

干细胞来源外泌体 miRNA 介导心脏修复的研究进展

陈鹏莉 宋紫微 张曼玉 李丽丽

(哈尔滨医科大学附属第二医院心内科, 黑龙江 哈尔滨 150081)

【摘要】 在过去几十年中,急性心肌梗死采用开通罪犯冠状动脉和血运重建术等新技术已经显著改善了心肌梗死的预后,但仍有许多患者心肌梗死后出现不良的心脏重构及心力衰竭。随着心力衰竭的流行,急需一种新的治疗方法,无细胞疗法是一种很有前途的治疗方法,在多种急慢性心脏病中调节并促进心脏修复。现就干细胞来源外泌体微 RNA 在心肌损伤后介导心脏修复的研究进展进行综述。

【关键词】 干细胞;外泌体;心脏修复;药物递送;纳米医学

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Exosomal miRNA Derived from Stem Cell in Mediating Cardiac Repair

CHEN Pengli, SONG Ziwei, ZHANG Manyu, LI Lili

(Department of Cardiology, The Second Affiliated Hospital of Harbin Medical University, Harbin 150081, Heilongjiang, China)

【Abstract】 Although new technologies such as opening criminal coronary arteries and revascularization have markedly enhanced the prognosis of acute myocardial infarction in recent decades, several patients still have poor cardiovascular remodeling and heart failure after myocardial infarction. With the prevalence of heart failure, there is an urgent need for a new approach to treatment. Cellular-free therapy is a promising therapeutic approach that has prospects for regulating and promoting cardiac repair in a variety of acute and chronic heart diseases. This review examines progress of exosomal miRNA derived from stem cell in mediating cardiac repair after myocardial lesion.

【Key words】 Stem cell; Exosomal; Cardiac repair; Drug delivery; Nanomedicine

心肌梗死是临床上常见的心血管疾病,虽然心肌梗死的治疗在不断地发展与完善,但心肌梗死后的并发症仍是心血管疾病死亡的主要原因。目前,干细胞移植已被证实可改善心功能,并且主要是通过旁分泌途径起作用。但由于干细胞移植存在伦理、安全和致瘤等问题,使其在临床应用上受到限制^[1]。目前研究认为外泌体含有大量的蛋白质和非编码 RNA,可传递至心脏改善心功能^[2]。现重点介绍和讨论基于干细胞来源的外泌体微 RNA (microRNA, miRNA) 修复受损心脏的机制及当前治疗进展,有望为心血管疾病临床诊疗提供有效的新靶点和治疗方法。

1 外泌体

1.1 外泌体特征

外泌体是一种直径为 40 ~ 160 nm、具有脂质双分子膜结构的细胞分泌型囊泡^[3],几乎可被所有类型的细胞分泌。外泌体最早是在显微镜下观察到的培养细胞脱落的具有酶活性的囊泡^[4]。随着研究的深

入,人们发现这种囊泡携带丰富的物质,包括核酸[信使 RNA (messenger RNA, mRNA)、miRNA 和 DNA 等]、蛋白质、脂质和遗传因子。到目前为止,ExoCarta exosome 数据库收集了 9 769 种蛋白质、3 408 个 mRNA、2 838 个 miRNA 和 1 116 种脂质体^[5]。外泌体表面有大量的跨膜蛋白及细胞黏附分子,可与靶细胞结合及穿透靶细胞膜^[6]。

1.2 miRNA

非编码 RNA 是包含大多数人类转录组的功能性 RNA,大约 1.5% 的人类基因组被转录成 mRNA。在非编码 RNA 中,miRNA 被广泛研究,它是一类由内源性、保守的 21 ~ 23 个核苷酸组成的单链非编码 RNA。miRNA 绝大多数与靶 mRNA 的 3' 非翻译区结合,抑制 mRNA 翻译来调节特定基因表达^[7]。此外,miRNA 还具有超出常规的调节功能,通过调控转录和核内基因启动子和增强子的表观遗传状态,以及靶向线粒体编码转录物,影响基因表达^[8]。

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通信作者:李丽丽, E-mail: Lisister1980@163.com

2 外泌体介导心脏修复的机制

心脏内稳态是通过细胞间的复杂相互作用网络实现的,包括血管内皮细胞、心肌细胞、成纤维细胞和炎症细胞等,心脏受损后,稳态被破坏造成心脏适应性不良重构,而干细胞来源的外泌体可以靶向这些细胞减少病理性重构。以下将分别阐述外泌体修复受损心脏的过程。

2.1 内皮细胞

外泌体被证实携带 miRNA 作用于靶组织,调节内皮细胞功能和血管生成^[7]。Wu 等^[9]报告在小鼠心肌梗死模型中,人多能干细胞来源的外泌体通过将外泌体内的 miR-497 携带入内皮细胞内,促进血管生成。Zhu 等^[10]发现脂肪干细胞的外泌体通过输送靶向缺氧诱导因子 1 的 miR-31 促进了小鼠心脏的血管生成,这有利于心肌梗死后心脏修复。另外,在大鼠脂肪干细胞外泌体中筛选出 miR-196a-5p 和 miR-425-5p,在缺氧环境下促进血管内皮生成,机制可能与靶向血管生成相关的 Spred1、SEPT7 和 PDGFRA 基因有关^[11]。以上研究表明在心脏损伤时,外泌体可通过改善血管内皮细胞导致心脏新血管生成,进而促进心脏修复。

2.2 心肌细胞

心肌细胞是心脏的主要细胞,缺血 30 min 以上心肌细胞发生水肿和结构改变,出现进行性死亡,心肌细胞减少,最终导致心力衰竭。已有研究证实,人诱导性多能干细胞外泌体通过向心肌细胞内传递心脏保护性 miRNA 如 miR-21^[12] 和 miR-210^[13],可减轻心肌细胞的损伤。此外,在猪的心肌梗死模型中,来自人诱导多功能干细胞外泌体可减轻心脏肥大并改善心脏功能^[14]。Song 等^[15]的研究发现携带具有抗凋亡作用的 miR-21 的细胞外囊泡转移至心肌细胞后,可通过降低程序性细胞死亡因子 4 的表达,减少心肌细胞凋亡。

既往认为成年心肌细胞不具有分化再生能力,但是近年来通过心肌细胞谱系追踪和同位素标记分析表明,心肌细胞具有很弱的增殖能力,可通过自我增殖而再生^[16]。使用全基因组 miRNA 文库筛选出诱导心脏再生潜力 hsa-miR-199a^[17], Gabisonia 等^[18]将 AAV6-miR-199a 注射到心肌梗死后猪的左心室室壁,通过刺激去分化和增殖,另外心脏内注射 miR-19a/19b 模拟物可增强心肌细胞增殖并刺激心肌再生以减少心肌损伤^[19],这种转染疗法可通过脂质体,或包裹在脂质或聚合物纳米颗粒中进行,另一种可能性是利用外泌体优先装载核酸,但这项技术还不成熟,可能成为进一步发展的途径。

2.3 成纤维细胞

心肌梗死时,坏死区域需要瘢痕沉积防止心脏破

裂并限制功能恶化,然而过度的纤维化会破坏正常的结构,最终导致心力衰竭^[20]。含有 miR-24 的间充质干细胞来源的外泌体可能通过抑制抗增殖蛋白 2 和线粒体自噬途径降低成纤维细胞的迁移和增殖能力,从而减轻心肌纤维化^[21]。Sun 等^[22]在心肌梗死模型中将过表达 miR-221-3p 外泌体注射到梗死边界区,观察到心肌纤维化减轻。此外,miR-222 也被证实通过调节 Wnt/ β -catenin 介导的内皮向间质转化,改善糖尿病小鼠心脏的心肌纤维化^[23]。由此可见,外泌体可通过 miRNA 介导细胞间通信,通过抗心肌纤维化促进心脏修复。

2.4 炎症细胞

心肌梗死后坏死的心肌细胞释放各种细胞因子以诱导炎症反应,在心肌缺血早期,炎症反应会清除坏死的心肌细胞和碎片^[24]。携带 miR-223-3p 的间充质干细胞的细胞外囊泡调节树突细胞成熟并促进其抗炎潜力^[25],Zhao 等^[26]发现间充质干细胞来源外泌体 (mesenchymal stem cell-derived exosome, MSC-Exo) 通过 miR-182 改变巨噬细胞的极化状态,减轻小鼠心肌缺血再灌注损伤。MSC-Exo 还被证实通过 miR-181a 影响 JAK1-STAT1/c-Fos 信号通路,减弱树突细胞的免疫炎症反应,并保护心肌细胞在缺氧条件下免于细胞死亡^[27]。Xu 等^[28]研究经脂多糖诱导的骨髓 MSC-Exo 可通过抑制核因子- κ B 信号通路及部分激活了 Akt1/Akt2 信号通路,影响巨噬细胞亚型的转化来减轻炎症。这些研究结果表明外泌体 miRNA 参与心肌损伤时的炎症反应。

3 治疗

外泌体具有免疫原性低、生物降解性低、毒性低等优点,但外泌体的临床转化面临着大量生产、标准分离、药物装载、稳定性和质量控制等诸多问题^[29-30]。目前已提出一系列新策略,例如修饰基因表达和改善心脏特异性摄取,提高外泌体的治疗效率。

3.1 外泌体的基因修饰与化学修饰

为了将治疗性外泌体运送到靶细胞或组织,可采用被动或主动的靶向策略,被动靶向利用外泌体的自然细胞趋向性,而主动靶向则通过各种技术手段靶向输送。Li 等^[31]构建 CD9-HuR 融合蛋白,富集 miR-155 的特定 RNA,识别内源靶标在受体细胞中起作用。Wang 等^[32]利用设计了与缺血心肌靶向肽融合的外泌体富集膜蛋白,可特异性靶向缺血心肌。Vandergriff 等^[33]利用心脏归巢肽与外泌体结合靶向梗死心肌,有研究^[34]将组织基质金属蛋白酶抑制剂 2 修饰人脐带血 MSC-Exo,通过激活 Akt/Sfrp2 通路减轻心肌梗死诱导的氧化应激和细胞外基质重构改善心功能。外泌

体通过 CRISPR/Cas9 修饰技术实现对靶目标的基因编辑,而且开发了一种光触发脂质体递送系统,通过 690 nm LED 光束照射组织表面,使 CRISPR/Cas9 基因编辑获得高度的空间和时间控制^[35]。

3.2 外泌体包装

近年来,人工外泌体更具有药物可接受性,经过化学改良,采用薄膜水合法分组的湿化学方法用于多层囊泡的合成是最经典的。Hammons 等^[36]使用薄膜水合方法将二油酰基磷脂酰胆碱和聚氧乙烯溶解在氯仿中,经过水合、去膜制备混合仿生囊泡。为了在体内将外泌体精确递送到受体细胞,模拟单核细胞募集特征,Zhang 等^[37]获得了从巨噬细胞分离的单核细胞膜,然后与来自间充质干细胞的细胞外囊泡通过融合获得单核细胞模拟的间充质干细胞的细胞外囊泡,增强缺血再灌注损伤后的心脏修复。Zhao 等^[38]将外泌体封装在海藻酸钠-盐水凝胶中,以促进心肌等组织的血管生成。Zhu 等^[39]在心肌梗死的动物模型中,通过微创心包内注射含有干细胞源性外泌体的水凝胶,可减轻免疫反应并增加药物在心脏滞留。另外,将外泌体与血小板膜混合^[40],利用生物材料的微创外泌体喷雾剂^[41]等新技术可以靶向受损心脏,发挥促进心脏修复的作用。

4 总结与展望

综上所述,外泌体携带来自其原始细胞的选择性生物分子并将其传递给受体细胞促进心脏修复。外泌体代表一种有前途的无细胞治疗,无生命但具有生物活性的外泌体在心脏受损时发挥独特作用,并且通过体外培养的方式,方便作为药物制剂提供给药,这可能潜在地应用于心血管疾病治疗。

上述研究强调了外泌体用于心血管疾病治疗的机制及治疗进展,但心脏修复方法仍局限于临床前水平,小动物实验主要揭示特定分子机制,而大型动物更适合建立在涉及混杂因素的研究,来自心肌梗死的大型临床前模型的数据仍然缺乏。目前外泌体的研究处于起步阶段,在临床应用中面临很多挑战,未来仍需做更深入研究。

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