遗传 » 2011, Vol. 33 » Issue (11) :1231-1238 DOI: 10.3724/SP.J.1005.2011.01231

小鼠孤雌胚胎干细胞的建立及其向运动神经元分化的初探

王振东[1,2], 薛媛[1], 单智焱[1], 郑重[1], 李雪[1], 吴嫣爽[1], 孙瑞珍[1], 石健[1], 李明杰[1], 刘忠华[2], 雷蕾[1]

1. 哈尔滨医科大学组织学与胚胎学教研室, 哈尔滨 150081 2. 东北农业大学生命科学学院, 哈尔滨 150030

WANG Zhen-Dong[1,2], XUE Yuan[1], SHAN Zhi-Yan[1], ZHENG Zhong[1], LI Xue[1], WU Yan-Shuang[1], SUN Rui-Zhen[1], SHI Jian[1], LI Ming-Jie[1], LIU Zhong-Hua[2], LEI Lei[1]

1. *Department of Histology and Embryology, Harbin Medical University, Harbin 150081, China* 2. *College of Life Science, Northeast Agricultural University, Harbin 150030, China*

- 摘要
- 参考文献
- 相关文章

Download: PDF (520KB) HTML (1KB) Export: BibTeX or EndNote (RIS) Supplementary data

摘要 文章采用小鼠的孤雌囊胚建立胚胎干细胞系，探究其向运动神经元分化的可能，为临床治疗以及研究基因组印记与神经分化的的关系提供理论基础。结果表明：卵母细胞孤雌激活率达到93.26%，成功建立了8个孤雌胚胎干细胞系，建系率达到23.53%。克隆表达多潜能标记Oct4及细胞表面标记SSEA-1，有高水平的碱性磷酸酶活性，在细胞第10代和第30代时核型分析检测显示为正常的40条染色体。体内、外均分化出三胚层来源的细胞。联合应用全反式维甲酸(RA)、音猬因子(Shh)及细胞外基质，小鼠孤雌胚胎干细胞可被诱导表达运动神经元的标志性标记HB9、Olig2。

关键词: 孤雌胚胎干细胞 印记基因 运动神经元 分化 维甲酸 音猬因子

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
- 王振东
- 单智焱
- 雷蕾
- 薛媛
- 郑重
- 刘忠华
- 孙瑞珍
- 李雪
- 吴嫣爽
- 石健
- 李明杰

Abstract: In this study, we generated embryonic stem cells from parthenogenetic embryos (PESCs), and induced them to differentiate to motor neurons, which could be an alternative source of histocompatible cells for replacement of therapy and theoretical foundation for studying the relationship of genome imprint and neural differentiation. The parthenogenetic activation rate of B6D2F1 mouse oocytes was 93.26%. We established eight parthenogenetic embryonic stem cell lines and the establishment rate from parthenogenetic embryos was 23.53%. The pluripotency marker Oct4, the cell surface marker SSEA-1, and alkaline phosphatase exhibited in PESCs. Karyotype analysis showed normal 40 chromosomes when examined at passages 10 and 30, which was in accordance with their oocyte origins. Three germinal layers were differentiated *in vivo* and *in vitro*. Mouse PESCs, which were treated by tretinoin and sonic hedgehog with extracellular matrix, could generate motor neurons that expressed the specific markers such as HB9 and Olig2.

Keywords: parthenogenetic embryonic stem cells, imprinted genes, motor neurons, differentiation, Tretinoin (RA), Sonic hedgehog (Shh)

收稿日期: 2011-01-25; 出版日期: 2011-11-25

基金资助:

国家自然科学基金项目(编号：30871431, 31000645)资助

通讯作者 雷蕾 Email: leil086@yahoo.com.cn

引用本文:

王振东，薛媛，单智焱，郑重，李雪，吴嫣爽，孙瑞珍，石健，李明杰，刘忠华，雷蕾. 小鼠孤雌胚胎干细胞的建立及其向运动神经元分化的初探. 遗传, 2011, 33(11): 1231-1238.

WANG Zhen-Dong, XUE Yuan, SHAN Zhi-Yan, ZHENG Zhong, LI Xue, WU Yan-Shuang, SUN Rui-Zhen, SHI Jian, LEI Lei. Generation of mouse parthenogenetic embryonic stem cells and preliminary study of the differentiation ability to motor neurons. HEREDITAS, 2011,V33(11): 1231-1238.

链接本文:

http://www.chinagene.cn/Jwk_yc/CN/10.3724/SP.J.1005.2011.01231 或 http://www.chinagene.cn/Jwk_yc/CN/Y2011/V33/I11/1231

[1] Brevini TAL, Pennarossa G, Antonini S, Gandolfi F. Parthenogenesis as an approach to pluripotency: advantages and limitations involved. *Stem Cell Rev*, 2008, 4(3): 127-135.

[2] Dighe V, Clepper L, Pedersen D, Byrne J, Ferguson B, Gokhale S, Penedo MCT, Wolf D, Mitalipov S. Heterozygous embryonic stem cell lines derived from nonhuman primate parthenotes. *Stem Cells*, 2008, 26(3): 756-766.

[3] Alexander B, Coppola G, Di Berardino D, Rho GJ, St John E, Betts DH, King WA. The effect of 6-dimethylaminopurine (6-DMAP) and

cycloheximide (CHX) on the development and chromosomal complement of sheep parthenogenetic and nuclear transfer embryos. *Mol Reprod Dev*, 2006, 73 (1): 20-30.

[4] Wada T, Honda M, Minami I, Tooi N, Amagai Y, Nakatsuji N, Aiba K. Highly efficient differentiation and enrichment of spinal motor neurons derived from human and monkey embryonic stem cells. *PLoS One*, 2009, 4(8): e6722.

[5] Yi YJ, Park CS. Parthenogenetic development of porcine oocytes treated by ethanol, cycloheximide, cytochalasin B and 6-dimethylaminopurine. *Anim Reprod Sci*, 2005, 86(3-4): 297-304.

[6] Jessell TM. Neuronal specification in the spinal cord: in-ductive signals and transcriptional codes. *Nat Rev Genet*, 2000, 1(1): 20-29.

[7] Wilson SI, Rydström A, Trimborn T, Willert K, Nusse R, Jessell TM, Edlund T. The status of Wnt signalling regulates neural and epidermal fates in the chick embryo. *Nature*, 2001, 411 (6835): 325-330.

[8] Muhr J, Graziano E, Wilson S, Jessell TM, Edlund T. Convergent inductive signals specify midbrain, hindbrain, and spinal cord identity in gastrula stage chick embryos. *Neuron*, 1999, 23(4): 689-702.

[9] Briscoe J, Ericson J. Specification of neuronal fates in the ventral neural tube. *Curr Opin Neurobiol*, 2001, 11(1): 43-49.

[10] Qian HR, Yang Y. Neuron differentiation and neuritogenesis stimulated by N-acetylcysteine (NAC). *Acta Pharmacol Sin*, 2009, 30(7): 907-912.

[11] Kawasaki H, Mizuseki K, Nishikawa S, Kaneko S, Kuwana Y, Nakanishi S, Nishikawa SI, Sasai Y. Induction of midbrain dopaminergic neurons from ES cells by stromal cell-derived inducing activity. *Neuron*, 2000, 28(1): 31-40.

[12] Anjomshoa M, Karbalaie K, Mardani M, Razavi S, Tanhaei S, Nasr-Esfahani MH, Baharvand H. Generation of motor neurons by coculture of retinoic acid-pretreated embryonic stem cells with chicken notochords. *Stem Cells Dev*, 2009, 18(2): 259-267.

[13] Shan ZY, Liu F, Lei L, Li QM, Jin LH, Wu YS, Li X, Shen JL. Generation of dorsal spinal cord GABAergic neurons from mouse embryonic stem cells. *Cell Reprogram*, 2011, 13(1): 85- 91.

[14] Nayak MS, Kim YS, Goldman M, Keirstead HS, Kerr DA. Cellular therapies in motor neuron diseases. *Biochim Biophys Acta*, 2006, 1762(11-12): 1128-1138.

[15] Fois AF, Wotton CJ, Yeates D, Turner MR, Goldacre MJ. Cancer in patients with motor neuron disease, multiple sclerosis and Parkinson's disease: record linkage studies. *J Neurol Neurosurg Psychiatry*, 2010, 81(2): 215-221.

[16] Karumbayaram S, Novitch BG, Patterson M, Umbach JA, Richter L, Lindgren A, Conway AE, Clark AT, Goldman SA, Plath K, Wiedau-Pazos M, Kornblum HI, Lowry WE. Directed differentiation of human-induced pluripotent stem cells generates active motor neurons. *Stem Cells*, 2009, 27 (4): 806-811.

[17] Rideout WM 3rd, Hochedlinger K, Kyba M, Daley GQ, Jaenisch R. Correction of a genetic defect by nuclear transplantation and combined cell and gene therapy. *Cell*, 2002, 109(1): 17-27.

[18] 孟国良, 滕路, 薛友纺, 尚克刚. BALB/c小鼠胚胎干细胞系建立的方法学探讨. 遗传学报, 2002, 29 (7): 581-588. 浏览

[19] Cibelli JB, Grant KA, Chapman KB, Cunniff K, Worst T, Green HL, Walker SJ, Gutin PH, Vilner L, Tabar V, Dominko T, Kane J, Wettstein PJ, Lanza RP, Studer L, Vrana KE, West MD. Parthenogenetic stem cells in non-human primates. *Science*, 2002, 295(5556): 819.

[20] Do JT, Joo JY, Han DW, Araúzo-Bravo MJ, Kim MJ, Gre-ber B, Zaehres H, Sobek-Klocke I, Chung HM, Schöler HR. Generation of parthenogenetic induced pluripotent stem cells from parthenogenetic neural stem cells. *Stem Cells*, 2009, 27(12): 2962-2968.

[21] Hu BY, Zhang SC. Differentiation of spinal motor neurons from pluripotent human stem cells. *Nat Protoc*, 2009, 4(9): 1295-1304.

[22] Vrana KE, Hipp JD, Goss AM, McCool BA, Riddle DR, Walker SJ, Wettstein PJ, Studer LP, Tabar V, Cunniff K, Chapman K, Vilner L, West MD, Grant KA, Cibelli JB. Nonhuman primate parthenogenetic stem cells. *Proc Natl Acad Sci USA*, 2003, 100(Suppl. 1): 11911-11916.

[23] Hikichi T, Wakayama S, Mizutani E, Takashima Y, Kishi-gami S, van Thuan N, Ohta H, Thuy Bui H, Nishikawa SI, Wakayama T. Differentiation potential of parthenogenetic embryonic stem cells is improved by nuclear transfer. *Stem Cells*, 2007, 25(1): 46-53.

[24] Peljto M, Dasen JS, Mazzoni EO, Jessell TM, Wichterle H. Functional diversity of ESC- derived motor neuron sub-types revealed through intraspinal transplantation. *Cell Stem Cell*, 2010, 7(3): 355-366.

[25] Cibelli JB, Cunniff K, Vrana KE. Embryonic stem cells from parthenotes. *Methods Enzymol*, 2006, 418: 117-135.

[26] Feinberg AP, Tycko B. The history of cancer epigenetics. *Nat Rev Cancer*, 2004, 4 (2): 143-153.

[27] Andrieu D, Watrin F, Niinobe M, Yoshikawa K, Muscatelli F, Fernandez PA. Expression of the Prader-Willi gene Necdin during mouse nervous system development corre-lates with neuronal differentiation and p75NTR expression. *Gene Expr Patterns*, 2003, 3(6): 761-765.

[28] Schumacher A. Doerfler W. Influence of in vitro manipu-lation on the stability of methylation patterns in the Snurf/ Snrpn-imprinting region in mouse embryonic stem cells. *Nucleic Acids Res*, 2004, 32(4): 1566-1576.

[29] Li C, Chen ZS, Liu Z, Huang JJ, Zhang W, Zhou LJ, Keefe DL, Liu L. Correlation of expression and methylation of imprinted genes with pluripotency of parthenogenetic em-bryonic stem cells. *Hum Mol Genet*, 2009, 18(12): 2177-2187.

[1] 官玲亮 侯凯 陈俊雯 徐应文 吴卫.**ω**-6和**ω**-3脂肪酸脱氢酶家族系统进化与功能分化[J]. 遗传, 2013,35(5): 643-654

[2] 徐冉冉 张从伟 曹羽 王强.缺失*mir122*抑制斑马鱼肝脏前体细胞向肝细胞分化[J]. 遗传, 2013,35(4): 488-494

[3] 佟静媛，柳星峰，贾顺姬.Rbb4l促进TGF-**β**/Nodal信号转导和斑马鱼胚胎的背部发育[J]. 遗传, 2013,35(4): 477-487

[4] 牧仁，边艳超，浦亚彬，李向臣，王凤龙，关伟军.北京油鸡胚胎肝脏来源间充质干细胞的分离培养及生物学特性[J]. 遗传, 2013,35(3): 365-372

[5] 谢翔，胡建军，王贵学.生物力学——胚胎血管系统发育研究新视野[J]. 遗传, 2012,34(9): 1123-1132

[6] 杨秀荣，蒋和生，杨宁.鸟类性别决定与性别分化机制[J]. 遗传, 2012,34(4): 407-411

[7] 王丽，那威，王宇祥，王彦博，王宁，王启贵，李玉茂，李辉.鸡*PPAR*$\boldsymbol{\gamma}$基因的表达特性及其对脂肪细胞增殖分化的影响[J]. 遗传, 2012,34(4): 454-464

[8] 李秀领，杨松柏，唐中林，李奎，刘榜，樊斌.大白猪和通城猪全基因组选择性清扫分析[J]. 遗传, 2012,34(10): 1271-1281

[9] 张莹，胡丽玲，谢伟，孙蓓，左爱军，张镜宇.重组人维甲酸X受体**α**的克隆与表达及其与甲状腺受体的结合[J]. 遗传, 2011,33(3): 246-250

[10] 周志军，张艳霞，常岩林，杨明茹.暗褐蝈螽不同地理种群间的遗传分化[J]. 遗传, 2011,33(1): 75-80

[11] 高娇,新龙，李韧，刘毅，贺文艳，孙圣坤，张毓，刘兵，熊加祥，毛宁.OP9的间充质干细胞特性[J]. 遗传, 2010,37(7): 475-482

[12] 李玥莹，何静，王凤超，鞠振宇，刘胜，张郁，寇朝辉，刘延风，程涛，高绍荣.胚胎干细胞在成体骨髓内的分化潜能[J]. 遗传, 2010,37(7): 431-439

[13] 张红宇，徐培洲，杨华，吴先军.拟南芥的印记基因和印记表达调控[J]. 遗传, 2010,32(7): 670-676

[14] 刘绍平，王珂，袁希平，汪登强，岳兴建，陈大庆.怒江扎那纹胸鮡的遗传多样性和遗传分化[J]. 遗传, 2010,32(3): 254-263

[15] 王丽娟，王友亮，杨晓.表皮干细胞研究进展[J]. 遗传, 2010,32(3): 198-204

Copyright 2010 by 遗传