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心脏重编程相关机制研究进展

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摘　要

心肌梗死占心血管疾病死亡率的50%。由于梗死部位发生纤维化重塑，在急性心肌梗死中幸存下来的人有着心力衰竭的显著风险。传统治疗方法可改善心脏血供，无法使已经受损的心肌细胞再生，而心脏再生医学的兴起为心肌梗死的治疗提供了新出路。心脏重编程可增加功能性心肌细胞数量并且减少心肌梗死后纤维化面积。本文主要介绍心脏直接重编程方法和相关机制研究，尤其是关于心脏重编程相关机制的研究进展。

关键词

心肌细胞，直接重编程，成纤维细胞，再生，重编程机制

Research Progress of Cardiac Reprogramming Mechanism

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Abstract

Myocardial infarction accounts for 50% of the mortality of cardiovascular diseases. People who survive an acute myocardial infarction are at significant risk of heart failure due to fibrotic remodeling at the infarct site. Traditional treatment methods can improve the blood supply to the heart, but cannot regenerate damaged myocardial cells. However, the rise of cardiac regenerative medicine provides a new way for the treatment of myocardial infarction. Cardiac reprogramming increases the number of functional cardiomyocytes and reduces the area of fibrosis after myocar-

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dial infarction. This paper mainly introduces the methods and mechanisms of cardiac direct reprogramming, especially the research progress on the mechanisms of cardiac reprogramming.

Keywords

Myocardial Cells, Direct Reprogramming, Fibroblasts, Regeneration, Reprogramming Mechanism

1. 引言

心肌梗死占心血管疾病死亡率的 50%。由于梗死部位发生纤维化重塑，在急性心肌梗死中幸存下来的人有着心力衰竭的显著风险。药物治疗、手术支架和冠脉搭桥可改善心脏血供，但无法使已经受损的心肌细胞再生。心脏移植可以改善预后，但供体相对较少且存在免疫排斥的风险。心脏再生疗法是一个迅速兴起修复受损心脏的领域。诱导成纤维细胞向心肌细胞转变可以改善心肌梗死后心功能和减少心脏纤维化的发生[1]。本篇文章主要介绍关于心脏重编程的主流方法，包括转录因子法、小分子法、miRNA 法。除此之外，还重点介绍了心脏重编程相关机制的研究进展。

2. 心脏直接重编程

2.1. 转录因子 GMT 以及优化

Ieda 等人提出利用转录因子 Gata4、Mef2c、Tbx5 (GMT)进行直接重编程，这也成为了直接重编程的重要基石[1]。与 GMT 相比，Song 等人利用 GMT 和 Hand2 四个转录因子诱导产生的心肌样细胞能够表达更多的 α-MHC-GFP+和 cTnT+ [2]。Addis 等人利 GMT 加 Hand2、Nkx2.5 组合的效率比单独使用 GMT 提高了 50 倍，使得心肌细胞标志物稳定表达，并且拥有钙振荡以及自发跳动的心肌样细胞，而这些心肌样细胞在转录因子失活后仍能持续数周[3]。Christoforoud 等人在 GMT 的基础上增加转录因子 MYOCD、SRF、Mesp1 和 SMARCD3，提高了心脏相关基因表达增加[4]。Zhao 等人从成年心肌梗死小鼠中分离出心脏成纤维细胞，利用转录因子组合 GMT 加 Myocd 和 Sall4 诱导其产生表达心脏相关蛋白的心肌样细胞[5]。以上研究通过优化转录因子 GMT 来提高心脏重编效率。

2.2. 小分子法

小分子化合物随后也展示出了优化重编程的能力，因其可避免在诱导心肌化进程中基因插入的致癌风险。HuangfuD 等人发现转录因子 Oct4 可以被小分子化合物所替代[6]。Hou 等人发现可诱导小鼠体细胞向多能干细胞细胞分化，该过程使用了七种小分子化合物[7]。而 Fu 等人仅使用了 CHIR99021、RepSox、Forskolin 和 VPA 诱导可产生心肌样细胞和多能干细胞(iPSC) [8]。

2.3. miRNA 法

miRNA 也可以通过调节心脏发育相关转录因子来调控心脏重编程的进程。Jayawardena 等人首次证实 miRNA 组合也可以进行体外重编程，而经过 JAK 抑制剂 I 处理后显著增强重编程效率[9]。Muraoka

等人发现 miR-133 通过直接靶向调控上皮间质转化调节因子 Snai1 来促进心脏重编程[10]。相比于 GMT，当加入 miR-133 后仅需三分之一的时间就能诱导产生自发跳动的心肌样细胞。除此之外，因环状 RNA (circRNA)可解除 miRNA 对靶基因的抑制从而提高靶基因表达水平也被用于心脏重编程。最近 Huang 等人证实沉默 circRNANfix 是通过抑制 Y-box 结合蛋白 1 (Ybx1)泛素依赖性降解和上调 miR-214 来促进心肌细胞增殖和血管生成[11]。

3. 心脏重编程相关机制

3.1. 抑制纤维化可以促进心脏重编程

众所周知，心脏重编程是为了心脏成纤维细胞在转分化过程中尽可能多的表达心肌细胞相关基因，减少成纤维细胞相关基因的表达，而抑制纤维化是促进细胞命运转化有力办法。TGF-β 超家族能够影响细胞的增殖，凋亡和分化。Ifkovits 等人发现在心脏重编程中使用 TGFβ 抑制剂 SB431542 (SB)可以提高 iCM 的产量[12]。Zhao 等人利用 TGF-β 或 Rho 相关激酶途径的小分子抑制促纤维化信号传导提高重编程效率 60%。在转录因子诱导后 2 周内出现自发收缩心肌细胞，而对照组需要 4 周。同样地，ROCK 抑制剂 Y-27632 也有类似作用[13]。Notch 是一种单通道跨膜受体，该受体激活需要 γ 分泌酶复合物触发 Notch 细胞内结构域。Abad 等人发现 DAPT 能够抑制 γ 分泌酶复合物与之结合进而增强转录因子组合介导的重编程。经过转录组分析表加入 DAPT 可显著增加心肌样细胞的钙通量和肌节结构，其机制为增加 Mef2c 与心脏基因位点启动子的结合诱导肌肉发育分化[14]。最近 Hashimoto 等人发现使用短发夹 RNA (shRNA)和化学物质抑制 EGFR 信号通路促进心脏重编程[15]。

3.2. 免疫机制

近年来，研究人员也开始关注免疫机制对于重编程过程的影响。Lee 等人在利用 CCP 诱导多能干细胞的产生时，偶然发现 Toll 样受体(TLRs)的激动剂与细胞渗透蛋白(CPP)相结合可以促进重编程。TLR3 介导的信号传导促进了表观遗传重塑，正是这种先天免疫激活导致细胞表型具有流动性，更有助于细胞核重编程的成功[16]。Sayed 等人研究发现对 RLR 和 TLR3 途径的双重敲低可导致 iPSC 显著减少，由此得出这两种途径对于核重编程来说具有累加效应，而 RLR 介导的先天免疫信号在核重编程中也起着关键作用[17]。Hu 等人证明了 RNA 传感受体配体 ICR2 通过 RNA 传感受体 RIG-I 和 TLR3 促进成纤维细胞重编程[18]。Zhou 等人近期利用单细胞转录组学测定发现免疫应答相关的 DNA 甲基化为人类心脏重编程诱导的必需品[19]。然而，也有研究表明抑制炎症的表达反而利于重编程。有研究筛选了 8400 种化合物发现双氯芬酸钠大大增强了心脏重编程[20]，这就产生了与之前矛盾的观点。对于重编程来说，我们还尚未明确免疫机制在其中所起的作用，需要投入更多精力把握二者的关系。可以肯定的是，免疫机制对于重编程来说不可或缺。

3.3. 细胞自噬

以往研究证实，重编程过程中也有细胞自噬的参与[21]。Ma 等人发现 Atg5 非依赖性参与自噬过程中的线粒体清除，当阻断自噬会抑制线粒体清除同时会降低多能干细胞的诱导效率[22]。Wang 等人证实敲低 Atg5 显著阻断了自噬并导致心肌样细胞的数量减少。除此之外，他们还发现敲低自噬限速因子 Becn1 能够提高心肌样细胞的质量和数量，改善小鼠心脏功能，减少心肌梗死后疤痕面积。通过进一步研究发现敲低 Becn1 并非依赖自噬参与重编程，而是通过激活 Wnt 通路促进心肌样细胞的成熟和诱导[23]。以上研究可以证实自噬也参与心脏重编程过程，但仍需要进一步深挖明确机制。

3.4. 表观遗传障碍

对于心脏重编程早期进行表观遗传重塑有望提高效率。Dal-Pra 等人发现经过 miR 组合处理后 H3K27me3 去甲基化，尤其是心脏转录因子启动子区域水平的下调。miR 组合可通过表观遗传机制启动直接心脏重编程[24]。Zhou 等人筛选了心脏重编程的表观遗传调节因子，发现敲低 polycomb 复合物基因 Bmi1 可以显著增强跳动的心肌样细胞。此外，Bmi1 缺失可以在心脏重编程期间代替转录因子 Gata4。因此得出 Bmi1 为心肌样细胞生产的关键表观遗传障碍[25]。Mll1 途径抑制剂小分子(MM408 和 MI503)直接抑制了参与脂肪细胞分化的 Mll1 靶基因 *Ebf1* 的表达，显著减少脂肪细胞的形成，提高胚胎成纤维细胞和心脏成纤维细胞转化为心肌样细胞的效率。因此，Mll1 抑制剂可能通过抑制替代谱系基因表达来促进心脏重编程[26]。以上研究通过早期调控表观遗传障碍以达到提高重编程效率的目的不失为一种有效策略。

3.5. 单细胞测序技术

scRNA-seq 数据使用降维手段后再根据需要进行差异基因表达、上下游信号通路富集、轨迹分析等处理。相比于传统的“区块样”RNA 测序(buik RNA-seq)分析，单细胞组学可以进一步分析出细胞亚群间的基因表达差异，不只是总体细胞基因表达的平均值。心脏重编程可利用单细胞 RNA 测序分析整个进程中转化轨迹[27] [28]，进而将心脏重编程过程中染色质调控与基因表达相结合[29] [30]。Wang 等人在早期重编程心肌样细胞就进行了 scRNA-seq 和 scATAC-seq 发现 Smad3 既参与重编程启动，又参与重编程中间阶段[31]。除此之外，Zhou 等人利用单细胞测序技术还发现小鼠和人类心脏重编程速度和命运的差异，这利于我们更明确基础研究和临床应用的关系[19]。由此看出单细胞测序技术可能是我们研究心脏重编程机制的突破口。

3.6. 细胞微环境

细胞微环境在心脏重编程的作用也不可小觑。相较于培养皿来说，心肌细胞在生物体内生存的基质硬度更为柔软，Kurotsu 等人研究了基质硬度对于心脏重编程的影响。他们发现软基质与天然心肌相当，可提高心脏重编程的效率和质量。从机制上讲，这是由于整合素、Rho/ROCK、肌动蛋白和 YAP/TAZ 信号传导的抑制以及成纤维细胞基因表达的抑制[32]。已有研究证实，预先使用心肌血管内皮生长因子可增强心脏重编程。Yamakawa 等人研究发现当使用 FGF2、FGF10 和 VEGF (简称 FFV)培养基时甚至不需要 Gata4，即可以通过 Mef2c 和 Tbx5 诱导心肌样细胞[33]。也有研究证实短期缺氧可提高心脏重编程效率[34]。

4. 展望与挑战

将成纤维细胞直接重编程为心肌样细胞已成为在心脏再生中具有吸引力的策略，但是该项技术仍存在许多挑战。第一，正如前文所说人类心脏成纤维细胞与小鼠存在异质性，然而很少有研究利用转录因子在成人心室成纤维细胞中进行测试，况且机体内衰老的成纤维细胞普遍存在慢性激活的炎症级联和坚定的表观遗传修饰，这对临床心脏重编程有着巨大阻力。第二，心肌细胞重编程的效率一直是难以解决的问题，更为高效的转录因子组合还有待发现。第三，各种研究对于心脏重编程效率没有统一的评价标准，无法对同类型研究做出横向对比，发掘出合适的评价系统也同样重要。尽管仍然存在众多挑战，但我们相信科学技术的发展定会解决心肌梗死后心力衰竭的难题，希望届时能为心肌梗死患者带来福音。

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