

综述

人诱导多能干细胞在扩张型心肌病模型建立及应用中的研究进展

谢曼婷, 谢冰冰, 向秋玲*

中山大学中山医学院, 广州 510080

摘要: 扩张型心肌病(dilated cardiomyopathy, DCM)是一种具有心肌结构和功能异常的非缺血性心肌病。利用DCM患者的心肌细胞进行细胞功能研究受伦理等多方面限制, 相应的动物模型难以构建, 疾病发生和发展的机制尚不清晰。人诱导多能干细胞(human induced pluripotent stem cells, hiPSCs)的出现为DCM的基础研究提供了新的方向。研究人员利用hiPSCs诱导分化获得心肌细胞(hiPSCs-derived cardiomyocytes, hiPSC-CMs)进行DCM疾病模型构建, 利用该模型进行药物筛选, 并进一步为DCM的病理机制探究及干预措施提供新的见解。本文对DCM患者特异性hiPSC-CMs (DCM-hiPSC-CMs)模型构建、药物筛选、机制研究等方面研究进展进行综述。

关键词: 扩张型心肌病; 诱导多能干细胞; 疾病模型; 研究进展

Research progress of human induced pluripotent stem cells in the establishment and application of dilated cardiomyopathy disease model

XIE Man-Ting, XIE Bing-Bing, XIANG Qiu-Ling*

Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou 510080, China

Abstract: Dilated cardiomyopathy (DCM) is a non-ischemic cardiomyopathy with abnormal myocardial structure and function. It is challenging to construct human primary cardiac myocytes from DCM patients due to ethical constraints. In addition, animal models failed to adequately replicate the complexity of the human disease. The mechanism of DCM remains unclear. The emergence of human induced pluripotent stem cells (hiPSCs) provides a new tool for basic research in DCM. Researchers have produced hiPSCs-derived cardiomyocytes (hiPSC-CMs) and applied them to drug screening, leading to new insight into the pathomechanism and treatment in DCM. This review summarizes the research progress in the establishment, drug screening and mechanism research of DCM patient-specific hiPSC-CMs (DCM-hiPSC-CMs) model.

Key words: dilated cardiomyopathy; induced pluripotent stem cells; disease model; research progress

扩张型心肌病 (dilated cardiomyopathy, DCM) 是一种以左室或双室扩张和收缩功能障碍为特征的异质性心肌病, 常引起心律失常、心力衰竭, 甚至猝死^[1]。遗传性因素在 DCM 中占主导地位。至今临床已发现超过 60 个 DCM 相关致病基因, 主要包括编码肌小节和细胞骨架蛋白基因, 如 *TTN*、*LMNA*、

MYH7、*MYH6*、*TNNT2*、*ACTC1* 和 *MYBPC3* 等^[2]。流行病学显示, DCM 的患病率为 1/250~1/400, 10 年生存率约为 60%。DCM 的全球患病率和致死率逐年升高, 已经成为危害人类健康的重大公共卫生问题^[3]。我国 DCM 的发病率也呈增长趋势, 约为 19~36.5/10 万^[4]。由于 DCM 的发病机制尚不完全

This work was supported by the National Natural Science Foundation of China (No. 82272164) and the Natural Science Foundation of Guangdong Province, China (No. 2021A1515011648).

*Corresponding author. E-mail: xiangql@mail.sysu.edu.cn

明确, 目前主要以对症治疗为主, 缺乏有效且特异的治疗方法^[3]。因此, 研究 DCM 的发病机制并开发出有效的治疗方法是当今心血管研究领域的热点, 具有重大医学价值和社会意义。

人诱导多能干细胞 (human induced pluripotent stem cells, hiPSCs) 是日本科学家山中伸弥通过导入 4 种特定转录因子至成人体细胞中得到的重编程多能干细胞^[5]。hiPSCs 具有与胚胎干细胞相似的自我更新和多向分化潜能, 可以在体外定向分化成终末分化细胞, 为难以从机体直接获取的细胞提供丰富的来源, 同时很好地避免了胚胎干细胞所面临的伦理和免疫排斥等问题。因此, hiPSCs 成为一个极具吸引力的种子细胞, 为获得患者特异性细胞并用于疾病建模、机制研究、个性化药物筛选提供了前所未有的机会^[6]。

本文就 DCM 患者特异性 hiPSCs 诱导分化获得心肌细胞 (DCM patient-specific hiPSCs-derived cardiomyocytes, DCM-hiPSC-CMs) 模型构建、药物筛选、机制研究方面的研究进展进行综述 (图 1)。

1 DCM-hiPSC-CMs模型的构建

疾病模型是医学研究中的重要工具, 它能够为

心血管疾病的发生 / 发展和疾病的分子机制提供重要见解。目前, 转基因动物和心肌细胞系已经成功地应用于人类心血管疾病的研究当中。然而, 由于人和小鼠心肌细胞的电生理性质不同, 小鼠模型难以充分再现人类疾病。人原代心肌细胞很难获取且在体外不易维持培养。长期缺乏合适的体外模型阻碍了人类心血管疾病研究的进一步发展^[7]。1998 年, 人胚胎干细胞 (human embryonic stem cells, hESCs) 的发现为人心血管发育生物学、药物发现和再生医学提供了巨大应用潜能^[8, 9]。同时基于 hESCs 的多项优势, 重编程获得的 hiPSCs 弥补了 hESCs 带来的免疫排斥和伦理等问题, 成为了更具应用前景的疾病模型^[5]。早期已有研究报道了多种从 hiPSCs 诱导分化获得心肌细胞 (hiPSCs-derived cardiomyocytes, hiPSC-CMs) 的方法^[10]。

1.1 拟胚体形成法

拟胚体是多能干细胞在体外一定条件下形成的含有内、中、外三个胚层的球状结构, 可模拟早期胚胎发育过程, 是获得心肌细胞的常用方法^[11]。2001 年, Kehat 等描述了一个可重复的心肌分化体系, 通过悬浮培养 hESCs 30 天获得具有早期心肌组织结构和自发收缩功能的心肌细胞的拟胚体^[11, 12]。

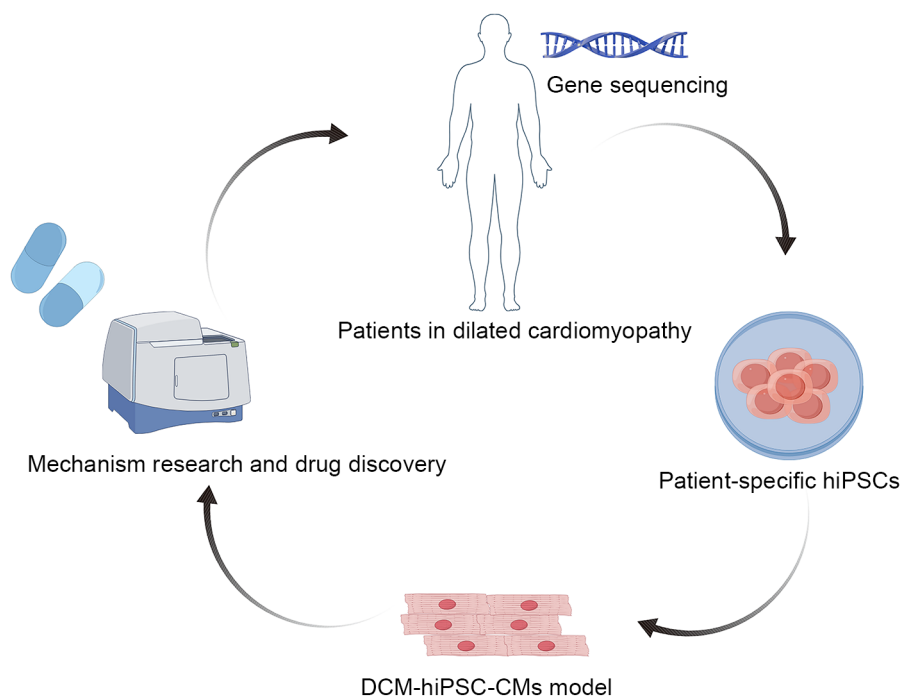


图 1. DCM-hiPSC-CMs模型建立及应用的研究路线

Fig 1. Process for generating dilated cardiomyopathy (DCM) patient-specific human induced pluripotent stem cells (hiPSCs)-derived cardiomyocytes (DCM-hiPSC-CMs) and using them for mechanism research and phenotypic drug discovery. By Figdraw.

基于拟胚体形成实验, hiPSCs 与 hESC 均可诱导分化为人心脏窦房结样、心房样和心室样心肌细胞^[13]。2012 年 Sun 等采用 Yang 等人前期建立的拟胚体形成法结合小分子定向诱导体系将 *TNNT2* 突变 DCM 患者来源的 hiPSCs 悬浮培养获得拟胚体, 并先后用 BMP4、 β FGF、激活素 A、DKK1 和 VEGF 小分子定向诱导, 构建了首个 DCM 家系 hiPSC-CMs 模型^[14, 15]。获得的 DCM-hiPSC-CMs 呈现异常肌节紊乱、对 β 肾上腺素能和机械应力刺激敏感、钙调控能力和收缩功能障碍等, 在一定程度上呈现了 DCM 疾病特征, 可能有助于研究疾病机制和药物筛选。然而, 该分化体系下, 不同细胞系之间的诱导分化效率各不相同, 限制了其进一步应用。

1.2 与内脏内胚层样细胞共培养法

内脏内胚层由原始内胚层发育而来, 参与胚胎外组织的形成和胚胎发育。2002 年 Mummery 等发现, 表达甲胎蛋白和细胞骨架蛋白 ENDO-A 内脏内胚层特异标志物的 END-2 细胞系与 hESC 共培养后可以获得心肌细胞^[16]。END-2 细胞分泌的因子具有与 FGF 相似的功能, 参与心肌细胞分化过程的诱导和调控^[17]。基于此共培养体系, Tse 和 Siu 等人先后将 *DES* 突变和 *LMNA* 突变 DCM 患者的 hiPSCs 悬浮培养成拟胚体, 再转移至与内胚层样细胞共培养, 获得自发跳动的 DCM-hiPSC-CMs^[16, 18, 19]。除了异常的钙调控能力, 获得的 *DES* 突变 DCM-hiPSC-CMs 呈现弥漫性结蛋白聚集, *LMNA* 突变 DCM-hiPSC-CMs 出现异常核改变。虽然采用该方法不同细胞系之间的分化效率无明显差异, 但仍然存在诸多问题, 例如获得跳动心肌细胞簇的时间节点跨度大以及操作步骤复杂繁多等。

1.3 hiPSCs 单层细胞诱导法

为了提高心肌细胞的产量和纯度, Laflamme 等人开发了通过激活素 A 和 BMP4 连续处理高密度未分化单层培养的 hESC 使其定向分化为心肌细胞的技术^[20]。大多数研究以 RPMI 1640 为基础培养基, 利用 GSK-3 β 抑制剂 CH99021、Wnt 信号通路抑制剂 IWR-1 或 IWP2 对单层培养的 hiPSCs 进行定向诱导获得患者特异 DCM-hiPSC-CMs。获得的 hiPSC-CMs 在第 8 到第 10 天即可出现搏动, 同时利用无糖培养基纯化 3 天可以获得 90% 以上纯度的 hiPSC-CMs^[21-23]。基于早期胚胎心脏发生, 使用不同的生长因子或小分子渐进诱导 hiPSCs 向心脏谱系分化并高效获得心肌细胞成为了常用的构建 DCM-hiPSC-

CMs 模型的方法^[24]。

与既往的疾病模型相比, hiPSC-CMs 具有获取简便且微创、可在体外模拟心肌细胞复杂的细胞生理学、可排除体内疾病状态下代偿机制的影响、涵盖了患者所有的基因组信息从而可以进行基因型-表型关联等多项优势^[25]。结合基因编辑技术将致病突变引入野生型细胞系中, DCM-hiPSC-CMs 模型将患者基因型与其细胞表型联系起来, 能够在患者相同遗传背景下精确分析疾病表型和药物反应, 更好地为患者提供个性化治疗。

2 DCM-hiPSC-CMs 在药物开发中的应用

2.1 药物筛选

药物筛选是药物创新研发的基础环节和重要手段, 旨在采用合适的筛选方法和模型, 以发现具有特定生物活性和药理作用的化合物, 实现药物从基础研究迈向临床应用。除了再现人心肌细胞相似的表型特征, hiPSC-CMs 还能表达人体内钠、钾、钙等重要离子通道, 具备与人心肌细胞相似的电生理特性, 日益成为药物筛选的理想模型^[26]。Sun 等采用 DCM 临床治疗药物 β 肾上腺素能阻滞剂美托洛尔处理 DCM-hiPSC-CMs 一周可显著改善其肌节结构, 证实利用该模型进行药物筛选的可行性^[14]。Siu 等发现 MEK1 信号通路阻滞剂 U106 和司美替尼均能有效减少电刺激后 *LMNA* 突变 DCM-hiPSC-CMs 的凋亡水平, 通过对比发现司美替尼以更低的药物浓度展现更好的治疗效果^[19]。此外, Broughton 等人首次报道了肌球蛋白激活剂小分子 omecamtiv mecarbil (OM) 可通过促进肌动蛋白和肌球蛋白的相互作用有效改善 *TNNT2* 突变引起的肌节收缩障碍, 并筛选出了更有效的药物剂量^[27]。

2.2 药物毒性筛查

心脏毒性是许多药物的副作用之一。为了尽量减少可能的药物心脏风险, 食品和药物管理局和制药行业都已强制要求在药物开发的早期进行体外心脏毒性筛查。hiPSC-CMs 可用于个性化的药物毒性检测, 以评估不同个体对药物诱导的心脏毒性的遗传敏感性^[28]。Liang 等在 2013 年利用 hiPSC-CMs 检测维拉帕米、阿夫唑嗪、西沙必利、尼可地尔等药物在 DCM 等心肌病中的心脏毒性, 证明患者特异性 DCM-hiPSC-CMs 比标准 hERG (the human ether-a-go-go-related gene) 试验更能准确地预测药物不良反应^[29]。此外, Andersson 等利用 hiPSC-CMs 结合

心脏损伤生物标志物心肌肌钙蛋白 T 和心型脂肪酸结合蛋白检测不同浓度抗癌药物的心脏毒性作用, 如常引起 DCM 的阿霉素^[30]。基于 DCM-hiPSC-CMs 表型和 DCM-hiPSC-CMs 释放的心脏损伤生物标志物等检测方法加入到临床前药物毒性筛选方案和药物开发中能够加速药物化合物安全地进入市场。

2.3 基于高通量技术药物发现

高通量筛选 (high throughput screening, HTS) 技术的出现为大规模的药物筛选提供了可能性^[31]。通过观察药物与细胞的相互作用, 在同一时间内对数以万计的样品进行检测, 在非常短的时间内完成数据采集和分析。在 DCM 的新药研究中, 研究人员利用表型药物筛选 (phenotypic drug screening), 以 DCM-hiPSC-CMs 的细胞活性或细胞收缩力作为表型进行初步筛选, 发现多种能有效改善 DCM 表型的化合物, 并进一步进行了验证^[32]。也有研究人员利用生物信息学形成的深度学习的成像分析方法在 5 500 种生物活性化合物和 siRNA 中对 *BAG3* 突变 DCM-hiPSC-CMs 进行表型筛选, 更高效且准确地筛选到组蛋白去乙酰化酶抑制剂可作为有效的治疗药物^[33, 34]。随着技术的发展, 高内涵筛选技术允许同时检测药物对心肌细胞凋亡、肌钙蛋白释放、线粒体跨膜电位、动作电位、钙瞬变形态、收缩力等多个环节的影响^[31]。结合多重检测结果, 高内涵筛选技术已成为更高效的药物发现的新模式和新策略。此外, 研究人员会根据自身前期研究结果和关注的研究方向选择不同的化合物库进行筛选。例如, Perea-Gil 等在含有 160 种化合物的激酶抑制剂库中发现两种激酶抑制剂的联合治疗能通过 ATF4 依赖的丝氨酸生物合成途径改善 DCM 引起的收缩和代谢功能障碍, 从而发现一种新的与基因型无关的遗传性 DCM 治疗策略^[35]。

3 DCM-hiPSC-CMs在致病机制和治疗策略中的价值

3.1 肌节蛋白功能障碍

肌节是心肌纤维结构和功能的基本单位。编码肌节蛋白相关基因突变可能会影响其转录和翻译, 从而影响参与收缩的肌节蛋白含量或结构。Fomin 等直接证明了在 *TTN* 截断突变的 DCM-hiPSC-CMs 中野生型肌联蛋白表达减少、蛋白酶体抑制作用减弱使得截断肌联蛋白聚集, 采用基因编辑或药物调

控肌联蛋白表达可以有效改善收缩功能障碍^[36]。Yang 等利用 DCM-hiPSC-CMs 模型发现 *MYH7* 基因突变破坏了肌球蛋白和肌球蛋白结合蛋白的相互作用, 影响心肌细胞收缩力, 提示恢复蛋白互作可能是有效的治疗策略^[37]。

3.2 细胞内离子失衡

Ca^{2+} 和 Na^{+} 参与心肌细胞动作电位产生和兴奋收缩耦联的过程, 在维持心脏正常生理功能中发挥了重要的作用^[38]。研究人员发现大多数 DCM-hiPSC-CMs 出现异常钙调控能力, 并借此研究 DCM 患者心律失常的离子机制。Jung 等发现携带 *TnT* 基因突变的 DCM-hiPSC-CMs 中进入到胞浆的 Ca^{2+} 无法有效地结合到肌钙蛋白上, 从而导致胞浆中游离 Ca^{2+} 含量增加^[39]。钙离子增敏剂可以增强肌钙蛋白 C 的敏感性, 有效地在表型上挽救 Ca^{2+} 瞬态。因此, 调节 Ca^{2+} 敏感性可能是一个有效的药物治疗方式^[39, 40]。此外, Sedaghat-Hamedani 等利用 hiPSC-CMs 发现心脏钠电压门控通道 *SCN5a* 基因突变同样会引起 DCM, 伴有心律失常发生^[41]。因此, Na^{+} 通道是 DCM 抗心律失常的一个重要靶点。

3.3 线粒体功能失调

线粒体作为心肌细胞的能量站, 提供维持心脏正常功能所必需的 ATP^[42]。有研究表明 DCM-hiPSC-CMs 出现线粒体功能障碍, Gö 6976 和 SB 203580 联合药物治疗能有效改善 DCM-hiPSC-CMs 的线粒体呼吸功能和 ATP 水平, 改善细胞收缩功能^[35]。线粒体还是细胞代谢的中心枢纽, 为生物合成提供代谢物。DCM-hiPSC-CMs 中线粒体数量下降或活动异常导致能量产生减少, 心肌细胞收缩力降低, ROS 产生增加从而导致病理状态^[43]。Dai 等发现 DCM-hiPSC-CMs 的内吞作用和运输作用受损导致的线粒体铁缺乏和线粒体功能障碍可能是携带遗传性基因突变 DCM 的相关病理机制^[44]。因此, 特异性靶向线粒体可能是减轻心肌细胞死亡和维持心肌细胞功能的一种方式。

3.4 细胞核内结构和功能异常

为了更深刻地了解 DCM 的致病机制, 研究人员运用不同测序方法寻找潜在的治疗靶点。Wu 实验室先后利用两种 DCM 常见的突变基因 *LMNA* 和 *FLNC* 在 hiPSC-CMs 中研究发现相关蛋白在核内出现异常分布^[45, 46]。转录组测序分析发现血小板衍生生长因子 (platelet-derived growth factor, PDGF) 信号通路的激活与 DCM 的发病机制相关, 通过抑制

PDGF 受体 β (PDGF receptor- β , PDGFRB) 可以改善 DCM-hiPSC-CMs 出现的心律失常表型。结合临床 DCM 患者数据, PDGFRB 可能是 DCM 一个共同的潜在治疗靶点。Chang 等发现遗传性 DCM-hiPSC-CMs 常伴随有端粒缩短, 可能会加速衰老过程。因此, 端粒缩短可能是遗传性心肌病的标志物。监测端粒损耗的动态变化使得未来机制研究和筛选新的治疗药物来阻止端粒缩短和疾病进展成为可能^[47]。因此, 细胞核内异常的信号、端粒和染色体结构可能成为有效的治疗靶点。

研究人员利用 DCM-hiPSC-CMs 模型探索 DCM 更多可能的发病机制, 加深了我们对 DCM 潜在机制的理解, 为 DCM 的临床治疗奠定了基础 (表 1)。

4 结论与展望

DCM 是严重的心肌疾病, 部分病因至今不明, 相关机制尚不清晰。hiPSCs 的广泛应用将对了解 DCM 的发生和发展、发现改善人类健康的药物作出巨大贡献。作为理想的研究工具, hiPSCs 为含个体遗传背景 DCM 患者建模、分析患者对不同治疗方式的特异性反应、确定相关的病理生理特征, 并发现适合干预的药物靶点提供了新的体外研究模型。

虽然 hiPSCs 在 DCM 疾病模型构建和应用上已

取得了重大进展, 但是基于 hiPSC-CMs 的研究仍然存在一些不足之处。首先, 传统的二维心肌细胞模型由于缺少细胞外基质成分, 无法模拟受 DCM 影响的细胞微环境, 无法准确再现三维立体结构对心肌细胞的结构和功能产生的影响, 不能完全适用于 DCM 的研究^[48]。其次, 获得的 hiPSC-CMs 表现出不成熟心肌细胞的形态和功能, 无法准确反映成熟心肌细胞的表型^[49]。另外, 通过诱导分化得到的 hiPSC-CMs 细胞种类多样, 各类细胞比例不一, 可能无法准确反映心脏的特异结构和功能^[50]。针对上述不足之处, 也有研究人员开发了基于 hiPSCs 的三维模型, 该模型更准确地保留了包括细胞-细胞之间和细胞-细胞外基质之间的相互作用等在内的体内细胞的生物学特性和功能, 为 DCM 的研究开辟了新的方向与思路^[51]。同时, 研究人员利用 hiPSC-CMs 对诱导产生的细胞类型和细胞成熟度等也在不断进行探索。目前, 由于全外显子基因测序技术费用昂贵, 未能落实到所有患者的病因诊断中, 再加上样本难以收集, 构建的 hiPSCs 疾病模型依然较少, 相关疾病研究仍不完善。我们期待更多 hiPSCs 疾病模型的构建, 以及模型构建技术整合和规范化, 对 DCM-hiPSC-CMs 的进一步研究有望促进 DCM 基础研究向临床转化迈一大步。

表 1. 遗传性 DCM 患者 hiPSC-CMs 应用的研究

Table 1. Studies reporting applications of hiPSC-CMs from patients with genetic DCM

Mutation	Mechanism research and drug discovery	Reference
<i>TNNT2</i>	Treatment with β -adrenergic blockers or overexpression of Serca2a improved the function of DCM-hiPSC-CMs	[14]
<i>LMNA</i>	Modulation of ERK1/2 pathway with MEK1/2 inhibitors, U0126 and selumetinib, significantly attenuated the pro-apoptotic effects of field electric stimulation on DCM-hiPSC-CMs	[19]
<i>RBM20</i>	<i>RBM20</i> mutation led to an inhibition of <i>TTN</i> alternative splicing and <i>TTN</i> isoform switch in hiPSC-CMs	[22]
<i>LMNA</i>	Abnormal cellular structure and functionality and an increased sensitivity to cellular stress were observed after hypoxia in DCM-hiPSC-CMs	[23]
<i>TNNT2</i>	The myosin activator omecamtiv mecarbil could restore function within DCM-hiPSC-CMs	[27]
<i>BAG3</i>	Using a phenotypic screen and deep learning, inhibiting HDAC6 was cardioprotective at the sarcomere level in DCM-hiPSC-CMs	[34]
<i>TNNT2</i>	Modulation of serine biosynthesis signaling with a combination of SMKIs ameliorated contractile and metabolic dysfunction in DCM-hiPSC-CMs	[35]
<i>MYH7</i>	The E848G allele disrupted the protein-protein interaction between β -myosin heavy chain and cardiac myosin binding protein C	[37]
<i>TNNT2</i>	Treatment of a Ca^{2+} desensitiser to DCM-hiPSC-CMs was able to phenotypically rescue Ca^{2+} dynamics	[39]
<i>LMNA</i>	Pharmacological and molecular inhibition of the PDGF signaling pathway ameliorated the arrhythmia phenotypes of DCM-hiPSC-CMs <i>in vitro</i>	[45]
<i>FLNC</i>	Treatment with the PDGFRA inhibitor, crenolanib, improved contractile function of DCM-hiPSC-CMs	[46]

DCM: dilated cardiomyopathy; hiPSC-CMs: human induced pluripotent stem cells-derived cardiomyocytes; SMKIs: small-molecule kinase inhibitors; PDGF: platelet derived growth factor; PDGFRA: platelet derived growth factor receptor α .

参考文献

- Hong K (洪葵), Su Y. Interpretation of the 2022 EHRA/HRS/APHRs/LAHRs expert consensus on genetic testing for cardiac diseases. *J Clin Cardiol* (临床心血管病杂志) 2023; 39(3): 163–167 (in Chinese).
- Rosenbaum AN, Agre KE, Pereira NL. Genetics of dilated cardiomyopathy: practical implications for heart failure management. *Nat Rev Cardiol* 2020; 17(5): 286–297.
- Schultheiss HP, Fairweather D, Caforio ALP, Escher F, Hersberger RE, Lipshultz SE, Liu PP, Matsumori A, Mazzanti A, McMurray J, Priori SG. Dilated cardiomyopathy. *Nat Rev Dis Primers* 2019; 5(1): 32.
- Section of Precision Cardiovascular Medicine of Chinese Society of Cardiology (中华医学会心血管病学分会精准心血管病学学组), Precision Cardiovascular Medicine Branch of China International Exchange, Promotive Association for Medical, Health Care Editorial Board of Chinese Journal of Cardiology. Guideline for the genetic diagnosis of monogenic cardiovascular diseases. *Chin J Cardiol* (中华心血管病杂志) 2019; 47(3): 175–196 (in Chinese).
- Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006; 126(4): 663–676.
- Smith AS, Macadangdang J, Leung W, Laflamme MA, Kim DH. Human iPSC-derived cardiomyocytes and tissue engineering strategies for disease modeling and drug screening. *Biotechnol Adv* 2017; 35(1): 77–94.
- Yoshida Y, Yamanaka S. Induced pluripotent stem cells 10 years later: for cardiac applications. *Circ Res* 2017; 120(12): 1958–1968.
- Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. *Science* 1998; 282(5391): 1145–1147.
- Reubinoff BE, Pera MF, Fong CY, Trounson A, Bongso A. Embryonic stem cell lines from human blastocysts: somatic differentiation *in vitro*. *Nat Biotechnol* 2000; 18(4): 399–404.
- Yoshida Y, Yamanaka S. iPS cells: a source of cardiac regeneration. *J Mol Cell Cardiol* 2011; 50(2): 327–332.
- Itskovitz-Eldor J, Schuldiner M, Karsenti D, Eden A, Yanuka O, Amit M, Soreq H, Benvenisty N. Differentiation of human embryonic stem cells into embryoid bodies compromising the three embryonic germ layers. *Mol Med* 2000; 6(2): 88–95.
- Kehat I, Kenyagin-Karsenti D, Snir M, Segev H, Amit M, Gepstein A, Livne E, Binah O, Itskovitz-Eldor J, Gepstein L. Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. *J Clin Invest* 2001; 108(3): 407–414.
- Zhang J, Wilson GF, Soerens AG, Koonce CH, Yu J, Palecek SP, Thomson JA, Kamp TJ. Functional cardiomyocytes derived from human induced pluripotent stem cells. *Circ Res* 2009; 104(4): e30–e41.
- Sun N, Yazawa M, Liu JW, Han L, Sanchez-Freire V, Abilez OJ, Navarrete EG, Hu SJ, Wang L, Lee A, Pavlovic A, Lin S, Chen R, Hajjar RJ, Snyder MP, Dolmetsch RE, Butte MJ, Ashley EA, Longaker MT, Robbins RC, Wu JC. Patient-specific induced pluripotent stem cells as a model for familial dilated cardiomyopathy. *Sci Transl Med* 2012; 4(130): 130ra47.
- Yang L, Soonpaa MH, Adler ED, Roepke TK, Kattman SJ, Kennedy M, Henckaerts E, Bonham K, Abbott GW, Linden RM, Field LJ, Keller GM. Human cardiovascular progenitor cells develop from a KDR⁺ embryonic-stem-cell-derived population. *Nature* 2008; 453(7194): 524–528.
- Mummery C, Ward D, van den Brink CE, Bird SD, Doeven-dans PA, Opthof T, Brutel de la Riviere A, Tertoolen L, van der Heyden M, Pera M. Cardiomyocyte differentiation of mouse and human embryonic stem cells. *J Anat* 2002; 200(Pt 3): 233–242.
- van den Eijnden-van Raaij AJ, van Achterberg TA, van der Kruijsen CM, Piersma AH, Huylebroeck D, de Laat SW, Mummery CL. Differentiation of aggregated murine P19 embryonal carcinoma cells is induced by a novel visceral endoderm-specific FGF-like factor and inhibited by activin A. *Mech Dev* 1991; 33(2): 157–165.
- Tse HF, Ho JC, Choi SW, Lee YK, Butler AW, Ng KM, Siu CW, Simpson MA, Lai WH, Chan YC, Au KW, Zhang J, Lay KW, Esteban MA, Nicholls JM, Colman A, Sham PC. Patient-specific induced-pluripotent stem cells-derived cardiomyocytes recapitulate the pathogenic phenotypes of dilated cardiomyopathy due to a novel DES mutation identified by whole exome sequencing. *Hum Mol Genet* 2013; 22(7): 1395–1403.
- Siu CW, Lee YK, Ho JC, Lai WH, Chan YC, Ng KM, Wong LY, Au KW, Lau YM, Zhang J, Lay KW, Colman A, Tse HF. Modeling of lamin A/C mutation premature cardiac aging using patient-specific induced pluripotent stem cells. *Aging (Albany NY)* 2012; 4(11): 803–822.
- Laflamme MA, Chen KY, Naumova AV, Muskheili V, Fugate JA, Dupras SK, Reinecke H, Xu C, Hassanipour M, Police S, O'Sullivan C, Collins L, Chen Y, Minami E, Gill EA, Ueno S, Yuan C, Gold J, Murry CE. Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. *Nat Biotechnol* 2007; 25(9): 1015–1024.
- Wu H, Lee J, Vincent LG, Wang Q, Gu M, Lan F, Churko JM, Sallam KI, Matsa E, Sharma A, Gold JD, Engler AJ,

- Xiang YK, Bers DM, Wu JC. Epigenetic regulation of phosphodiesterases 2A and 3A underlies compromised β -adrenergic signaling in an iPSC model of dilated cardiomyopathy. *Cell Stem Cell* 2015; 17(1): 89–100.
- 22 Streckfuss-Bömeke K, Tiburcy M, Fomin A, Luo X, Li W, Fischer C, Özcelik C, Perrot A, Sossalla S, Haas J, Vidal RO, Rebs S, Khadjeh S, Meder B, Bonn S, Linke WA, Zimmermann WH, Hasenfuss G, Guan K. Severe DCM phenotype of patient harboring RBM20 mutation S635A can be modeled by patient-specific induced pluripotent stem cell-derived cardiomyocytes. *J Mol Cell Cardiol* 2017; 113: 9–21.
- 23 Shah D, Virtanen L, Prajapati C, Kiamehr M, Gullmets J, West G, Kreutzer J, Pekkanen-Mattila M, Heliö T, Kallio P, Taimen P, Aalto-Setälä K. Modeling of *LMNA*-related dilated cardiomyopathy using human induced pluripotent stem cells. *Cells* 2019; 8(6): 594.
- 24 Burridge PW, Keller G, Gold JD, Wu JC. Production of *de novo* cardiomyocytes: human pluripotent stem cell differentiation and direct reprogramming. *Cell Stem Cell* 2012; 10(1): 16–28.
- 25 Karakikes I, Ameen M, Termglinchan V, Wu JC. Human induced pluripotent stem cell-derived cardiomyocytes insights into molecular, cellular, and functional phenotypes. *Circ Res* 2015; 117(1): 80–88.
- 26 Paci M, Hyttinen J, Rodriguez B, Severi S. Human induced pluripotent stem cell-derived versus adult cardiomyocytes: an in silico electrophysiological study on effects of ionic current block. *Br J Pharmacol* 2015; 172(21): 5147–5160.
- 27 Broughton KM, Li J, Sarmah E, Warren CM, Lin YH, Henze MP, Sanchez-Freire V, Solaro RJ, Russell B. A myosin activator improves actin assembly and sarcomere function of human-induced pluripotent stem cell-derived cardiomyocytes with a troponin T point mutation. *Am J Physiol Heart Circ Physiol* 2016; 311(1): H107–H117.
- 28 Deshmukh RS, Kovács KA, Dinnyés A. Drug discovery models and toxicity testing using embryonic and induced pluripotent stem-cell-derived cardiac and neuronal cells. *Stem Cells Int* 2012; 2012: 379569.
- 29 Liang P, Lan F, Lee AS, Gong TY, Sanchez-Freire V, Wang YM, Diecke S, Sallam K, Knowles JW, Wang PJ, Nguyen PK, Bers DM, Robbins RC, Wu JC. Drug screening using a library of human induced pluripotent stem cell-derived cardiomyocytes reveals disease-specific patterns of cardiotoxicity. *Circulation* 2013; 127(16): 1677–1691.
- 30 Andersson H, Steel D, Asp J, Dahlenborg K, Jonsson M, Jeppsson A, Lindahl A, Kågedal B, Sartipy P, Mandenius CF. Assaying cardiac biomarkers for toxicity testing using biosensing and cardiomyocytes derived from human embryonic stem cells. *J Biotechnol* 2010; 150(1): 175–181.
- 31 Fang LH (方莲花), Wang YH, Du GH. Application progress of high throughput screening technology in drug discovery. *Chin Pharm J (中国药理学杂志)* 2023; 58(4): 289–295 (in Chinese).
- 32 Hnatiuk AP, Briganti F, Staudt DW, Mercola M. Human iPSC modeling of heart disease for drug development. *Cell Chem Biol* 2021; 28(3): 271–282.
- 33 Serrano R, Feyen DAM, Bruyneel AAN, Hnatiuk AP, Vu MM, Amatya PL, Perea-Gil I, Prado M, Seeger T, Wu JC, Karakikes I, Mercola M. A deep learning platform to assess drug proarrhythmia risk. *Cell Stem Cell* 2023; 30(1): 86–95.e4.
- 34 Yang J, Grafton F, Ranjbarvaziri S, Budan A, Farshidfar F, Cho M, Xu E, Ho J, Maddah M, Loewke KE, Medina J, Sperandio D, Patel S, Hoey T, Mandegar MA. Phenotypic screening with deep learning identifies HDAC6 inhibitors as cardioprotective in a BAG3 mouse model of dilated cardiomyopathy. *Sci Transl Med* 2022; 14(652): eab15654.
- 35 Perea-Gil I, Seeger T, Bruyneel AAN, Termglinchan V, Monte E, Lim EW, Vadgama N, Furihata T, Gavidia AA, Ataam JA, Bharucha N, Martinez-Amador N, Ameen M, Nair P, Serrano R, Kaur B, Feyen DAM, Diecke S, Snyder MP, Metallo CM, Mercola M, Karakikes I. Serine biosynthesis as a novel therapeutic target for dilated cardiomyopathy. *Eur Heart J* 2022; 43(36): 3477–3489.
- 36 Fomin A, Gärtner A, Cyganek L, Tiburcy M, Tuleta I, Wellers L, Folsche L, Hobbach AJ, Von Frieling-Salewsky M, Unger A, Hucke A, Koser F, Kassner A, Sielemann K, Streckfuss-Bömeke K, Hasenfuss G, Goedel A, Laugwitz KL, Moretti A, Gummert JF, Dos Remedios CG, Reinecke H, Knöll R, van Heesch S, Hubner N, Zimmermann WH, Milting H, Linke WA. Truncated titin proteins and titin haploinsufficiency are targets for functional recovery in human cardiomyopathy due to mutations. *Sci Transl Med* 2021; 13(618): eabd3079.
- 37 Yang KC, Breitbart A, De Lange WJ, Hofsteen P, Futakuchi-Tsuchida A, Xu J, Schopf C, Razumova MV, Jiao A, Boucek R, Pabon L, Reinecke H, Kim DH, Ralphe JC, Regnier M, Murry CE. Novel adult-onset systolic cardiomyopathy due to MYH7 E848G mutation in patient-derived induced pluripotent stem cells. *JACC Basic Transl Sci* 2018; 3(6): 728–740.
- 38 Bers DM. Cardiac excitation-contraction coupling. *Nature* 2002; 415(6868): 198–205.
- 39 Jung P, Seibert F, Fakuade FE, Ignatyeva N, Sampathkumar S, Ritter M, Li H, Mason FE, Ebert A, Voigt N. Increased cytosolic calcium buffering contributes to a cellular arrhythmogenic substrate in iPSC-cardiomyocytes from patients with dilated cardiomyopathy. *Basic Res Cardiol* 2022;

- 117(1): 5.
- 40 Badone B, Ronchi C, Lodola F, Knaust AE, Hansen A, Eschenhagen T, Zaza A. Characterization of the PLN p.Arg-14del mutation in human induced pluripotent stem cell-derived cardiomyocytes. *Int J Mol Sci* 2021; 22(24): 13500.
- 41 Sedaghat-Hamedani F, Rebs S, El-Battrawy I, Chasan S, Krause T, Haas J, Zhong R, Liao Z, Xu Q, Zhou X, Akin I, Zitron E, Frey N, Streckfuss-Bomeke K, Kayvanpour E. Identification of SCN5a p.C335R variant in a large family with dilated cardiomyopathy and conduction disease. *Int J Mol Sci* 2021; 22(23): 12990.
- 42 Ramaccini D, Montoya-Urbe V, Aan FJ, Modesti L, Potes Y, Wieckowski MR, Krga I, Glibetic M, Pinton P, Giorgi C, Matter ML. Mitochondrial function and dysfunction in dilated cardiomyopathy. *Front Cell Dev Biol* 2020; 8: 624216.
- 43 Li A, Shami GJ, Griffiths L, Lal S, Irving H, Braet F. Giant mitochondria in cardiomyocytes: cellular architecture in health and disease. *Basic Res Cardiol* 2023; 118(1): 39.
- 44 Dai YY, Ignatyeva N, Xu H, Wali R, Toischer K, Brandenburg S, Lenz C, Pronto J, Fakuade FE, Sossalla S, Zeisberg EM, Janshoff A, Kutschka I, Voigt N, Urlaub H, Rasmussen TB, Mogensen J, Lehnart SE, Hasenfuss G, Ebert A. An alternative mechanism of subcellular iron uptake deficiency in cardiomyocytes. *Circ Res* 2023; 133(2): E19–E46.
- 45 Lee J, Termglinchan V, Diecke S, Itzhaki I, Lam CK, Garg P, Lau E, Greenhaw M, Seeger T, Wu H, Zhang JZ, Chen X, Gil IP, Ameen M, Sallam K, Rhee JW, Churko JM, Chaudhary R, Chour T, Wang PJ, Snyder MP, Chang HY, Karakikes I, Wu JC. Activation of PDGF pathway links LMNA mutation to dilated cardiomyopathy. *Nature* 2019; 572(7769): 335–340.
- 46 Chen SN, Lam CK, Wan YW, Gao S, Malak OA, Zhao SR, Lombardi R, Ambardekar AV, Bristow MR, Cleveland J, Gigli M, Sinagra G, Graw S, Taylor MRG, Wu JC, Mestroni L. Activation of PDGFRA signaling contributes to filamin C-related arrhythmogenic cardiomyopathy. *Sci Adv* 2022; 8(8): eabk0052.
- 47 Chang ACY, Chang ACH, Kirillova A, Sasagawa K, Su W, Weber G, Lin J, Termglinchan V, Karakikes I, Seeger T, Dainis AM, Hinson JT, Seidman J, Seidman CE, Day JW, Ashley E, Wu JC, Blau HM. Telomere shortening is a hallmark of genetic cardiomyopathies. *Proc Natl Acad Sci U S A* 2018; 115(37): 9276–9281.
- 48 Camman M, Joanne P, Agbulut O, Helary C. 3D models of dilated cardiomyopathy: Shaping the chemical, physical and topographical properties of biomaterials to mimic the cardiac extracellular matrix. *Bioact Mater* 2022; 7: 275–291.
- 49 Gomez-Garcia MJ, Quesnel E, Al-Attar R, Laskary AR, Laflamme MA. Maturation of human pluripotent stem cell derived cardiomyocytes *in vitro* and *in vivo*. *Semin Cell Dev Biol* 2021; 118: 163–171.
- 50 Protze SI, Lee JH, Keller GM. Human pluripotent stem cell-derived cardiovascular cells: from developmental biology to therapeutic applications. *Cell Stem Cell* 2019; 25(3): 311–327.
- 51 Cho J, Lee H, Rah W, Chang HJ, Yoon YS. From engineered heart tissue to cardiac organoid. *Theranostics* 2022; 12(6): 2758–2772.