

论著·基础研究

doi:10.3969/j.issn.1671-8348.2020.17.020

网络首发 [https://kns.cnki.net/kcms/detail/50.1097.R.20200629.1041.018.html\(2020-06-29\)](https://kns.cnki.net/kcms/detail/50.1097.R.20200629.1041.018.html(2020-06-29))

小鼠毛囊干细胞向心肌细胞的诱导分化及其鉴定*

席海燕¹,丁海麦¹,李晓晶¹,王步云¹,周蕾²

(包头医学院基础医学与法医学院;1.生物化学与分子生物学教研室;

2.组织胚胎教研室,内蒙古包头 014060)

[摘要] **目的** 探究小鼠毛囊隆突部分离培养的毛囊干细胞,诱导分化成为心肌细胞的能力。**方法** 显微镜下剥离结构完整的小鼠毛囊,只保留隆突部,Ⅳ型胶原黏附法分离、培养隆突部细胞,对其进行形态学和分子免疫荧光检测;第二代细胞的单细胞克隆通过有限稀释法纯化,并筛选 K15、K19 强阳性的细胞;加入心肌细胞诱导液进行诱导分化并做心肌细胞标志物肌红蛋白的免疫荧光鉴定。**结果** 小鼠毛囊隆突部干细胞经成心肌诱导后,细胞呈现典型的心肌细胞样形态,K15、K19 呈阳性;诱导后的细胞能特异表达心肌细胞的标志物肌红蛋白。**结论** 小鼠毛囊干细胞有分化成为心肌细胞的能力。

[关键词] 干细胞;毛囊;隆突部;细胞分化;肌细胞,心脏**[中图法分类号]** R310.11 **[文献标识码]** A **[文章编号]** 1671-8348(2020)17-2867-03

Induction and differentiation of mouse hair follicle stem cells into cardiomyocytes*

XI Haiyan¹,DING Haimai¹,LI Xiaojing¹,WANG Buyun¹,ZHOU Lei²

(1. Department of Biochemistry and Molecular Biology;2. Department of Tissue Embryology,

School of Basic Medicine and Forensic Science, Baotou Medical College, Baotou, Inner Mongolia 014060, China)

[Abstract] **Objective** To investigate the ability of hair follicle stem cells isolated and cultured from mouse hair follicle bulge to induce and differentiate into cardiomyocytes. **Methods** The dissected hair follicles with complete structure were peeled off under the microscope, and only the bulge was retained. Cells of the bulge were isolated and cultured by the type Ⅳ collagen adhesion method, and their morphology were observed and molecular immunofluorescence were done. The single cell clone of the second generation cells was pontificated by limiting dilution method, K15 and K19 strong positive cells were screened. Cardiomyocyte induction solution was added into the culture medium to induce and differentiation, and immunofluorescence identification was done to indentify mygotobin which was the marker of cardiomyocytes. **Results** The stem cells of the hair follicle of the mice were induced into cardiomyocytes, and the cells showed typical cardiomyocyte-like morphology with positive expression of K15 and K19. Myoglobin, the marker of cardiomyocyte, were specifically expressed after induction. **Conclusion** Mouse hair follicle stem cells have the ability to differentiate into cardiomyocytes.

[Key words] stem cells; hair follicle; bulge; cell differentiation; myocytes, cardiac

毛囊干细胞(hair follicle stem cells, HFSCs)是一类位于毛囊隆突部(bulge)的成体干细胞,表现为 BrdU 或 3 H-TdR 标记滞留,对基底膜有快速黏附的特性^[1]。HFSCs 在毛囊的形成及其生长周期中起着非常重要的作用^[2]。HFSCs 的增殖分化与其周围的微环境密不可分,这个特殊的影响干细胞增殖分化的微环境被称为壁龛(niche)^[3]。HFSCs 具有多向分化潜能,不仅能分化成各种上皮组织^[4],还能被诱导分化形成脂肪细胞^[5]、神经细胞^[6]等其他类型细胞。本实验以模式生物小鼠为研究对象,体外分离、培养其

隆突部的 HFSCs,诱导分化成心肌细胞并对诱导细胞进行初步鉴定,旨在探讨小鼠 HFSCs 多分化的潜能,也为心肌细胞再生提供更多的思路。

1 材料与方法

1.1 动物及主要试剂

昆明小鼠,4 周龄,体重 60 g 左右,由内蒙古大学实验动物中心提供。DMEM/F12(货号 12500-039)、F12(货号 21700-026)、DMEM(货号 12800-017)购自美国 Gibco 公司;分离酶 dispase Ⅱ(货号 SCM133)、Ⅳ型胶原(货号 C5533)、胰酶(货号 P3292)、多聚甲醛

* 基金项目:内蒙古自然科学基金项目(2011BS1111);包头医学院博士科研启动基金项目(BSJ201628)。 作者简介:席海燕(1975—),副教授,博士,主要从事皮肤干细胞生物学相关研究。

(货号 P6148)、Triton-X100(货号 P9284)、L-谷氨酰胺(货号 G8540)、5-氮胞苷(货号 A2385)、胰岛素(货号 I0516)、青霉素(货号 P7794)、链霉素(货号 S1277)、氢化可的松(货号 H0888)购自美国 Sigma 公司;一抗 K15 和 K19(鼠抗单克隆抗体)购自美国 Lab Vision 公司;二抗兔抗鼠 IgG(RPE)购自英国 Serotec 公司;胎牛血清(FBS,货号 TBD0032HYP)购自上海 TBD 公司;肌红蛋白鼠抗单克隆抗体购自美国 ZMED 公司。

1.2 主要仪器

显微镜购自日本 Olympus 公司;细胞培养箱购自德国 Binder 公司;荧光显微镜购自德国 Zeiss 公司;激光共聚焦显微镜购自美国 Bio-Red 公司;细胞培养皿购自美国 Falcon 公司;细胞培养板购自美国 Corning 公司。

1.3 方法

1.3.1 HFSCs 的分离、培养和纯化

脊椎脱臼法处死小鼠,用 75%乙醇消毒,0.01 mol/L PBS 洗涤 3 次。取小鼠触须,用 0.01 mol/L PBS 溶液冲洗干净后,在显微镜下剥离完整的带有结缔组织鞘的毛囊,放在 PBS 溶液中,去除皮脂腺及毛囊下部,将毛囊的毛囊隆突部直接植入涂有 IV 型胶原的培养板中,在细胞培养箱(培养条件:37℃、5% CO₂、空气饱和湿度)中放置 15 min,将悬浮细胞去掉,添加 HFSCs 培养液继续培养。每天在显微镜下观察细胞的形态及增殖情况,2 d 更换 1 次培养基。当细胞长至 80%~90%时,用 IV 型胶原快速黏附法进行传代培养^[7]。HFSCs 的纯化采用有限稀释法:用 0.25%胰酶消化 IV 型胶原黏附后的第二代细胞,制成单细胞悬液;台盼蓝染色并计数,制备成 5×10^3 /mL 的细胞悬液;依次稀释 100 倍、5 倍和 2 倍,制成浓度为 50/mL、10/mL 和 5/mL 的细胞悬液,分别接种于 IV 型胶原处理过的培养板中进行培养。次日镜检,对只含有 1 个细胞的培养孔继续培养,待克隆长至 1/3~1/2 底面积时接种于培养板中继续扩大培养^[7]。

1.3.2 免疫荧光染色鉴定 HFSCs

将纯化的细胞悬液接种于盖玻片上培养 2 d,用 PBS 洗涤后用 4%多聚甲醛中固定 0.5 h, Triton-X100 透化 5 min,分别加入 HFSCs 标志物 K15、K19 一抗孵育 1 h, PBS 冲洗后加入 IgG 二抗,遮光染色 0.5 h, PBS 冲洗后激光共聚焦显微镜检测。

1.3.3 HFSCs 定向诱导分化为心肌细胞

(1)诱导,在培养皿中接种第二代生长良好的纯化 HFSCs,当 HFSCs 生长至 50%后添加心肌细胞的预诱导液(2 mmol/L 5-氮胞苷+DMEM-F12+10% FBS)。诱导 2 d 后弃掉预诱导液,用 DMEM-F12 培养液清洗培养皿,再加入诱导培养基继续培养,2 d 更换 1 次培养液。心肌细胞条件培养基制备:取小鼠心脏,除去周围的脂肪等杂质,剪成尽量小的碎块,移入

培养皿,加入 2 000 U/mL 的胶原酶和无血清培养液,37℃培养过夜。次日,吹打组织使之分散,200 目滤网过滤,制成单细胞悬液,离心去上清液。悬浮细胞接种培养(培养基:DMEM-F12+10% FBS+293.2 mg/L L-谷氨酰胺+5 μg/mL 胰岛素+100 IU/mL 青霉素+100 μg/mL 链霉素),2 d 更换 1 次培养液。细胞生长至约 80%融合度时传代培养,收集第三代培养液,离心,0.22 μm 滤膜过滤,分装于-20℃保存备用。诱导培养基:DMEM-F12+5 μg/mL 胰岛素+1 μg/mL 氢化可的松+10% FBS+20%条件培养基。(2)鉴定,用特异的心肌细胞标志物肌红蛋白行细胞免疫荧光染色鉴定 HFSCs 诱导分化情况。

2 结果

2.1 HFSCs 的分离和纯化

IV 型胶原黏附 10 min 的细胞,2 d 左右贴壁,镜下可以观察到明显的铺路石状克隆,符合 HFSCs 的特征,见图 1。

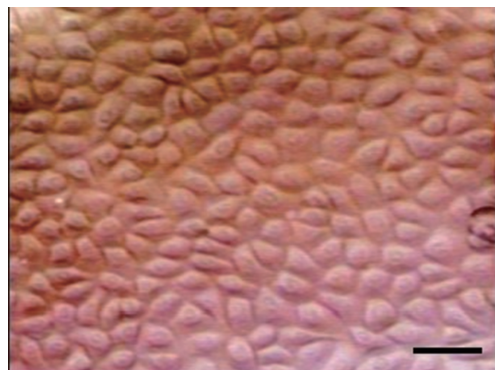
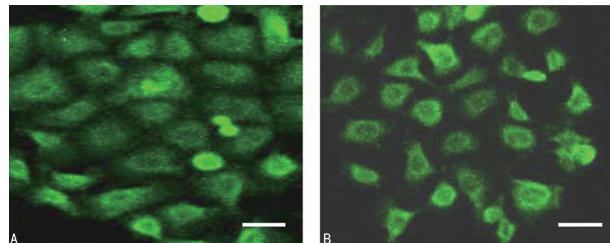


图 1 HFSCs 的铺路石状克隆(普通光学显微镜×150)

2.2 HFSCs 的 K15、K19 免疫荧光鉴定

K15、K19 细胞免疫荧光染色结果表明,二者均阳性表达于细胞质中,说明培养的细胞就是 HFSCs,见图 2。



A:K15;B:K19。

图 2 HFSCs 的免疫荧光染色鉴定(荧光显微镜×150)

2.3 HFSCs 向心肌细胞定向诱导分化

用胶原酶过夜消化为单细胞悬液,这些细胞生长旺盛且能有规律的收缩,加入 5-氮胞苷 7 d 左右,部分贴壁的多角形细胞开始出现轻微拉长现象。2 周后,细胞拉伸变长,细胞呈现形成长梭状、棒球状和椭圆状等。3 周后形成心肌细胞样典型的梭形纤维样结构,见图 3。

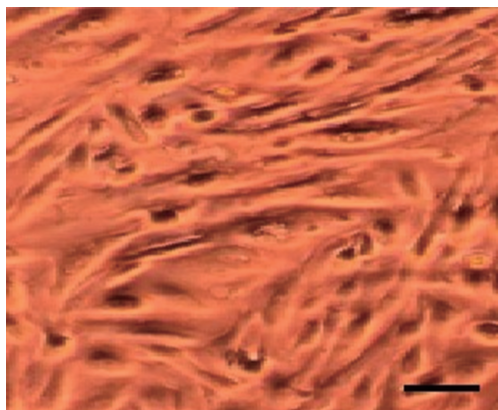


图3 HFSCs 诱导 3 周后生成的心肌样细胞
(普通光学显微镜×150)

2.4 心肌细胞的鉴定

免疫荧光染色检测诱导 2 周的细胞,标志物肌红蛋白表达于诱导细胞的细胞质中,说明 HFSCs 能够分化成心肌细胞,见图 4。

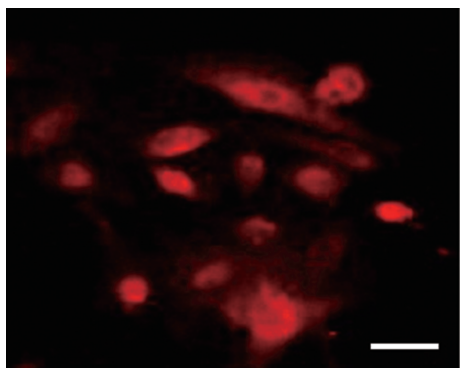


图4 HFSCs 诱导心肌细胞的免疫荧光染色
鉴定(荧光显微镜×200)

3 讨论

HFSCs 是定位于毛囊隆突部的具有多向分化潜能、来源广泛的一类成体干细胞,具有表皮干细胞的显著特征:高度的增殖潜能、慢周期性及快速黏附基底膜,可以用Ⅳ型胶原黏附法在体外分离培养,并表现出多向分化潜能^[8]。HFSCs 在体外不仅可诱导分化成角质化细胞、汗腺细胞及皮脂腺细胞等^[9],还能诱导分化形成神经细胞^[5]、尿路上皮细胞^[10]、血管内皮细胞^[11-12]、成骨细胞^[13]等跨胚层的细胞。本实验的特色在于,没有利用常规的“贴壁法”和“酶解法”直接分离 HFSCs;而是先在显微镜下分离得到毛囊隆突部,再用Ⅳ型胶原黏附分离 HFSCs,并用有限稀释法对其纯化,得到了较为理想的细胞系,细胞呈现典型的铺路石样的形态,同时细胞质 K15 和 K19 呈阳性表达,表明分离培养的细胞具备 HFSCs 的性质^[14-15]。培养液中添加 5-氮胞苷定向诱导形成心肌细胞样结构^[16],免疫荧光鉴定诱导后的细胞表达心肌细胞的标志物肌红蛋白^[17],说明 HFSCs 能够分化成心肌细胞。总之, HFSCs 作为再生医学的一种较为理想的干细胞源,其应用会越来越广泛。

参考文献

- [1] GONZALES K A U, FUCHS E. Skin and its regenerative powers: an alliance between stem cells and their niche[J]. *Dev Cell*, 2017, 43(4): 387-401.
- [2] LEI M, CHUONG C M. Aging, alopecia and stem cells[J]. *Science*, 2016, 351(6273): 559-560.
- [3] HSU Y C, LI L, FUCHS E. Emerging interactions between skin stem cells and their niches[J]. *Nature medicine*, 2014, 20(8): 847-856.
- [4] ABBASI S, BIERNASKIE J. Injury modifies the fate of hair follicle dermal stem cell progeny in a hair cycle-dependent manner[J]. *Exp Dermatol*, 2019, 28(4): 419-424.
- [5] DREWA T, JOACHIMIAK R, BAJEK A, et al. Hair follicle stem cells can be driven into a urothelial-like phenotype: An experimental study[J]. *Int J Urol*, 2013, 20(5): 537-542.
- [6] LIU F, LIN H, ZHANG C. Construction of tissue-engineered nerve conduits seeded with neurons derived from hair-follicle neural crest stem cells[J]. *Methods Mol Biol*, 2016, 1453: 33-38.
- [7] 王瑶. 毛囊干细胞的多分化潜能及其向汗腺细胞分化的实验研究[D]. 天津:南开大学, 2013.
- [8] QIU W, LEI M, ZHOU L, et al. Hair follicle stem cell proliferation, Akt and Wnt signaling activation in TPA-induced hair regeneration[J]. *Histochem Cell Biol*, 2017, 147(6): 749-58.
- [9] BABAKHANI A, HASHEMI P, MOHAJER ANSARI J, et al. In vitro differentiation of hair follicle stem cell into keratinocyte by simvastatin[J]. *Iran Biomed J*, 2019, 23(6): 404-411.
- [10] YANG R, XU X. Isolation and culture of neural crest stem cells from human hair follicles[J]. *Methods Mol Biol*, 2016, 1453: 49-55.
- [11] QUAN R F, DU W B, ZHENG X, et al. VEGF165 induces differentiation of hair follicle stem cells into endothelial cells and plays a role in vivo angiogenesis[J]. *J Cell Mol Med*, 2017, 21(8): 1593-1604.
- [12] 杜伟斌, 全仁夫, 郑宣, 等. 毛囊干细胞体外诱导成血管内皮细胞的实验研究[J/CD]. *中华细胞与干细胞杂志(电子版)*, 2016, 6(5): 284-291.
- [13] MA D, LEE S T, CHUA A W C. Isolation and culture of hair follicle dermal sheath mesenchymal stromal cells[J]. *Methods Mol Biol*, 2019, 1993: 61-70.

- miR-204 in the regulation of adipocyte proliferation, differentiation, and apoptosis [J]. *J Cell Physiol*, 2019, 234(7):11037-11046.
- [5] DU J, ZHANG P, GAN M, et al. MicroRNA-204-5p regulates 3T3-L1 preadipocyte proliferation, apoptosis and differentiation [J]. *Gene*, 2018, 668: 1-7.
- [6] KARBIENER M, FISCHER C, NOWITSCH S, et al. MicroRNA miR-27b impairs human adipocyte differentiation and targets ppargamma [J]. *Biochem Biophys Res Commun*, 2009, 390(2):247-251.
- [7] 张丽华, 欧阳丹, 徐立凤, 等. miR-92a-3p 对 3T3-L1 前体脂肪细胞增殖与分化的影响 [J]. *中国兽医科学*, 2016, 46(11):1450-1455.
- [8] CASCIO S, D'ANDREA A, FERLA R, et al. miR-20b modulates VEGF expression by targeting HIF-1 alpha and STAT3 in MCF-7 breast cancer cells [J]. *J Cell Physiol*, 2010, 224(1): 242-249.
- [9] SHIN Y, KIM D Y, KO J Y, et al. Regulation of KLF12 by microRNA-20b and microRNA-106a in cystogenesis [J]. *FASEB J*, 2018, 32(7): 3574-3582.
- [10] LI G, NING C, MA Y, et al. miR-26b promotes 3T3-L1 adipocyte differentiation through targeting PTEN [J]. *DNA Cell Biol*, 2017, 36(8): 672-681.
- [11] CHEN C, XIANG H, PENG Y L, et al. Mature miR-183, negatively regulated by transcription factor GATA3, promotes 3T3-L1 adipogenesis through inhibition of the canonical Wnt/ β -catenin signaling pathway by targeting LRP6 [J]. *Cell Signal*, 2014, 26(6):1155-1165.
- [12] NING C, LI G, YOU L, et al. MiR-185 inhibits 3T3-L1 cell differentiation by targeting SREBP-1 [J]. *Biosci Biotechnol Biochem*, 2017, 81(9):1747-1754.
- [13] CHEN H, MO D, LI M, et al. miR-709 inhibits 3T3-L1 cell differentiation by targeting GSK3 β of Wnt/ β -catenin signaling [J]. *Cell Signal*, 2014, 26(11):2583-2589.
- [14] STRYCHARZ J, SWIDERSKA E, WRÓBLEWSKI I A, et al. Hyperglycemia affects miRNAs expression pattern during adipogenesis of human visceral adipocytes memorization involved? [J]. *Nutrients*, 2018, 10(11):E1774.
- [15] SIERSB K R, NIELSEN R, MANDRUP S. PPAR γ in adipocyte differentiation and metabolism—novel insights from genome-wide studies [J]. *FEBS Lett*, 2010, 584(15):3242-3249.
- [16] CHOI S K, PARK S, JANG S, et al. Cascade regulation of PPAR γ (2) and C/EBP α signaling pathways by celastrol impairs adipocyte differentiation and stimulates lipolysis in 3T3-L1 adipocytes [J]. *Metabolism*, 2016, 65(5):646-654.
- [17] ROSE S E, HEMATI N, LONGO K A, et al. Inhibition of adipogenesis by Wnt signaling [J]. *Science*, 2010, 289(5481):950-953.
- [18] ZHAO C, WU H, QIMUGE N, et al. MAT2A promotes porcine adipogenesis by mediating H3K27me3 at Wnt10b locus and repressing Wnt/ β -catenin signaling [J]. *Biochim Biophys Acta Mol Cell Biol Lipids*, 2018, 1863(2):132-142.

(收稿日期:2020-03-27 修回日期:2020-04-27)

(上接第 2869 页)

- [14] HOFFMAN R M, AMOH Y. Hair follicle-associated pluripotent(HAP) stem cells [J]. *Prog Mol Biol Transl Sci*, 2018, 160:23-28.
- [15] ABBASI S, BIERNASKIE J. Injury modifies the fate of hair follicle dermal stem cell progeny in a hair cycle-dependent manner [J]. *Exp Dermatol*, 2019, 28(4):419-424.
- [16] JOSHI J, BRENNAN D, BEACHLEY V, et al. Cardiomyogenic differentiation of human bone marrow-derived mesenchymal stem cellspheroids within electrospun collagen nanofiber mats [J]. *J Biomed Mater Res A*, 2018, 106(12):3303-3312.
- [17] PETERS E L, OFFRINGA C, KOS D, et al. Regulation of myoglobin in hypertrophied rat cardiomyocytes in experimental pulmonary hypertension [J]. *Pflugers Arch*, 2016, 468(10):1697-1707.

(收稿日期:2020-03-05 修回日期:2020-05-16)