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## • 基础研究 •

## 不同浓度白花丹素对骨肉瘤细胞 MG-63 凋亡迁移、基质金属蛋白酶及 Bcl-2、Bax、Ezrin 蛋白表达的影响\*

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**摘要 目的:**研究不同浓度白花丹素对骨肉瘤细胞 MG-63 凋亡迁移、基质金属蛋白酶(MMP)及 Bcl-2、Bax、Ezrin 蛋白表达的影响。**方法:**取对数生长期的骨肉瘤 MG-63 细胞,传代培养成细胞株后以随机法分成对照组、低剂量组、中剂量组、高剂量组。其中对照组加入到 0.1%浓度的 DMSO 完全培养基中培养,低剂量组、中剂量组、高剂量组分别加入到浓度为 5、10、20  $\mu\text{mol/L}$  的白花丹素的有关培养基中培养。培养 24 h 后,采用 Transwell 法检测 MG-63 细胞迁移率、Hoechst33342 染色法检测 MG-63 细胞凋亡率、Western blot 法检测四组 MG-63 细胞的 MMP-2、MMP-9、Bcl-2、Bax、Ezrin 蛋白表达水平。**结果:**培养 24 h 后,低剂量组、中剂量组、高剂量组的骨肉瘤细胞 MG-63 凋亡率及 Bax 蛋白表达水平均较对照组升高( $P<0.05$ ),且随白花丹素浓度的增加而升高( $P<0.05$ );骨肉瘤细胞 MG-63 的细胞迁移率、MMP-2、MMP-9、Bcl-2 及 Ezrin 蛋白表达水平较对照组降低( $P<0.05$ ),且随白花丹素浓度的增加而降低( $P<0.05$ )。**结论:**白花丹素对骨肉瘤细胞 MG-63 凋亡的促进作用以及迁移的抑制作用明显,其作用机制可能与抑制骨肉瘤细胞 MG-63 中的 MMP-2、MMP-9、Bcl-2、Ezrin 蛋白表达及促进 Bax 蛋白表达有关,且浓度越高,抑制或促进作用越明显。

**关键词:**骨肉瘤;白花丹素;MG-63 细胞;基质金属蛋白酶;凋亡;迁移

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## Effects of Different Concentrations of Baihuadansu on MG-63 Apoptosis Migration, Matrix Metalloproteinase and Expression of Bcl-2, Bax and Ezrin in Osteosarcoma Cells\*

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**ABSTRACT Objective:** To study the effects of different concentrations of baihuadansu on MG-63 apoptosis migration, matrix metalloproteinase and expression of Bcl-2, Bax and Ezrin in osteosarcoma cells. **Methods:** MG-63 cells of osteosarcoma at logarithmic growth stage were taken. After subculture, the cells were randomly divided into control group, low dose group, medium dose group and high dose group. The complete DMSO medium with 0.1% concentration was added to control group, and culture medium containing 5 mol/L, 10 mol/L and 20 mol/L of baihuadansu were added to the low dose group, medium dose group and high dose group, respectively. After 24 hours of culture, the migration rate of MG-63 cells was determined by Transwell method, the apoptosis rate of MG-63 cells was determined by Hoechst33342 staining, and the expression levels of MMP-2, MMP-9, bcl-2, Bax and Ezrin of MG-63 cells in the four groups were detected by Western blot. **Results:** After 24 hours of culture, the apoptosis rate and Bax protein expression level of osteosarcoma cells in low dose group, middle dose group and high dose group were higher than those in the control group ( $P<0.05$ ), and increased with the increase of baihuadansu concentration ( $P<0.05$ ). The cell migration rate, MMP-2, MMP-9, Bcl-2 and ezrin expression level of osteosarcoma MG-63 were lower than those of the control group ( $P<0.05$ ), and decreased with the increase of the concentration of baihuadansu ( $P<0.05$ ). **Conclusion:** The effect of baihuadansu on the apoptosis and migration of osteosarcoma cell line MG-63 is obvi-

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ous. Its mechanism may be related to the inhibition of MMP-2, MMP-9, Bcl-2, Ezrin protein expression and the promotion of Bax protein expression in osteosarcoma cell line MG-63. The higher the concentration, the more obvious the inhibition or promotion.

**Key words:** Osteosarcoma; Baihuadansu; MG-63 cells; Matrix metalloproteinase; Apoptosis; Migration

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## 前言

骨肉瘤属于骨原发性恶性肿瘤之一,多见于儿童以及青少年人群,往往发生在长管状骨的干骺端。骨肉瘤具有高度恶性以及高侵袭性的特点,因此转移是导致临床治疗失败,甚至死亡的重要原因之一<sup>[1,2]</sup>。相关研究表明<sup>[3,4]</sup>,基质金属蛋白酶-2(Matrix metalloproteinase-2, MMP-2)、基质金属蛋白酶-9(Matrix metalloproteinase-9, MMP-9)和肿瘤转移存在密切相关,且在骨肉瘤以及转移灶内表达显著升高。另有相关研究报道表明<sup>[5,6]</sup>, Bcl-2、Bax、Ezrin 蛋白在多种恶性肿瘤中均存在明显异常表达,其中 Bcl-2、Ezrin 具有抑制细胞凋亡的作用,而 Bax 具有促进细胞凋亡的作用。白花丹素主要是指对中药白花丹进行提取后获得的含醌类化合物,可对多类肿瘤细胞产生杀伤效果<sup>[7,8]</sup>。鉴于此,本文通过研究不同浓度白花丹素对骨肉瘤细胞 MG-63 凋亡迁移、基质金属蛋白酶及 Bcl-2、Bax、Ezrin 蛋白表达的影响并进行分析,旨在明确白花丹素影响骨肉瘤细胞 MG-63 凋亡迁移的可能机制,现作以下报道。

## 1 资料与方法

### 1.1 材料与仪器

① 白花丹素、Hoechst33342 以及细胞培养基二甲基亚砜(DMSO)均购自美国 Sigma 公司。② 将白花丹素溶解于 DMSO 内配成 0.1mmol/L 溶液,并贮存于 -20℃ 冰箱内。③ 骨肉瘤细胞 MG-63 购自中国科学院下属的上海细胞库。④ 小牛血清(美国 Hyclone 公司)。⑤ 青/链霉素双抗及高糖细胞培养基(美国 Gibco 公司)。⑥ ECL 发光试剂盒和凝胶试剂盒、硝酸纤维素膜和兔抗人一抗、羊抗兔二抗(武汉博士德生物公司)。⑦ 显微镜(日本 Nikon 公司)。

### 1.2 研究方法

(1)细胞传代培养:取对数生长期的骨肉瘤 MG-63 细胞,采用含有 10%胎牛血清、100 μg/mL 青霉素以及 0.1 mg/mL 链霉素的 RPMI 1640 培养基培养,培养条件:37℃、5% CO<sub>2</sub>, 2~3 d 更换一次培养液。用倒置相差显微镜观察细胞生长状况,待其铺满培养基 80%时,采用 0.25%胰酶实施消化处理,按照 1:3 的比例予以传代培养。将传代培养后的细胞株(n=95)以随机法分成对照组(n=20)、低剂量组(n=25)、中剂量组(n=25)、高剂量组(n=25)。(2)Transwell 法检测 MG-63 细胞迁移率:待细胞传代培养结束后制备成 10<sup>6</sup> mL 的细胞悬液,取 200 μL 接种在上层小室,其中对照组加入到 0.1%浓度的 DMSO 完全培养基中培养,低剂量组、中剂量组、高剂量组分别加入到含 5、10 及 20 μmol/L 白花丹素的有关培养基中培养。下层小室内滴加 300 μL 含有 10%胎牛血清培养基为化学诱导物,每组设 3 个复孔。于 37℃ 条件下培养 24 h,取出吸干培养基,加入 4%的多聚甲醛固定 30 min,以 PBS 重复 3 次洗涤, Gemisa 溶液染色,

于高倍镜下随机采取 5 个视野计数细胞,取平均数作为实验结果。(3)Hoechst33342 染色法检测 MG-63 细胞凋亡率:取 Hoechst33342 溶解于 PBS 内,配制成浓度为 10 μg/mL 的储存液。按照上述(2)中的条件进行细胞培养以及相关干预。24 h 后添加 1 μg/mL Hoechst33342 型工作液进行 10 min 的染色。任选 10 个视野于 100 倍的显微镜下对总细胞数和凋亡细胞数实施计数,而后统计细胞凋亡率。(4)Western blot 法检测四组 MG-63 细胞的 MMP-2、MMP-9、Bcl-2、Bax、Ezrin 蛋白表达水平:取处于对数生长期内的部分骨肉瘤细胞,将每孔约 10<sup>6</sup> 个细胞常规接种于 6 孔板,在培养过夜之后通过上述(2)步骤实施分组干预,每组分别设置 3 个复孔,在 24 h 后通过 PBS 进行重复性漂洗共 3 次,通过 0.25%的胰酶进行消化后再采集细胞,而后添入 200 μL 组织裂解液,置于冰上裂解约 0.5 h,于 4℃ 条件下,以 12000 r/min 离心 15min,获取上清液,采用考马斯亮蓝法完成蛋白浓度的测定,保存于 -20℃ 条件下备用。调节蛋白浓度并进行热变形,取总量相同的蛋白质通过 10% SDS-聚丙烯酰胺的凝胶进行电泳,电泳结束之后将蛋白转移至硝酸纤维素膜,通过 5%的脱脂奶粉实施 2 h 封闭,再添入经 5%的脱脂奶粉稀释后的一抗,在 4℃ 下孵育过夜,另用 TBST 实施重复漂洗共 3 次,15 min/ 次,随后加入 HRP 标记的二抗,于室温下孵育 2 h 后采用 TBST 重复漂洗 3 次,15 min/ 次。在暗室中用 ECL 发光液常规发光,并在压片之后经曝光盒曝光,图片照相后借助 Quantity One 软件实施灰度值分析,以 GAPDH 为内参,对比目的蛋白和内参蛋白的灰度值,二者比值为目的蛋白相对表达量。

### 1.3 观察指标

比较各组细胞凋亡率、细胞迁移率, MMP-2 及 MMP-9 蛋白表达水平, Bcl-2、Bax、Ezrin 蛋白表达水平。

### 1.4 统计学方法

数据的分析借助 SPSS20.0 软件完成,计量资料以( $\bar{x} \pm s$ )表示,予以 LSD-t 检验,多组间对比采用单因素方差进行分析。 $P < 0.05$  表示差异有统计学意义。

## 2 结果

### 2.1 各组细胞凋亡率、细胞迁移率对比

对照组、低剂量组、中剂量组、高剂量组的细胞凋亡率呈逐渐升高趋势,而细胞迁移率呈逐渐降低趋势(均  $P < 0.05$ ),见表1。

### 2.2 各组 MMP-2 及 MMP-9 蛋白表达水平对比

对照组、低剂量组、中剂量组、高剂量组的 MMP-2 及 MMP-9 蛋白表达水平均呈逐渐降低趋势(均  $P < 0.05$ ),见表2。

### 2.3 各组 Bcl-2、Bax、Ezrin 蛋白表达水平对比

对照组、低剂量组、中剂量组、高剂量组的 Bcl-2、Ezrin 蛋白表达水平均呈逐渐下降趋势,而 Bax 蛋白表达水平呈逐渐升高趋势(均  $P < 0.05$ ),见表3。

表 1 各组细胞凋亡率、细胞迁移率对比( $\bar{x} \pm s, \%$ )Table 1 Comparison of cell apoptosis rate and cell migration rate in each group( $\bar{x} \pm s, \%$ )

| Groups            | n  | Cell apoptosis rate                               | Cell migration rate                               |
|-------------------|----|---------------------------------------------------|---------------------------------------------------|
| Control group     | 20 | 7.74 $\pm$ 2.34                                   | 100.00 $\pm$ 0.00                                 |
| Low dose group    | 25 | 12.39 $\pm$ 2.61 <sup>#</sup>                     | 76.22 $\pm$ 10.42 <sup>#</sup>                    |
| Middle dose group | 25 | 18.74 $\pm$ 2.77 <sup>**</sup>                    | 50.71 $\pm$ 8.67 <sup>**</sup>                    |
| High dose group   | 25 | 30.27 $\pm$ 4.68 <sup>**<math>\nabla</math></sup> | 31.62 $\pm$ 7.49 <sup>**<math>\nabla</math></sup> |
| F                 | -  | 36.293                                            | 41.582                                            |
| P                 | -  | 0.000                                             | 0.000                                             |

Note: compared with the control group, <sup>#</sup> $P < 0.05$ ; compared with the low dose group, <sup>\*</sup> $P < 0.05$ ; compared with the middle dose group,  <sup>$\nabla$</sup>  $P < 0.05$ .

表 2 各组 MMP-2 及 MMP-9 蛋白表达水平对比( $\bar{x} \pm s$ )Table 2 Comparison of MMP-2 and MMP-9 protein expression levels in each group( $\bar{x} \pm s$ )

| Groups            | n  | MMP-2                                            | MMP-9                                            |
|-------------------|----|--------------------------------------------------|--------------------------------------------------|
| Control group     | 20 | 0.71 $\pm$ 0.13                                  | 0.54 $\pm$ 0.09                                  |
| Low dose group    | 25 | 0.52 $\pm$ 0.11 <sup>#</sup>                     | 0.37 $\pm$ 0.08 <sup>#</sup>                     |
| Middle dose group | 25 | 0.26 $\pm$ 0.09 <sup>**</sup>                    | 0.24 $\pm$ 0.08 <sup>**</sup>                    |
| High dose group   | 25 | 0.15 $\pm$ 0.07 <sup>**<math>\nabla</math></sup> | 0.17 $\pm$ 0.04 <sup>**<math>\nabla</math></sup> |
| F                 | -  | 29.745                                           | 18.374                                           |
| P                 | -  | 0.000                                            | 0.000                                            |

Note: compared with the control group, <sup>#</sup> $P < 0.05$ ; compared with the low dose group, <sup>\*</sup> $P < 0.05$ ; compared with the middle dose group,  <sup>$\nabla$</sup>  $P < 0.05$ .

表 3 各组 Bcl-2、Bax、Ezrin 蛋白表达水平对比( $\bar{x} \pm s$ )Table 3 Comparison of protein expression levels of Bcl-2, Bax and Ezrin in each group( $\bar{x} \pm s$ )

| Groups            | n  | Bcl-2                                            | Bax                                              | Ezrin                                            |
|-------------------|----|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|
| Control group     | 20 | 0.83 $\pm$ 0.04                                  | 0.05 $\pm$ 0.01                                  | 0.71 $\pm$ 0.03                                  |
| Low dose group    | 25 | 0.61 $\pm$ 0.02 <sup>#</sup>                     | 0.09 $\pm$ 0.01 <sup>#</sup>                     | 0.52 $\pm$ 0.02 <sup>#</sup>                     |
| Middle dose group | 25 | 0.40 $\pm$ 0.03 <sup>**</sup>                    | 0.14 $\pm$ 0.02 <sup>**</sup>                    | 0.34 $\pm$ 0.01 <sup>**</sup>                    |
| High dose group   | 25 | 0.15 $\pm$ 0.01 <sup>**<math>\nabla</math></sup> | 0.20 $\pm$ 0.04 <sup>**<math>\nabla</math></sup> | 0.17 $\pm$ 0.01 <sup>**<math>\nabla</math></sup> |
| F                 | -  | 44.292                                           | 28.475                                           | 11.844                                           |
| P                 | -  | 0.000                                            | 0.000                                            | 0.000                                            |

Note: compared with the control group, <sup>#</sup> $P < 0.05$ ; compared with the low dose group, <sup>\*</sup> $P < 0.05$ ; compared with the middle dose group,  <sup>$\nabla$</sup>  $P < 0.05$ .

### 3 讨论

临床上大多数恶性肿瘤的致命性在于早期发生转移,骨肉瘤的早期转移主要表现为血行转移,且存在转移出现早、发生率高特点,一旦发生会显著缩短患者生存期<sup>[9-11]</sup>。随着新辅助化疗的出现,骨肉瘤患者的 5 年生存率得以显著提高,但近年来未见明显突破,加之化疗药物可能引发一系列严重不良反应,因此寻找一种新的安全性更高的抗肿瘤药物显得尤为重要<sup>[12-14]</sup>。白花丹素主要是源自中药白花丹的根茎,在我国已有数千年的应用历史,具有抗炎、抗菌等多种作用<sup>[15,16]</sup>。已有不少研究报道证实,白花丹素可通过改变微小蛋白质表达,从而在前列腺癌细胞的凋亡过程中起着至关重要的作用<sup>[17,18]</sup>。另有研究学者发现,白花丹素可有效抑制大肠癌的细胞增殖,促进细胞凋亡<sup>[19,20]</sup>。而 Chen H 等<sup>[21]</sup>、Li YC 等<sup>[22]</sup>人的研究报道发现,白花

丹素可通过 MAPK 以及 PI3K/Akt/mTOR 信号通路,在鳞状细胞癌中发挥至关重要的作用。

本研究结果表明,对照组、低剂量组、中剂量组、高剂量组的细胞凋亡率呈逐渐升高趋势,而细胞迁移率呈逐渐降低趋势,这提示了白花丹素可诱导骨肉瘤细胞 MG-63 的凋亡,抑制其迁移,其中主要原因可能在于白花丹素可降低蛋白激酶 B 和哺乳动物的雷帕霉素蛋白的磷酸化程度,因此可诱导肿瘤细胞的自噬性凋亡<sup>[23,24]</sup>。此外,肿瘤转移属于极其复杂的过程,涉及肿瘤细胞的解离、细胞外基质的降解、浸润以及转移等多种重要过程,而 MMPs 家族在上述过程中起着至关重要的作用,其中 MMP-2、MMP-9 是通过降解细胞外的重要成分-IV 型胶原的途径参与恶性肿瘤的转移过程<sup>[25,26]</sup>。本文结果发现,对照组、低剂量组、中剂量组、高剂量组的 MMP-2 及 MMP-9 蛋白表达水平呈逐渐降低趋势,这提示了白花丹素可有效抑制 MMP-2

及 MMP-9 蛋白的表达,且随着药物浓度的不断增加,该抑制作用越明显,因此笔者推测白花丹素抑制骨肉瘤细胞 MG-63 迁移、促进其凋亡的可能机制与抑制 MMP-2、MMP-9 蛋白表达有关,这与田林强等人的研究报道基本相符<sup>[27]</sup>。Bcl-2 使细胞存活时间延长,其发生表达降低可能导致细胞活力也下降,但 Bax 蛋白能够诱导细胞凋亡,二者均分布在细胞的线粒体中,可通过影响细胞代谢从而影响细胞的凋亡<sup>[28,29]</sup>。本文结果显示:对照组、低剂量组、中剂量组、高剂量组的 Bcl-2、Ezrin 蛋白表达水平均呈逐渐下降趋势,而 Bax 蛋白表达水平呈逐渐升高趋势,这提示了白花丹素可能是通过诱导线粒体死亡途径激活,进一步发挥促进细胞凋亡的作用,这是因为 Ezrin 蛋白属于膜细胞骨架连接蛋白,主要介导微绒毛形成过程,同时对细胞运动、黏附、细胞骨架重塑等过程具有极其重要的作用。有相关研究报道证实<sup>[30]</sup>,Ezrin 蛋白和糖蛋白结合,进一步促使骨肉瘤发生多重耐药,从而降低临床治疗效果,因此抑制 Ezrin 蛋白的表达,可能降低骨肉瘤细胞对抗肿瘤药物的耐药性,这可能是白花丹素促进骨肉瘤 MG-63 细胞凋亡的重要途径之一。

综上所述,白花丹素对骨肉瘤 MG-63 细胞凋亡具有显著促进作用,且对该细胞迁移具有显著的抑制作用,其作用机制可能与抑制 MMP-2、MMP-9、Bcl-2、Ezrin 蛋白表达以及促进 Bax 蛋白表达有关。

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