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论著

体外诱导小鼠胚胎干细胞分化为心肌细胞的初步研究

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摘要 目的：5-氮胞苷作为分化诱导剂，初步探讨其单独或联合全反式维甲酸应用时对小鼠胚胎干细胞（mESC）分化为心肌细胞的影响，旨在建立一种体外诱导mESC分化为心肌细胞的实验方法。方法： 采用MTT法确定5-氮胞苷的非细胞毒性参考剂量。设计不同条件培养基（5-氮胞苷单独或配伍全反式维甲酸应用）对mESC 进行诱导分化，并通过免疫组化技术及RT-PCR方法等对分化细胞进行鉴定。结果： 5-氮胞苷的非细胞毒性参考剂量为8 μmol/L，能够诱导mESC分化为心肌合胞体（与阴性对照组比较，P<0.01），诱导分化率可达50%。配伍全反式维甲酸持续诱导的结果等同于单独应用全反式维甲酸的作用效果（P>0.05）：即对ESC向心肌细胞的诱导分化没有促进作用。结论：5-氮胞苷能够诱导mESC分化为心肌合胞体，从而得以建立一种体外诱导mESC分化为心肌细胞的方法。

关键词 胚胎干细胞； 心肌； 分化； 阿扎胞苷； 维甲酸

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Study on inducing differentiation of mouse embryonic stem cells into cardiomyocytes in vitro

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Abstract

<FONT face=Verdana>AIM: To set up a method of inducing mouse embryonic stem cells (mESC) to differentiate into cardiomyocyte after treatment with 5-azacytidine. METHODS: Cytotoxicity of 5-azacytidine was measured by MTT assay. Treatment of mESC with conditioned culture mediums, which were composed of 5-azacytidine alone or combined with retinoic acid, induced the cell differentiation to cardiomyocytes. The cells induced were identified by detecting the expression of cardiac proteins (myosin, desmin, α-actin and α-actinin). Gene MLC-2v, a specific gene of ventricular-like cardiomyocyte, was also detected by RT-PCR. RESULTS: The non-cytotoxic dose of 5-azacytidine was 8 μmol/L, which was able to induce mESC to differentiate into cardiac syncytiums. Cells induced expressed many cardiac proteins and MLC-2v mRNA. However, combined with retinoic acid inhibited mESC differentiation into cardiomyocyte. CONCLUSION: 5-azacytidine is able to promote mESC differentiation into cardiomyocytes. A method of inducing mESC to differentiate into cardiomyocytes in vitro has been established. </FONT>

Key words Embryonic stem cells Myocardium Differentiation Azacitidine Tretinoin

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