

• 综述 •

间充质干细胞来源外泌体在骨组织修复中的应用

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[摘要] 间充质干细胞(mesenchymal stem cell, MSC)是骨组织修复中细胞治疗的重要来源之一,研究表明, MSC 衍生的外泌体可以规避干细胞移植的局限性。在骨微环境中,外泌体已被证明在体内外均能促进成骨和成骨分化。外泌体携带 DNA、mRNA、miRNA、蛋白质和脂质,在细胞间交流中发挥重要作用,直接在转录水平调节其靶细胞。其中,miRNA 在分化的各个阶段都是主要调节因子,通过降解 mRNA 或阻断翻译来调节生理和病理过程。最近, MSC 衍生的外泌体在骨修复和再生中取得重大进展,文章讨论 MSC 衍生的外泌体的形成以及其在骨组织缺损修复中的应用与潜在治疗机制。

[关键词] 间充质干细胞;外泌体;miRNA;免疫调节;骨修复

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Application of mesenchymal stem cell-derived exosomes in bone tissue repair

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[Abstract] Mesenchymal stem cell (MSC) is one of the most significant sources of cell-based therapeutics for bone tissue regeneration. Investigations have demonstrated that MSC-derived exosomes can get beyond the restrictions of stem cell transplantation. Exosomes have been demonstrated to stimulate osteogenesis and osteogenic differentiation in the bone microenvironment both *in vivo* and *in vitro*. Exosomes play as a crucial role in intercellular communication by directly influencing the transcriptional level of their target cells. They carry DNA, mRNA, miRNA, protein, and lipids. The miRNAs are major regulators at various stages of cell differentiation, and modulate physiological and pathological processes through mRNA degradation or translation blockade. MSC-derived exosomes have advanced significantly in bone regeneration and repair recently. This article addresses the formation of MSC-derived exosomes, their application, and possible therapeutic procedures in the restoration of bone tissue defects.

[Key words] mesenchymal stem cell; exosomes; miRNA; immunomodulation; bone repair

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由于严重创伤、肿瘤切除、手术、炎症性疾病和先天性缺损引起的骨缺损,其修复治疗一直是临床骨科面临的巨大挑战。传统骨缺损生物治疗方法包括自体骨移植、骨移植替代物和细胞治疗等,其中自体骨移植是修复骨缺损的金标准^[1-3],但因其来

源有限且存在供区并发症等问题,极大限制了其临床应用。

将干细胞作为种子细胞并联合骨移植材料,是骨组织修复领域中提高骨移植材料疗效的重要手段之一。间充质干细胞(mesenchymal stem cell, MSC)已广泛用于多种组织损伤修复的基础研究与临床试验^[4-5]。MSC 可从许多不同组织中获得,包括骨髓、脐带血、胚胎、胎膜和脂肪,或是通过各种培养方法进行诱导,分化为成骨细胞、软骨细胞和脂肪细胞,产生骨、软骨、脂肪组织和其他胚胎谱系细

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胞,是具有自我更新能力的多能前体细胞^[1,6]。虽然MSC具有免疫调节性和低免疫原性,但其在循环中易被快速清除,且只有1%的MSC可定位于靶组织^[7-9]。最近研究表明, MSC通过旁分泌机制发挥治疗作用,而外泌体(exosome, Exo)是重要的旁分泌介质,可将某些生物活性分子转移到靶细胞以调节细胞活动^[6,10]。MSC来源外泌体(MSC-Exo)与亲本细胞具有相似的生物学特征,其再生效应在肾损伤^[11]、肺损伤^[12]、心肌梗死^[13]和肝脏损伤^[14]的临床前模型中有广泛报道。Exo的具体数量和含量会随微环境信号的不同而不同^[15],较亲本细胞更稳定,与MSC移植相比,Exo具备安全性更高,更易储存、输送和管理的优势^[16]。当机体存在临界大小组织缺损时,可以直接将Exo注射到缺损组织中^[4,17-18]。因此, MSC-Exo可能是MSC治疗的潜在替代方案。

1 Exo的定义与形成

Exo是由不同细胞释放的双层脂膜胞外小泡,直径为30~150 nm。1981年,Trams等^[19]首次提出“外泌体”的概念,于1983年首次在绵羊网织红细胞中发现,为了与其他类型的细胞外囊泡区分开,将其命名为Exo^[20]。由内体囊泡通过质膜内陷形成, 释放到细胞外^[21-22]。多囊泡体(multivesicular body, MVB)与质膜融合并释放腔内小泡(intraluminal vesicle, ILV)、Exo或经溶酶体降解^[23-24](图1)。MSC-Exo由多种分子组成,包括微小RNA(microRNA, miRNA)、DNA、mRNA、蛋白质等。Exo的双层膜结构可以保持其内容物稳定,并允许它们在组织和局

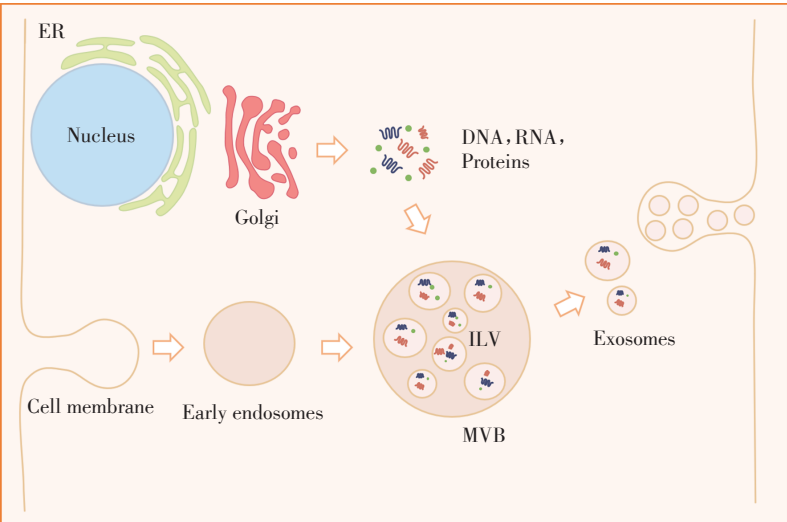
部细胞内移动^[25]。Exo膜上富含表面蛋白,如ALG-2相互作用蛋白X(ALG-2-interacting protein X, Alix)、肿瘤易感基因101(tumor susceptibility gene 101, TSG101)和跨膜蛋白,包括白细胞分化抗原9(cluster of differentiation 9, CD9)、CD63和CD81等^[26-27]。MSC-Exo可通过表面的配体/受体分子进行信号传递,或通过网状蛋白和脂筏介导的内吞、巨胞饮和吞噬与细胞膜融合,将内容物释放到靶细胞^[10,28],充当细胞外信使,调节细胞间通讯。

2 MSC-Exo与骨修复

骨缺损修复过程可分为3个阶段:血肿形成、纤维愈伤组织形成和骨重塑。许多研究表明,骨再生的启动依赖于炎症反应和新血管的生成^[29-30]。近来, MSC-Exo已被确定为用于组织再生的新兴纳米级无细胞治疗剂,常被用于皮肤伤口愈合、缺血性脑损伤、肾损伤、骨关节炎、骨折愈合和退行性骨病等^[31-32]。Exo可以从不同来源的细胞中分离出来,Exo的内容物已被证实反映其亲代细胞的功能, MSC-Exo在骨修复中的作用见表1。

2.1 骨髓间充质干细胞(bone marrow mesenchymal stem cell, BMSC)源性Exo与骨修复

BMSC是骨髓中含量最丰富的细胞,其分泌的Exo已在各种实验模型中验证^[33]。应用BMSC来源的Exo(BMSC-Exo)治疗,可使成骨生长因子和骨相关蛋白,如骨桥蛋白(osteopontin, OPN)、Runt相关转录因子2(Runt-related transcription factor 2, RUNX2)、I型胶原蛋白(collagen I, COL I)、转化



ER: endoplasmic reticulum; ILV: intraluminal vesicle; MVB: multivesicular body.

图1 外泌体生物形成示意图
Figure 1 Schematic of the biogenesis of exosomes

生长因子(transforming growth factor, TGF)- β 1等表达水平增加,还可增加体外钙沉积和基质矿化^[16,34-35]。体外实验中,BMSC-Exo可促进靶细胞的增殖和迁移。Zhang等^[3]每周往大鼠关节内注射人BMSC(hBMSC)-Exo,可有效修复大鼠临界大小的软骨缺损。另外,在股骨头坏死^[35]、放射性骨丢失^[16]、股骨骨折模型中^[36],植入BMSC-Exo后,骨形成和成骨标志物水平增加。骨愈合是个漫长的过程,常规的递送Exo策略无法持续到骨损伤后的血管生成阶段。Yang等^[37]通过制备基质金属蛋白酶-1敏感的可注射微球,实现Exo的有效控释,以响应新生血管形成并加速骨愈合早期的组织再生。此外,研究者们通过化学修饰或工程化来增强BMSC及其Exo的治疗效果,如镁可通过调节巨噬细胞的免疫反应来增强BMSC的成骨作用^[38];在磁性纳米颗粒和静态磁场的刺激下,BMSC-Exo表现出更强的成骨和血管生成作用^[39];以及锂可刺激BMSC-Exo增强成骨作用等^[40]。

2.2 脂肪间充质干细胞(adipose-derived stem cell, ASC)源性Exo与骨修复

脂肪相比胎盘、羊膜、骨髓等组织来源更丰富,便于提取且供体创伤小、可自体移植、安全性高,被认为是MSC临床应用的理想来源^[41]。ASC可以在体外和体内进行快速有效的成骨分化,最近研究表明人ASC(hASC)衍生的Exo(hASC-Exo)可以发挥与hASC相似的生物学功能,并在血管生成和愈合中起重要作用^[42-43]。Zhu等^[44]用成骨诱导后的hASC分泌的Exo培养hASC,发现该Exo可以促进hASC的增殖和迁移,且成骨相关蛋白表达与对照组相比显著上调。Li等^[45]用含有hASC-Exo的聚乳酸-羟基乙酸共聚物支架治疗小鼠颅骨缺损,该无细胞纳米载体具有骨诱导作用,通过促进MSC在新形成的骨组织中迁移和归巢来显著增强骨再生。缺氧预处理ASC所衍生的外泌体(hypo-ASC-Exo)可能在骨质疏松性骨质愈合过程中起着更重要的促血管生成作用。Li等^[46]将hypo-ASC-Exo负载于甲基丙烯酸酯化明胶中,证实其相比ASC-Exo,血管容积和新骨形成量显著增加。上述研究表明ASC-Exo在骨修复中具有相当大的应用潜力。

2.3 脐带间充质干细胞(umbilical mesenchymal stem cell, uMSC)源性外泌体与骨修复

在现有MSC来源中,脐带来源安全且多产。与其他MSC相比,uMSC促血管生成能力更强,这些特点有助于骨再生过程中的快速组织重建^[47-48]。最近一项研究表明,uMSC来源Exo(uMSC-Exo)可加速内

皮细胞增殖、迁移和管状细胞形成,进一步促进血管生成,移植uMSC-Exo显著促进股骨骨折大鼠模型的血管生成和骨愈合,因此,Exo可能通过促进血管生成来加速骨折愈合^[25]。Hu等^[49]用人uMSC(huMSC)-Exo处理软骨细胞与BMSC,发现其促进细胞迁移、增殖和分化,将huMSC-Exo包覆在水凝胶中,可持续释放Exo治疗软骨缺损。分别采用增殖培养基(proliferation medium, PM)和成骨诱导培养基(osteogenic induction medium, OM)培养huMSC以获取Exo。体外结果显示,PM-huMSC-Exo和OM-huMSC-Exo均显著促进hBMSC的增殖与迁移。将Exo掺入3D打印磷酸三钙支架中,OM-huMSC-Exo在修复颅颌面骨缺损方面效果更为明显^[50]。因此,功能化Exo在骨修复方面也具有广阔的应用潜力。

2.4 其他MSC源性Exo与骨修复

人牙髓干细胞分泌多种生长因子,据报道这些生长因子参与MSC的成骨。Jin等^[51]将人牙髓干细胞外泌体与hASC共培养,促进hASC的成骨分化;在下颌骨缺损大鼠模型中,该Exo可促进骨缺损修复。另外,牙周韧带干细胞衍生的Exo可以在炎症环境下挽救内源性干细胞的成骨能力,促进牙槽骨的再生。Lei等^[52]证实牙周韧带干细胞衍生的Exo可在体外显著增强炎症性牙周韧带干细胞的成骨分化能力;在体内促进牙周炎大鼠骨缺损的修复。胎盘间充质干细胞(placental mesenchymal stem cell, PMSC)具有强分化潜力和免疫调节特性,且可以诱导脊髓、心脏和骨骼等各种组织再生。PMSC来源的Exo已被证明可改善心肌纤维化、抑制炎症反应以及加速伤口瘢痕愈合等^[53]。其在骨缺损修复方面的治疗作用有待进一步探索。

综上,MSC-Exo主要通过增强靶细胞的增殖与迁移能力,促进成骨或血管生成,为Exo应用于骨缺损组织修复与再生提供了新方向。但不同MSC-Exo在促成骨或成血管能力上差异较大,将来需要投入更多的时间去探索最适合骨组织再生的Exo来源。

3 MSC-Exo在骨修复中的作用机制

MSC-Exo促成骨作用可能由于:①调节免疫功能,改善微环境;②刺激血管生成,优化微环境;③直接调节邻近靶细胞成骨分化过程,为骨再生创造理想条件(图2)。

3.1 调节免疫功能,改善微环境

骨再生和愈合是一个复杂的过程,免疫细胞的持续或异常激活、促炎因子的分泌都不利于骨再

表1 干细胞衍生的外泌体在骨修复中的功能
Table 1 The function of stem cell derived exosomes in bone tissue repair

Parent cell	Target cell	Advantage	<i>In vitro</i>	Model	<i>In vivo</i>	Reference
BM-MSC	BM-MSC	Reduced oxidative stress, and prevented bone loss	Rescued proliferation inhibition and reduced related aging protein expression	Radiation-induced bone loss in rats	Mitigated radiation - induced bone loss	[16]
hMSC	hMSC	Osteoinduction	Upregulated osteogenic genes	Calvarial defect in rats	Enhanced osteogenesis	[33]
BMSC	Osteoblast	Delayed/avoided collapse of femoral head	Increased proliferation, and enhanced osteogenic differentiation	Femoral necrosis in rabbits	Promoted local angiogenesis, and prevented bone loss	[34]
mBMSC	Osteoblast	Regenerated defect tissue	Enhanced osteogenic differentiation	Fracture in mice	Promoted fracture recovery	[35]
BMSC	BMSC	Recruited stem cells	Increased migration, and enhanced osteogenic differentiation	Calvarial defect in rats	Promoted angiogenesis	[37]
hASC	hBMSC	Promoted angiogenesis and wound healing	Increased proliferation, increased migration, and enhanced osteogenic differentiation	Calvarial defect in mice	Increased bone formation	[42]
hASC	hASC	Rich source, obtained easily, ideal cell source	Increased proliferation, increased migration, and upregulated osteogenic protein/gene	—	—	[44]
hASC	U937	High yield, low invasion rate	Inhibited M1 marker expression, and upregulated M2 marker expression	Calvarial defect in rats	Increased bone formation	[45]
huMSC	hBMSC	Higher pluripotency potential of bone tissue engineering	Upregulated osteogenic genes	Calvarial defect in rats	Increased bone formation	[47]
huMSC	HUVEC	Obtained easily, excellent proliferation and differentiation ability	Increased migration and proliferation, and promoted angiogenesis	Femoral fracture in rats	Increased angiogenesis, and accelerated bone healing	[25]
huMSC	hBMSC	Rich source of tissues, and painless collection	Increased migration and proliferation, and enhanced differentiation	Knee cartilage defect in rats	Promoted cartilage regeneration	[49]
huMSC	hBMSC	High cell yield	Increased migration and proliferation	Critical-sized calvarial bone defect in rats	Enhanced osteogenesis	[50]
hDPSC	hADSC	Secreted multiple growth factors	Enhanced osteogenic differentiation, and increased migration	Mandibular defect in rats	Increased bone formation	[51]

BM-MSC: bone marrow derived-mesenchymal stem cells; hMSC: human mesenchymal stem cells; BMSC: bone marrow mesenchymal stem cells; mBMSC: mouse bone marrow mesenchymal stem cells; U937: human monocyte cell line; hASC: human adipose derived stem cells; huMSC: human umbilical cord mesenchymal stem cells; HUVEC: human umbilical vein endothelial cells; hDPSC: human dental pulp stem cells.

生。免疫细胞包括T细胞、B细胞、巨噬细胞和中性粒细胞,在骨缺损中起着重要作用^[54]。研究表明, MSC-Exo 具有持续的炎症调节能力,可降低白介素(interleukin, IL)-1 β 、IL-6和肿瘤坏死因子(tumor necrosis factor, TNF)- α 的表达,并抑制炎症中巨噬细胞的M1表型标志物 mRNA 的表达^[55-57]。其中, Exo 的 miRNA146、miRNA-34 和 miRNA-181a 可以通过促进巨噬细胞的 M2 极化来减少 M1 相关细胞因

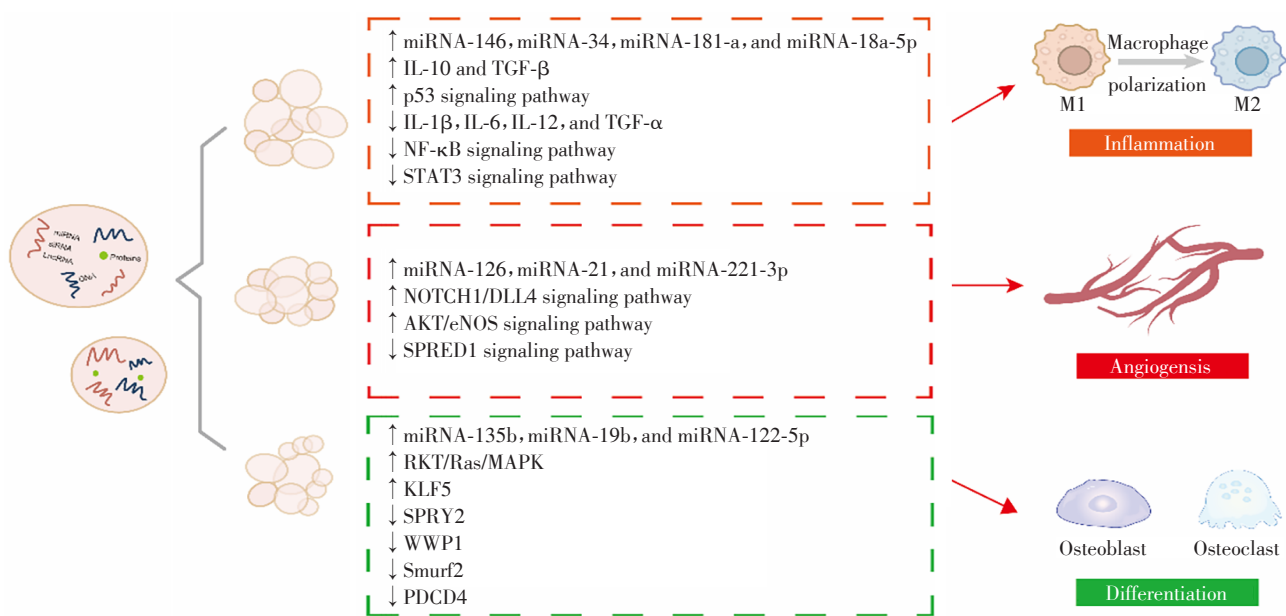


图2 外泌体在骨修复中的主要机制

Figure 2 The mechanisms of exosomes in bone repair

子,如 IL-6、IL-12 和 TNF- α ,增强 M2 相关细胞因子,如 IL-10 和 TGF- β ^[58-60]。Li 等^[61]证明 MSC-Exo 中的 miRNA-18a-5p 通过靶向共济失调毛细血管扩张突变基因激活 p53 信号通路,并诱导巨噬细胞 M2 表型极化。此外,MSC-Exo 可以通过 NF- κ B 和 STAT3 通路诱导巨噬细胞 M2 极化诱导骨再生^[62]。因此,MSC-Exo 的免疫调节潜力可用于骨缺损修复。

3.2 刺激血管生成,优化微环境

血管生成与复杂的骨修复和再生密切相关,血管可将营养物质和氧气带到高度代谢活跃区域^[63-64]。Liu 等^[65]证实低氧条件可通过缺氧诱导因子(hypoxia inducible factor, HIF)-1 α 介导 hBMSC-Exo 中的 miRNA-126 增加,miRNA-126 可抑制靶基因 SPRED1(Sprouty 相关蛋白家族,调控造血)表达,促进 HUVEC 的增殖和迁移。多项研究表明,MSC-Exo 通过加速血管生成促进骨愈合和修复。Zhang 等^[66]从 uMSC 中分离出 Exo 并封装在水凝胶中,该复合材料在体外能促进内皮祖细胞的增殖、迁移和血管生成,在体内能促进临界大小骨缺损模型的血管生成与成骨。机制研究表明,Exo 所含的 miRNA-21 是潜在的细胞间信使,通过上调 NOTCH1/DLL4 通路促进血管生成。此外,AKT 磷酸化在血管生成中起重要作用,miRNA-221 的直接靶点 PTEN 是 AKT 的抑制剂,而 miRNA-221 的下调会显著抑制 HUVEC 的功能,进一步损害血管生成。Yu 等^[67]用阿托伐他汀(atorvastatin, ATV)预处理 BMSC 获取 ATV-Exo,结果显示

该 Exo 可通过上调 miRNA-221-3p 激活 AKT/eNOS 通路促进血管生成。因此,Exo 中 miRNA 通过激活或去激活不同细胞信号通路来调节成骨分化与血管生成,从而调节其靶蛋白的基因转录和表达。

3.3 直接调节靶细胞成骨分化过程

Xu 等^[9]发现 BMSC-Exo 在成骨诱导过程中有 9 个 miRNA 显著上调,5 个 miRNA 显著下调,在调控靶细胞 MSC 成骨过程中起重要作用。其中 miRNA-135b 通过抑制程序性细胞死亡因子 4(programmed cell death factor 4, PDCD4)表达来增强 MSC-Exo 的作用,从而减轻大鼠股骨坏死症状^[68]。BMSC-Exo 可促进骨折愈合,在亲本细胞中过表达 miRNA-19b 的体内外实验中,骨折愈合增加。miRNA-19b 通过 Wnt/ β -catenin 信号通路抑制含有 WW 结构域的 E3 泛素蛋白连接酶 1(WW domain-containing E3 ubiquitin protein ligase 1, WWP1)或 Smad 泛素调节因子 2(Smad ubiquitin regulatory factor 2, Smurf2)表达,并上调 Kruppel 样因子 5(Kruppel-liked factor 5, KLF5)表达来促进 hBMSC 向成骨细胞分化^[69]。恢复 Wnt/ β -catenin 信号有助于 BMSC 成脂和成骨分化之间的平衡^[16],携带 miRNA 的 Exo 选择性调节成骨细胞活性影响骨质疏松过程。Liao 等^[35]通过功能获得性实验,研究 miRNA-122-5p 对成骨细胞和 BMSC 的影响以及含 miRNA-122-5p 的 Exo 对股骨头坏死的影响。结果显示 miRNA-122-5p 负调控发芽的 RTK 信号拮抗因子 2(sprouty RTK signaling antagonist 2,

SPRY2)并提高RTK的活性,从而促进成骨细胞的增殖和分化。另外,在Exo内过表达miRNA-122-5p后,股骨头的骨矿物质密度、骨小梁体积和平均小梁板厚度增加,且观察到坏死股骨头明显愈合。携带miRNA-122-5p的Exo可能通过RTK/Ras/MAPK信号通路下调SPRY2以减轻股骨头坏死症状。这些发现表明相关miRNA与Exo的结合可成为促进骨再生的有前景的策略之一。

Exo通过释放特定成骨蛋白、细胞外基质蛋白或骨相关miRNA,调节靶细胞基因表达^[70]。因此,可对亲本细胞进行预处理或修饰亲本细胞以表达特定蛋白质,有针对性地将刺激因子输送到邻近细胞。

4 结论与展望

肿瘤、创伤、手术或炎症性疾病可能会导致临界大小骨缺损,骨缺损及外科重建已成为全球卫生保健系统的主要财务负担之一^[71]。骨缺损修复涉及骨组织再生与血管生成,骨环境中的MSC通过分泌Exo参与骨修复调节^[72]。与细胞治疗相比,Exo治疗具有安全性高、易于储存运输、免疫原性低、可作为药物载体和治疗效率高等优势,可规避细胞疗法的局限性^[16, 73]。许多研究已证明不同MSC-Exo可促进成骨或成血管^[74]以及免疫调节^[75],在骨缺损和其他疾病(如骨坏死、骨折和骨质疏松症)动物模型中有助于骨再生,这可能与Exo的内容物miRNA有关^[76-77]。由于Exo中生物活性分子的复杂性,多数研究仅限于Exo中的单一功能分子。因此,需要更多探索以深入了解Exo参与旁分泌的治疗机制,以便选择适当的细胞源。Exo治疗虽是一种新型且具有前景的骨修复治疗策略,但在将其安全应用于人体之前,还需进行大量研究,尤其是Exo的临床疗效和生产效率方面。

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