骨髓单个核细胞移植治疗心肌梗死的机制及临床研究现状

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摘要 心肌梗死(myocardial infarction, MI)是心血管疾病中导致患者死亡的主要病因。自体骨髓单个核细胞(bone marrow mononuclear cells, BMMNCs)因其具有易分离获得和无免疫排斥等优点, 成为治疗心肌梗死的候选种子细胞之一。前期动物实验已经证明, BMMNCs修复受损心肌的主要机制是通过促进梗死部位血管新生、调节免疫系统平衡以及分化为心肌细胞等, 从而改善缺血心肌的血液循环和收缩功能, 但在临床试验中的结论不尽一致。因此, 该文就BMMNCs在治疗心肌梗死的主要机制、临床研究现状以及影响BMMNCs治疗心肌梗死疗效的因素分别予以综述。

关键词 骨髓单个核细胞; 心肌梗死; 机制; 移植; 微环境

The Mechanism and Clinical Status of Bone Marrow Mononuclear Cell Therapy for Myocardial Infarction

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Abstract Myocardial infarction is a leading cause of death among all cardiovascular diseases. Bone marrow mononuclear cells (BMMNCs) have become one of the candidate seed cells in treatment of myocardial infarction because of its advantages of easy separation and no immune rejection. The previous experiments *in vivo* have proved the improved cardiac blood circulation and systolic function in animal models after BMMNCs transplantation by producing the secretions of growth factors and other proteins to promote angiogenesis, balancing the immunological system and stimulating cardiac muscle regeneration. Although the animal experiments have demonstrated the effectiveness of BMMNCs, the conflicting results have occurred in many clinical trials. Therefore, this article will respectively review the progress of the primary mechanism of BMMNCs, the clinical status of BMMNCs and the major influencing factors in the treatment of myocardial infarction.

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心肌梗死(myocardial infarction, MI)后左室瘢痕形成和心肌重构是导致心力衰竭的重要因素。早期的心脏重塑是心脏在异常缺血状态下的血液动力学变化, 即心脏成纤维细胞增殖是为保护心脏正常出血量的适应性改变。心脏重塑伴随着心肌细胞的肥大、坏死、凋亡和心肌成纤维细胞的增殖以及细胞外基质如I型胶原(约占心肌胶原80%)、III型胶原(约占心肌胶原11%)、糖蛋白、蛋白聚糖等水平的升高。其中, 基质金属蛋白酶抑制剂(tissue inhibitor of metalloproteinases, TIMPs)和降解胶原的基质金属蛋白酶(matrix metalloproteinases, MMPs)的调节失衡是影响细胞外基质重构的重要因素。心梗早期以细小的III型胶原填充修复, 后期以粗大的I型纤维为主, 增加了心肌的强硬度, 这种不同类型胶原纤维合成与降解的失衡加强了梗死后心肌的纤维化程度和疤痕形成[1]。传统治疗心肌梗死的手段主要包括: 心血管药物溶栓治疗(包括β受体阻滞剂和他汀类药物等)、内科介入及外科手术治疗等。这些手段均可以使血管再通, 使急性期死亡率下降, 暂时延长了病人寿命, 但是无法从根本上弥补丢失的心肌细胞。心脏移植作为最有效的治疗手段, 其面临的严峻问题就是供体有限、异体排斥、伦理问题和高昂的费用等, 从而限制了其在临床中的应用。近些年的研究表明, 干细胞具有的组织再生和旁分泌作用为心肌梗死的治疗带来了希望[2-4]。多数研究移植的干细胞是经体外传代培养之后再回输至患者体内, 而骨髓单个核细胞(bone marrow mononuclear cells, BMMNCs)是直接从患者骨髓中分离提取的单个核细胞群, 可最大程度保留其中包含的骨髓间充质干细胞、造血干细胞和内皮祖细胞等干细胞的干性。因而, BMMNCs成为干细胞移植治疗心肌梗死研究中的重要候选种子细胞之一, 具有广阔的应用前景。本文就BMMNCs的主要作用机制、临床研究现状以及影响BMMNCs治疗心肌梗死疗效的因素综述如下。

1 骨髓单个核细胞治疗心肌梗死的主要机制

骨髓单个核细胞是骨髓中具有单个细胞核的细胞总称, 其中含有骨髓间充质干细胞(bone marrow mesenchymal stem cells, BMSCs)、内皮祖细胞和造血干细胞(hematopoietic stem cells, HSCs)的异质性细胞群, 尽管多数BMMNCs不是干细胞, 但是其发挥修复心肌的作用主要由干细胞来完成, 尤其是造血干细胞和骨髓间充质干细胞。它们修复受损心肌的主要机制是以旁分泌或自分泌形式分泌促血管形成因子和免疫调节因子, 间接促进血管形成和调节机体免疫平衡, 也可通过分化成心肌细胞、血管内皮细胞和平滑肌细胞促进血管形成。

1.1 旁分泌作用

BMMNCs所包含的各种干细胞可分泌多种细胞因子, 如血管内皮生长因子(vascular endothelial growth factor, VEGF)、表皮生长因子(epidermal growth factor, EGF)、碱性成纤维细胞生长因子(fibroblast growth factor, FGF)、胰岛素样生长因子-1(insulin-like growth factor-1, IGF-1)、肝细胞生长因子(hepatocyte growth factor, HGF)、血小板源性生长因子(platelet-derived growth factor, PDGF)等[5-6], 在干细胞的增殖、趋化、分化和抗凋亡等过程中发挥重要作用。最近的研究表明, 心肌内注射BMSCs和心脏干细胞(cardiac stem cells, CSCs)至心肌梗死大鼠体内28 d时, 可使心脏射血分数从42.63%升高至59.73%, 心梗面积由31.42%降至18.87%, 心肌纤维化的比例由29.82%降至4.09%, 最终使心功能恢复和心肌重塑的改善, 显著优于单独移植BMSCs或CSCs组[7-8]。尽管只有很小比例的干细胞分化为心肌细胞或内皮细胞, 但BMSCs和CSCs共移植导致细胞因子(如VEGF、EGF、HGF和IGF)水平升高, 是显著提升移植干细胞在梗死心肌中增殖能力和滞留率并降低凋亡率的关键, 最终使共移植组心肌中供体干细胞数仅降低22.32%, 显著低于单独移植BMSCs或CSCs时干细胞比例(分别为70.84%和67.44%)[7-8]。将HGF/IGF与BMSCs共植入心肌梗死兔子体内4周后, HGF/IGF+BMSCs组与BMSCs组相比, 心肌中干细胞存活数目(D值分别为26 937 pixels/hpf和10 931 pixels/hpf)和转分化为心肌细胞的数目(分别为9.9 cell/hpf和2.6 cell/hpf, P<0.001)显著升高。同时, 利用体外实验证明, HGF可促进干细胞的分化和迁移作用, IGF能够促进BMSCs的增殖和迁移并抑制其凋亡, 这提示

HGF/IGF共移植BMSCs可抑制干细胞凋亡并促进其迁移和转分化作用[9]。心肌梗死大鼠实验提示, HGF除了促进BMSCs的转分化作用, 还将I型胶原和III胶原的含量降低1/3~1/2(P<0.001), 有效改善了心肌纤维化程度[10]。此外, 将BMMNCs植入心肌梗死动物体内可促进梗死区VEGF和FGF等因子的表达, 使梗死区血管密度升高约2倍[11-12]。由此可见, BMSCs可通过旁分泌作用诱导干细胞迁移并归巢至损伤心肌部位[13-14], 调节心肌代谢、抑制基质降解和细胞凋亡[15], 也可间接促进心肌细胞、血管内皮细胞和平滑肌细胞的增殖, 促进心脏干细胞在梗死心肌的归巢、增殖和分化能力[16]。可见, BMMNCs中的干细胞可作为生产和运输各种细胞因子的工具, 将其传送到动物体内, 从而发挥各种心肌保护作用[17]。也有证据表明, 利用细胞因子基因修饰的干细胞可有效改善心功能和心肌重塑[18]。由此可见, BMMNCs通过旁分泌作用对梗死心肌的修复是一个复杂、多效的信号调节机制, 通过基因修饰或药物刺激等方式可有效提高某些特定细胞因子基因表达和分泌, 实现受损心肌和血管的修复以及达到改善心肌重塑的目的。

1.2 外泌体机制

干细胞源外泌体(exosome)是在特定的应激条件下分泌释放的、可从干细胞培养液中提取获得的、直径在30~100 nm的膜性小囊泡。其中, 含有多种与细胞生物学功能密切相关的蛋白质分子、脂质分子、细胞特异性mRNA、细胞特异性miRNA及信号分子等, 是干细胞旁分泌效应的特殊形式。它可避免某些大分子蛋白质不易通过细胞膜屏障的困难, 使其能顺利地通过细胞膜, 直接或间接参与多种细胞生物学功能的调节[19-20]。BMSCs来源外泌体能够促进急性心肌梗死(acute myocardial infarction, AMI)患者梗死心肌区毛细血管密度的升高[21-22], 抑制I型和III型胶原在心脏的沉积以及MMP-1和TIMP-1的表达, 并且降低促炎因子肿瘤坏死因子-α(tumor necrosis factor-α, TNF-α)、白介素-1b(interleukin-1b, IL-1b)和IL-6的表达, 从而抑制心脏基质的降解和炎症程度, 最终达到抑制MI患者心肌纤维化并有效改善心功能的目的[23]。CD34造血干细胞来源的外泌体能够通过增加内皮细胞的活性、增殖和管状结构的形成而促进心肌梗死区的血管形成[24]。还有研究表明, 缺血预处理的BMSCs外泌体含大量miR-22, 可通过下调心肌细胞甲基CpG结合蛋白的表达而抑制心肌细胞凋亡[25]。将锌指转录因子-4(GATA binding factor-4, *GATA-4*)基因转入BMSCs可促进BMSCs外泌体中miR-19a水平, 而miR-19a可减少靶蛋白PTEN的表达并激活丝氨酸/苏氨酸蛋白激酶(serine/threonine-protein kinases)和细胞外调节蛋白激酶(extracellular regulated protein kinase, Erk)信号途径, 最终达到保护心肌的目的[26]。由此可见, 干细胞外泌体的特性能够代表相应干细胞的基本功能, 缺血或缺氧等条件预处理、抗凋亡基因或miRNA的转入均可促进干细胞对梗死微环境的适应和对心肌的保护作用提高。目前对于干细胞外泌体修复梗死心肌机制的研究还处于初期阶段, 外泌体中复杂成分如何分析及其作用机制如何解析是面临的重要问题, 或许在体外特殊条件下获得的干细胞外泌体将会替代干细胞移植, 发挥心脏修复的作用。因此, 干细胞源外泌体中包含的某些分子是细胞间信号传递的重要介质, 在心肌梗死后对心肌的保护中起着至关重要的作用, 有望为梗死心肌的再生修复治疗提供新策略。

1.3 调节炎症平衡

急性心梗后促炎因子, 如IL-1-ra和TNF-α的升高与细胞凋亡和射血分数下降有关, 且该炎症期与心室重塑和适宜的自修复功能相关[27], 而持久的促炎作用就会导致不利的心室重塑和心脏畸形的发生[28]。研究表明, BMSCs的植入能够降低心梗患者促炎因子(如IL-6、IL-1β、TNF-α、IFN-γ)的水平, 促进抗炎因子(如IL-10)的水平升高, 从而有效升高射血分数, 降低左室舒张末期内径、左室舒张末期容积和左心室舒张末压(P<0.05)[23]。该作用可能与通过MSCs植入后导致的M2型巨噬细胞升高从而使其分泌的抗炎因子水平升高有关[29]。可见, 抗炎与促炎因子的平衡在心肌修复的过程中发挥着关键作用。Alestalo等[30]对BMMNCs移植后2 d和4 d的促炎因子(IL-6、TNF-α、IL-1b、IL-1-ra、IFN-γ)和抗炎因子(IL-4、IL-10、IL-13)的分泌水平分别进行测量发现, BMMNCs治疗组在各个时间点的抗炎和促炎因子的Kendall's tau值均为0.7(P=0.001), 而与对照组相差很大(分别为Kendall's tau 0.6, P=0.01和Kendall's tau 0.3, P=0.17), 说明BMMNCs治疗组的抗炎因子与促炎因子有很大的相关性, 处于相对平衡状态。可见BMMNCs植入AMI后可使抗炎和促炎因子的分泌保

持平衡, 这是BMMNCs治疗AMI的重要机制之一。

1.4 转分化作用

前期研究表明, 骨髓来源干细胞可被5-AZA等因子诱导分化为心肌样细胞[31-32]。间充质干细胞、造血干细胞和内皮祖细胞均具有分化为心肌细胞、内皮细胞和平滑肌细胞的能力[33], 达到改善心功能的目的[34]。据报道, BMSCs在心肌梗死动物体内的转分化效率极低(<1%), 尽管经药物(如吡格列酮、坎地沙坦和5-氮杂胞苷等)处理后或细胞因子(FGF、IGF-1和骨形成蛋白2等)刺激后, 可将转分化效率提高2~3倍[35-38], 但目前并不是其发挥心肌修复的重要机制。另外一项研究表明, BMMNCs修复心肌的机制是通过促进血管生成相关因子的表达而促进血管新生, 并对BMMNCs分化为心肌细胞这一机制提出质疑[39]。近些年报道显示, BMMNCs对梗死心肌的修复主要通过旁分泌作用实现, BMMNCs分泌的多种细胞因子可直接或间接促进血管生成、抑制心肌细胞凋亡、调节心肌炎症系统平衡等。因此, BMMNCs修复梗死心肌的机制还需要在动物体内做进一步验证。

2 BMMNCs治疗心肌梗死的临床研究进展

自从Strauer等[40]证明BMMNCs在临床应用的可能性后, BMMNCs移植便广泛应用于治疗心肌梗死的临床研究中。Jeevanantham等[41]对50个研究中的2 625例患者(包括急性心梗和慢性缺血性心脏病患者)进行了系统全面的分析。分析结果显示, 骨髓单个核细胞移植组较对照组更能显著升高左室射血分数(left ventricular ejection fraction, LVEF)(3.96%), 降低梗死面积(–4.03%)、左室收缩末期容积(–8.91 mL)和舒张末期容积(–5.23 mL)(P<0.000 01), 最终改善左室功能和缓解心室重塑, 而且减少死亡事件、心梗事件以及支架内血栓形成事件的发生率。这种改善作用与2014年的另一个Meta分析结果[42]一致。2014年的这项研究对32项临床研究中的2 306例急性冠脉综合征或稳定型冠心病受试患者进行了分析。结果显示, BMMNCs治疗组较对照组LVEF分别升高4.6%(P<0.001)和2.8%(P=0.001), 灌注后心肌损伤面积分别减小9.5%(P<0.001)和3.8%(P=0.002)[42], 证实了BMMNCs具备治疗心梗的潜在价值。尽管有些研究表明, BMMNCs对心肌梗死的改善作用非常微弱甚至无显著作用[6]。但是值得肯定的是, BMMNCs对MI的治疗并没有严重的副作用(如心律失常和心血管再狭窄等)。导致治疗效果不一致或显著程度有差别的结果与多种因素相关, 包括移植方式、移植时间、细胞的分离程序等。讨论特定条件下BMMNCs对MI的改善情况, 能够更针对性地分析不同因素对疗效的影响, 也将为今后的研究提供理论依据。

2.1 移植途径

用于BMMNCs治疗心肌梗死的移植途径主要有冠脉注射、心肌内注射和静脉注射三种方式, 冠脉注射BMMNCs是最常用的移植方式。冠脉注射BMMNCs的临床随机对照实验证明, BMMNCs可显著减少血液中IL-1β和TNF-α的水平, 而它们与心功能的严重程度成正比[43], 冠脉植入BMMNCs可显著提高左室射血分数和改善心肌重塑, 减少心血管事件的发生[44-47], 因此, 这些实验已经表明BMMNCs治疗心肌梗死的可行性与安全性。2012年, Ye等[48]对10项临床试验涵盖757例心肌梗死患者的BMMNCs治疗研究结果进行了Meta分析, 发现冠脉注射BMMNCs可以有效提升LVEF(4.04%, P<0.01)、降低心肌梗死面积(–2.47%, P=0.000 2)和左室舒张末期容积(6.13 mL, P=0.007), 从而能够有效改善左心室功能和心脏重塑, 而且没有导致主要不良反应事件的发生。2014年, 另一项更大规模的涵括16项研究1 641例ST-段升高的心肌梗死患者的Meta分析表明, BMMNCs能够显著减少左室舒张末容积指数(–3.17 mL/m², P<0.01)和左室收缩末期容量指数(–3.17 mL/m², P<0.01), 且LVEF在55岁以下的较年轻患者(3.38%, P=0.03)的改善较55岁以上患者(1.77%, P=0.03)更显著。在LVEF<40%的患者的升高(5.30%, P<0.001)较LVEF≥40%的患者(1.45%, P<0.001)更明显, 可见冠脉注射BMMNCs能够改善MI患者的左室心功能和心脏重塑[49]。近期的一项BONAMI临床试验同样证明, 冠脉注射自体BMMNCs 3个月至1年有效提高了患者的生活质量, 尽管在1年的随访期内没有观测到心功能的显著改善[50]。可见, 冠脉注射BMMNCs治疗心肌梗死的疗效和可行性是毋庸置疑的, 但是注入细胞归巢于心肌梗死部位的数量有限, 这也是限制其疗效发挥的重要因素。

近几年, 心肌内注射的方式移植BMMNCs越来越受到关注。Charwat等[3]研究表明, 心肌梗死患者接受经心肌内注射的自体骨髓单个核细胞3个月后

可将患者心脏射血分数从38.0%升至41.5%(P<0.001), 心梗面积从27.2%减至24.1%(P<0.001), 心肌活力指数(单相电压)从7.9 mV增加至9.9 mV(P<0.001), 局部室壁运动距离指数从11.0%提升到12.7%(P=0.01)。虽然9个随机临床试验的Meta分析显示, BMMNCs经心内膜移植后的的治疗效果并不显著[51], 但是并不能否定心内膜移植BMMNCs的治疗效果, 因为细胞治疗的效果与多种因素相关, 这就提示研究者们要用更大规模的临床试验并进行长时间的随访, conger确定该方法的可行性。最近的一项临床研究显示, 经皮冠状动脉联合心肌内注射BMMNCs后对心肌梗死的改善效果可以持续到治疗后5年(左室EF 5.7%、右室EF 8.4%), 且该效果与随访1年时(左室EF 5.3%、右室EF 5.4%)的效果相差不多, 可见BMMNCs的疗效具有持久性的特点[52]。

静脉注射是最方便和安全的移植方法, 羊镇宇等[53]研究表明, 经静脉注射BMMNCs 1个月后, 移植组LVEF由术前的37.26%升到54.42%(P<0.05), 而对照组差异不显著(38.86% *vs* 40.28%, P>0.05), 移植组术后6个月与术前相比LVEF差异不显著, 但并未加重疾病严重程度, 而对照组患者心脏呈现扩大趋势。尽管目前对于静脉注射疗效并没有前两种方法疗效显著, 但静脉注射BMMNCs具有其自身的优点, 尤其对一些特殊患者, 如病情严重或年老心梗患者的治疗更为安全, 然而该方法的有效性还需要临床试验进一步验证。因此, 对不同程度心肌梗死的患者选择最适宜的移植方式, 将为BMMNCs在临床治疗中的应用提供合理的方案。

2.2 移植时间

BMMNCs移植时间从急性心梗发生后几天到几周均已被研究, 心梗后5~7 d和3~4周分别将BMMNCs植入200例ST段升高的心肌梗死患者, 在4个月[54]和12个月[55]时没有使AMI患者的心功能得到显著性改善。而另外一项研究表明, 冠脉植入BMMNCs至心梗患者12个月时与对照组相比, 介入术后24 h内和3~7 d时移植BMMNCs均可显著提高LVEF(分别为7.9%和6.9%, P<0.001)和降低左心室收缩末期容积(分别为–20.5 mL和–19.6 mL, P<0.001), 而对照组和介入术后7~30 d移植BMMNCs组并没有显著改善MI患者LVEF(分别为3.4%和4.7%)和降低左心室收缩末期容积(分别为–6.4 mL和–9.4 mL)。这表明, 介入术后7 d以内移植BMMNCs较7 d以后移植更能有效改善MI患者心功能和心室重塑状态[56]。而Ronak Delewi等[49]的Meta-analysis表明, 经皮冠状动脉介入术后小于7 d和大于7 d移植BMMNCs对LVEF的改善没有显著差别(分别为1.46%和2.69%, P=0.08)。可见BMMNCs的植入时间并不确定, 而需要更大规模的临床试验[如正在进行的BAMI试验(NCT 01569178)]进一步证明在移植术后2年是否能观察到BMMNCs的有效性。

2.3 移植数目

除以上因素外, 移植细胞的数量与BMMNCs治疗MI的疗效密切相关。Ye等[48]进行的Meta-analysis表明, >1×10^{8}个BMMNCs较<1×10^{8}个细胞的治疗的LVEF升高更明显(分别为4.20%和3.27%, P<0.01)、心肌梗死面积减少更显著(分别为–3.37和–2.77, P<0.01)。BMMNCs移植后LVEF在1年和2年分别升高4.00%和4.52%(P<0.01), 较3~5年时升高的3.51%(P<0.01)更显著, 心肌梗死面积在治疗1年时(–3.57, P<0.01)较2年(–3.00, P=0.10)和3~5年(–0.58, P=0.74)降低更明显。而另一项分析表明, BMMNCs移植数量的多数与LVEF的改变并没有直接关系[49]。Messori也对细胞数目对LVEF影响的结构提出质疑[57], 且随访时间由短期几个月至几年所得的结果也不一致。这就需要大规模的基础或临床试验在规范前提研究条件的基础上, 开展更详细、严格的研究。由此可见, 细胞治疗是一项非常复杂的过程, 只有将所有条件最佳化和规范化才有可能得到有意义的评价结果, 这对今后临床应用提供重要的参考意义。

2.4 细胞制备方法

细胞制备技术对BMMNCs的治疗效果也至关重要, 不同的制备流程和试剂组分对BMMNCs中干细胞的活性和比例均具有较大影响[58]。分离过程中红细胞污染同样降低细胞活性、迁移能力、集落形成和血管形成的能力等, 而这些能力正是BMMNCs疗效发挥的重要方面[59]。因此, 规范、合理、成熟、稳定的细胞制备技术对BMMNCs治疗心肌梗死的疗效非常重要。

3 心脏微环境对BMMNCs治疗心肌梗死效果的影响及处理措施

虽然干细胞移植治疗心肌梗死已经取得了一定成效, 但疗效的不稳定和不显著还与移植干细胞的低驻留率和低存活率密切相关[60]。因此, 如何提

高移植细胞的驻留率和存活率是解决这一问题的关键。

据报道, 细胞外基质分子、纳米纤维、透明质酸水凝胶及纤维蛋白胶与BMMNCs共同移植入心梗模型动物后, 可将BMMNCs固定在疤痕及其周边组织部位, 防止大量流失, 并且基质当中含有的某些成分可促进干细胞的增殖、分化或黏附能力等, 能有效改善治疗效果[61-63]。然而, 干细胞的存活率并没有在移植后得到显著改善, 因为缺血部位血流中断导致移植的干细胞处在缺血和缺氧微环境中, 干细胞的存活率受到极大影响。目前, 解决这一问题的策略主要包括使用药物[64-66]或细胞因子[67-69]处理、抗凋亡基因修饰[70-71]或多种干细胞共移植[8,72-73]等。由此可见, 干细胞移植治疗心肌梗死的临床研究还处于初期阶段, 仍有许多问题需要解决和探讨。

4 结语与展望

BMMNCs是含有多种细胞成分的单个核细胞群, 分离后可直接植入患者体内, 避免了体外扩增带来的干细胞分化能力和迁移能力的下降, 保留了其最初始的干性, 且自体BMMNCs不存在免疫排斥反应, 是临床研究中的最佳选择之一。虽然BMMNCs移植治疗心肌梗死安全可靠, 但是在最终疗效方面还存在一些争议, 这与多种因素如移植的BMMNCs数目、移植方法和时间以及与梗死心肌微环境等密切相关。近些年, 将传统治疗方法和干细胞移植结合起来协同改善心脏功能已经越来越受到重视。临床中常用的他汀类药物(阿托伐他汀、辛伐他汀和瑞舒伐他汀等)[74-75]和通心络[76]等可有效改善干细胞的归巢能力、抗凋亡能力以及促进血管新生的作用, 结合冠脉手术或心肌注射方法移植干细胞, 既有益于患者的疾病恢复又能促进干细胞功能效应的发挥。因此, BMMNCs与药物或手术联合治疗心肌梗死疗效的评价还需要许多基础和大规模临床试验进一步验证。在基础方面主要包括干细胞归巢、干细胞抗凋亡和抗炎以及促血管形成等机制研究。同时, 开发一些适用于临床的有效保护心肌和干细胞的药物, 再利用大规模、多中心的临床试验进一步验证该药物在辅助提升BMMNCs治疗心肌梗死的疗效中是否发挥重要作用, 将会进一步改善BMMNCs治疗心肌梗死的疗效, 也将为心肌梗死患者的治疗带来新的希望。

参考文献 (References)

1 Sun Y, Zhang JQ, Zhang J, Lamparter S. Cardiac remodeling by fibrous tissue after infarction in rats. J Lab Clin Med 2000; 135(4): 316-23.

2 Kovacic JC, Fuster V. Cell therapy for patients with acute myocardial infarction: Accrued evidence to date. Circ Res 2015; 116(8): 1287-90.

3 Charwat S, Lang I, Dettke M, Graf S, Nyolczas N, Hemetsberger R, *et al*. Effect of intramyocardial delivery of autologous bone marrow mononuclear stem cells on the regional myocardial perfusion. NOGA-guided subanalysis of the MYSTAR prospective randomised study. Thromb Haemost 2010; 103(3): 564-71.

4 Luo J, Lee SO, Cui Y, Yang R, Li L, Chang C. Infiltrating bone marrow mesenchymal stem cells (BM-MSCs) increase prostate cancer cell invasion via altering the CCL5/HIF2alpha/androgen receptor signals. Oncotarget 2015; 6(29): 27555-65.

5 Fedak PW. Paracrine effects of cell transplantation: Modifying ventricular remodeling in the failing heart. Semin Thorac Cardiovasc Surg 2008; 20(2): 87-93.

6 Lionetti V, Bianchi G, Recchia FA, Ventura C. Control of autocrine and paracrine myocardial signals: An emerging therapeutic strategy in heart failure. Heart Fail Rev 2010; 15(6): 531-42.

7 Bao L, Meng Q, Li Y, Deng S, Yu Z, Liu Z, *et al*. C-kit positive cardiac stem cells and bone marrow-derived mesenchymal stem cells synergistically enhance angiogenesis and improve cardiac function after myocardial infarction in a paracrine manner. J Card Fail 2017; 23(5): 403-15.

8 Williams AR, Hatzistergos KE, Addicott B, Mc Call F, Carvalho D, Suncion V, *et al*. Enhanced effect of combining human cardiac stem cells and bone marrow mesenchymal stem cells to reduce infarct size and to restore cardiac function after myocardial infarction. Circulation 2013; 127(2): 213-23.

9 Zhang GW, Gu TX, Guan XY, Sun XJ, Qi X, Li XY, *et al*. HGF and IGF-1 promote protective effects of allogeneic BMSC transplantation in rabbit model of acute myocardial infarction. Cell Prolif 2015; 48(6): 661-70.

10 Chen H, Xia R, Li Z, Zhang L, Xia C, Ai H, *et al*. Mesenchymal stem cells combined with hepatocyte growth factor therapy for attenuating ischaemic myocardial fibrosis: Assessment using multimodal molecular imaging. Sci Rep 2016; 6: 33700.

11 Lin GS, Lu JJ, Jiang XJ, Li XY, Li GS. Autologous transplantation of bone marrow mononuclear cells improved heart function after myocardial infarction. Acta Pharmacol Sin 2004; 25(7): 876-86.

12 唐 洁, 陈 涛, 米 杰, 徐爱国, 王勇德, 张 健, 等. 骨髓单个核细胞移植后心肌梗死区的血管生成及细胞因子分泌. 中国组织工程研究(Tang Jie, Chen Tao, Mi Jie, Xu Aiguo, Wang Yongde, Zhang Jian, *et al*. Effect of bone marrow mononuclear cell transplantation on angiogenesis and expression of cytokines following myocardial infarction. Chinese Journal of Tissue Engineering Research) 2013; 17(32): 5840-6.

13 Makela J, Ylitalo K, Lehtonen S, Dahlbacka S, Niemela E, Kiviluoma K, *et al*. Bone marrow-derived mononuclear cell transplantation improves myocardial recovery by enhancing cellular recruitment and differentiation at the infarction site. J

Thorac Cardiovasc Surg 2007; 134(3): 565-73.
14 Makela J, Anttila V, Ylitalo K, Takalo R, Lehtonen S, Makikallio T, *et al*. Acute homing of bone marrow-derived mononuclear cells in intramyocardial *vs*. intracoronary transplantation. Scand Cardiovasc J 2009; 43(6): 366-73.
15 Cai M, Shen R, Song L, Lu M, Wang J, Zhao S, *et al*. Bone marrow mesenchymal stem cells (BM-MSCs) improve heart function in swine myocardial infarction model through paracrine effects. Sci Rep 2016; 6: 28250.
16 Liu N, Qi X, Han Z, Liang L, Kong D, Han Z, *et al*. Bone marrow is a reservoir for cardiac resident stem cells. Sci Rep 2016; 6: 28739.
17 D'Souza N, Rossignoli F, Golinelli G, Grisendi G, Spano C, Candini O, *et al*. Mesenchymal stem/stromal cells as a delivery platform in cell and gene therapies. BMC Med 2015; 13: 186.
18 Li N, Pasha Z, Ashraf M. Reversal of ischemic cardiomyopathy with Sca-1$^+$ stem cells modified with multiple growth factors. PLoS One 2014; 9(4): e93645.
19 Feng Y, Lu SH, Wang X, Cui JJ, Li X, Du WJ, *et al*. Biological characteristics of exosomes secreted by human bone marrow mesenchymal stem cells. Zhongguo Shi Yan Xue Ye Xue Za Zhi 2014; 22(3): 595-9.
20 Sun D, Zhuang X, Zhang S, Deng ZB, Grizzle W, Miller D, *et al*. Exosomes are endogenous nanoparticles that can deliver biological information between cells. Adv Drug Deliv Rev 2013; 65(3): 342-7.
21 Teng X, Chen L, Chen W, Yang J, Yang Z, Shen Z. Mesenchymal stem cell-derived exosomes improve the microenvironment of infarcted myocardium contributing to angiogenesis and anti-inflammation. Cell Physiol Biochem 2015; 37(6): 2415-24.
22 Bian S, Zhang L, Duan L, Wang X, Min Y, Yu H. Extracellular vesicles derived from human bone marrow mesenchymal stem cells promote angiogenesis in a rat myocardial infarction model. J Mol Med (Berl) 2014; 92(4): 387-97.
23 Guo J, Lin GS, Bao CY, Hu ZM, Hu MY. Anti-inflammation role for mesenchymal stem cells transplantation in myocardial infarction. Inflammation 2007; 30(3/4): 97-104.
24 Sahoo S, Klychko E, Thorne T, Misener S, Schultz KM, Millay M, *et al*. Exosomes from human CD34$^+$ stem cells mediate their proangiogenic paracrine activity. Circ Res 2011; 109(7): 724-8.
25 Feng Y, Huang W, Wani M, Yu X, Ashraf M. Ischemic preconditioning potentiates the protective effect of stem cells through secretion of exosomes by targeting Mecp2 via miR-22. PLoS One 2014; 9(2): e88685.
26 Yu B, Kim HW, Gong M, Wang J, Millard RW, Wang Y, *et al*. Exosomes secreted from GATA-4 overexpressing mesenchymal stem cells serve as a reservoir of anti-apoptotic microRNAs for cardioprotection. Int J Cardiol 2015; 182: 349-60.
27 Frangogiannis NG. Regulation of the inflammatory response in cardiac repair. Circ Res 2012; 110(1): 159-73.
28 Frangogiannis NG, Smith CW, Entman ML. The inflammatory response in myocardial infarction. Cardiovasc Res 2002; 53(1): 31-47.
29 Ben-Mordechai T, Holbova R, Landa-Rouben N, Harel-Adar T, Feinberg MS, Abd Elrahman I, *et al*. Macrophage subpopulations are essential for infarct repair with and without stem cell therapy. J Am Coll Cardiol 2013; 62(20): 1890-901.
30 Alestalo K, Miettinen JA, Vuolteenaho O, Huikuri H, Lehenkari P. Bone marrow mononuclear cell transplantation restores inflammatory balance of cytokines after ST segment elevation myocardial infarction. PLoS One 2015; 10(12): e0145094.
31 Hou J, Lu AL, Liu BW, Xing YJ, Da J, Hou ZL, *et al*. Combination of BMP-2 and 5-AZA is advantageous in rat bone marrow-derived mesenchymal stem cells differentiation into cardiomyocytes. Cell Biol Int 2013; 37(12): 1291-9.
32 Xing Y, Lv A, Wang L, Yan X. The combination of angiotensin II and 5-azacytidine promotes cardiomyocyte differentiation of rat bone marrow mesenchymal stem cells. Mol Cell Biochem 2012; 360(1/2): 279-87.
33 Guan K, Hasenfuss G. Do stem cells in the heart truly differentiate into cardiomyocytes? J Mol Cell Cardiol 2007; 43(4): 377-87.
34 Orlic D, Kajstura J, Chimenti S, Bodine DM, Leri A, Anversa P. Bone marrow stem cells regenerate infarcted myocardium. Pediatr Transplant 2003; 7 Suppl 3: 86-8.
35 Shinmura D, Togashi I, Miyoshi S, Nishiyama N, Hida N, Tsuji H, *et al*. Pretreatment of human mesenchymal stem cells with pioglitazone improved efficiency of cardiomyogenic transdifferentiation and cardiac function. Stem Cells 2011; 29(2): 357-66.
36 Bartunek J, Croissant JD, Wijns W, Gofflot S, de Lavareille A, Vanderheyden M, *et al*. Pretreatment of adult bone marrow mesenchymal stem cells with cardiomyogenic growth factors and repair of the chronically infarcted myocardium. Am J Physiol Heart Circ Physiol 2007; 292(2): H1095-104.
37 Numasawa Y, Kimura T, Miyoshi S, Nishiyama N, Hida N, Tsuji H, *et al*. Treatment of human mesenchymal stem cells with angiotensin receptor blocker improved efficiency of cardiomyogenic transdifferentiation and improved cardiac function via angiogenesis. Stem Cells 2011; 29(9): 1405-14.
38 Ling SK, Wang R, Dai ZQ, Nie JL, Wang HH, Tan YJ, *et al*. Pretreatment of rat bone marrow mesenchymal stem cells with a combination of hypergravity and 5-azacytidine enhances therapeutic efficacy for myocardial infarction. Biotechnol Prog 2011; 27(2): 473-82.
39 Agbulut O, Mazo M, Bressolle C, Gutierrez M, Azarnoush K, Sabbah L, *et al*. Can bone marrow-derived multipotent adult progenitor cells regenerate infarcted myocardium? Cardiovasc Res 2006; 72(1): 175-83.
40 Strauer BE, Brehm M, Zeus T, Gattermann N, Hernandez A, Sorg RV, *et al*. Intracoronary, human autologous stem cell transplantation for myocardial regeneration following myocardial infarction. Dtsch Med Wochenschr 2001; 126(34/35): 932-8.
41 Jeevanantham V, Butler M, Saad A, Abdel-Latif A, Zuba-Surma EK, Dawn B. Adult bone marrow cell therapy improves survival and induces long-term improvement in cardiac parameters: A systematic review and meta-analysis. Circulation 2012; 126(5): 551-68.
42 Sadat K, Ather S, Aljaroudi W, Heo J, Iskandrian AE, Hage FG. The effect of bone marrow mononuclear stem cell therapy on left ventricular function and myocardial perfusion. J Nucl Cardiol 2014; 21(2): 351-67.
43 Karpov RS, Popov SV, Markov VA, Suslova TE, Ryabov VV, Poponina YS, *et al*. Autologous mononuclear bone marrow cells

during reparative regeneratrion after acute myocardial infarction. Bull Exp Biol Med 2005; 140(5): 640-3.

44 Assmus B, Rolf A, Erbs S, Elsasser A, Haberbosch W, Hambrecht R, *et al*. Clinical outcome 2 years after intracoronary administration of bone marrow-derived progenitor cells in acute myocardial infarction. Circ Heart Fail 2010; 3(1): 89-96.

45 Dill T, Schachinger V, Rolf A, Mollmann S, Thiele H, Tillmanns H, *et al*. Intracoronary administration of bone marrow-derived progenitor cells improves left ventricular function in patients at risk for adverse remodeling after acute ST-segment elevation myocardial infarction: Results of the reinfusion of enriched progenitor cells and infarct remodeling in acute myocardial infarction study (REPAIR-AMI) cardiac magnetic resonance imaging substudy. Am Heart J 2009; 157(3): 541-7.

46 Santoso T, Siu CW, Irawan C, Chan WS, Alwi I, Yiu KH, *et al*. Endomyocardial implantation of autologous bone marrow mononuclear cells in advanced ischemic heart failure: A randomized placebo-controlled trial (END-HF). J Cardiovasc Transl Res 2014; 7(6): 545-52.

47 Heldman AW, DiFede DL, Fishman JE, Zambrano JP, Trachtenberg BH, Karantalis V, *et al*. Transendocardial mesenchymal stem cells and mononuclear bone marrow cells for ischemic cardiomyopathy: the TAC-HFT randomized trial. JAMA 2014; 311(1): 62-73.

48 Ye Z, Zhang BL, Zhao XX, Qin YW, Wu H, Cao J, *et al*. Intracoronary infusion of bone marrow-derived mononuclear cells contributes to longstanding improvements of left ventricular performance and remodelling after acute myocardial infarction: A meta-analysis. Heart Lung Circ 2012; 21(11): 725-33.

49 Delewi R, Hirsch A, Tijssen JG, Schachinger V, Wojakowski W, Roncalli J, *et al*. Impact of intracoronary bone marrow cell therapy on left ventricular function in the setting of ST-segment elevation myocardial infarction: A collaborative meta-analysis. Eur Heart J 2014; 35(15): 989-98.

50 Lamirault G, de Bock E, Sebille V, Delasalle B, Roncalli J, Susen S, *et al*. Sustained quality of life improvement after intracoronary injection of autologous bone marrow cells in the setting of acute myocardial infarction: Results from the BONAMI trial. Qual Life Res 2017; 26(1): 121-5.

51 Zhu K, Li J, Wang Y, Luo J, Zhang W, Guo C, *et al*. Intramyocardial autologous bone marrow-derived stem cells injection for ischemic heart disease ineligible for revascularization: A systematic review and meta-analysis. Arch Med Res 2015; 46(4): 286-95.

52 Gyongyosi M, Giurgea GA, Syeda B, Charwat S, Marzluf B, Mascherbauer J, *et al*. Long-term outcome of combined (percutaneous intramyocardial and intracoronary) application of autologous bone marrow mononuclear cells post myocardial infarction: the 5-Year MYSTAR study. PLoS One 2016; 11(10): e0164908.

53 羊镇宇, 钱大均, 吴小庆, 王如兴, 薄小平, 王 强, 等. 经静脉心肌超声造影对心肌梗死患者自体骨髓单个核细胞移植后心肌微循环的评价. 中国微循环(Yang Zhenyu, Qian Dajun, Wu Xiaoxing, Wang Ruxing, Bo Xiaoping, Wang Qiang, *et al*. Evaluation of myocardial mircrocirculation aafter transplantation of autologous bone marrow mononuclear cell in patients with acute myocardial infarction by intravenous myocardial contrast echocardiography. Journal of Chinese Microcirculation) 2009; 13(2): 197-9.

54 Surder D, Manka R, Lo Cicero V, Moccetti T, Rufibach K, Soncin S, *et al*. Intracoronary injection of bone marrow-derived mononuclear cells early or late after acute myocardial infarction: Effects on global left ventricular function. Circulation 2013; 127(19): 1968-79.

55 Surder D, Manka R, Moccetti T, Lo Cicero V, Emmert MY, Klersy C, *et al*. Effect of bone marrow-derived mononuclear cell treatment, early or late after acute myocardial infarction: Twelve months CMR and long-term clinical results. Circ Res 2016; 119(3): 481-90.

56 Huang R, Yao K, Sun A, Qian J, Ge L, Zhang Y, *et al*. Timing for intracoronary administration of bone marrow mononuclear cells after acute ST-elevation myocardial infarction: A pilot study. Stem Cell Res Ther 2015; 6: 112.

57 Messori A, Fadda V, Maratea D, Trippoli S. Intracoronary infusion of bone-marrow derived mononuclear cells in acute myocardial infarction: Are outcomes influenced by the number of infused cells? Heart Lung Circ 2013; 22(9): 786-7.

58 Seeger FH, Tonn T, Krzossok N, Zeiher AM, Dimmeler S. Cell isolation procedures matter: A comparison of different isolation protocols of bone marrow mononuclear cells used for cell therapy in patients with acute myocardial infarction. Eur Heart J 2007; 28(6): 766-72.

59 Lapi D, Vagnani S, Sapio D, Mastantuono T, Boscia F, Pignataro G, *et al*. Effects of bone marrow mesenchymal stem cells (BM-MSCs) on rat pial microvascular remodeling after transient middle cerebral artery occlusion. Front Cell Neurosci 2015; 9: 329.

60 Kurtz A. Mesenchymal stem cell delivery routes and fate. Int J Stem Cells 2008; 1(1): 1-7.

61 Schmuck EG, Mulligan JD, Ertel RL, Kouris NA, Ogle BM, Raval AN, *et al*. Cardiac fibroblast-derived 3D extracellular matrix seeded with mesenchymal stem cells as a novel device to transfer cells to the ischemic myocardium. Cardiovasc Eng Technol 2014; 5(1): 119-31.

62 Santhakumar R, Vidyasekar P, Verma RS. Cardiogel: A nano-matrix scaffold with potential application in cardiac regeneration using mesenchymal stem cells. PLoS One 2014; 9(12): e114697.

63 Mayfield AE, Tilokee EL, Latham N, McNeill B, Lam BK, Ruel M, *et al*. The effect of encapsulation of cardiac stem cells within matrix-enriched hydrogel capsules on cell survival, post-ischemic cell retention and cardiac function. Biomaterials 2014; 35(1): 133-42.

64 Wang XY, Fan XS, Cai L, Liu S, Cong XF, Chen X. Lysophosphatidic acid rescues bone mesenchymal stem cells from hydrogen peroxide-induced apoptosis. Apoptosis 2015; 20(3): 273-84.

65 Zhang Q, Wang H, Yang YJ, Dong QT, Wang TJ, Qian HY, *et al*. Atorvastatin treatment improves the effects of mesenchymal stem cell transplantation on acute myocardial infarction: the role of the RhoA/ROCK/ERK pathway. Int J Cardiol 2014; 176(3): 670-9.

66 Liu X, Hou J, Shi L, Chen J, Sang J, Hu S, *et al*. Lysophosphatidic acid protects mesenchymal stem cells against ischemia-induced apoptosis *in vivo*. Stem Cells Dev 2009; 18(7): 947-54.

67 Luo Y, Wang Y, Poynter JA, Manukyan MC, Herrmann JL,

Abarbanell AM, *et al*. Pretreating mesenchymal stem cells with interleukin-1beta and transforming growth factor-beta synergistically increases vascular endothelial growth factor production and improves mesenchymal stem cell-mediated myocardial protection after acute ischemia. Surgery 2012; 151(3): 353-63.

68 Herrmann JL, Wang Y, Abarbanell AM, Weil BR, Tan J, Meldrum DR. Preconditioning mesenchymal stem cells with transforming growth factor-alpha improves mesenchymal stem cell-mediated cardioprotection. Shock 2010; 33(1): 24-30.

69 Takehara N, Tsutsumi Y, Tateishi K, Ogata T, Tanaka H, Ueyama T, *et al*. Controlled delivery of basic fibroblast growth factor promotes human cardiosphere-derived cell engraftment to enhance cardiac repair for chronic myocardial infarction. J Am Coll Cardiol 2008; 52(23): 1858-65.

70 Yin PT, Han E, Lee KB. Engineering stem cells for biomedical applications. Adv Healthc Mater 2016; 5(1): 10-55.

71 Park JS, Suryaprakash S, Lao YH, Leong KW. Engineering mesenchymal stem cells for regenerative medicine and drug delivery. Methods 2015; 84: 3-16.

72 Fujita T, Sakaguchi T, Miyagawa S, Saito A, Sekiya N, Izutani H, *et al*. Clinical impact of combined transplantation of autologous skeletal myoblasts and bone marrow mononuclear cells in patients with severely deteriorated ischemic cardiomyopathy. Surg Today 2011; 41(8): 1029-36.

73 Miyagawa S, Matsumiya G, Funatsu T, Yoshitatsu M, Sekiya N, Fukui S, *et al*. Combined autologous cellular cardiomyoplasty using skeletal myoblasts and bone marrow cells for human ischemic cardiomyopathy with left ventricular assist system implantation: Report of a case. Surg Today 2009; 39(2): 133-6.

74 Yang YJ, Qian HY, Huang J, Geng YJ, Gao RL, Dou KF, *et al*. Atorvastatin treatment improves survival and effects of implanted mesenchymal stem cells in post-infarct swine hearts. Eur Heart J 2008; 29(12): 1578-90.

75 Yang YJ, Qian HY, Huang J, Li JJ, Gao RL, Dou KF, *et al*. Combined therapy with simvastatin and bone marrow-derived mesenchymal stem cells increases benefits in infarcted swine hearts. Arterioscler Thromb Vasc Biol 2009; 29(12): 2076-82.

76 Li N, Yang YJ, Cui HH, Zhang Q, Jin C, Qian HY, *et al*. Tongxinluo decreases apoptosis of mesenchymal stem cells concentration-dependently under hypoxia and serum deprivation conditions through the AMPK/eNOS pathway. J Cardiovasc Pharmacol 2014; 63(3): 265-73.