外泌体对骨关节炎的影响

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摘要 骨关节炎(osteoarthritis, OA)的发病主要与关节内生物力学改变、关节软骨磨损以及合成分解代谢紊乱有关。关节内代谢平衡主要依靠一系列细胞内分子(激酶级联、自噬、转录因子和表观遗传机制等)和细胞外刺激(细胞因子、激素和机械应力等)的调节。外泌体作为一种分泌到细胞外发挥作用的囊泡状物质, 广泛参与细胞间信息交流。外泌体能够通过内部含有的mRNA、miRNA和蛋白质等生物活性分子对骨关节炎病程发展产生一定的影响, 且不同干细胞来源的外泌体对OA的作用存在一定的差异。该文通过综述近年来有关外泌体对骨关节炎疗效研究的文献报道, 为后续进一步探究外泌体对OA作用机制及其相关研究提供理论参考和依据。

关键词 外泌体; 骨关节炎; 间充质干细胞

Effect of Exosomes on Osteoarthritis

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Abstract The occurrence of osteoarthritis (OA) mainly associated with intra-articular biomechanics changes, cartilage damage and synthetic catabolic dysfunction. The homeostatic balance of joint is regulated by intracellular molecules such as kinase cascades, autophagy, and transcription factors, epigenetic mechanisms and extracellular stimuli including cytokines, hormones and mechanical stress. Exosomes, as a vesicle-like substance secreted into the extracellular environment, are widely involved in intercellular communication. Exosomes contain mRNAs, microRNAs and proteins and other bioactive molecules which can affect the progression of OA. Different stem cell-derived exosomes have some differences in their effects on OA. This article summarizes literatures about the effect of exosomes on OA in recent years to provide a further basis for the research of OA pathogenesis and treatment.

Keywords exosomes; osteoarthritis; mesenchymal stem cells

骨关节炎(osteoarthritis, OA)是一种以关节软骨退变、软骨下骨重塑、骨赘形成、滑膜炎症反应和血管生成为特征的慢性退行性疾病[1-2]。OA的发生可能由遗传、环境、代谢和生物化学等多种因素共同影响, 具体发病机制尚不明确[3]。目前针对OA的治疗手段以非甾体类药物治疗和手术治疗为主, 但是药物治疗具有明显的副作用, 且对疼痛的控制效果有限, 最终仍将导致关节软骨退变[4-5]。对于晚期OA患者只能采取手术治疗, 手术治疗能够矫正关节畸形, 治疗关节疼痛。然而老年人手术风险较大, 存

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在术后并发感染、血栓以及二次手术可能性等潜在风险[6-8]。细胞移植是一种新兴的OA治疗方式, 主要使用自体软骨细胞和间充质干细胞(mesenchymal stem cells, MSCs)移植。但是自体软骨细胞在体外扩增过程中容易出现去分化丧失功能。因此, OA间充质干细胞疗法成为热门研究领域之一[9-10]。近年来, 外泌体在肿瘤、心血管、骨组织等领域的相关研究被大量报道, 部分学者开始关注各种类型MSCs分泌的外泌体在OA中的作用[11-12]。但是由于不同干细胞来源的外泌体在功能上表现不同, 目前有关外泌体对OA的研究结论之间差异较大, 且深入到作用机制方面的研究尚少。本文通过综述近几年有关外泌体对OA作用疗效的相关研究, 为进一步探究外泌体对OA的作用机制提供参考和依据。

1 外泌体的来源及结构功能

胞外囊泡(extracellular vesicles, EVs)是一种几乎所有细胞都能分泌的磷脂双分子层囊泡, 由质膜直接向外“出芽”形成[13]。在血液、尿液、母乳和唾液等体液中均能找到EVs[14-16]。早在1969年, 有学者就发现在软骨细胞中含有羟基磷灰石晶体的EVs[17]。OA病变软骨细胞中的EVs直径为50~250 nm, 主要存在于关节软骨潮线附近, 其功能可能与碱性磷酸酶活性增强有关[18]。根据EVs的直径、成分和来源可以将它们分为外泌体、细胞微泡和凋亡小体三种类型[19]。其中, 外泌体是一种较小的直径介于30~100 nm的囊泡[19], 由细胞内溶酶体微粒内陷形成, 表达细胞黏附分子CD9(cluster of differentiation 9)、CD63、CD81、热休克蛋白60(heat-shock proteins 60, Hsp60)、Hsp70和Hsp90等标志物[20]。目前研究中使用的外泌体提纯技术即是以外泌体直径大小和表面分子标志物检测作为依据, 取细胞培养上清液经过一系列离心去掉死亡的细胞和较大的碎片物质, 再超速离心获取外泌体后检测特异性标志物[11,21]。在上个世纪八十年代, 外泌体第一次在羊网织红细胞的离体实验研究中被发现并报道[22], 然而外泌体的分泌以往一直被误认为只是用于清除细胞内容物中的“垃圾”, 直到近年来发现外泌体中包含mRNA、miRNA(microRNA)和蛋白质等多种生物活性分子, 并能够和胞质膜融合通过细胞内吞发挥作用, 参与细胞间的信息传递, 与各种疾病的发病机理密切相关[23-24]。随着对外泌体内容物研究的不断深入, 研究发现, 外泌体通过囊泡内多种生物活性分子作用于各种受体细胞, 在免疫监视、血管形成、肿瘤发展、合成代谢和炎症老化等过程中起到重要作用。第一, 外泌体可以通过配体作用同时结合两个细胞进行信息交流而不需要细胞间的直接接触。第二, 外泌体能够与靶细胞膜结合从而使细胞获得新的黏附性能。第三, 外泌体还能与靶细胞直接融合通过物质传递在细胞内发挥生物学作用[25]。这可能为多种疾病的机制通路研究提供新的思路和为疾病治疗提供靶点。目前, 外泌体已成为重要的热点研究对象之一。

2 外泌体对骨关节炎的影响

OA的发生可能造成关节功能受限。周围组织慢性炎症改变还会出现关节疼痛等症状, 进而严重影响患者的日常生活质量[26]。研究表明, 早期OA病变出现软骨细胞凋亡[27]和软骨基质降解[28]。蛋白多糖分解也被认为是早期OA软骨破坏的表现之一[29]。随着OA病变的发展, 关节软骨细胞坏死脱落, 潮线向软骨层推进, 严重扭曲、断裂甚至消失, 血管组织新生侵入钙化层, 囊腔样结构大量出现[30-31]。最近研究显示, OA血管生成与关节结构破坏、疼痛以及炎症等病变密切相关[32]。血管侵袭同时伴行感觉神经和交感神经末梢, 其中感觉神经的侵入可能是引起OA疼痛的重要原因之一[33-35]。关节慢性炎症组织中含有大量炎症细胞、新生血管和炎性介质, 炎性介质直接或间接地促进血管生成, 新生血管通过传送炎症细胞、供氧而有利于炎症的发展[36]。OA的发生发展受到多种因素共同影响, 具体机制复杂且尚不明确[3], 外泌体作为当今研究的热点之一, 无论是在OA发病机制还是治疗领域的研究都逐渐显示出巨大的潜力和优势。

目前, 外泌体在皮肤[37]、四肢[38]、心脏[39]等各种组织器官中的修复治疗作用已有广泛报道, 但是外泌体对OA影响的有关研究报道较少, 且尚无统一定论。有研究发现, 外泌体可以缓解OA的病理变化[11]。另有研究显示, 外泌体能够促进OA发生发展[40]。这可能跟外泌体的来源密切相关。外泌体中成分复杂, 具体生物学效应可能与外泌体内含有的蛋白质、mRNA、miRNA等种类和含量密切相关。同样, 来源于MSCs的外泌体, 多能干细胞诱导的间充质干细胞和滑膜间充质干细胞都能够促进OA软骨修复, 但

滑膜间充质干细胞分泌的外泌体软骨表面呈现轻度不规则、浅表纤维化、蛋白聚糖丢失、表层软骨缺失。多能干细胞诱导的间充质干细胞分泌的外泌体治疗小鼠的软骨表面无上述表现, 与正常软骨几乎一致[41]。以上结果说明, 不同细胞分泌的外泌体作用效果可能存在一定的差异, 甚至完全相反。有研究报道, 来自OA患者的滑膜囊液体含有滑膜细胞和软骨细胞分泌的EVs, 然而OA患者和正常人关节中含有的EVs直径大小和浓度差异较大[42]。这也提示, 不同来源的外泌体作用机制不同。

2.1 外泌体缓解骨关节炎病理进程

近年来, 基于间充质干细胞疗法治疗OA患者疼痛和炎症反应的临床报道逐渐增多[43-44], 图1为各种干细胞来源的外泌体对OA软骨细胞作用研究的基本实验步骤图示。Wang等[11]将人胚胎干细胞诱导分化成间充质干细胞(mesenchymal stem cells derived from pluripotent embryonic stem cells, ESC-MSCs), 然后离心提纯ESC-MSCs分泌的外泌体, 通过离体实验(将外泌体添加到OA样改变的关节软骨细胞培养基中)和在体实验(将外泌体注射到OA小鼠膝关节腔内)分别探讨ESC-MSCs分泌的外泌体对OA病变的影响。体外细胞培养的基因表达和蛋白印迹检测结果一致显示, ESC-MSCs分泌的外泌体能够显著上调II型胶原蛋白(collagen-II, COL-II), 下调I型血小板结合蛋白基序的解聚蛋白样金属蛋白酶-5(a disintegrin and metalloprotease with thrombospondinmotifs-5, ADAMTS-5)。动物实验表明, 关节腔注射外泌体显著降低小鼠关节软骨OARSI评分, 有效延缓OA小鼠软骨病变和细胞外基质降解。此外, 通过追踪定位ESC-MSCs分泌的外泌体作用途径, 在COL-II表达的软骨细胞中发现外泌体的存在, 这暗示外泌体可能通过与软骨细胞融合直接作用于该细胞。除了使用胚胎干细胞诱导的间充质干细胞, Tofiño-Vian等[12]尝试使用脂肪间充质干细胞分泌提纯的外泌体来干预白细胞介素-1β(interleukin-1β, IL-1β)刺激下的成骨细胞。IL-1β可以增强破骨细胞生成与骨吸收并抑制成骨分化和骨形成[45-46], 慢性炎症也能促进骨关节炎细胞老化[47]。因此, IL-1β刺激下的成骨细胞出现代谢失调、炎症反应和细胞衰老等OA病变特征[48]。Tofiño-Vian等[12]研究发现, 脂肪间充质干细胞分泌的外泌体下调IL-1β诱导下OA成骨细胞中的衰老因子表达, 实验表明, 外泌体能够在OA成骨细胞中起到抗炎症反应和抗氧化应激的作用。

2001年, 滑膜间充质干细胞第一次从关节周围滑膜中被分离出来[49]。滑膜间充质干细胞具有使软骨再生的组织特异性, 在软骨再生研究中具有巨大的潜力[50]。最近研究发现, 外泌体可能是Wnt信号通路中一种潜在的细胞外运输分泌物[51-52]。Wnt5a/b通过Wnt信号通路激活Hippo-YAP信号通路中YAP(Yes-associated protein)。Wnt5a/b-YAP能够对抗经典的Wnt/β-catenin信号, 减少包括转录因子SOX9(sex determining region Y box 9)在内的主链

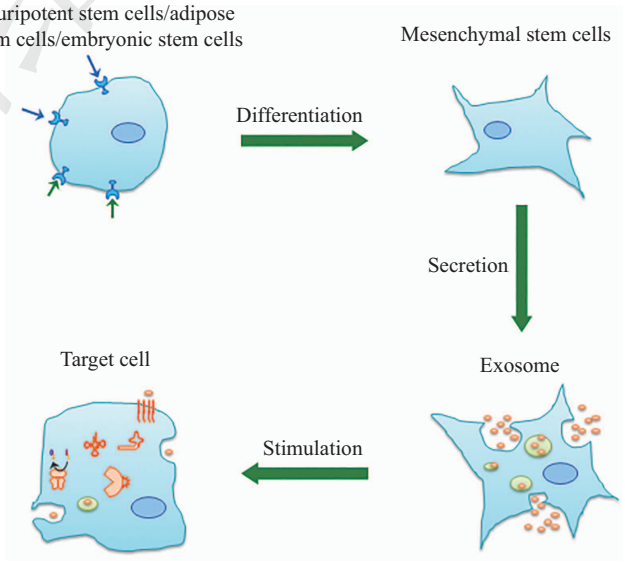

图1 实验步骤

Fig.1 Experimental procedure

β-catenin/TCF基因表达[53], 从而调控软骨细胞外基质相关基因表达和软骨形成[54-55]。Tao等[21]研究发现, 滑膜间充质干细胞中Wnt5a和Wnt5b高表达, 并且在滑膜间充质干细胞分泌的外泌体中也富含Wnt5a和Wnt5b。滑膜间充质干细胞分泌的外泌体通过Wnt5a和Wnt5b激活YAP, 激活的YAP导致软骨细胞增殖迁移, 但是引起SOX9表达和细胞外基质蛋白合成分泌降低。近年来, 有关miR-140-5p能够通过增强SOX9和蛋白聚糖(aggrecan, ACAN)维持软骨内稳态进而预防缓解OA的相关研究吸引了大量学者关注[56-60]。Tao等[21]据此设计进一步实验改良外泌体疗效。通过添加核酸使滑膜间充质干细胞高表达miR-140-5p, 并在滑膜间充质干细胞分泌的外泌体中检测到高表达的miR-140-5p。结果表明, 高表达miR-140-5p的外泌体能够增加SOX9和细胞外基质分泌。这一实验结果说明, 滑膜间充质干细胞分泌的高表达miR-140-5p的外泌体可以用来预防和缓解OA病变, 在临床实践中具有巨大潜力, 可用于治疗OA药物开发研究[21]。

2.2 外泌体促进骨关节炎发生发展

IL-1β是介导OA软骨退化和关节炎症的一种关键因子, IL-1β刺激滑膜成纤维细胞能够产生OA样基因表达[61-62]。Kato等[40]从人的正常膝关节中提取滑膜成纤维细胞和关节软骨细胞, 离心提纯正常滑膜成纤维细胞和IL-1β刺激下滑膜成纤维细胞分泌的外泌体, 分别将两种提纯的外泌体加入到正常关节软骨细胞培养液中进行干预。结果在IL-1β刺激下滑膜成纤维细胞分泌的外泌体使关节软骨细胞中基质金属蛋白酶-3(matrix metalloproteinase-3, MMP-3)、MMP-13、IL-1β、ADAMTS-5和血管内皮生长因子表达显著上调, COL2A1和ACAN表达显著下调。IL-1β刺激下滑膜成纤维细胞分泌的外泌体能够诱导人脐静脉内皮细胞迁移和血管形成。小鼠动物实验发现, IL-1β刺激下滑膜成纤维细胞分泌的外泌体蛋白聚糖丢失明显增加。上述结果表明, IL-1β刺激下滑膜成纤维细胞分泌的外泌体可能通过新的信号通路传导途径参与OA细胞外基质降解和血管生成等病变进程。此外, 有研究显示, 提取自风湿性骨关节炎滑膜成纤维细胞的细胞微泡中含有的大量ADAMTS-5可以促进蛋白聚糖降解[63]。来自风湿性关节炎和OA患者EVs中的己糖胺酶-D能够导致关节软骨退变[64-65]。上述研究表明, OA病变下的关节内环境能够调节滑膜成纤维细胞和其他细胞分泌外泌体, 反过来, 外泌体的分泌也能够反应OA具体的病理变化并参与OA病程发展。因此, 外泌体有可能作为一种潜在的OA诊断标志, 并为OA发病机理调控通路的研究提供新的方向。

综上所述, 外泌体既可以缓解OA病理变化, 又能够促进OA发生发展, 可能是由于外泌体来源不同所导致。来自间充质干细胞分泌的外泌体[11-12]起到治疗OA的作用, 来自患者关节内细胞分泌的外泌体[40]起到加重OA的作用。此外, 外泌体的作用差异还可能与提取纯度有关。外泌体提取一般在离心过滤后, 通过CD9、CD63和CD81等相关分子标记物表达检测和囊泡直径大小来确定外泌体的存在[11-12,21], 这可能存在外泌体纯度问题。目前研究使用最多的滑膜间充质干细胞很难获取, 滑膜仅能通过侵入性操作从OA患者关节中获得[66]。不排除来自患者体内滑膜间充质干细胞提取物中本身就含有对软骨有害的炎症因子和分解代谢介质等干扰。但是, 无论是加重OA病变还是缓解OA进程, 外泌体对OA未来研究的潜力巨大。在OA治疗方面, 在动物实验中, 虽然不同造模方式形成的OA发病机制有所差异, 但外泌体对于手术创伤[21]、药物诱导[66]等各种OA动物模型均有明显缓解作用。

3 小结

干细胞来源的外泌体能够减轻OA软骨损伤, 促进细胞外基质合成代谢从而预防和缓解OA病理变化, 可用于OA药物治疗方面的研究。OA患者滑膜成纤维细胞来源的外泌体可能通过某种新的信号通路介导OA病变促进病情发展, 外泌体有可能作为一种潜在的OA诊断标志物, 对其进一步深入研究可能为OA发病机制的解释提供新的思路。

无论是对OA发病机制的研究还是对OA药物治疗的研究, 外泌体都展现出无限的潜能。但不可否认的是, 目前有关外泌体对OA影响的研究尚不深入, 仍然存在例如外泌体注射剂量单一、最适剂量不确定、外泌体来源有限和分离提取困难等诸多缺陷, 外泌体具体的作用机制更是鲜有报道。因此, 需要对外泌体进行更加深入的研究探索, 外泌体对OA的作用和机制研究将是未来一段时间内的重要热点之一。

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