

基础医学

脂多糖所致小鼠睾丸支持细胞GDNF表达减少对精原干细胞的影响

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摘要:

目的 探讨脂多糖(LPS)处理后的小鼠睾丸支持细胞(SCs)中胶质细胞源性神经营养因子(GDNF)表达的变化及该变化对精原干细胞(SSCs)的影响。方法 取5~6周龄昆明小白鼠睾丸组织,分离纯化并培养SCs,将其分为4组:正常对照组(Con组),添加LPS处理1d组(1d组)、2d组(2d组)和3d组(3d组),收集4组培养液作为条件培养液,采用RT-PCR和Western blot检测各组SCs GDNF表达的变化;取5~7d龄昆明小乳鼠睾丸组织,筛选SSCs后分为4组:Con组、I组、II组和III组,分别用前面的4组培养液培养96h。通过免疫荧光染色和RT-PCR检测细胞中PLZF、oct4、PCNA和c-kit、sohlh2的表达变化,并通过RT-PCR检测SSCs中Gfra1、c-ret和Bcl6b、Etv5的表达变化。结果 RT-PCR与Western blot结果显示,SCs GDNF表达按Con、1d、2d、3d组的顺序降低;RT-PCR和免疫荧光染色结果显示,SSCs PLZF、oct4和PCNA的表达按Con、I、II、III组顺序降低,而c-kit和sohlh2的表达按Con组、I、II、III组顺序升高;SSCs中Gfra1、c-ret、Bcl6b与Etv5的表达I、II和III组均低于Con组。结论 LPS处理导致小鼠SCs GDNF表达减少,该变化可使SSCs分化加速而其自我更新受到抑制,其机制可能与Bcl6b和Etv5的表达下调有关。

关键词: 脂多糖; 睾丸支持细胞; 胶质细胞源性神经营养因子; 精原干细胞; 分化; 自我更新

Expression of GDNF in LPS-treated mouse Sertoli cells and its effects on spermatogonial stem cells

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Abstract:

Objective To investigate the changes of GDNF expression in sertoli cells(SCs) of LPS-treated Kunming (KM) mice and its effects on spermatogonial stem cells (SSCs). Methods SCs were isolated and purified from testes of 5~6 weeks old KM mice. The SCs were cultured and divided into four groups: the normal culture medium(group con), the medium with LPS treatment 1 day(group 1d), treatment 2 days(group 2d) and treatment 3 days(group 3d). The medium of four groups were collected respectively as the conditioned medium. RT-PCR and Western blotting were performed to investigate the changes of GDNF expressions of four groups SCs. The SSCs from the testes of 5~7days KM mice were isolated and cultured, and divided into four groups: con, group I , group II and group III. The SSCs were cultured for 96 hours in the conditioned medium respectively. Immunofluorescent staining was performed on SSCs to investigate the expressions of PLZF, oct4, PCNA, c-kit and sohlh2. RT-PCR was performed to investigate the expressions of Gfra1, c-ret, Bcl6b and Etv5. Results RT-PCR and Western blotting results revealed that the expression of GDNF in SCs was decreased in the order of group con, group 1d, group 2d and group 3d. Immunofluorescence and RT-PCR results revealed that the expressions of PLZF, oct4 and PCNA in SSCs were decreased in the order of group con, group 1d, group 2d and group 3d, and the expressions of c-kit and sohlh2 were increased in the order of group con, group 1d, group 2d and group 3d. RT-PCR results revealed that the expressions of Gfra1, c-ret, Bcl6b and Etv5 in group 1d, group 2d and group 3d were decreased compared with group con. Conclusion The expression of GDNF in LPS-treated mouse SCs is decreased. The differentiation and self-renewal of SSCs can be stimulated and inhibited respectively by the reduction of GDNF expression in SCs via down-regulation of Bcl6b and Etv5 expression.

Keywords: Lipopolysaccharide; Sertoli cells; Glial cell-line derived neurotrophic factor; Spermatogonial stem cells; Differentiation; Self-renewal

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