

熊果酸联合顺铂对卵巢癌干细胞的增殖、凋亡、侵袭和迁移的影响

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Title: Effect of ursolic acid combined with cisplatin on proliferation, apoptosis, invasion and migration of ovarian cancer stem cells

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关键词: 熊果酸; 卵巢癌干细胞; 凋亡; 增殖; 侵袭; 迁移; 上皮间质转化

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摘要: 目的: 观察熊果酸(ursolic acid, UA)联合顺铂(DDP)对卵巢癌干细胞增殖、凋亡、侵袭及迁移能力的影响及其作用机制。方法: 通过体外无血清悬浮培养人卵巢癌SKOV3干细胞并进行细胞鉴定。实验分为SKOV3干细胞组、熊果酸组、熊果酸联合顺铂组。MTT法检测干细胞的增殖, Transwell实验检测干细胞侵袭与迁移能力, 采用Annexin V/PI双染法流式细胞术检测干细胞凋亡。Real-time PCR检测上皮间质转化(epithelial-mesenchymal transition, EMT)相关标记分子Vimentin、N-cadherin、E-cadherin、Fibronectin、Twist的mRNA表达情况, Western-blot检测E-cadherin、Vimentin、Twist蛋白表达情况。结果: 熊果酸对SKOV3干细胞增殖有显著抑制作用, 呈剂量依赖性($P < 0.05$); 流式细胞术显示熊果酸组、熊果酸联合顺铂组均可提高SKOV3干细胞的凋亡率, 与SKOV3干细胞组相比有统计学差异($P < 0.05$); 熊果酸组、熊果酸联合顺铂组可有效抑制SKOV3干细胞的侵袭和迁移能力($P < 0.05$); Real-time PCR测定熊果酸组、熊果酸联合顺铂组作用SKOV3干细胞后EMT基因表达水平, 结果显示Fibronectin、Twist、Vimentin、N-cadherin表达降低, E-cadherin表达升高($P < 0.05$); Western-blot结果显示熊果酸组、熊果酸联合顺铂组可上调E-cadherin蛋白的表达, 下调Vimentin、Twist蛋白的表达; 与熊果酸组相比, 熊果酸联合顺铂组对干细胞凋亡率更高, 迁移和侵袭能力更低, E-cadherin表达更高, Vimentin和Twist表达更低, 以上差异均有统计学意义($P < 0.05$)。结论: 熊果酸对卵巢癌干细胞有抑制增殖、侵袭和迁移, 诱导凋亡的作用, 联合顺铂作用效果更优, 其机制可能与逆转EMT有关。

Abstract: Objective: To observe the effect of ursolic acid (UA) combined with cisplatin (DDP) on proliferation, apoptosis, invasion and migration of ovarian cancer stem cells, and to explore its mechanism. Methods: Ovarian cancer SKOV3 stem cells were cultured in serum-free suspension in vitro and identified. There were three groups, including SKOV3 stem cell group, ursolic acid group, ursolic acid combined with cisplatin group. MTT assay was used to detect the proliferation of stem cells. Transwell assay was used to detect the invasion and migration of stem cells. Annexin V/PI double staining flow cytometry was used to detect the apoptosis of stem cells. Real time-PCR was used to detect the mRNA expression of Vimentin, N-cadherin, E-cadherin, Fibronectin, Twist and Western-blot was used to detect the protein expression of E-cadherin, Vimentin and Twist. Results: Ursolic acid significantly inhibited the proliferation of SKOV3 stem cells in a dose-dependent manner ($P < 0.05$). Flow cytometry showed that both ursolic acid group and ursolic acid combined with cisplatin group could increase the apoptotic rate of SKOV3 stem cells, which was significantly different from the SKOV3 stem cells group ($P < 0.05$). Ursolic acid group and ursolic acid combined with cisplatin group could effectively inhibit the invasion and migration of SKOV3 stem cells ($P < 0.05$). The mRNA expression

of Fibronectin, Twist, Vimentin and N-cadherin was decreased, while the expression of E-cadherin was increased ($P < 0.05$). Western-blot results showed that ursolic acid group and ursolic acid combined with cisplatin group could up-regulate the expression of E-cadherin and down-regulate the expression of Vimentin and Twist protein. Compared with ursolic acid group, the ursolic acid combined with cisplatin group had higher apoptotic rate, lower migration and invasion ability, higher E-cadherin expression and lower Vimentin and Twist expression, with statistical significance ($P < 0.05$). Conclusion: Ursolic acid can inhibit the proliferation, invasion and migration of ovarian cancer stem cells, and induce apoptosis and ursolic acid combined with cisplatin has a better effect. Its mechanism may be related to the reversal of epithelial-mesenchymal transition.

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