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菟丝子含药血清对大鼠肢芽细胞、骨形成蛋白2及Ⅱ型胶原表达的影响

刘星¹, 李啸红², 王燕¹, 邹聪聪² ✓

Effect of *Cuscuta chinensis* Lam medicated serum on rat embryo limb bud cells and expressions of BMP-2 and CollagenⅡ

LIU Xing¹, LI Xiaohong², WANG Yan¹, ZOU Congcong² ✓



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摘要/Abstract

摘要：目的 探讨菟丝子含药血清对SD大鼠胚胎肢芽细胞增殖与分化、细胞中骨形成蛋白2 (BMP-2) mRNA表达、Ⅱ型胶原mRNA和蛋白表达的影响。方法 采用中药血清药理学方法制备高、中、低剂量 (40、20、10 g/kg) 菟丝子含药血清和正常血清。取E15后肢芽制成细胞悬液，接种到细胞培养板中，分别加入高、中、低剂量菟丝子含药血清和正常血清，作为高、中、低剂量组和阴性对照组。体外培养72 h，MTT法检测肢芽细胞的增殖，阿利新蓝染色检测肢芽细胞的分化，RT-PCR法检测肢芽细胞中BMP-2、Ⅱ型胶原mRNA的表达，Western blotting检测Ⅱ型胶原蛋白的表达。结果 低、中剂量组细胞增殖较快，吸光度值高于阴性对照组 ($P<0.05$)；中剂量组软骨细胞集落数多于阴性对照组 ($P<0.05$)，高剂量组软骨细胞集落数低于阴性对照组 ($P<0.05$)；低、中剂量组BMP-2 mRNA表达水平高于阴性对照组 ($P<0.05$)，高剂量组BMP-2 mRNA低于阴性对照组 ($P<0.05$)；各剂量组Ⅱ型胶原mRNA和蛋白的表达水平均高于阴性对照组 ($P<0.05$)。结论 不同剂量菟丝子含药血清均可促进肢芽细胞的增殖和Ⅱ型胶原mRNA及蛋白的表达；高剂量菟丝子含药血清可下调BMP-2 mRNA的表达水平，抑制肢芽细胞的分化。

关键词：骨形成蛋白2, Ⅱ型胶原, 菟丝子, 肢芽细胞, 含药血清

Abstract: Objective To explore the effect of *Cuscuta chinensis* Lam medicated serum on the proliferation and differentiation of limb bud cells, the mRNA expressions of bone morphogenetic protein 2 (BMP-2) and CollagenⅡ, and the protein expression of CollagenⅡ. **Methods** Different doses of *Cuscuta chinensis* Lam medicated serum and normal serum were prepared by serological pharmacological method. The hindlimb buds were amputated from SD rat embryos on day 15, then were made into cell suspension. The cells were cultured by the normal serum and the high, middle and low doses of *Cuscuta Chinensis* Lam medicated serum *in vitro* for 72 h, and were divided into the negative control group and high, middle and low dose groups, respectively. Limb bud cells proliferation was evaluated by MTT. Cells differentiation was evaluated by the cartilage cell colony numbers, which were stained with alcian blue. The mRNA expressions of BMP-2 and CollagenⅡ were analyzed by RT-PCR, and protein expression of CollagenⅡ was analyzed by Western blotting. **Results** Compared with the negative control group, the cells differentiation in low and middle dose groups were faster ($P<0.05$); the cartilage cell colony numbers in middle dose group were more, while those in high dose group were less (all $P<0.05$); the expressions of BMP-2 mRNA in low and middle dose groups were higher and that in high dose group was lower (all $P<0.05$); the expressions of CollagenⅡ mRNA and protein in high, middle and low dose groups were higher ($P<0.05$). **Conclusion** Different doses of *Cuscuta chinensis* Lam medicated serum can promote cell proliferation and increase expressions of CollagenⅡ mRNA and protein. High dose of *Cuscuta Chinensis* Lam medicated serum can inhibit limb bud cells differentiation by decreasing BMP-2 mRNA expression.

Key words: Limb bud cell, *Cuscuta Chinensis* Lam, Bone morphogenetic protein 2, Medicated serum, CollagenⅡ

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