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论著

人成纤维细胞饲养层的制备及其对胚胎干细胞生长支持作用

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摘要

目的 为建立不含动物细胞成分的人胚胎干细胞(human embryonic stem cell, hESc)体外培养系统，本实验欲用人包皮成纤维细胞(human postnatal foreskin fibroblasts, hPFF)制备细胞饲养层并观察其对hESc体外生长的支持功能及生物学特性的维持作用。方法 利用儿童阴茎包皮环切术后遗弃包皮组织分离培养成纤维细胞，纯化增殖细胞，采用35Gyγ射线照射灭活法制备成饲养层。将hESc（HS181细胞株）接种在该饲养层上，连续传代至20代，倒置显微镜观察其形态学变化，测定其细胞碱性磷酸酶活性，类胚体形成实验及干细胞转录因子表达，严重联合免疫缺陷的小鼠(severe combined immune deficiency mice, SCID)体内畸胎瘤形成及体内全能分化特性实验对hESc生物学特性进行鉴定。 结果 hPFF体外培养过程中生长增殖旺盛，连续传20代以上能保持正常细胞形态学和生物学特性。经γ射线照射使其停止增殖，24h内能较好地保持细胞活力和形态学特征，具备饲养层细胞基本条件。HS181 hESc在hPFF上传到20代任能较好地保持未分化状态，细胞呈克隆性生长，高度表达碱性磷酸酶及Oct-4、Nanog等胚胎干细胞转录因子；悬浮法培养连续传20代的hESc可获得由3个胚层细胞所形成的类胚体（Embryoid bodies, EB）结构, 可检测到CD90、Flt-1、Nestin基因的表达，证明得到的类胚体中含了3个胚层细胞，说明hESc具有体外多能干性特征。体内全能分化实验显示：将第20代的hESc接种到SCID小鼠体内,6周后可形成畸胎瘤,组织学切片分析其包含了源于全部3个胚层的各种分化细胞,直接证明其具有体内多向分化的全能性。结论 hPFF可作为一种来源丰富，取材方便的人源化饲养层细胞，能有效地支持hESc体外生长。克服了目前hESc建系和体外培养过程中使用动物细胞饲养层带来的异种蛋白污染和致病微生物的危险，初步解决了hESc临床应用的生物安全性问题。

关键词 胚胎干细胞；成纤维细胞；饲养层；人类

分类号

Human postnatal foreskin fibroblasts as feeder cells to support human embryonic stem cell growth

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Abstract

Objective To investigate whether human fibroblasts cells as feeder layers were capable of supporting the growth of human embryonic stem cells in vitro. We now describe the hESc lines (HS181) that had been derived from the beginning using human postnatal foreskin fibroblasts (hPFF) as feeder cells, serum replacement (SR) medium and continued undifferentiated growth. And then we characterize hESc through their biological characteristics. Methods Foreskins were obtained from the postnatal babies after circumcision and were donated by their parents. After the primary cell culture with enzymatic digestion method and purification, we got the human postnatal foreskin fibroblasts cell lines. Cellular morphologies were observed under inverted phase contrast microscopy with hematoxylin-eosin staining. The cultured cells were identified by vimentin immunofluorescence cell staining, and they were used as feeder cells to culture hESc. The culture medium applied to culture of the hESc (HS181) consisted of Knockout Dulbecco's modified Eagle's medium (Knockout DMEM) supplemented with SR medium and basic fibroblast growth factor (bFGF). After exponentially growing HS181 cells from passage 7 to passage 20 were identified hESc through their biological characteristics. The lines expressed markers of pluripotent hESCs (alkaline phosphatase, Oct-4 and Nanog). The pluripotency has been shown in embryoid bodies in vitro, and the pluripotency of line181 has also been shown in vivo by teratoma formation in severe combined immunodeficiency/ beige mice. Results By morphological and vimentin immunocytochemical staining identification, we got the stable hPFF cell lines. The coactions of SR with bFGF culture medium and hPFF as feeder cells play an

important role in proliferation and undifferentiation of hESc in vitro. To identify growth situation of the Passage 20th HS181 cell line from morphological observation,its pluripotency and undifferentiation in vitro has been analyzed, and alkaline posphatase (AKP)，the markers Oct-4 and Nanog ,was expressed. The embryoid bodies formed with the suspending method from lines HS181 expressed CD90, Flt-1 and Nestin as constituents of the three germ cell layers by using reverse transcription–polymerase chain reaction (RT-PCR). This means that the Passage 20th hESc still kept pluripotency in vitro. Pluripotency in vivo of the Passage 20th HS181 cell line was shown by teratoma formation in severe combined immune deficiency (SCID) mice. Conclusion The culture of hESc in SR with bFGF medium and on hPFF feeder cells as an alternative is one of optimal culture systems. The culture system that we applied can keep hESc undifferentiated after more than 20 passages propagation. This is a step toward xeno-free conditions and facilitates the usage of these hESc in transplantation.
Key words Embryonic stem cell foreskin fibroblast feeder cells human

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