



## 非病毒诱导体系高效诱导脐带来源间充质干细胞向胰岛细胞分化

李晶, 朱莉, 赵春华\*

中国医学科学院 北京协和医学院 基础医学研究所组织工程研究中心, 北京100005

## Virus Free Induction of Umbilical Cord Derived Mesenchymal Stem Cells into Islet-like Cells

LI Jing, ZHU Li, ZHAO Chun-hua\*

Center of Excellence in Tissue Engineering, Institute of Basic Medical Sciences, CAMS and PUMC, Beijing, 100005, China

摘要

参考文献

相关文章

Download: PDF (738KB) HTML 1KB Export: BibTeX or EndNote (RIS) Supporting Info

**摘要** 目的 探讨非病毒法诱导脐带来源间充质干细胞(UC-MSC)向胰岛素分泌细胞分化的可行性,为胰岛细胞移植治疗糖尿病提供临床移植数量级的细胞。方法 从人脐带分离间充质干细胞,体外分阶段诱导分化为胰岛细胞。采用RT-PCR方法比较胰岛细胞分化过程中转录因子foxa2、sox17、pdx1、ngn3、pax4、insulin和glut-2在诱导组和非诱导组中的表达水平;免疫荧光染色方法检测胰岛素和C-肽在诱导终末阶段细胞中的表达定位;酶联免疫吸附实验(ELISA)检测胰岛素及C-肽的分泌以及细胞对葡萄糖刺激的反应性。结果 诱导第1阶段末,诱导后细胞foxa2和sox17的表达明显高于未诱导细胞( $P < 0.05$ );诱导第2阶段末,诱导细胞pdx1、ngn3和pax4的表达明显高于未诱导细胞( $P < 0.05$ );诱导第3阶段末,诱导细胞的insulin和glut-2表达明显高于未诱导细胞( $P < 0.05$ )。免疫荧光染色结果显示,胰岛素和C-肽均表达于诱导终末分化阶段的细胞,效率可达90%以上。ELISA检测结果显示,诱导第3阶段末细胞胰岛素总量为(346.3-739±32.5-149)μU/ml,明显高于未诱导细胞的(17.69±1.46)μU/ml( $P < 0.01$ );诱导后细胞置于5.5-mmol/L葡萄糖环境检测到的基础C-肽释放量为(195.10±8.88)pmol/L/h( $P < 0.01$ ),细胞置于22-mmol/L葡萄糖环境测定葡萄糖刺激C-肽释放量达到(340.99±7.91)pmol/L/h( $P < 0.01$ )。结论 以UC-MSC作为种子细胞,采用体外非病毒诱导体系获得的分化终末阶段细胞是具有胰岛素分泌功能的成熟胰岛细胞。

**关键词:** 脐带 间充质干细胞 胰岛细胞 转录因子

**Abstract:** Objective To explore the feasibility of using a virus-free system in the induction of umbilical cord derived mesenchymal stem cells (UC-MSCs) into insulin-secreting cells. Methods MSCs were isolated from human umbilical cord and induced into insulin-secreting cells with a three-stage method. The mRNA expression levels of foxa2, sox17, pdx1, ngn3, pax4, insulin, and glut-2 were compared between induced and non-induced groups by RT-PCR in each stage. The distribution pattern of insulin and c-peptide were detected by immunofluorescence staining and observed by fluorescence microscopy. Insulin and c-peptide secretion and glucose responsiveness were detected by enzyme-linked immunosorbent assay (ELISA). Results Transcription factors foxa2, sox17, pdx1, ngn3, pax4, insulin, and glut-2 were expressed in the induced cells. The mRNA expression levels of foxa2 and sox17 were significantly higher in the induced group than those in non-induced group in the first stage (all  $P < 0.05$ ), pdx1, ngn3, and pax4 were significantly higher in the induced cells than those in non-induced cells in the second stage (all  $P < 0.05$ ), and insulin and glut-2 expressions were significantly up-regulated in the induced group at last stage (all  $P < 0.05$ ). Immunofluorescence staining showed that insulin and c-peptide were located in the cytoplasm of more than 90% of induced cells. ELISA showed that total intracellular insulin content of the induced cells contained up to (346.3-739±32.5-149)μU/ml, which was significantly higher than insulin in non-induced cells (17.69±1.46)μU/ml ( $P < 0.01$ ). C-peptide content of the induced cells measured up to (195.10±8.88)pmol/L/h ( $P < 0.01$ ), when exposed to 5.5 mmol/L glucose ( $P < 0.01$ ). When stimulated with 22 mmol/L glucose, the c-peptide content of the induced cells increased to (340.99±7.91)pmol/L/h ( $P < 0.01$ ). Conclusion The umbilical cord derived MSCs can be efficiently induced into insulin-secreting cells via a virus-free system.

**Keywords:** umbilical cord mesenchymal stem cell insulin transcription factor

Received 2011-11-11;

Fund:

国家重大科学研究计划项目(2011CB964901)

Corresponding Authors: 赵春华 Email: zhaoch16@hotmail.com

引用本文:

### Service

- ▶ 把本文推荐给朋友
- ▶ 加入我的书架
- ▶ 加入引用管理器
- ▶ Email Alert
- ▶ RSS

### 作者相关文章

- ▶ 李晶
- ▶ 朱莉
- ▶ 赵春华

LI Jing , ZHU Li, ZHAO Chun-hua.Virus Free Induction of Umbilical Cord Derived Mesenchymal Stem Cells into Islet-like Cells[J] CAMS, 2011,V33(6): 675-678

链接本文:

[http://www.actacams.com/Jwk\\_yxkxy/CN/10.3881/j.issn.1000-503X.2011.06.018](http://www.actacams.com/Jwk_yxkxy/CN/10.3881/j.issn.1000-503X.2011.06.018) 或

[http://www.actacams.com/Jwk\\_yxkxy/CN/Y2011/V33/I6/675](http://www.actacams.com/Jwk_yxkxy/CN/Y2011/V33/I6/675)