

实验方法

## 人淀粉样蛋白前体695基因及人烟碱乙酰胆碱受体 $\alpha 4\beta 2$ 亚单位基因共表达细胞模型的构建

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**摘要** 目的 为满足寻找可能影响淀粉样 $\beta$ 蛋白(A $\beta$ )在阿尔茨海默病过程中的药物实验的需求,建立共表达人淀粉样蛋白前体(APP)695基因和烟碱乙酰胆碱受体(nAChR) $\alpha 4\beta 2$ 亚单位基因的细胞模型。方法 用脂质体转染方法把APP 695基因转染进已稳定转染nAChR  $\alpha 4\beta 2$ 亚单位基因的SH-EP1细胞。用500 mg·L<sup>-1</sup> G418加压筛选,并挑选细胞单克隆。用RT-PCR和Western蛋白印迹法对转染后的细胞单克隆进行鉴定,挑取成功转染并高表达APP 695的细胞进行克隆。膜片钳检测所挑细胞克隆的 $\alpha 4\beta 2$ 受体功能。结果 挑出成功稳定转染人APP 695基因及人nAChR  $\alpha 4\beta 2$ 亚单位基因的共表达细胞克隆株。膜片钳方法检测到此细胞克隆株上nAChR  $\alpha 4\beta 2$ 存在活性。结论 成功制备了共表达人APP 695基因及人nAChR  $\alpha 4\beta 2$ 亚单位基因的细胞模型,为探讨神经元nAChR  $\alpha 4\beta 2$ 亚型对A $\beta$ 加工影响的药物实验提供了条件。

**关键词** [淀粉样 \$\beta\$ 蛋白前体](#) [受体](#), [烟碱](#)

**分类号** [R965.2](#)

## Construction of coexpression cell model of human amyloid protein precursor 695 gene and human nicotinic acetylcholinergic $\alpha 4\beta 2$ subunit genes

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### Abstract

**AIM** For the need of cell model in drugs research that may have effect on amyloid  $\beta$ -protein in Alzheimer's disease processing, the cells coexpressed with human amyloid protein precursor (APP) 695 gene and human nicotinic acetylcholine receptor(nAChR)  $\alpha 4\beta 2$  subunit genes were constructed. **METHODS** Liposome was used to transfect human APP 695 gene into SH-EP1 cells which stably expressed nAChR  $\alpha 4\beta 2$  genes. Neomycin (500 mg·L<sup>-1</sup>) was used to ensure stable expression of APP 695, and limiting dilution assay was used to obtain single transfected cell clones. RT-PCR and Western blot were used to verify the clones. The highly expressed cell clones were selected, and the activity of nAChR  $\alpha 4\beta 2$  was justified by patch clamp. **RESULTS** Cell clone with stable coexpression of APP 695 gene and nAChR  $\alpha 4\beta 2$  genes was constructed successfully, and the activity of  $\alpha 4\beta 2$  receptors in the cell clone was confirmed by patch clamp. **CONCLUSION** Coexpression cell model of human APP 695 and nAChR  $\alpha 4\beta 2$  genes was constructed success fully, which may provide good method for pharmaceutical experiments on effects of nAChR  $\alpha 4\beta 2$  on amyloid processing.

**Key words** [amyloid  \$\beta\$ -protein precursor](#) [receptors](#) [nicotinic](#)

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