间充质干细胞来源外泌体治疗骨关节炎和软骨损伤的研究进展

张厚建[1],张维新[1],童培建[2]
（1. 浙江中医药大学第一临床医学院,浙江　杭州　310053;
2. 浙江省中医院,浙江　杭州　310006）

摘　要　骨关节炎是临床常见病,以关节软骨损伤、软骨下骨硬化及滑膜炎症等为特征,可导致患肢疼痛、畸形和功能障碍。外泌体是细胞间通讯的主要载体,在细胞间转运具有生物活性的脂质、核酸及蛋白质,从而改变受体细胞的生物学功能。间充质干细胞来源外泌体具有抗炎、免疫调节及促进组织修复和再生等作用,是治疗骨关节炎和软骨损伤的新靶点。本文对间充质干细胞及外泌体进行了概述,并对间充质干细胞来源外泌体在骨关节炎和软骨损伤治疗中的应用及其作用机制进行了综述。

关键词　骨关节炎;软骨;间质干细胞;外泌体;综述

骨关节炎（osteoarthritis,OA）是临床常见病,以关节软骨进行性破坏及丢失为特征,主要由软骨细胞及细胞外基质合成和分解代谢失衡所致[1-2]。软骨损伤在 OA 疾病进展中扮演着重要角色[3-5],因此恢复关节软骨的完整性及功能,对于延缓甚至逆转 OA 的疾病进展起着至关重要的作用[6]。目前 OA 的治疗方法较多,虽然均可缓解临床症状、在一定程度上延缓软骨的退变,但无法完全修复受损的软骨,且远期疗效欠佳[7-8]。间充质干细胞来源外泌体（mesenchymal stem cells - derived exosomes,MSC - EXO）具有抗炎、免疫调节及促进组织修复和再生等作用[9],是治疗 OA 和软骨损伤的新靶点。本文对 MSC - EXO 治疗 OA 和软骨损伤的研究进展进行了综述,以期为后续研究提供参考。

1　间充质干细胞概述

间充质干细胞（mesenchymal stem cell,MSC）最初由 Friedenstein 等[10]从骨髓组织中分离出来,现在可以从成人或胚胎的组织[11]中获取,包括脂肪[12]、脐带[13]、滑膜[14]等。MSC 是具有自我更新和多向分化能力的多能祖细胞,可以分化成脂肪细胞、成骨细胞和软骨细胞[15]。近年来,有关 MSC 治疗软骨损伤[16]和 OA[17-18]的报道逐渐增多。MSC 具有良好的组织再生和免疫调节功能[19-22],能够恢复 OA 软骨细胞的合成和分解代谢平衡。虽然 MSC 疗法可以有效缓解关节疼痛、改善关节运动功能、修复关节软骨[23-27],但该法仍存在有待进一步解决的问题:如 MSC 在体外传代和扩增后,会出现细胞表型异常,导致其增殖分化能力下降[28];为了维持 MSC 的活性,植入前对其储存条件要求较高[26];植入后新生软骨可能会出现肥大和骨化[29]。最初学者们推测 MSC 主要通过直接分化为软骨细胞来替代损伤组织,但近年来的研究发现,MSC 是通过旁分泌作用[30-32]来调控损伤组织的微环境,并启动自我修复程序,而非直接进行细胞替换[33],Wu 等[34-35]的研究结果（MSC 分泌蛋白可促进软骨细胞的增殖和基质的合成）也证明了这一观点。随着研究的深入,学者们发现由 MSC 分泌的外泌体（exosome,EXO）才是促进组织修复和再生的关键[36-39]。

2　EXO 概述

所有的细胞均可在其正常和获得性异常状态下释放细胞外囊泡,细胞外囊泡大致分为 2 类,即胞外体和 EXO。胞外体主要通过细胞膜向外出芽的方式形成囊泡。EXO 来源于细胞内,直径 40 ~ 160 nm,主要通过细胞膜内陷形成多泡体（multivesicular bodies,MVBs）,MVBs 内含大量腔内囊泡（intraluminal vesicles,ILVs）,MVBs 与细胞膜融合,向外释放包括 ILVs 的物质,这些被释放到细胞外的 ILVs 物质即 EXO[40]（图 1）。EXO 内含脂质、蛋白质及核酸等物质[41],EXO 的内含物水平代表了其供体母细胞的生物学状态[42]。目前 EXO 的生理意义尚不明确,可能与去除细胞中多余的成分以维持细胞稳态、在细胞间通讯等有关。EXO 主要通过旁分泌、近分泌及内分泌

通讯作者:童培建　E-mail:tongpeijian@163.com

来实现细胞间通讯[43]。EXO 内的信使 RNA 可在受体细胞内通过 EXO 与细胞间的相互作用进行转化，调控基因的表达[41]。EXO 可将供体母细胞的基因信息传递至受体细胞，使受体细胞的生物学功能发生改变。

3　MSC – EXO 在 OA 和软骨损伤治疗中的应用

目前，多数 MSC – EXO 在 OA 和软骨损伤治疗中的应用尚处于实验研究阶段。在小鼠 OA 模型中，EXO 可促进软骨细胞的合成代谢、抑制炎症反应，且能延缓软骨退化及 OA 病情进展[44-50]。EXO 在体外具有免疫调节和保护软骨的作用，还可影响软骨细胞和多种免疫细胞的表达[51-54]。在小鼠[38,55]和兔[53]软骨损伤模型中，采用 MSC – EXO 治疗后，损伤区域均出现了富含Ⅱ型胶原物质的类透明样软骨。上述研究均采用了 MSC 作为 EXO 的供体细胞，且各种组织的 MSC 均有涉及，如骨髓[44-45,47]、脂肪[52]、滑膜[46,49]、胚胎[46,48]等。MSC 与 MSC – EXO 的免疫调节、保护软骨及促进组织再生作用相当，但在治疗 OA 和软骨损伤方面 MSC – EXO 更具优势，如直径小、低免疫原性、易于生产和储存等[54-55]。此外，MSC – EXO 疗法的安全性较高，即使反复注射[56]或使用不同物种细胞来源的 EXO[57]均不会引起免疫排斥反应。

4　MSC – EXO 治疗 OA 及软骨损伤的作用机制

MSC – EXO 可对靶细胞产生特定的作用，包括新抗原呈递、免疫调节和药物有效载荷传递等。EXO 调控靶细胞的作用机制较为复杂，可能与胞饮作用、吞噬作用、脂筏及质膜微囊等有关[40]（图 2）。EXO 是一类具有高度异质性的物质，根据其对受体细胞功能的影响（功能异质性）可以分为 3 类：①给予受体细胞促存活信号；②给予受体细胞促凋亡信号；③介导免疫调节[40]。综合 OA 的病机及 EXO 的功能异质性，MSC – EXO 治疗 OA 及软骨损伤的作用机制可能与细胞数量、软骨细胞的新陈代谢、免疫调节、细胞能量平衡及内源性 miRNA 等有关。

4.1　促进软骨细胞的增殖、迁移并抑制其凋亡　软骨细胞死亡是 OA 的重要病理特征，其原因主要包括细胞凋亡、炎症反应、氧化应激和线粒体功能失调等[58]。软骨的损伤通常会因软骨细胞的死亡及其引起的细胞外基质降解、局部结构功能失调而越发严重[59]。因此，OA 的治疗应注重恢复软骨细胞的稳态，其中又以恢复软骨细胞数量平衡为主。

软骨细胞的增殖、分化和凋亡之间的平衡，受复杂的信号通路调控。蛋白激酶 B（protein kinase B，PKB）和细胞外信号调节激酶（extracellular signal regulated kinase，ERK）是调控细胞增殖和存活的重要信

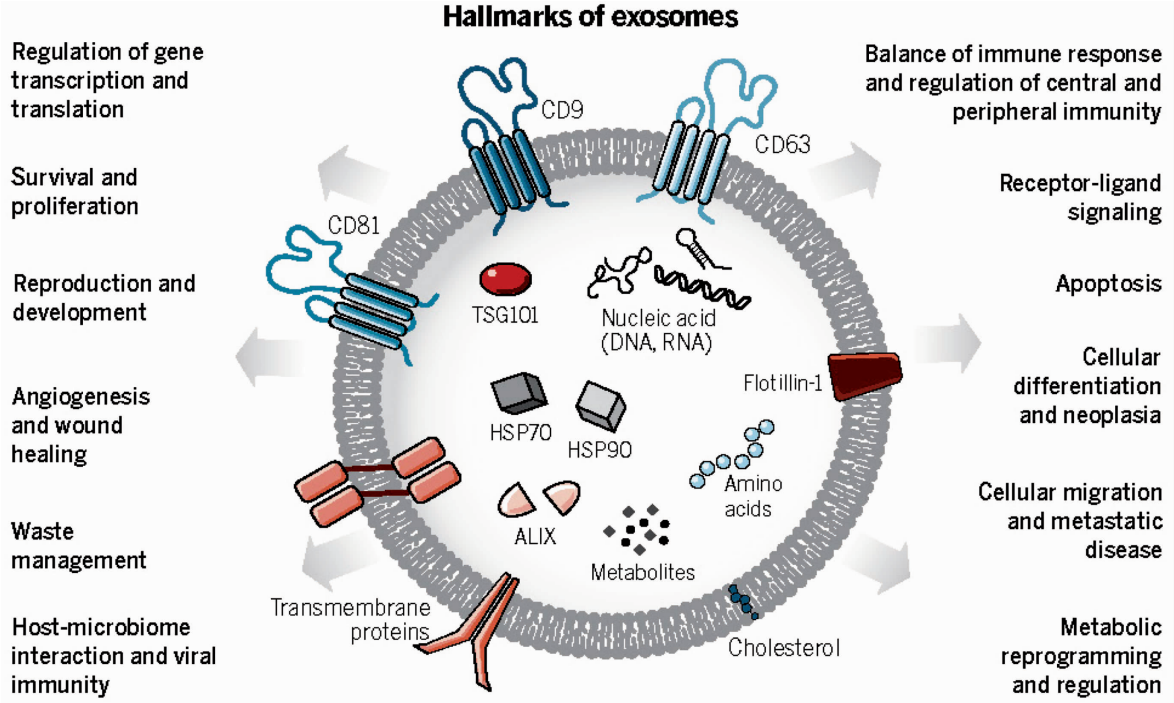

图 1　外泌体的组成和功能示意图[40]

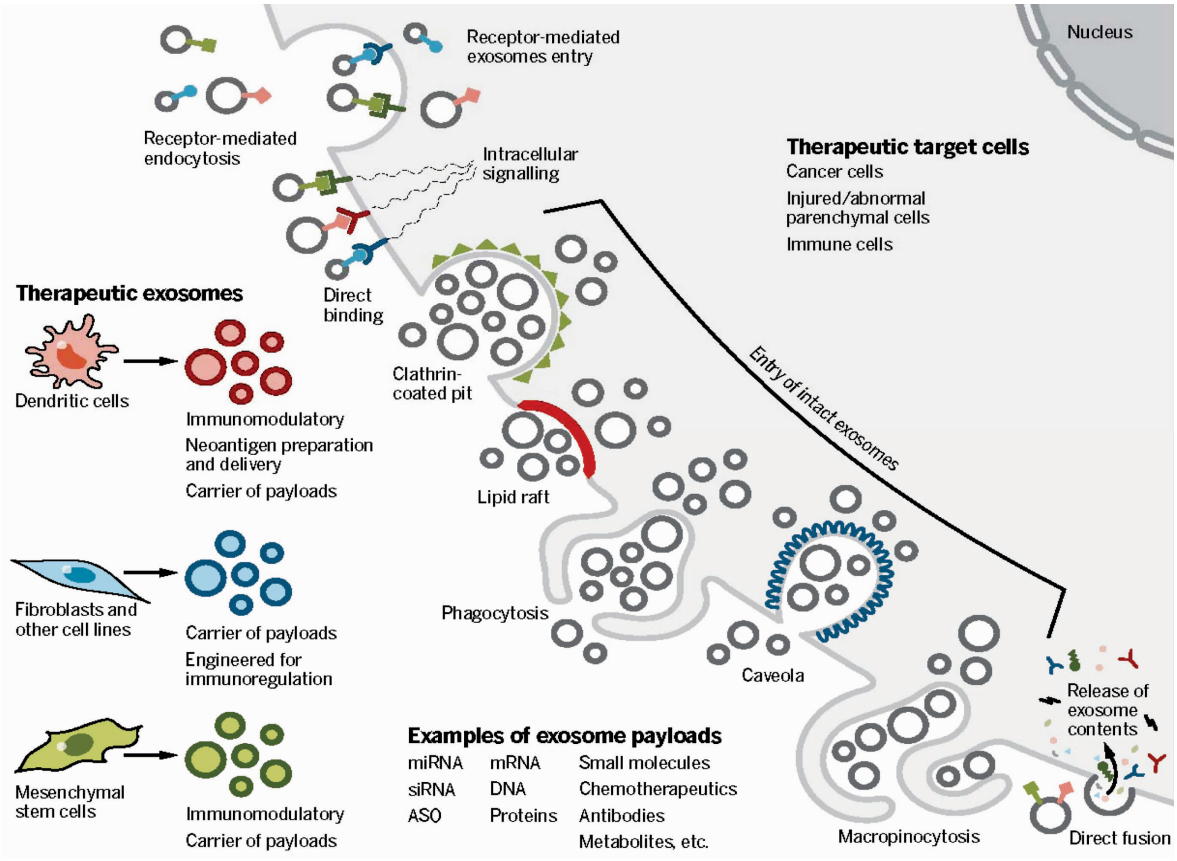

图 2　外泌体的作用机制示意图[40]

号通路[60]，PKB 信号通路主要通过抑制促凋亡基因、促进抗凋亡基因的表达影响细胞凋亡[61]，ERK 信号通路主要通过促进调控细胞增殖因子的磷酸化影响细胞增殖[62]。CD73 是目前唯一已知的能将细胞外一磷酸腺苷转化为腺苷的细胞外 5’－核苷酸酶，其可以通过与腺苷受体的相互作用来激活 PKB 和 ERK 信号通路[63]。研究表明，MSC－EXO 中富含 CD73 并且具有较强的酶活性[63]。笔者认为，MSC－EXO 可能主要通过 PKB 和 ERK 信号通路激活其磷酸化，从而起到促进软骨细胞增殖、迁移并抑制其凋亡的作用。Qi 等[64] 研究发现，在白细胞介素（interleukin，IL）－1β 介导的软骨细胞凋亡模型中加入 MSC－EXO 后，P38 丝裂原活化蛋白激酶和 ERK 的磷酸化水平降低，而 PKB 的磷酸化水平升高，软骨细胞的凋亡率也明显降低。Zhang 等[37] 研究发现，MSC－EXO 可以促进 PKB 和 ERK 的表达，并激活其磷酸化，从而促进软骨细胞的增殖和迁移；在培养基中加入 PKB 和 ERK 信号通路抑制剂后，软骨细胞的增殖和迁移能力下降；在培养基中加入 CD73 抑制剂后，PKB 和 ERK 的磷酸化水平降低，同时软骨细胞的增殖和迁移能力也随之下降。

维持软骨细胞数量平衡的过程较为复杂，除了 PKB 和 ERK 信号通路外，还存在其他的信号通路。Wnt 蛋白已被证实可以通过调控软骨细胞的增殖和分化来影响细胞的稳态，而 MSC－EXO 则可能是通过 Wnt 蛋白来调控软骨细胞[65]。Tao 等[46] 研究发现，EXO 携带的 Wnt5 和 Wnt5b 可以通过 Wnt 信号通路激活 Yes 相关蛋白，从而起到促进软骨细胞增殖和迁移的作用。Mao 等[45] 研究发现，miR－92a－3p 过表达的 MSC－EXO 可以通过作用于 Wnt5a 促进软骨细胞的增殖。此外，一些研究发现，MSC－EXO 调控软骨细胞增殖和凋亡的作用机制还可能与 1－磷酸鞘氨醇[66]、miR－206/G 蛋白偶联受体激酶结合蛋白 1[67]、哺乳动物雷帕霉素靶蛋白（mammalian target of rapamycin，mTOR）[68] 等信号通路有关。

4.2　促进软骨细胞的合成并抑制其分解　软骨退化也是 OA 的重要病理特征，可能由软骨细胞产生过量的基质降解酶所致[69]，主要包括基质金属蛋白酶（matrix metalloproteinases，MMPs）和解聚蛋白样金属蛋白酶（a disintegrin and metalloproteinase with thrombospondin motifs，ADAMTS），其中 MMP－13 对Ⅱ型胶原的降解有重要作用、ADAMTS－4 及 ADAMTS－5

对聚蛋白多糖的降解有重要作用[70-71]。这些酶可以通过激活特定的信号通路[71]并下调某些 miRNA 的表达水平,从而调控某些细胞因子、趋化因子及炎症介质的表达[70,72-74],使软骨细胞向肥大样表型分化、细胞外基质中出现钙盐沉积,最终使骺板软骨发生软骨内骨化。如激活素受体样激酶(activin receptor-like kinase,ALK)-5,其介导的 Smad 2/3 信号通路可以调控聚蛋白多糖和Ⅱ型胶原的合成[75-77];ALK-1 介导的 Smad 1/5/8 信号通路可以通过 Runt 相关转录因子-2(runt-related transcription factor-2,RUNX-2)调控 MMP-13 和 X 型胶原等软骨肥大标志物的表达[78-79]。此外,性别决定区 Y 框蛋白-9(sex determining region Y-related high mobility group-box gene-9,SOX-9)对软骨细胞的分化和形成也有重要作用[62]。

笔者认为,MSC-EXO 可能通过对多种信号通路及其相关蛋白、细胞因子的调控,抑制软骨基质降解、促进软骨细胞合成,从而恢复并维持软骨细胞的稳态。近年来的一些有关 MSC-EXO 的研究也证实了笔者的观点。经 MSC-EXO 治疗后,OA 软骨细胞内的 MMP-13[44-45,52,67-68,80-82]、ADAMTS-5[45,48,68]、RUNX-2[45,47,67,82]、X 型胶原[45,47,70,83]的表达水平均下降,SOX-9[45,47,81]、Ⅱ型胶原[48,52,68]、聚蛋白多糖[44-46,48,52,68,81-84]、Ⅱ型前胶原基因[45,67-68,81-82]的表达水平均上升;此外,正常[47,80]或 miR-92a-3p[45]及 miR-95-5p[83]过表达的 MSC-EXO 均能使 X 型胶原的表达水平下降。

4.3　通过恢复线粒体功能促进软骨细胞的能量平衡　线粒体功能障碍不仅是 OA 软骨退变的典型标志,也与 OA 的病情进展密切相关[85]。与健康软骨细胞相比,OA 软骨细胞中线粒体的生物合成[86]及电子传递链(electron transport chain,ETC)[87]均较少,且随着 OA 病情进展逐渐减少。ETC 是线粒体通过氧化磷酸化产生三磷酸腺苷(adenosine triphophate,ATP)的核心蛋白,线粒体数量减少及 ETC 活性下降,均会打破软骨细胞的能量平衡,导致氧化应激和炎症反应增加、软骨基质钙化、细胞凋亡等[88-90]。因此,恢复 OA 软骨细胞的能量平衡对于启动软骨细胞的修复和再生程序至关重要。MSC-EXO 富含 ATP 结合蛋白及糖酵解相关酶(葡萄糖激酶、丙酮酸激酶、腺苷酸激酶)[91-92],能有效调节糖酵解和 ATP 合成的平衡,维持细胞稳态。此外,有研究发现 MSC-EXO 中含有线粒体的多种组分(内膜、外膜、膜间隙等)[93]和 DNA[94]。

笔者认为,MSC-EXO 可能通过促进 OA 软骨细胞中 ATP 的合成,修复线粒体的功能障碍或损伤,从而恢复软骨细胞的能量平衡。Chen 等[93]研究发现,在使用线粒体 ETC 复合体Ⅰ抑制剂模拟的线粒体损伤模型中,与对照组相比,MSC-EXO 组线粒体的质量和拷贝数增加、活性氧产量减少,软骨细胞中 ATP 水平增加了 21%;这表明 MSC-EXO 可能通过直接向线粒体提供其合成所需的蛋白,而修复软骨细胞中线粒体的损伤。Qi 等[95]研究发现,在 IL-1B 诱导的软骨细胞凋亡模型中,线粒体膜电位水平较低,而在加入 MSC-EXO 后,线粒体膜电位显著增高;这表明 MSC-EXO 可以促进线粒体的氧化磷酸化和 ATP 的合成,有利于恢复软骨细胞的能量平衡。

4.4　通过调控巨噬细胞极化抑制炎症反应并促进软骨修复　最初 OA 被认为是一种非炎症性关节退行性疾病,但是越来越多的证据表明,长期、低程度的滑膜炎症在 OA 的病理变化和病情进展过程中发挥着重要作用[96-97]。受损软骨产生的代谢产物激活了巨噬细胞,也促进了一些免疫细胞释放炎症因子(包括 IL-1β、IL-6、IL-8、MMP-3 等),因此引起了软骨细胞功能障碍[98]。在滑膜炎症中巨噬细胞扮演着重要角色,OA 滑膜中的巨噬细胞已被证明是 IL-1、肿瘤坏死因子-α(tumor necrosis factor,TNF-α)等促炎因子的重要来源[99]。Bondeson 等[100-101]研究发现,人 $CD14^+$ 滑膜巨噬细胞还可以促进 MMPs 和其他基质降解酶的产生。根据与 T 细胞的相互作用可以将巨噬细胞分为 M1 型和 M2 型 2 类,M1 型主要由 Th1 细胞激活,具有抗菌和促炎作用;M2 型主要由 Th2 细胞激活,具有抗炎[102]和促进损伤组织修复[103]等作用。若 M1 型和 M2 型巨噬细胞所占比例平衡被打破,可能会引起长期低程度的炎症反应[102]。因此,调控炎症微环境对 OA 及软骨损伤的治疗至关重要[104]。Spiller 等[105]研究发现,巨噬细胞经特定的环境或炎症介质刺激,会从 M1 型向 M2 型极化。Lo 等[106]研究发现,MSC-EXO 可以诱导巨噬细胞向 M2 型极化。

笔者认为,MSC-EXO 可能通过调控巨噬细胞向 M2 型极化而发挥抑制炎症反应并修复软骨损伤的作

用（图 3）。近年来，对 MSC－EXO 的一些研究也证实了笔者的观点。Zhang 等[37]研究发现，在培养基中加入 MSC－EXO 后，M1 型巨噬细胞标志物 $CD86^+$ 细胞的数量减少，而 M2 型巨噬细胞标志物 $CD163^+$ 细胞的数量显著增加。Cosenza 等[44]研究发现，MSC－EXO 可以使巨噬细胞标志物 $CD86^+$、$CD40^+$、主要组织相容性复合物Ⅱ的表达水平下降。这些研究结果表明，MSC－EXO 可以抑制巨噬细胞活化并调控其向 M2 型极化。有研究发现，经 MSC－EXO 治疗后，炎症因子 IL－6[47,52,84,98]、TNF－α[37,44,52]、IL－1β[37,47]、环氧合酶－2[47,52]、前列腺素－2[52,98]、诱导型一氧化氮合酶[44,52]、IL－1A、IL－8、IL－17[47]的表达水平下降，抗炎因子 IL－10[44,52,98]的表达水平升高；这表明 MSC－EXO 可以通过调控免疫细胞（包括巨噬细胞）抑制炎症因子的表达、促进抗炎因子的表达，从而发挥免疫调节和抗炎作用。

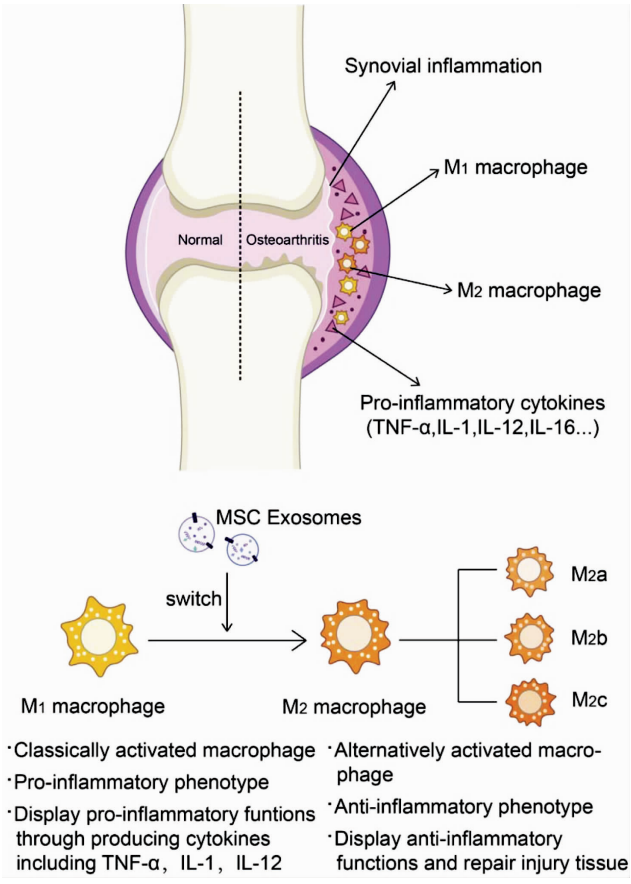

图 3　间充质干细胞来源外泌体的免疫调节机制示意图[107]

4.5　通过内源性 miRNA 调控软骨修复　EXO 中包含 150 种左右的 miRNA[108－109]，这些 miRNA 又称 EXO 源性 miRNA。Carthew 等[110]研究发现，EXO 中的 miRNA 含量高于其亲代细胞。miRNA 主要通过与靶基因 3’端非翻译区不完全配对，在转录后水平抑制靶基因的翻译过程，从而抑制相关蛋白的表达[111]。miRNA 与软骨细胞的新陈代谢、OA 的病理进展密切相关[112]，如 miR－320 可以抑制 MMP－13 的表达，并调控 IL－1β 诱导的软骨细胞代谢[113]；miR－92 可以调控 co19a2 和聚蛋白多糖的表达[114]；miR－140 在软骨的生长发育、稳态维持及损伤修复过程中具有重要作用[115]。

笔者认为，MSC－EXO 可能通过其内源性的 miRNA 进行信号传导，调控相关蛋白的表达，从而促进软骨修复。Tao 等[46]研究发现，与正常滑膜间充质干细胞（synovium－derived mesenchymal stem cells，SMSC）－EXO 相比，miR－140－5p 过表达的 SMSC－EXO 可以通过 Wnt 信号通路促进 OA 软骨细胞的增殖和迁移。Sun 等[81]研究发现，与正常骨髓间充质干细胞（bone marrow－derived mesenchymal stem cells，BMSC）－EXO 相比，miR－320c 过表达的 BMSC－EXO 可以有效促进软骨细胞的增殖及 SOX－9 的表达，并抑制 MMP－13 的表达。Wu 等[68]研究发现，miR－100－5p 过表达的髌下脂肪垫来源 EXO 可以通过靶向 mTOR mRNA 下调 mTOR 蛋白的表达水平，从而抑制 OA 软骨细胞凋亡和分解代谢，并促进其合成代谢。Mao 等[45]研究发现，与正常的 BMSC－EXO 相比，miR－92a－3p 过表达的 BMSC－EXO 可以通过 Wnt5a 促进软骨细胞增殖及基质合成。这些研究结果表明，内源性 miRNA 在 MSC－EXO 修复软骨的过程中具有重要作用，miRNA 过表达的 MSC－EXO 具有更强的软骨修复作用。因此，基因修饰后的 MSC－EXO可能会成为下一个研究热点。

5　小　结

OA 和软骨损伤的治疗一直是再生医学的热点和难点问题。现有的研究结果表明，MSC－EXO 具有增强软骨细胞活性和增殖能力、抑制炎症反应、促进损伤组织修复或再生、延缓 OA 病情进展等作用。但是目前与 MSC－EXO 相关的临床试验尚未启动，且 EXO 疗法尚存在作用机制不明确、最佳给药剂量及频率不确定等问题，因此需要对 MSC－EXO 进行更深入的研究。

参考文献

[1]　MURRAY C J，VOS T，LOZANO R，et al. Disability-adjusted life years（DALYs）for 291 diseases and injuries in 21 regions，1990－2010：a systematic analysis for the Global Bur-

den of Disease Study 2010[J]. Lancet,2012,380(9859):2197 – 2223.

[2] FELLOWS C R,MATTA C,MOBASHERI A. Applying proteomics to study crosstalk at the cartilage – subchondral bone interface in osteoarthritis:current status and future directions[J]. EBioMedicine,2016,11:2 – 4.

[3] VINATIER C,BOUFFI C,MERCERON C,et al. Cartilage tissue engineering:towards a biomaterial – assisted mesenchymal stem cell therapy[J]. Curr Stem Cell Res Ther,2009,4(4):318 – 329.

[4] IM G I. Regeneration of articular cartilage using adipose stem cells[J]. J Biomed Mater Res A,2016,104(7):1830 – 1844.

[5] IM G I. Clinical use of stem cells in orthopaedics[J]. Eur Cell Mater,2017,33:183 – 196.

[6] TOH W S,LAI R C,HUI J H P,et al. MSC exosome as a cell – free MSC therapy for cartilage regeneration:Implications for osteoarthritis treatment[J]. Semin Cell Dev Biol,2017,67:56 – 64.

[7] MAKRIS E A,GOMOLL A H,MALIZOS K N,et al. Repair and tissue engineering techniques for articular cartilage[J]. Nat Rev Rheumatol,2015,11(1):21 – 34.

[8] GAO L,GOEBEL L,ORTH P,et al. Subchondral drilling for articular cartilage repair:a systematic review of translational research[J]. Dis Model Mech,2018,11(6):dmm034280.

[9] MIYAKI S,LOTZ M K. Extracellular vesicles in cartilage homeostasis and osteoarthritis[J]. Curr Opin Rheumatol,2018,30(1):129 – 135.

[10] FRIEDENSTEIN A J,GORSKAJA J F,KULAGINA N N. Fibroblast precursors in normal and irradiated mouse hematopoietic organs[J]. Exp Hematol,1976,4(5):267 – 274.

[11] CRISAN M,YAP S,CASTEILLA L,et al. A perivascular origin for mesenchymal stem cells in multiple human organs[J]. Cell Stem Cell,2008,3(3):301 – 313.

[12] ZUK P A,ZHU M,ASHJIAN P,et al. Human adipose tissue is a source of multipotent stem cells[J]. Mol Biol Cell,2003,13(12):4279 – 4295.

[13] HUANG L,NIU C,WILLARD B,et al. Proteomic analysis of porcine mesenchymal stem cells derived from bone marrow and umbilical cord:implication of the proteins involved in the higher migration capability of bone marrow mesenchymal stem cells[J]. Stem Cell Res Ther,2015,6(1):77.

[14] DE BARI C,DELL'ACCIO F,VANDENABEELE F,et al. Skeletal muscle repair by adult human mesenchymal stem cells from synovial membrane[J]. J Cell Biol,2003,160(6):909 – 918.

[15] PITTENGER M F,MACKAY A M,BECK S C,et al. Multilineage potential of adult human mesenchymal stem cells[J]. Science,1999,284(5411):143 – 147.

[16] LEE W Y,WANG B. Cartilage repair by mesenchymal stem cells:Clinical trial update and perspectives[J]. J Orthop Translat,2017,9:76 – 88.

[17] MENDICINO M,BAILEY A M,WONNACOTT K,et al. MSC – based product characterization for clinical trials:an FDA perspective[J]. Cell Stem Cell,2014,14(2):141 – 145.

[18] BASTOS R,MATHIAS M,ANDRADE R,et al. Intra – articular injections of expanded mesenchymal stem cells with and without addition of platelet – rich plasma are safe and effective for knee osteoarthritis[J]. Knee Surg Sports Traumatol Arthrosc,2018,26(11):3342 – 3350.

[19] VEGA A,MARTIN – FERRERO M A,DEL C F,et al. Treatment of knee osteoarthritis with allogeneic bone marrow mesenchymal stem cells:a randomized controlled trial[J]. Transplantation,2015,99(8):1681 – 1690.

[20] UCCELLI A,DE ROSBO N K. The immunomodulatory function of mesenchymal stem cells:mode of action and pathways[J]. Ann N Y Acad Sci,2015,1351:114 – 126.

[21] ROELOFS A J,ROCKE J P,DE BARI C. Cell – based approaches to joint surface repair:a research perspective[J]. Osteoarthritis Cartilage,2013,21(7):892 – 900.

[22] MAUMUS M,GUÉRIT D,TOUPET K,et al. Mesenchymal stem cell – based therapies in regenerative medicine:applications in rheumatology[J]. Stem Cell Res Ther,2011,2(2):14.

[23] AL – NAJAR M,KHALIL H,AL – AJLOUNI J,et al. Intra-articular injection of expanded autologous bone marrow mesenchymal cells in moderate and severe knee osteoarthritis is safe:a phase I/II study[J]. J Orthop Surg Res,2017,12(1):190.

[24] LAMO – ESPINOSA J M,MORA G,BLANCO J F,et al. Intra – articular injection of two different doses of autologous bone marrow mesenchymal stem cells versus hyaluronic acid in the treatment of knee osteoarthritis:long – term follow up of a multicenter randomized controlled clinical trial(phase I/II)[J]. J Transl Med,2018,16(1):213.

[25] LOPA S,COLOMBINI A,MORETTI M,et al. Injective mesenchymal stem cell – based treatments for knee osteo-

arthritis:from mechanisms of action to current clinical evidences[J]. Knee Surg Sports Traumatol Arthrosc,2019,27(6):2003 - 2020.

[26] ROFFI A,NAKAMURA N,SANCHEZ M,et al. Injectable systems for intra - articular delivery of mesenchymal stromal cells for cartilage treatment:a systematic review of preclinical and clinical evidence[J]. Int J Mol Sci,2018,19(11):3322.

[27] JEVOTOVSKY D S,ALFONSO A R,EINHORN T A,et al. Osteoarthritis and stem cell therapy in humans:a systematic review[J]. Osteoarthritis Cartilage,2018,26(6):711 - 729.

[28] SIDDAPPA R,LICHT R,VAN BLITTERSWIJK C,et al. Donor variation and loss of multipotency during in vitro expansion of human mesenchymal stem cells for bone tissue engineering[J]. J Orthop Res,2007,25(8):1029 - 1041.

[29] MUSIC E,FUTREGA K,DORAN M R. Sheep as a model for evaluating mesenchymal stem/stromal cell(MSC) - based chondral defect repair[J]. Osteoarthritis Cartilage,2018,26(6):730 - 740.

[30] TOH W S,FOLDAGER C B,PEI M,et al. Advances in mesenchymal stem cell - based strategies for cartilage repair and regeneration[J]. Stem Cell Rev Rep,2014,10(5):686 - 696.

[31] MEIRELLES LDA S,FONTES A M,COVAS D T,et al. Mechanisms involved in the therapeutic properties of mesenchymal stem cells[J]. Cytokine Growth Factor Rev,2009,20(5/6):419 - 427.

[32] HOFER H R,TUAN R S. Secreted trophic factors of mesenchymal stem cells support neurovascular and musculoskeletal therapies[J]. Stem Cell Res Ther,2016,7(1):131.

[33] LIANG X,DING Y,ZHANG Y,et al. Paracrine mechanisms of mesenchymal stem cell - based therapy:current status and perspectives[J]. Cell Transplant,2014,23(9):1045 - 1059.

[34] WU L,LEIJTEN J C,GEORGI N,et al. Trophic effects of mesenchymal stem cells increase chondrocyte proliferation and matrix formation[J]. Tissue Eng Part A,2011,17(9/10):1425 - 1436.

[35] LAI J H,KAJIYAMA G,SMITH R L,et al. Stem cells catalyze cartilage formation by neonatal articular chondrocytes in 3D biomimetic hydrogels[J]. Sci Rep,2013,3:3553.

[36] YEO R W Y,LIM S K. Exosomes and their therapeutic applications[J]. World Scientific,2015:477 - 501.

[37] ZHANG S,CHUAH S J,LAI R C,et al. MSC exosomes mediate cartilage repair by enhancing proliferation,attenuating apoptosis and modulating immune reactivity[J]. Biomaterials,2018,156:16 - 27.

[38] ZHANG S,CHU W C,LAI R C,et al. Exosomes derived from human embryonic mesenchymal stem cells promote osteochondral regeneration[J]. Osteoarthritis Cartilage,2016,24(12):2135 - 2140.

[39] BJØRGE I M,KIM S Y,MANO J F,et al. Extracellular vesicles,exosomes and shedding vesicles in regenerative medicine - a new paradigm for tissue repair[J]. Biomater Sci,2017,6(1):60 - 78.

[40] KALLURI R,LEBLEU V S. The biology,function,and biomedical applications of exosomes[J]. Science,2020,367(6478):eaau6977.

[41] VALADI H,EKSTRÖM K,BOSSIOS A,et al. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells[J]. Nat Cell Biol,2007,9(6):654 - 659.

[42] CHAPUT N,FLAMENT C,VIAUD S,et al. Dendritic cell derived - exosomes:biology and clinical implementations[J]. J Leukoc Biol,2006,80(3):471 - 478.

[43] LO CICERO A,STAHL P D,RAPOSO G. Extracellular vesicles shuffling intercellular messages:for good or for bad[J]. Curr Opin Cell Biol,2015,35:69 - 77.

[44] COSENZA S,RUIZ M,TOUPET K,et al. Mesenchymal stem cells derived exosomes and microparticles protect cartilage and bone from degradation in osteoarthritis[J]. Sci Rep,2017,7(1):16214.

[45] MAO G,ZHANG Z,HU S,et al. Exosomes derived from miR - 92a - 3p - overexpressing human mesenchymal stem cells enhance chondrogenesis and suppress cartilage degradation via targeting WNT5A[J]. Stem Cell Res Ther,2018,9(1):247.

[46] TAO S C,YUAN T,ZHANG Y L,et al. Exosomes derived from miR - 140 - 5p - overexpressing human synovial mesenchymal stem cells enhance cartilage tissue regeneration and prevent osteoarthritis of the knee in a rat model[J]. Theranostics,2017,7(1):180 - 195.

[47] VONK L A,VAN DOOREMALEN S F J,LIV N,et al. Mesenchymal stromal/stem cell - derived extracellular vesicles promote human cartilage regeneration in vitro[J]. Theranostics,2018,8(4):906 - 920.

[48] WANG Y,YU D,LIU Z,et al. Exosomes from embryonic mesenchymal stem cells alleviate osteoarthritis through balancing synthesis and degradation of cartilage extracellular matrix[J]. Stem Cell Res Ther,2017,8(1):189.

[49] ZHU Y,WANG Y,ZHAO B,et al. Comparison of exosomes secreted by induced pluripotent stem cell – derived mesenchymal stem cells and synovial membrane – derived mesenchymal stem cells for the treatment of osteoarthritis[J]. Stem Cell Res Ther,2017,8(1):64.

[50] COSENZA S,TOUPET K,MAUMUS M,et al. Mesenchymal stem cells – derived exosomes are more immunosuppressive than microparticles in inflammatory arthritis[J]. Theranostics,2018,8(5):1399 – 1410.

[51] HEADLAND S E,JONES H R,NORLING L V,et al. Neutrophil – derived microvesicles enter cartilage and protect the joint in inflammatory arthritis[J]. Sci Transl Med,2015,7(315):315ra190.

[52] TOFIÑO – VIAN M,GUILLÉN M I,PÉREZ DEL CAZ M D,et al. Microvesicles from human adipose tissue-derived mesenchymal stem cells as a new protective strategy in osteoarthritic chondrocytes[J]. Cell Physiol Biochem,2018,47(1):11 – 25.

[53] LIU X,YANG Y,LI Y,et al. Integration of stem cell-derived exosomes with in situ hydrogel glue as a promising tissue patch for articular cartilage regeneration[J]. Nanoscale,2017,9(13):4430 – 4438.

[54] LI J J,HOSSEINI – BEHESHTI E,GRAU G E,et al. Stem cell-derived extracellular vesicles for treating joint injury and osteoarthritis[J]. Nanomaterials(Basel),2019,9(2):261.

[55] MIANEHSAZ E,MIRZAEI H R,MAHJOUBIN – TEHRAN M,et al. Mesenchymal stem cell – derived exosomes:a new therapeutic approach to osteoarthritis? [J]. Stem Cell Res Ther,2019,10(1):340.

[56] MENDT M,KAMERKAR S,SUGIMOTO H,et al. Generation and testing of clinical – grade exosomes for pancreatic cancer[J]. JCI Insight,2018,3(8):e99263.

[57] MUNAGALA R,AQIL F,JEYABALAN J,et al. Bovine milk-derived exosomes for drug delivery[J]. Cancer Lett,2016,371(1):48 – 61.

[58] HASLAUER C M,ELSAID K A,FLEMING B C,et al. Loss of extracellular matrix from articular cartilage is mediated by the synovium and ligament after anterior cruciate ligament injury[J]. Osteoarthritis Cartilage,2013,21(12):1950 – 1957.

[59] HEARD B J,BARTON K I,CHUNG M,et al. Single intra-articular dexamethasone injection immediately post-surgery in a rabbit model mitigates early inflammatory responses and post – traumatic osteoarthritis – like alterations[J]. J Orthop Res,2015,33(12):1826 – 1834.

[60] BEIER F,LOESER R F. Biology and pathology of Rho GTPase,PI – 3 kinase – Akt,and MAP kinase signaling pathways in chondrocytes[J]. J Cell Biochem,2010,110(3):573 – 580.

[61] MARTINI M,DE SANTIS M C,BRACCINI L,et al. PI3K/AKT signaling pathway and cancer:an updated review[J]. Ann Med,2014,46(6):372 – 383.

[62] MA D,KOU X,JIN J,et al. Hydrostatic compress force enhances the viability and decreases the apoptosis of condylar chondrocytes through Integrin – FAK – ERK/PI3K pathway[J]. Int J Mol Sci,2016,17(11):1847.

[63] LAI R C,TAN S S,TEH B J,et al. Proteolytic potential of the MSC exosome proteome:implications for an exosome-mediated delivery of therapeutic proteasome[J]. Int J Proteomics,2012,2012:971907.

[64] QI H,LIU D P,XIAO D W,et al. Exosomes derived from mesenchymal stem cells inhibit mitochondrial dysfunction-induced apoptosis of chondrocytes via p38,ERK,and Akt pathways[J]. In Vitro Cell Dev Biol Anim,2019,55(3):203 – 210.

[65] LOGAN C Y,NUSSE R. The Wnt signaling pathway in development and disease[J]. Annu Rev Cell Dev Biol,2004,20:781 – 810.

[66] XIANG C,YANG K,LIANG Z,et al. Sphingosine – 1 – phosphate mediates the therapeutic effects of bone marrow mesenchymal stem cell – derived microvesicles on articular cartilage defect[J]. Transl Res,2018,193:42 – 53.

[67] LIU Y,LIN L,ZOU R,et al. MSC – derived exosomes promote proliferation and inhibit apoptosis of chondrocytes via lncRNA – KLF3 – AS1/miR – 206/GIT1 axis in osteoarthritis[J]. Cell Cycle,2018,17(21 – 22):2411 – 2422.

[68] WU J,KUANG L,CHEN C,et al. miR – 100 – 5p – abundant exosomes derived from infrapatellar fat pad MSCs protect articular cartilage and ameliorate gait abnormalities via inhibition of mTOR in osteoarthritis[J]. Biomaterials,2019,206:87 – 100.

[69] LIU H,LI Z,CAO Y,et al. Effect of chondrocyte mitochondrial dysfunction on cartilage degeneration:a possible pathway for osteoarthritis pathology at the subcellular level[J]. Mol Med Rep,2019,20(4):3308 – 3316.

[70] LI H,WANG D,YUAN Y,et al. New insights on the MMP – 13 regulatory network in the pathogenesis of early osteoarthritis[J]. Arthritis Res Ther,2017,19(1):248.

[71] SAITO T,TANAKA S. Molecular mechanisms underlying osteoarthritis development:Notch and NF – kappaB[J]. Arthritis Res Ther,2017,19(1):94.

[72] MARCU K B,OTERO M,OLIVOTTO E,et al. NF - kappaB signaling:multiple angles to target OA[J]. Curr Drug Targets,2010,11(5):599 - 613.

[73] OGANDO J,TARDÁGUILA M,DÍAZ - ALDERETE A,et al. Notch - regulated miR - 223 targets the aryl hydrocarbon receptor pathway and increases cytokine production in macrophages from rheumatoid arthritis patients[J]. Sci Rep,2016,6:20223.

[74] MARIANI E, PULSATELLI L, FACCHINI A. Signaling pathways in cartilage repair[J]. Int J Mol Sci, 2014, 15(5):8667 - 8698.

[75] BI W,DENG J M,ZHANG Z,et al. Sox9 is required for cartilage formation[J]. Nat Genet,1999,22(1):85 - 89.

[76] YANG X,CHEN L,XU X,et al. TGF - beta/Smad3 signals repress chondrocyte hypertrophic differentiation and are required for maintaining articular cartilage[J]. J Cell Biol,2001,153(1):35 - 46.

[77] LI T F,DAROWISH M,ZUSCIK M J,et al. Smad3 - deficient chondrocytes have enhanced BMP signaling and accelerated differentiation[J]. J Bone Miner Res, 2006, 21(1):4 - 16.

[78] HELLINGMAN C A,DAVIDSON E N,KOEVOET W,et al. Smad signaling determines chondrogenic differentiation of bone - marrow - derived mesenchymal stem cells:inhibition of Smad1/5/8P prevents terminal differentiation and calcification[J]. Tissue Eng Part A,2011,17(7/8):1157 - 1167.

[79] VAN DER KRAAN P M,BLANEY DAVIDSON E N,VAN DEN BERG W B. Bone morphogenetic proteins and articular cartilage:to serve and protect or a wolf in sheep clothing's?[J]. Osteoarthritis Cartilage,2010,18(6):735 - 741.

[80] NIADA S,GIANNASI C,GOMARASCA M,et al. Adipose-derived stromal cell secretome reduces TNFalpha-induced hypertrophy and catabolic markers in primary human articular chondrocytes[J]. Stem Cell Res,2019,38:101463.

[81] SUN H,HU S,ZHANG Z,et al. Expression of exosomal microRNAs during chondrogenic differentiation of human bone mesenchymal stem cells[J]. J Cell Biochem,2019, 120(1):171 - 181.

[82] LIU Y,ZOU R,WANG Z,et al. Exosomal KLF3 - AS1 from hMSCs promoted cartilage repair and chondrocyte proliferation in osteoarthritis[J]. Biochem J,2018,475(22):3629 - 3638.

[83] MAO G,HU S,ZHANG Z,et al. Exosomal miR - 95 - 5p regulates chondrogenesis and cartilage degradation via histone deacetylase 2/8[J]. J Cell Mol Med,2018,22(11): 5354 - 5366.

[84] RAGNI E,PERUCCA O C,DE LUCA P,et al. Interaction with hyaluronan matrix and miRNA cargo as contributors for in vitro potential of mesenchymal stem cell - derived extracellular vesicles in a model of human osteoarthritic synoviocytes[J]. Stem Cell Res Ther,2019,10(1):109.

[85] LOESER R F,COLLINS J A,DIEKMAN B O. Ageing and the pathogenesis of osteoarthritis[J]. Nat Rev Rheumatol, 2016,12(7):412 - 420.

[86] WANG Y,ZHAO X,LOTZ M,et al. Mitochondrial biogenesis is impaired in osteoarthritis chondrocytes but reversible via peroxisome proliferator - activated receptor γ coactivator 1α[J]. Arthritis Rheumatol,2015,67(8):2141 - 2153.

[87] RUIZ - ROMERO C,CALAMIA V,MATEOS J,et al. Mitochondrial dysregulation of osteoarthritic human articular chondrocytes analyzed by proteomics:a decrease in mitochondrial superoxide dismutase points to a redox imbalance[J]. Mol Cell Proteomics,2009,8(1):172 - 189.

[88] VAAMONDE - GARCIA C,RIVEIRO - NAVEIRA R R, VALCÁRCEL - ARES M N,et al. Mitochondrial dysfunction increases inflammatory responsiveness to cytokines in normal human chondrocytes[J]. Arthritis Rheum,2012, 64(9):2927 - 2936.

[89] TERKELTAUB R,JOHNSON K,MURPHY A,et al. Invited review:the mitochondrion in osteoarthritis[J]. Mitochondrion,2002,1(4):301 - 319.

[90] LOESER R F. Aging and osteoarthritis[J]. Curr Opin Rheumatol,2011,23(5):492 - 496.

[91] LAI R C,YEO R W,TAN K H,et al. Mesenchymal stem cell exosome ameliorates reperfusion injury through proteomic complementation[J]. Regen Med,2013,8(2): 197 - 209.

[92] ARSLAN F,LAI R C,SMEETS M B,et al. Mesenchymal stem cell - derived exosomes increase ATP levels,decrease oxidative stress and activate PI3K/Akt pathway to enhance myocardial viability and prevent adverse remodeling after myocardial ischemia/reperfusion injury[J]. Stem Cell Res,2013,10(3):301 - 312.

[93] CHEN P,ZHENG L,WANG Y,et al. Desktop - stereolithography 3D printing of a radially oriented extracellular matrix/mesenchymal stem cell exosome bioink for osteochondral defect regeneration[J]. Theranostics, 2019, 9(9):2439 - 2459.

[94] SANSONE P,SAVINI C,KURELAC I,et al. Packaging and

transfer of mitochondrial DNA via exosomes regulate escape from dormancy in hormonal therapy-resistant breast cancer[J]. Proc Natl Acad Sci U S A,2017,114(43):E9066 – E9075.

[95] QI H,LIU D P,XIAO D W,et al. Exosomes derived from mesenchymal stem cells inhibit mitochondrial dysfunction-induced apoptosis of chondrocytes via p38,ERK,and Akt pathways[J]. In Vitro Cell Dev Biol Anim,2019,55(3):203 – 210.

[96] GOLDRING M B,OTERO M,PLUMB D A,et al. Roles of inflammatory and anabolic cytokines in cartilage metabolism:signals and multiple effectors converge upon MMP – 13 regulation in osteoarthritis[J]. Eur Cell Mater,2011,21:202 – 220.

[97] MANFERDINI C,PAOLELLA F,GABUSI E,et al. From osteoarthritic synovium to synovial – derived cells characterization:synovial macrophages are key effector cells[J]. Arthritis Res Ther,2016,18:83.

[98] VAN LENT P L,GREVERS L,BLOM A B,et al. Myeloid-related proteins S100A8/S100A9 regulate joint inflammation and cartilage destruction during antigen – induced arthritis[J]. Ann Rheum Dis,2008,67(12):1750 – 1758.

[99] LEWIS J S Jr,FURMAN B D,ZEITLER E,et al. Genetic and cellular evidence of decreased inflammation associated with reduced incidence of posttraumatic arthritis in MRL/MpJ mice[J]. Arthritis Rheum,2013,65(3):660 – 670.

[100] BONDESON J,WAINWRIGHT S D,LAUDER S,et al. The role of synovial macrophages and macrophage – produced cytokines in driving aggrecanases,matrix metalloproteinases,and other destructive and inflammatory responses in osteoarthritis[J]. Arthritis Res Ther,2006,8(6):R187.

[101] BONDESON J,BLOM A B,WAINWRIGHT S,et al. The role of synovial macrophages and macrophage – produced mediators in driving inflammatory and destructive responses in osteoarthritis[J]. Arthritis Rheum,2010,62(3):647 – 657.

[102] XIE J,HUANG Z,YU X,et al. Clinical implications of macrophage dysfunction in the development of osteoarthritis of the knee[J]. Cytokine Growth Factor Rev,2019,46:36 – 44.

[103] DAS A,SINHA M,DATTA S,et al. Monocyte and macrophage plasticity in tissue repair and regeneration[J]. Am J Pathol,2015,185(10):2596 – 2606.

[104] UTOMO L,VAN OSCH G J,BAYON Y,et al. Guiding synovial inflammation by macrophage phenotype modulation:an in vitro study towards a therapy for osteoarthritis[J]. Osteoarthritis Cartilage,2016,24(9):1629 – 1638.

[105] SPILLER K L,ANFANG R R,SPILLER K J,et al. The role of macrophage phenotype in vascularization of tissue engineering scaffolds[J]. Biomaterials,2014,35(15):4477 – 4488.

[106] LO SICCO C,REVERBERI D,BALBI C,et al. Mesenchymal stem cell-derived extracellular vesicles as mediators of anti-inflammatory effects:endorsement of macrophage polarization[J]. Stem Cells Transl Med,2017,6(3):1018 – 1028.

[107] WU X,WANG Y,XIAO Y,et al. Extracellular vesicles:potential role in osteoarthritis regenerative medicine[J]. J Orthop Translat,2019,21:73 – 80.

[108] CHEN T S,LAI R C,LEE M M,et al. Mesenchymal stem cell secretes microparticles enriched in pre – microRNAs[J]. Nucleic Acids Res,2010,38(1):215 – 224.

[109] GOLDIE B J,DUN M D,LIN M,et al. Activity – associated miRNA are packaged in Map1b – enriched exosomes released from depolarized neurons[J]. Nucleic Acids Res,2014,42(14):9195 – 9208.

[110] CARTHEW R W,SONTHEIMER E J. Origins and Mechanisms of miRNAs and siRNAs[J]. Cell,2009,136(4):642 – 655.

[111] LE LT,SWINGLER T E,CLARK I M. Review:the role of microRNAs in osteoarthritis and chondrogenesis[J]. Arthritis Rheum,2013,65(8):1963 – 1974.

[112] MENG F,ZHANG Z,CHEN W,et al. MicroRNA – 320 regulates matrix metalloproteinase – 13 expression in chondrogenesis and interleukin – 1β – induced chondrocyte responses[J]. Osteoarthritis Cartilage,2016,24(5):932 – 941.

[113] HOU C,ZHANG Z,ZHANG Z,et al. Presence and function of microRNA – 92a in chondrogenic ATDC5 and adipose – derived mesenchymal stem cells[J]. Mol Med Rep,2015,12(4):4877 – 4886.

[114] MIYAKI S,SATO T,INOUE A,et al. MicroRNA – 140 plays dual roles in both cartilage development and homeostasis[J]. Genes Dev,2010,24(11):1173 – 1185.

[115] TOFIÑO – VIAN M,GUILLÉN M I,PÉREZ DEL CAZ M D,et al. Extracellular vesicles from adipose – derived mesenchymal stem cells downregulate senescence features in osteoarthritic osteoblasts[J]. Oxid Med Cell Longev,2017,2017:7197598.

(收稿日期:2020-03-13　本文编辑:郭毅曼)