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人参皂苷Rg_1延缓造血干细胞衰老与 $p16^{INK4a}$表达关系的研究

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中文摘要:目的: 探讨人参皂苷单体Rg_1延缓造血干细胞(HSC)衰老与$p16^{INK4a}$的表达调控关系,为寻找延缓HSC衰老途径提供理论和实验依据。 方法 :免疫磁性分选法分离纯化Sca-1^+HSC后分组。对照组常规培养;衰老组运用三丁基过氧化氢 (t-BHP) 复制衰老模型;Rg_1组在对照组基础上加入10 μmol·L^{-1} Rg_1共培养;Rg_1延缓衰老组给予10 μmol·L^{-1} Rg_1预处理后,复制衰老模型;Rg_1治疗衰老组,衰老模型复制后,给予10 μmol·$L^{-1}$$Rg_1$抗衰老处理。衰老相关$\beta$-半乳糖苷酶(SA-$\beta$-gal)细胞化学染色、流式细胞术分析细胞周期和造血祖细胞混合集落(CFU-Mix)培养确定Rg_1延缓或治疗Sca-1^+HSC衰老生物学作用。RT-PCR及Western blotting检测衰老相关基因$p16^{INK4a}$ mRNA及蛋白的表达。 结果 :Rg_1延缓衰老组,Rg_1治疗衰老组与衰老组相比,SA-β-gal染色阳性细胞百分比降低,G_1期细胞比例下降,生成造血祖细胞混合集落数增加,$p16^{INK4a}$ mRNA及蛋白的表达下降;Rg_1延缓衰老组的SA-β-gal染色阳性细胞百分比、G_1期比例、$p16^{INK4a}$ mRNA及蛋白的表达均低于Rg_1治疗衰老组,形成造血祖细胞混合集落数高于Rg_1治疗衰老组。 结论 :Rg_1具有延缓和治疗Sca-1^+HSC衰老的作用,Rg_1延缓衰老比治疗衰老效果更好。Rg_1可能通过调控$p16^{INK4a}$的表达发挥其对抗t-BHP诱导的Sca-1^+HSC衰老的作用。

中文关键词:人参皂苷Rg_1 $p16^{INK4a}$ 造血干细胞 衰老

Experimental study of relationship between effect of ginsenoside Rg_1 to delay hematopoietic stem cell senescence and expression of $p16^{INK4a}$

Abstract: Objective : To investigate the relation between the effect of ginsenoside Rg_1 to delay hematopoietic stem cell senescence and the expression of $p16^{INK4a}$. The purpose is to provide the theory and experimental foundation for searching the methods of how to delay HSC senescence. Method : Sca-1^+HSC was isolated by magnetic cell sorting(MACS) and divided into five groups.The control group cells were routinely cultured, the aging group cells were induced aging by tert-butylhydroperoxide(t-BHP,final concentration of 100 μmol·L^{-1}) to establish the aging model, the Rg_1 group cells were co-cutured with Rg_1 (final concentration is 10 μmol·L^{-1}).To Rg_1 delay aging group, Sca-1^+HSC were established aging model after pretreatment of Rg_1 (final concentration is 10 μmol·L^{-1}) . To Rg_1 treat aging group, Sca-1^+HSC gave Rg_1 (final concentration is 10 μmol·L^{-1}) antiaging treatment after the aging model was established.The changes of cells observed by senescence-associated β-galactosidase(SA-β-gal) staining,cell cycle analysis and culture of mixed hematopoietic progenitor cell were used to investigate the antiaging and delay aging effect of ginsenoside Rg_1.The expression of senescence associated $p16^{INK4a}$ mRNA and $p16^{INK4a}$ protein was examined by RT-PCR and western blotting. Result : Compared with aging group, the percentage of positive cells expressed SA-β-gal and cells in G_1 phase decreased and the number of forming colony of mixed hematopoietic progenitor increased and it showed higher expression of $p16^{INK4a}$ mRNA and $p16^{INK4a}$ protein in Rg_1 treat aging group and Rg_1 delay aging group. Furthermore the percentage of positive cells expressed SA-β-gal,cells in G_1 phase,the number of forming colony of mixed hematopoietic progenitor and the expression of $p16^{INK4a}$ mRNA and protein decreased in Rg_1 delay aging group compared with Rg_1 treat aging group. Conclution : Rg_1 can significantly delay and treat the senescence of Sca-1^+HSC.The effect of Rg_1 delaying aging is better than treatment.$p16^{INK4a}$ may be play a key role in the antiaging effect of Rg_1 to Sca-1^+HSC senescence induced by t-BHP.

keywords:ginsenoside Rg_1 $p16^{INK4a}$ Sca-1^+HSC senescence

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