53蛋白表达水平　与对照组比较，低、中和高剂量扶正软坚抗癌方组HepG2细胞中PCNA、MMP-2、MMP-9、p-Akt和p-MDM2蛋白表达水平均明显降低（$P<0.05$），并呈剂量依赖性；Caspase-3和P53蛋白表达水平均明显升高（$P<0.05$），并呈剂量依赖性；SC79组HepG2细胞中PCNA、MMP-2、MMP-9、p-Akt和p-MDM2蛋白表达水平均明显升高（$P<0.05$），Caspase-3和P53蛋白表达水平均明显降低（$P<0.05$）。与高剂量扶正软坚抗癌方组比较，高剂量扶正软坚抗癌方＋SC79组HepG2细胞中PCNA、MMP-2、MMP-9、p-Akt和p-MDM2蛋白表达水平均明显升高（$P<0.05$），Caspase-3和P53蛋白



A: Control group; B: Low dose of Fuzheng Ruanjian Anticancer Formula group; C: Medium dose of Fuzheng Ruanjian Anticancer Formula group; D: High dose of Fuzheng Ruanjian Anticancer Formula group; E: SC79 group; F: High dose of Fuzheng Ruanjian Anticancer Formula＋SC79 group.

图2　流式细胞术检测各组HepG2细胞凋亡情况

Fig. 2　Apoptosis of HepG2 cells in various groups detected by flow cytometry

表3　各组HepG2细胞凋亡率

Tab. 3　Apoptotic rates of HepG2 cells in various groups

（$n=6, \bar{x}\pm s, \eta/\%$）

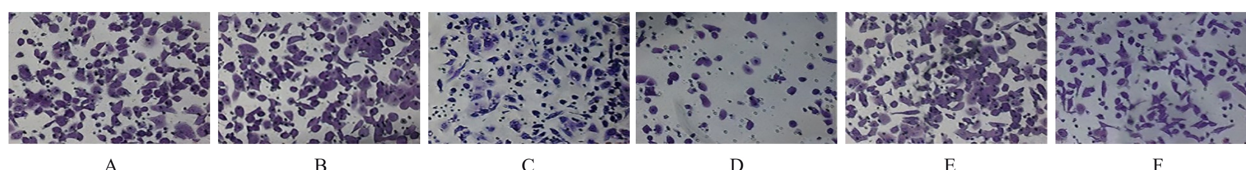
Group	Apoptotic rate
Control	54.45±2.18
Fuzheng Ruanjian Anticancer Formula	
Low dose	48.82±2.09*
Medium dose	41.13±2.11*△
High dose	26.68±1.26*△#
SC79	68.83±3.15*
High dose of Fuzheng Ruanjian Anticancer Formula＋SC79	43.38±2.13○

*$P<0.05$ compared with control group; △$P<0.05$ compared with low dose of Fuzheng Ruanjian Anticancer Formula group; #$P<0.05$ compared with medium dose of Fuzheng Ruanjian Anticancer Formula group; ○$P<0.05$ compared with high dose of Fuzheng Ruanjian Anticancer Formula group.

表达水平均明显降低（$P<0.05$）。见图5和表5。

3　讨　论

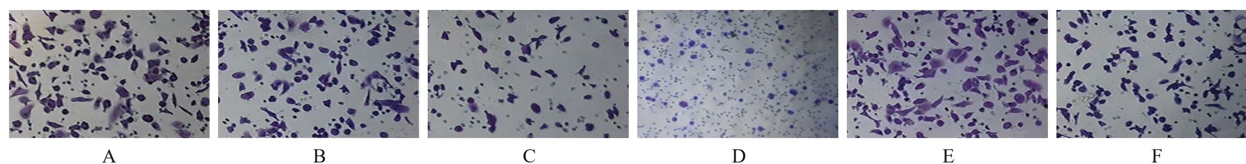
2020年全球癌症统计数据[7]显示：全球每年约有90.6万例肝癌新发病例和83万例死亡病例。肝癌起病隐匿，摄入真菌代谢物黄曲霉毒素B1、慢性乙型或丙型肝炎病毒感染及过度饮酒均为肝癌的主要危险因素[8]。肝癌具有放疗和化疗干预、早期手术治疗和肝移植等多种治疗策略，但其5年生存率仍低于5%[9]。因此，需要寻找新的有效抗肿瘤药物。中医药在世界范围内被广泛接受，主要原因是其在临床治疗和预防癌症方面的有效性和安全性[10]。扶正软坚抗癌方由人参、黄芪和当归、黄精、莪术、白花蛇舌草和山慈菇7味中药组成。其单药或其中几味药组合治疗癌症的研究较多，如人参皂苷可抑制肝癌细胞增殖[11]，黄芪和莪术配伍可抑制肝癌裸鼠肿瘤新生血管生成[12]，当归多糖纳米粒靶向给药系统可有效治疗肝癌并增强抗肿瘤活性[13]，山慈菇含药血清可明显抑制肝癌HepG2细胞的生长，诱导细胞凋亡并抑制其上皮-间质转化[14]。扶正软坚抗癌方的核心药物具有扶正和抗癌等作用。本研究结果显示：扶正软坚抗癌方可呈剂量依赖性地抑制HepG2细胞增殖、迁移和侵袭，促进细胞凋亡。此外，PCNA是细胞异常增殖的关键蛋白，其表达在细胞复制过程中呈周期性变化，与细胞有丝分裂过程中DNA含量的变化相吻合，并在细胞有丝分裂的S期达到峰值[15]。Caspase-3是细胞凋亡的关键执行蛋白，可切割对细胞活性有重要作用的蛋白质[16]。MMP-2和MMP-9的主要生物学功能是降解细胞外基质，参与细胞迁移和侵袭等过程[17]。本研究结果显示：扶正软坚抗癌方可呈剂



A: Control group; B: Low dose of Fuzheng Ruanjian Anticancer Formula group; C: Medium dose of Fuzheng Ruanjian Anticancer Formula group; D: High dose of Fuzheng Ruanjian Anticancer Formula group; E: SC79 group; F: High dose of Fuzheng Ruanjian Anticancer Formula＋SC79 group.

图3　Transwell小室实验检测各组HepG2细胞迁移情况（结晶紫，×400）

Fig. 3　Migration of HepG2 cells in various groups detected by Transwell chamber assay (Crystal violet, ×400)



A: Control group; B: Low dose of Fuzheng Ruanjian Anticancer Formula group; C: Medium dose of Fuzheng Ruanjian Anticancer Formula group; D: High dose of Fuzheng Ruanjian Anticancer Formula group; E: SC79 group; F: High dose of Fuzheng Ruanjian Anticancer Formula＋SC79 group.

图4　Transwell小室实验检测各组HepG2细胞侵袭情况（结晶紫，×400）

Fig. 4　Invasion of HepG2 cells in various groups detected by Transwell chamber assay (Crystal violet, ×400)

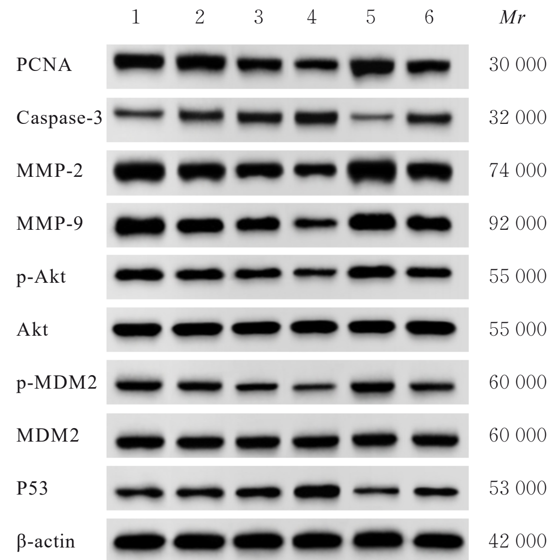
表4　各组HepG2细胞迁移细胞数和侵袭细胞数

Tab. 4　Numbers of migration and invasion HepG2 cells in various groups　(n=6, $\bar{x}\pm s$)

Group	Number of migration cells	Number of invasion cells
Control	116.67±3.85	78.65±2.36
Fuzheng Ruanjian Anticancer Formula		
Low dose	103.32±2.63*	65.51±2.14*
Medium dose	89.94±3.08*△	46.69±2.03*△
High dose of	42.26±1.86*△#	21.18±1.14*△#
SC79	139.94±5.67*	93.35±3.54*
High dose of Fuzheng Ruanjian Anticancer Formula＋SC79	97.73±3.45○	57.72±2.13○

*P<0.05 compared with control group; △P<0.05 compared with low dose of Fuzheng Ruanjian Anticancer Formula group; #P<0.05 compared with medium dose of Fuzheng Ruanjian Anticancer Formula group; ○P<0.05 compared with high dose of Fuzheng Ruanjian Anticancer Formula group.

量依赖性地抑制HepG2细胞中PCNA、MMP-2和MMP-9蛋白表达，上调Caspase-3蛋白表达，再次从蛋白质水平上证实了扶正软坚抗癌方对HepG2细胞增殖、迁移和侵袭的抑制作用及其对细胞凋亡的促进作用，提示扶正软坚抗癌方可能成为治疗肝癌的潜在有效药物。研究[18]显示：扶正抗癌方可抑制肝癌细胞增殖和转移。扶正抑瘤汤在原发性肝癌根治切除术患者术后治疗中应用效果良好，可有效地延长患者生存期，降低术后复发风险，在扶正抗癌方的基础上，再加以软坚（即肿瘤包块软化，消散结节）之功效，为肝癌的临床治疗提供了坚实的理论基础[19]。



Lane 1: Control group; Lane 2: Low dose of Fuzheng Ruanjian Anticancer Formula group; Lane 3: Medium dose of Fuzheng Ruanjian Anticancer Formula group; Lane 4: High dose of Fuzheng Ruanjian Anticancer Formula group; Lane 5: SC79 group; Lane 6: High dose of Fuzheng Ruanjian Anticancer Formula＋SC79 group.

图5　Western blotting法检测各组HepG2细胞中Akt/MDM2/P53信号通路相关蛋白表达电泳图

Fig. 5　Electrophoregram of expressions of Akt/MDM2/P53 signaling pathway-related proteins in HepG2 cells in various groups detected by Western blotting method

表5　各组HepG2细胞中Akt/MDM2/P53信号通路相关蛋白表达水平

Tab. 5　Expression levels of Akt/MDM2/P53 signaling pathway-related proteins in HepG2 cells in various groups　(n=6, $\bar{x}\pm s$)

Group	PCNA	Caspase-3	MMP-2	MMP-9	p-Akt	p-MDM2	P53
Control	0.97±0.09	0.21±0.02	1.38±0.11	1.26±0.10	0.82±0.08	0.73±0.06	0.36±0.02
Fuzheng Ruanjian Anticancer Formula							
Low dose	0.84±0.08*	0.38±0.03*	1.06±0.09*	0.96±0.09*	0.71±0.06*	0.62±0.05*	0.48±0.03*
Medium dose	0.67±0.05*△	0.57±0.05*△	0.86±0.07*△	0.72±0.06*△	0.56±0.05*△	0.43±0.04*△	0.59±0.05*△
High dose	0.32±0.03*△#	0.86±0.07*△#	0.54±0.05*△#	0.31±0.03*△#	0.28±0.02*△#	0.16±0.01*△#	0.96±0.08*△#
SC79	1.29±0.10*	0.12±0.01*	1.61±0.15*	1.43±0.11*	0.93±0.08*	0.83±0.07*	0.16±0.01*
High dose of Fuzheng Ruanjian Anticancer Formula＋SC79	0.72±0.06○	0.45±0.04○	0.96±0.07○	0.81±0.07○	0.67±0.06○	0.55±0.04○	0.52±0.04○

*P<0.05 compared with control group; △P<0.05 compared with low dose of Fuzheng Ruanjian Anticancer Formula group; #P<0.05 compared with medium dose of Fuzheng Ruanjian Anticancer Formula group; ○P<0.05 compared with high dose of Fuzheng Ruanjian Anticancer Formula group.

Akt/MDM2/P53信号通路失调与多种恶性肿瘤有关，Akt磷酸化可促进转录因子MDM2的稳定和核转位，进而引发肿瘤抑制因子P53的泛素化和降解[20]。近年来，靶向Akt/MDM2/P53通路被认为是对抗人类恶性肿瘤的一种有效治疗方式，如抑制Akt/MDM2通路的激活且上调P53蛋白表达可抑制胶质瘤细胞增殖和侵袭[21]，抑制Akt/MDM2通路的激活且上调P53蛋白表达可诱导胃癌细胞凋亡[22]，提示抑制Akt/MDM2通路的激活且上调P53蛋白表达可抑制肿瘤细胞的恶性进展，与本研究结果一致。本研究结果显示：与对照组比较，SC79组HepG2细胞中p-Akt和p-MDM2蛋白表达水平升高，P53蛋白表达水平降低，HepG2细胞增殖、迁移和侵袭能力增强，细胞凋亡能力减弱，证实Akt/MDM2/P53通路参与HepG2细胞增殖、凋亡、迁移和侵袭。此外，扶正软坚抗癌方可呈剂量依赖性地抑制HepG2细胞中p-Akt和p-MDM2蛋白表达，上调P53蛋白表达，提示扶正软坚抗癌方可能通过调控Akt/MDM2/P53通路抑制HepG2细胞增殖、迁移和侵袭，促进细胞凋亡。本研究在高剂量扶正软坚抗癌方作用的基础上联合Akt激活剂SC79干预HepG2细胞，结果显示：SC79可减弱高剂量扶正软坚抗癌方对HepG2细胞增殖、迁移和侵袭的抑制作用及其对细胞凋亡的促进作用，提示扶正软坚抗癌方可能通过抑制Akt/MDM2通路的激活且上调P53蛋白表达，从而抑制HepG2细胞增殖、迁移和侵袭，促进细胞凋亡。

综上所述，扶正软坚抗癌方可能通过抑制Akt/MDM2通路的激活且上调P53蛋白表达，从而抑制HepG2细胞增殖、迁移和侵袭，促进细胞凋亡。本研究仅在体外细胞水平上验证了扶正软坚抗癌方对肝癌HepG2细胞进展的抑制作用，未选取不同肝癌细胞株来验证实验结果，本课题组后期将会进一步深入探索。

利益冲突声明：

所有作者声明不存在利益冲突。

作者贡献声明：

娄静参与研究设计、实验操作和论文撰写，赵雷、朱岩洁和袁帅强参与实验操作、数据收集及整理，王菲、张杭洲、徐娇娇和余晓珂参与数据统计学分析及论文修改，侯留法参与实验设计及研究指导。

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