MSC来源的外泌体在OA中潜在的免疫调节及抗炎作用

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摘要 骨性关节炎(osteoarthritis, OA)是一种整体的“器官”疾病, 伴随着复杂的病理改变。大量研究表明, OA中存在一种慢性、轻度的炎症, 伴随相关免疫反应, 并作为中心环节贯穿其病理变化过程。间充质干细胞(mesenchymal stem cell, MSC)因其抗炎免疫调节的能力而备受关注。间充质干细胞经旁分泌途径分泌外泌体(exosomes), 是其发挥抗炎及免疫调节作用的主要机制之一。MSC来源的外泌体介导传递具有抗炎及免疫活性的分子, 尤其是miRNA, 协调炎症微环境并促进组织修复重建。该文就MSC来源外泌体在OA中潜在的免疫调节、抗炎作用及其可塑性进行探讨, 以期为OA的治疗提供新思路。

关键词 骨性关节炎; 间充质干细胞; 外泌体; 抗炎; 免疫调节

The Emerging Immunomodulatory and Anti-Inflammatory Activity of Mesenchymal Stem Cell-Derived Exosomes in Osteoarthritis

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Abstract Osteoarthritis (OA), as a disease of the joint as an “organ”, which is accompanied with complex pathologic changes. Accumulating evidence indicate that a critical role of chronic and low-grade inflammation and concomitant immunoreaction in the pathogenesis of osteoarthritis. Mesenchymal stem cell attracted the most attention due to its immunomodulatory and anti-inflammatory properties. Immunomodulatory and anti-inflammatory abilities of MSC are mainly attributed to the paracrine secretion, particularly exosomes, which is the main mechanism of MSC. MSC-derived exosomes could transfer bioactive substances, especially miRNA, which orchestrate inflammatory microenvironment and promote repairing effect in tissue injury. In this review, we discuss potential immunomodulatory and anti-inflammatory activity roles and plasticity of mesenchymal stem cell derived exosomes in OA. This may shed new light on osteoarthritis treatment.

Keywords OA; MSC; exosomes; anti-inflammatory; immunomodulatory

骨性关节炎以往一直被认为是一种因软骨磨损的退行性疾病[1]。但随着对骨性关节炎的研究进一步深入和发展, 人们渐渐认识到骨性关节炎实际上影响了整个关节结构, 伴随着复杂的病理过程, 包

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括软骨的退行性病变及降解, 还涉及周围滑膜的炎症、韧带及半月板的退变、软骨下骨的重塑及异位骨化的形成和关节囊的水肿——是一个整体的关节“器官”疾病[2]。大量研究表明, 在骨性关节炎中, 存在一种不同于类风湿性关节炎的慢性的、低度的炎症, 正是这种低度炎症, 在上述病理变化过程中扮演重要的角色, 驱使上述病理变化进一步加重[3]。而炎症的过程伴随免疫系统的激活, 并诱发免疫反应[4]。

近年来, 间充质干细胞因其有效的免疫调节和抗炎能力吸引了广泛的关注。大量研究显示, 在一些炎性疾病中, 如脓毒血症[5]、移植物抗宿主反应疾病[6]和多发性硬化[7]等疾病, 间充质干细胞治疗是一种有效的治疗手段。然而, 间充质干细胞的免疫调节及抗炎功能是具有可塑性的。不同程度的炎性刺激决定了间充质干细胞的抗炎免疫调节功能, 影响间充质干细胞的治疗效果[8]。研究表明, 在移植物抗宿主反应疾病的治疗中, 间充质干细胞的治疗效果取决于受体的炎症状态。当炎症程度较高时, 间充质干细胞的治疗效果更好; 反之在疾病早期阶段, 炎症程度较低时疗效较差[6,9]。

间充质干细胞的旁分泌途径是其发挥治疗作用的主要机制之一[10], 而其旁分泌途径是通过分泌外泌体实现的[11]。外泌体是一种能被大多数细胞分泌的直径为40~100 nm微小囊泡, 具有脂质双层膜结构, 可携带生物活性脂质、核酸(mRNA and microRNA)和蛋白等, 作用于靶细胞, 介导细胞间交流的重要介质[12]。间充质外泌体携带多种活性分子及超过150种miRNA, 涉及众多生化及细胞过程, 并可能在很多疾病的生理病理发挥功效[13-14]。最近, 研究表明在组织修复中, 外泌体是间充质干细胞发挥抗炎免疫调节功能的基础[15]。此外, 间充质干细胞的可塑性为进一步塑造外泌体提供了可能。外泌体具有特殊的双侧膜结构, 可作为稳定的载体, 保护其包裹的物质, 并易于被靶细胞接受。相较于间充质干细胞, 因其分子直径小, 易于滤过除菌; 且不可复制, 而难以因周围环境改变而改变; 加之其更小的免疫原性, 可减少干细胞移植引起的免疫反应, 更易应用于治疗[11]。

1 骨性关节炎的炎症及免疫过程

尽管骨性关节炎的危险因素众多, 包括年龄、性别、肥胖、劳损、创伤、遗传因素及关节畸形先天异常等[16], 但不同因素导致的骨性关节炎表现相似的病理改变, 影响到整个关节结构[2]。关节结构的各个组分(如滑膜、软骨组织及软骨下骨)继发改变, 且之间互有关联并相互作用, 导致关节内环境的失衡, 最终诱发骨性关节炎。而炎症伴随着免疫反应是整个骨性关节炎过程的中心环节。

1.1 固有免疫反应机制

固有免疫即非特异性免疫, 是机体针对外源性侵入物的第一线防御机制。在骨性关节炎中, 这种免疫机制通常由模式识别受体(pattern recognition receptor, PRR)识别损伤相关的分子模式激活。正常情况下, 一旦损伤相关的分子模式(damage assciated molecular pattern, DAMP)——模式识别受体通路激活, 将促进免疫系统生产一系列炎症介质激发免疫反应, 促进组织修复。然而, 病理情况(如骨性关节炎)下, 失调的损伤相关的分子模式–模式识别受体通路–炎症介质可能带来不良的后果, 造成关节结构的破坏[17], 而这种破坏是一种慢性损伤的过程[18]。

1.1.1 损伤相关分子模式——模式识别受体(DAMP-PRR) 损伤相关分子模式包括由坏死、损伤的细胞来源的警报素、来源于损伤的细胞外基质、炎症引起血管通透性改变或损伤造成血管渗漏血浆蛋白及软骨组织因反复磨损或创伤而释放的纤维结晶等。在骨性关节炎中, 通常识别损伤相关分子模式的模式识别受体是Toll样受体。人体中共有10种Toll样受体(1-10), 表达于多种细胞尤其是巨噬细胞上, 以及软骨细胞[19]。有研究报道, 在骨性关节炎病人的滑膜上均可检测到Toll样受体1-7及Toll样受体9。Toll样受体的激活与滑膜炎的形成、软骨组织的退变及对骨性关节炎易感性的增加相关[20]。此外, Toll样受体的激活经募集髓样分化因子88(myeloid differentiation factor 88, MyD88)最终引起炎症转录程序的激活, 特别是核因子-κB(nuclear factor-kappa B, NF-κB)途径, 继而诱发炎症基因表达[18]。

1.1.2 NF-κB通路 NF-κB通路是骨性关节炎中基因调控的枢纽, 可控制骨性关节炎(osteoarthritis, OA)中相关蛋白表达, 继而影响OA的炎症及免疫反应过程[21]。在鼠关节炎模型中, 利用腺病毒载体装载siRNA特异性抑制NF-κB-p56, 可减少关节液中两种重要炎症因子白介素-1β(IL-1β)及肿瘤坏死因子-α(tumor necrosis factor-α, TNF-α)的表达, 并减少软骨降解及缓解滑膜炎[22]。在体外培养的滑膜成纤维细胞中, 特异性过表达核因子-κB的抑制蛋白(inhibitor of

nuclear factor-kappa B, IκB), 可减少IL-6、IL-8、核细胞趋化蛋白-1(monocytic chemoattractant protein-1, MCP-1)、趋化因子2(CCL2)及基质金属蛋白酶(matrix metalloproteinase, MMPs)的水平[23]。

1.1.3 补体系统 越来越多研究表明, 补体系统在骨性关节炎发病过程中扮演了重要的角色[24]。骨性关节炎中相关的损伤相关分子模式, 诸如凋亡细胞碎片、钙结晶, 都能结合并激活补体系统[3]。此外, 血液的渗漏、软骨及滑膜巨噬细胞均是补体的重要来源[25]。目前关于补体系统与骨性关节炎发病的关系尚不明确。但有研究表明, 一种补体成分1s(C1s)可降解软骨保护营养因子[26]。相反, 抑制C1s可改善骨性关节炎症状[27]。提示针对补体系统可能是治疗骨性关节炎的手段。

1.1.4 巨噬细胞 巨噬细胞是渗入滑膜中最主要的细胞之一。在骨性关节炎滑膜中, 可检测到丰富的巨噬细胞[28]。如上述, 巨噬细胞与Toll样受体和补体系统密切相关。补体系统及损伤相关分子模式均能激活固有免疫细胞如巨噬细胞[29-30]。在骨性关节炎中, 激活的巨噬细胞介导众多可溶性分子释放, 包括生长因子如转化生长因子(transforming growth factor-β, TGF-β)[31]、血管内皮生长因子(vascular endothelial growth factor, VEGF)[32]、MMPs[33]、IL-1β及TNF-α[34]及相关促炎因子(如IL-6, IL-8)[35], 从而介导滑膜纤维化及血管形成、骨赘形成及软骨降解等骨性关节炎相关病理过程。此外, 研究表明, 极化的M2巨噬细胞可促进IL-10分泌从而起抗炎作用[36], 然而极化的M1巨噬细胞可经IL-6抑制间充质干细胞的成软骨分化[37]。关于巨噬细胞在骨性关节炎中的作用值得进一步研究。

1.2 适应性免疫反应机制

目前, 适应性免疫反应机制在骨性关节炎的机制仍未完全阐明。Fernandez-Madrid等[38]在骨性关节炎患者术后组织活检标本中, 发现滑膜中渗入了B淋巴细胞及T淋巴细胞(其中以CD4阳性T淋巴细胞为主), 提示骨性关节炎与适应性免疫反应机制相关。在骨性关节炎中, 坏死的细胞碎片、成纤维细胞膜及降解的软骨组织成分[39-41], 都可能成为潜在的抗原来源, 诱发适应性免疫反应的激活, 可能导致整个关节内环境紊乱, 加重骨性关节炎。

1.3 骨性关节炎中相关炎症介质

如上述, 许多小分子可能作为炎症介质参与了骨性关节炎的过程。诸如促炎因子(TNF、IL-1β、IL-6、IL-15、IL-17、IL-18、IL-21)[42]、趋化因子(IL-8、CCL5、CCL19、MCP-1)[20]、生长因子[TGF-β、成纤维细胞生长因子(fibroblast growth factor, FGF)、VEGF、神经生长因子(nerve growth factor, NGF)][3]、脂肪因子(瘦素、脂联素、抵抗素等)[3]及一氧化碳(NO)与前列腺素(protaglandin E2, PGE2)[42]等, 它们可能来源于软骨、骨组织、滑膜、周围脂肪组织及渗入滑膜的免疫细胞。研究显示, 靶向抑制VEGF对骨性关节炎相关的炎症及滑膜血管形成有效[43]。但靶向针对如TNF及IL-1β的治疗效果是不确切的或者收效甚微, 有的只是缓解了疼痛症状[44], 有的甚至毫无作用[45]。单一针对某一炎症介质的治疗是有局限性的。充分理解骨性关节炎中复杂的炎症网络系统, 进一步研究相关炎症介质的作用机制, 探明关于靶向针对其他相关炎症介质的疗效, 有助于更加有效系统治疗骨性关节炎。

2 间充质干细胞的免疫调节及抗炎作用

间充质干细胞因其抗炎免疫调节的作用被广泛运用于诸多自免性疾病模型中, 并取得了良好的治疗效果。总体来说, 间充质干细胞作用在于调整炎症与组织重建的平衡, 使受损组织处于相对稳态的环境中, 促进组织修复与重建[46]。在骨性关节炎中, 间充质干细胞调节关节内炎症微环境, 促进软骨组织修复重建[47]。但干细胞疗法面临诸多问题。值得注意的是, 在治疗过程中间充质干细胞可能存在分化现象[48], 然而分化的间充质干细胞是否保留原有的抗炎免疫调节功能仍存在争议[49-50], 待进一步研究证实。此外, 间充质干细胞是具有可塑性的。间充质干细胞的抗炎免疫调节功能依赖于受体的炎症状态[46]。故在运用干细胞疗法时, 需评估受体的炎症程度, 相应调整或综合其他治疗手段, 以达到治疗效果。相较间充质干细胞, 外泌体因其特殊的双层脂质膜结构在炎症环境中更具稳定性, 加之其更小的免疫原性, 易于被靶细胞接受; 分子直径小, 易于滤过除菌; 并很好地模仿其来源母体细胞的功能等特性。因此, 外泌体疗法将拥有广阔的前景。

3 外泌体潜在的免疫调节及抗炎作用

3.1 外泌体协调炎症微环境及其可塑性

关于外泌体的抗炎免疫调节能力在一些体外

实验中已经得到了阐述。如在一个外周血单核细胞—外泌体共培养体系中, 外泌体减少了促炎因子TNF-α及IL-1β的分泌, 并增加TGF-β的含量; 促进辅助性T细胞1(Th1)向辅助性T细胞2(Th2)转化, 并提高了调节性T细胞(regulatory cells, Treg)的含量[51]。Zhang等[52]发现, 外泌体有助于Treg的极化激活, 引起人急性白血病单核细胞(THP1)呈现M2巨噬细胞类似表型及增加抗炎因子IL-10的表达, 并证明了间充质干细胞来源外泌体可能与Toll样受体尤其是Toll样受体4(Toll-like receptor 4, TLR4)有关。间充质干细胞外泌体被运用于多种疾病模型的治疗之中, 并展现出协调炎症微环境的能力。在严重烧伤的鼠模型中, 人脐带间充质干细胞外泌体可显著减少中性粒细胞和巨噬细胞(CD68)的数量及TLR4蛋白的水平(而不影响TLR4 mRNA的水平), 并增加抗炎因子IL-10的表达, 降低炎症因子TNF-α及IL-1β的水平。此外, 研究表明, miR-181c可与TLR4 mRNA的3′-UTR段结合, 降低TLR4蛋白的表达, 进而限制炎症反应; 而相较假手术组, 严重烧伤鼠组的miR-181c表达显著降低。故进一步研究发现, 将miR-181c转染于人脐带间充质干细胞后可获得富集miR-181c的外泌体。且相较于未经转染的外泌体, 富集miR-181c的外泌体能更加显著减少TLR4蛋白的水平(而不影响TLR4 mRNA的水平), 并增加IL-10的表达, 降低TNF-α及IL-1β的水平。由此可见, 经富集miR-181c的人脐带间充质干细胞来源外泌体能更加有效抑制TLR4信号通路和减轻炎症反应[53]。

间充质干细胞是具有可塑性的。经处理过的间充质干细胞分泌特性不同的外泌体。进一步塑造外泌体可能获得更好的治疗效果。经TNF-α处理的人骨髓间充质干细胞来源外泌体具有更好的抗炎效果[54]。外泌体特有的双层脂质结构使其成为药物的一种很好的载体, 特别是某些水溶性及脂溶性药物。利用外泌体呈递脂溶性药物姜黄素不仅保证了在血液中的稳定性及浓度, 且靶向作用于炎症细胞, 不对周围正常细胞产生副作用, 甚至加强了姜黄素的抗炎作用[55]。此外, 近年来的研究表明, 外泌体与miRNA具有很强的关联性。外泌体介导miRNA传递是细胞之间交流的重要机制[56]。miRNA是一系列长度21~24个核苷酸的非编码RNA分子, 它们参与转录后基因表达调控[57]。Zou等[58]的研究发现, 人脐带间充质及其外泌体具有相似的miRNA组分。其中一小部分在外泌体内富集的miRNA与氧化应激、T细胞的激活及Toll样受体通路相关。

3.2 外泌体与骨性关节炎

3.2.1 *衰老相关分泌表型*(senescence-associated secretory phenotype, SASP) 近来, 软骨细胞的衰老被认为在骨性关节炎发病过程中扮演重要的角色[59]。OA中软骨细胞常展现出SASP, 分泌SASP相关分子(如促炎因子IL-6等), 对临近细胞造成不良影响, 进一步加重OA[60]。有趣的是, 衰老细胞分泌的外泌体也可能作为SASP之一[61]。针对软骨细胞的衰老可有效延缓骨性关节炎。有研究表明, 在IL-1β诱发软骨细胞衰老体系中, 间充质干细胞有效对抗软骨细胞的衰老[62]。而且前关于外泌体与软骨细胞衰老的机制尚不明确, 值得进一步研究。

3.2.2 *抗炎免疫调节与软骨组织重建* 协调骨性关节炎中炎症及免疫反应和组织重建的平衡是治疗骨性关节炎的关键。首先, 关节软骨主要由软骨细胞和软骨基质构成, 且软骨细胞在维持软骨形态和功能方面发挥重要作用, 软骨基质的合成与更新主要依靠软骨细胞[63]。正常软骨细胞外基质的合成和降解也处于动态平衡。在骨性关节炎的炎症微环境中, 正常的动态平衡被打破。

研究表明, 间充质干细胞来源的外泌体通过增加了细胞外基质II型胶原蛋白(Collagen II, Col II)、蛋白聚糖的合成, 降低解聚蛋白样金属蛋白酶-5(a disintegrin and metalloprotease with thrombospondin motifs-5, ADAMTS-5)、MMPs及胶原蛋白酶的表达, 促进了细胞外基质的沉积, 从而加强软骨组织重建[64-67]。进一步研究发现, 在经IL-1β处理的软骨细胞中添加胚胎间充质干细胞来源的外泌体后, 免疫组化结果显示, Col II表达明显增加, 且Col II阳性的细胞显著多于对照组。此外, 标记后的外泌体可在表达Col II的软骨细胞内检测到, 因此表明外泌体和软骨细胞间存在共定位的可能[65]。在软骨缺损的鼠模型中, PBS对照组中软骨大部分表现为纤维化软骨修复, 而外泌体组则表现为表面更为规整的透明软骨修复, 与邻近软骨组织紧密结合[64]。

外泌体表现出抗炎作用。外泌体减少了促炎因子TNF-α、IL-6、PGE2及NO的含量, 这可 能 与NF-κB通路有关, 并增加了抗炎因子IL-10的表达[66]; 并遏制了TNF-α引起的炎症反应如促炎因子白介素及环氧化酶-2(cyclooxygenase-2, COX-2)的表达。然

而, 外泌体匮乏的间充质干细胞上清液对COX-2表达的抑制作用明显更弱。这说明, 外泌体是间充质干细胞抗炎旁分泌途径的重要成分。为进一步理解外泌体的抗炎作用, 研究检测了NF-κB亚单位p65的亚细胞定位情况。经TNF-α处理可引起p65由细胞质向细胞核易位, 诱使NF-κB途径激活。而外泌体可抑制NF-κB的亚单位IκBα的磷酸化, 进而阻断NF-κB途径的激活[67]。此外, Zhang等[68]的研究发现外泌体具有免疫调节作用, 可吸引M2巨噬细胞大量渗入OA骨缺损及滑膜处, 而减少了M1巨噬细胞的渗入, 且下调M1巨噬细胞相关的促炎因子IL-1β和TNF-α的含量。综上所述, 间充质外泌体展现出潜在的抗炎免疫能力, 协调了炎症免疫与组织重建的平衡。

3.2.3 *塑造外泌体* 通过塑造外泌体可带来不同的效果。经IL-1β处理的滑膜成纤维细胞使关节软骨细胞展现出似骨性关节炎的表现[69]。经特异性转染miR-140-5p的滑膜间充质干细胞, 获得过表达miR-140-5p的外泌体, 并成功抑制了鼠骨性关节炎模型的进程[70]。利用外泌体特殊的结构(粒径小、双层脂质结构), 包含众多调节小分子(超过150种miRNA、一系列蛋白质组[71]), 并靶向作用于细胞, 对周围正常细胞不产生干扰, 且其更小的免疫原性及高稳定性等特性, 为外泌体疗法提供了可能。通过进一步塑造间充质细胞(如转染即siRNA沉默相关miRNA), 以克服原外泌体的缺点, 获得目的外泌体, 有助于更针对性地运用于治疗。

4 结论

骨性关节炎是整体的关节疾病。在骨性关节炎中, 关节的各个组分伴随着炎症及免疫的过程, 互有关联并相互作用。间充质干细胞来源外泌体能协调骨性关节炎中的炎症及免疫过程, 为治疗骨性关节炎提供了可能。通过相关手段塑造外泌体, 获得目的外泌体, 更加针对有效地运用于骨性关节炎治疗之中, 是未来的研究方向。但关于外泌体与骨性关节炎的炎症免疫过程的关系需进一步研究。此外, 如何有效、快速且低耗费制备大量外泌体是目前的难题。且关于外泌体在临床应用的可行性及安全性需进一步验证。

参考文献 (References)

1 Felson DT. Clinical practice. Osteoarthritis of the knee. N Engl J Med 2006; 354: 841-8.

2 Loeser RF, Goldring SR, Scanzello CR, Goldring MB. Osteoarthritis: a disease of the joint as an organ. Arthritis Rheum 2012; 64: 1697-707.

3 Robinson WH, Lepus CM, Wang Q, Raghu H, Mao R, Lindstrom TM, *et al*. Low-grade inflammation as a key mediator of the pathogenesis of osteoarthritis. Nat Rev Rheumatol 2016; 12(10): 580-92.

4 Zhuo Q, Yang W, Chen J, Wang Y. Metabolic syndrome meets osteoarthritis. Nat Rev Rheumatol 2012; 8: 729-37.

5 Nemeth K, Leelahavanichkul A, Yuen PS, Mayer B, Parmelee A, Doi K, *et al*. Bone marrow stromal cells attenuate sepsis via prostaglandin E(2)-dependent reprogramming of host macrophages to increase their interleukin-10 production. Nat Med 2009; 15(1): 42-9.

6 Ren G, Zhang L, Zhao X, Xu G, Zhang Y, Roberts AI, *et al*. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. Cell Stem Cell 2008; 2(2): 141-50.

7 Zappia E, Casazza S, Pedemonte E, Benvenuto F, Bonanni I, Gerdoni E, *et al*. Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T cell anergy. Blood 2005; 106(5): 1755-61.

8 Wang Y, Chen X, Cao W, Shi Y. Plasticity of mesenchymal stem cells in immunomodulation: pathological and therapeutic implications. Nat Immunol 2014; 15(11): 1009-16.

9 Tisato V, Naresh K, Girdlestone J, Navarrete C, Dazzi F. Mesenchymal stem cells of cord blood origin are effective at preventing but not treating graft-versus-host disease. Leukemia 2007; 21(9): 1992-9.

10 Gnecchi M, Danieli P, Malpasso G, Ciuffreda MC. Paracrine mechanisms of mesenchymal stem cells in tissue repair. Methods Mol Biol 2016; 1416: 123-46.

11 Kordelas L, Rebmann V, Ludwig AK, Radtke S, Ruesing J, Doeppner TR, *et al*. MSC-derived exosomes: a novel tool to treat therapy-refractory graft-versus-host disease. Leukemia 2014; 28(4): 970-3.

12 Lai RC, Yeo RW, Lim SK. Mesenchymal stem cell exosomes. Semin Cell Dev Biol 2015; 40: 82-8.

13 Lai RC, Tan SS, Teh BJ, Sze SK, Arslan F, de Kleijn DP, *et al*. Proteolytic potential of the MSC exosome proteome: implications for an exosome-mediated delivery of therapeutic proteasome. Int J Proteomics 2012; 2012: 971907.

14 Chen TS, Lai RC, Lee MM, Choo ABH, Lee CN, Lim SK. Mesenchymal stem cell secretes microparticles enriched in pre-microRNAs. Nucleic Acids Res 2010; 38: 21524.

15 Huss R, Guenther C, Hauser A. Advances in pharmaceutical cell therapy (Principles of Cell-Based Biopharmaceuticals). New Jersey: World Scientific 2015; 10.1142/9240: 401-13.

16 Felson DT, Lawrence RC, Dieppe PA, Hirsch R, Helmick CG, Jordan JM, *et al*. Osteoarthritis: new insights. Ann Intern Med 2000; 133(8): 635-46.

17 Orlowsky EW, Kraus VB. The role of innate immunity in osteoarthritis: when our first line of defense goes on the offensive. J Rheumatol 2015; 42: 363-71.

18 Scanzello CR, Plaas A, Crow MK. Innate immune system activation in osteoarthritis: is osteoarthritis a chronic wound? Curr

Opin Rheumatol 2008; 20(5): 565-72.
19 Janeway CA Jr, Medzhitov R. Innate immune recognition. Annu Rev Immunol 2002; 20: 197-216.
20 Scanzello CR, Goldring SR. The role of synovitis in osteoarthritis pathogenesis. Bone 2012; 51: 249-57.
21 Roman-Blas JA, Jimenez SA. NF-kappaB as a potential therapeutic target in osteoarthritis and rheumatoid arthritis. Osteoarthritis Cartilage 2006; 14(9): 839-48.
22 Chen LX, Lin L, Wang HJ, Wei XL, Fu X, Zhang JY, *et al*. Suppression of early experimental osteoarthritis by in vivo delivery of the adenoviral vector-mediated NF-kappaBp65-specific siRNA. Osteoarthritis Cartilage 2008; 16(2): 174-84.
23 Amos N, Lauder S, Evans A, Feldmann M, Bondeson J. Adenoviral gene transfer into osteoarthritis synovial cells using the endogenous inhibitor IkappaBalpha reveals that most, but not all, inflammatory and destructive mediators are NFkappaB dependent. Rheumatology(Oxford) 2006; 45(10): 1201-9.
24 Wang Q, Rozelle AL, Lepus CM, Scanzello CR, Song JJ, Larsen DM, *et al*. Identification of a central role for complement in osteoarthritis. Nat Med 2011; 17: 1674-9.
25 Bradley K, North J, Saunders D, Schwaeble W, Jeziorska M, Woolley DE, *et al*. Synthesis of classical pathway complement components by chondrocytes. Immunology 1996; 88: 648-56.
26 Clemmons DR, Jr BW, Garmong A, Schultz DR, Howell DS, Altman RD, *et al*. Inhibition of insulin-like growth factor binding protein 5 proteolysis in articular cartilage and joint fluid results in enhanced concentrations of insulin-like growth factor 1 and is associated with improved osteoarthritis. Arthritis Rheum 2002; 46(3): 694-703.
27 Busby WH, Yocum SA, Rowland M, Kellner D, Lazerwith S, Sverdrup F, *et al*. Complement 1s is the serine protease that cleaves IGFBP-5 in human osteoarthritic joint fluid. Osteoarthritis Cartilage 2009; 17(4): 547-55.
28 Revell PA, Mayston V, Lalor P, Mapp P. The synovial membrane in osteoarthritis: a histological study including the characterisation of the cellular infiltrate present in inflammatory osteoarthritis using monoclonal antibodies. Ann Rheum Dis 1988; 47(4): 300-7.
29 Foell D, Wittkowski H, Roth J. Mechanisms of disease: a 'DAMP' view of inflammatory arthritis. Nat Clin Pract Rheumatol 2007; 3(7): 382-90.
30 Blom AB, Lent PLEMV, Libregts S, Holthuysen AEM, Kraan PMVD, Rooijen NV, *et al*. Crucial role of macrophages in matrix metalloproteinase-mediated cartilage destruction during experimental osteoarthritis: involvement of matrix metalloproteinase 3. Arthritis Rheum 2007; 56: 147-57.
31 van Lent PL, Blom AB, van der Kraan P, Holthuysen AE, Vitters E, van Rooijen N, *et al*. Crucial role of synovial lining macrophages in the promotion of transforming growth factor beta-mediated osteophyte formation. Arthritis Rheum 2004; 50: 103-11.
32 Haywood L, McWilliams DF, Pearson CI, Gill SE, Ganesan A, Wilson D, *et al*. Inflammation and angiogenesis in osteoarthritis. Arthritis Rheum 2003; 48: 2173-7.
33 Bondeson J, wainwright SD, Lauder S, Amos N, Hughes CE. The role of synovial macrophages and macrophage-produced cytokines in driving aggrecanases, matrix metalloproteinases, and other destructive and inflammatory responses in osteoarthritis. Arthritis Res Ther 2006; 8(6): R187.
34 Blom AB, van Lent PL, Libregts S, Holthuysen AE, van der Kraan PM, van Rooijen N, *et al*. Crucial role ofmacrophages in matrix metalloproteinase-mediated cartilage destruction during experimental osteoarthritis: involvement of matrix metalloproteinase 3. Arthritis Rheum 2007; 56: 147-57.
35 Bondeson J, Blom AB, Wainwright S, Hughes C, Caterson B, Berg WBVD. The role of synovial macrophages and macrophage-produced mediators in driving inflammatory and destructive responses in osteoarthritis. Arthritis Rheum 2010; 62: 647-57.
36 Ding J, Chen B, Lv T, Liu X, Fu X, Wang Q, *et al*. Bone marrow mesenchymal stem cell-based engineered cartilage ameliorates polyglycolic acid/polylactic acid scaffold-induced inflammation through M2 polarization of macrophages in a pig model. Stem Cells Transl Med 2016; 5(8): 1079-89.
37 Fahy N, de Vries-van Melle ML, Lehmann J, Wei W, Grotenhuis N, Farrell E, *et al*. Human osteoarthritic synovium impacts chondrogenic differentiation of mesenchymal stem cells via macrophage polarisation state. Osteoarthritis Cartilage 2014; 22(8): 1167-75.
38 Fernandez-Madrid F, Karvonen RL, Teitge RA, Miller PR, An T, Negendank WG. Synovial thickening detected by MR imaging in osteoarthritis of the knee confirmed by biopsy as synovitis. Magn Reson Imaging 1995; 13(2): 177-83.
39 Alsalameh S, Mollenhauer J, Hain N. Cellular immune response toward human articular chondrocytes. T cell reactivities against chondrocyte and fibroblast membranes in destructive joint diseases, Arthritis Rheum 1990; 33(10): 1477-86.
40 Guerassimov A, Zhang Y, Cartman A, Rosenberg LC, Esdaile J, Fitzcharles MA, *et al*. Immune responses to cartilage link protein and the G1 domain of proteoglycan aggrecan in patients with osteoarthritis. Arthritis Rheum 1999; 42(3): 527-33.
41 De Jong H, Berlo SE, Hombrink P, Otten HG, Van Eden W, Lafeber FP, *et al*. Cartilage proteoglycan aggrecan epitopes induce proinflammatory autoreactive T-cell responses in rheumatoid arthritis and osteoarthritis. Ann Rheum Dis 2010; 69(01): 255-62.
42 Kapoor M, Martel-Pelletier J, Lajeunesse D, Pelletier JP, Fahmi H. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. Nat Rev Rheumatol 2011; 7: 33-42.
43 Henrotin Y, Pesesse L, Lambert C. Targeting the synovial angiogenesis as a novel treatment approach to osteoarthritis. Ther Adv Musculoskelet Dis 2014; 6(1): 20-34.
44 Chevalier X, Goupille P, Beaulieu AD, Burch FX, Bensen WG, *et al*. Intraarticular injection of anakinra in osteoarthritis of the knee: a multicenter, randomized, double-blind, placebo-controlled study. Arthritis Rheum 2009; 61(3): 344-52.
45 Magnano MD, Chakravarty EF, Broudy C, Chung L, Genovese MC. A pilot study of tumor necrosis factor inhibition in erosive/inflammatory osteoarthritis of the hands. J Rheumatol 2007; 34(6): 1323-7.
46 Yufang S, Yu W, Qing L, Keli L, Jianquan H, Changshun S, *et al*. Immunoregulatory mechanisms of mesenchymal stem and stromal cells in inflammatory diseases. Nat Rev Nephrol 2018; 14(8): 493-507.
47 Pers YM, Ruiz M, No LD, Jorgensen C. Mesenchymal stem cells for the management of inflammation in osteoarthritis: state of the art and perspectives. Osteoarthritis Cartilage 2015; 23(11): 2027-35.

48 Jorgensen C, Noël D. Mesenchymal stem cells in osteoarticular diseases. Regen Med 2011; 6(6 Suppl): 44-51.

49 Zheng ZH, Li XY, Ding J, Jia JF, Zhu P. Allogeneic mesenchymal stem cell and mesenchymal stem cell-differentiated chondrocyte suppress the responses of type II collagen-reactive T cells in rheumatoid arthritis. Rheumatology (Oxford) 2008; 47(1): 22-30.

50 Chen X, McClurg A, Zhou GQ, McCaigue M, Armstrong MA, Li G. Chondrogenic differentiation alters the immunosuppressive property of bone marrow-derived mesenchymal stem cells, and the effect is partially due to the upregulated expression of B7 molecules. Stem Cells 2007; 25(2): 364-70.

51 Chen W, Huang Y, Han J, Yu L, Li Y, Lu Z, *et al*. Immunomodulatory effects of mesenchymal stromal cells-derived exosome. Immunol Res 2016; 64(4): 831-40.

52 Zhang B, Yin Y, Lai RC, Tan SS, Choo ABH, Lim SK. Mesenchymal stem cell secretes immunologically active exosomes. Stem Cells Dev 2014; 23(11): 1233-44.

53 Li X, Liu L, Yang J, Yu Y, Chai J, Wang L, *et al*. Exosome derived from human umbilical cord mesenchymal stem cell mediates miR-181c attenuating burn-induced excessive inflammation. EBiomedicine 2016; 8(C): 72-82.

54 Harting MT, Srivastava AK, Zhaorigetu S, Bair H, Prabhakara KS, Furman NET, *et al*. Inflammation-stimulated mesenchymal stromal cell-derived extracellular vesicles attenuate inflammation. Stem Cells 2018; 36(1): 79-90.

55 Sun D, Zhuang X, Xiang X, Liu Y, Zhang S, Liu C, *et al*. A novel nanoparticle drug delivery system: the anti-inflammatory activity of curcumin is enhanced when encapsulated in exosomes. Mol Ther 2010; 18(9): 1606-14.

56 Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 2007; 9(6): 654-9.

57 Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116(2): 281-97.

58 Zou XY, Yu Y, Lin S, Zhong L, Sun J, Zhang G, *et al*. Comprehensive miRNA analysis of human umbilical cord-derived mesenchymal stromal cells and extracellular vesicles. Kidney Blood Press Res 2018; 43(1): 152-61.

59 Loeser RF, Collins JA, Diekman BO. Ageing and the pathogenesis of osteoarthritis. Nat Rev Rheumatol 2016; 12(7): 412-20.

60 Zhu Y, Tchkonia T, Pirtskhalava T, Gower AC, Ding H, Giorgadze N, *et al*. The Achilles' heel of senescent cells: from transcriptome to senolytic drugs. Aging Cell 2015; 14: 644-58.

61 Kadota T, Fujita Y, Yoshioka Y, Araya J, Kuwano K, Ochiya T. Emerging role of extracellular vesicles as a senescence-associated secretory phenotype: insights into the pathophysiology of lung diseases. Mol Aspects Med 2017; 60: 92-103.

62 Platas J, Guillén MI, Pérez Del Caz MD, Gomar F, Castejón MA, Mirabet V, *et al*. Paracrine effects of human adipose-derived mesenchymal stem cells in inflammatory stress-induced senescence features of osteoarthritic chondrocytes. Aging (Albany NY) 2016; 8(8): 1703-17.

63 Tian J, Zhang FJ, Lei GH. Role of integrins and their ligands in osteoarthritic cartilage. Rheumatol Int 2015; 35(5): 787-98.

64 Zhang S, Chu WC, Lai RC, Lim SK, Hui JH, Toh WS. Exosomes derived from human embryonic mesenchymal stem cells promote osteochondral regeneration. Osteoarthritis Cartilage 2016; 24(12): 2135-40.

65 Wang Y, Yu D, Liu Z, Zhou F, Dai J, Wu B, *et al*. Exosomes from embryonic mesenchymal stem cells alleviate osteoarthritis through balancing synthesis and degradation of cartilage extracellular matrix. Stem Cell Res Ther 2017; 8(1): 189.

66 Tofiño-Vian M, Guillén MI, Pérez Del Caz MD, Silvestre A, Alcaraz MJ. Microvesicles from human adipose tissue-derived mesenchymal stem cells as a new protective strategy in osteoarthritic chondrocytes. Cell Physiol Biochem 2018; 47(1): 11-25.

67 Vonk LA, van Dooremalen SFJ, Liv N, Klumperman J, Coffer PJ, Saris DBF, *et al*. Mesenchymal stromal/stem cell-derived extracellular vesicles promote human cartilage regeneration in vitro. Theranostics 2018; 8(4): 906-20.

68 Zhang S, Chuah SJ, Lai RC, Hui JHP, Lim SK, Toh WS. MSC exosomes mediate cartilage repair by enhancing proliferation, attenuating apoptosis and modulating immune reactivity. Biomaterials 2018; 156: 16-27.

69 Kato T, Miyaki S, Ishitobi H, Nakamura Y, Nakasa T, Lotz MK, *et al*. Exosomes from IL-1β stimulated synovial fibroblasts induce osteoarthritic changes in articular chondrocytes. Arthritis Res Ther 2014; 16(4): R163.

70 Tao SC, Yuan T, Zhang YL, Yin WJ, Guo SC, Zhang CQ. 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. Theranostics 2017; 7(1): 180-95.

71 Lai RC, Tan SS, Teh BJ, Sze SK, Arslan F, de Klejin DP, *et al*. Proteolytic potential of the MSC exosome proteome: implications for an exosome-mediated delivery of therapeutic proteasome. Int J Proteomics 2012; 2012: 971907.