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论著

Dynein抑制剂对卵母细胞体外成熟及cyclinB1 mRNA水平的影响

王晓梅，黄天华,谢庆东，张清健，阮 烨

(汕头大学医学院生殖医学研究中心，广东 汕头 515041

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摘要 背景与目的：胞浆Dynein(动力蛋白)做为微管负极方向的马达蛋白在细胞增殖进程中发挥重要作用。本实验采用原钒酸钠特异抑制Dynein生物活性后,观察卵母细胞体外培养成熟率和cyclinB1基因转录水平变化，探讨Dynein功能异常对卵母细胞成熟分裂进程的影响及相关机制。材料与方法：应用卵母细胞体外培养成熟技术、半定量RT-PCR和单细胞RT-PCR方法，分别检测加入Dynein抑制剂前后卵母细胞成熟率和cyclinB1基因转录水平的变化。结果：12 h量效实验结果证实，不同浓度原钒酸钠作用后，5 μmol/L原钒酸钠即可明显降低小鼠卵母细胞成熟率，0～400 μmol/L卵母细胞成熟率随着剂量的增加而显著减少；同时检测发现，50～500 μmol/L原钒酸钠组卵母细胞cyclinB1 mRNA表达呈剂量依赖性增加。时程结果表明，卵母细胞与400 μmol/L原钒酸钠作用1 h 以上，卵母细胞成熟率明显减少；体外分别培养4 h或8 h后再放入含有原钒酸钠培养基中培养至12 h，仍可明显抑制卵母细胞第一极体排放。400 μmol/L原钒酸钠作用后，卵母细胞内cyclinB1 mRNA时程表达水平与对照组相比，呈明显反向变化。结论：Dynein抑制剂诱导体外培养的卵母细胞减数分裂阻滞，成熟促进因子(MPF)活性改变可能是Dynein功能丧失后引起减数分裂停滞的一个机制。

关键词 动力蛋白；卵母细胞；细胞周期素B1；原钒酸钠

Effect of Dynein Inhibitor on Mouse Oocyte in vitro Maturation and Its CyclinB1 mRNA Level

WANG Xiao-mei, HUANG Tian-hua , XIE Qing-dong, et al

Research Center for Reproductive Medicine,Shantou University Medical College,Shantou 515041, China

Abstract BACKGROUND & AIM: Cytoplasmic dynein is a kind of microtubule-based related protein involving in many fundamental cellular process including organelles transport, spindles assembly and spindle checkpoint inactivity.To elucidate the effect of dynein dysfunction on oocytes meiosis process and the relative mechanism of premature oocytes, we observed the changes in oocytes maturation rate and its cyclinB1 gene transcription level after exposed to a known dynein inhibitor-sodium orthovanadate(SOV) in vitro. MATERIAL AND METHODS: The technique of oocytes cultured in vitro maturation(CVM)was employed to detect oocytes maturation rate. Single cell RT-PCR and semi-quantitative RT-PCR methods were applied to measure cyclinB1 mRNA level in oocytes. RESULTS: The maturation rates of oocytes were significantly different between 5 μmol/L SOV and control groups($P<0.05$) and decreased with SOV increasing doses. However,the elevation of cyclinB1 mRNA level of immatured oocytes cultured for 12 h depended on SOV concentrations ranging from 50～500 μmol/L. In incontinuity SOV exposure experiments, the maturation rates of oocytes markedly reduced after first incubation with 400 μmol/L SOV at least for 1 h and first cultured in SOV-free medium for 4 h or 8 h then exposed to SOV ($P<0.05$).In time-course experiment, the opposite changes of cyclinB1 mRNA level of oocytes between SOV and control groups were observed. CONCLUSION: Dynein inhibitor might delay oocytes meiosis process, and cause the ectopic expression of cyclinB1inoocytes. The possible mechanism of meiosis arrest induced by SOV is related to dynein dysfunction and the abnormal transcription expression of cyclinB1 that caused changes of mature promoting factor (MPF) activity.

Keywords dynein； oocytes； cyclinB1； sodium orthovanadate(SOV

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通讯作者 黄天华 thhuang@stu.edu.cn