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Research Progress of Exosomes in Cardiovascular Diseases

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Abstract

Exosomes are micro-vesicles secreted by a variety of cells under certain conditions, ranging in diameter from 30 to 150 nm. The exosomes contain abundant proteins, lipids and nucleic acids. In recent years, studies have found that exosomes can participate in the development of cardiovascular diseases and play an important role in diagnosis and treatment of cardiovascular diseases. Exosomes can promote the occurrence and development of cardiovascular diseases by affecting cell proliferation, apoptosis and autophagy, regulating the relevant cellular microenvironment, and promoting the regeneration of blood vessels. Exosomes can also be used as biomarkers for cardiovascular diseases, as therapeutic targets for cardiovascular diseases. This article reviews the formation, composition and function of exosomes and the role of exosomes in cardiovascular disease in recent years.

Keywords

Exosomes, Cardiovascular Disease, Diagnosis, Treatment

外泌体在心血管疾病中的研究进展

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摘　要

本研究旨在综述外泌体在心血管疾病中的研究进展，为完善细胞治疗在心血管疾病中的机制提供理论基础。外泌体是多种细胞在一定条件下分泌的微小囊泡，直径在30~150 nm，外泌体中包含了丰富的蛋白质、脂质与核酸。近几年的研究发现外泌体可参与心血管疾病的发生发展，在心血管疾病诊断和治疗中发挥重要的作用。外泌体可以通过影响细胞增殖、凋亡和自噬，调节相关细胞微环境，促进血管的新生等多方面促进心血管疾病的发生与发展。外泌体还可以作为心血管疾病的生物标志物，作为心血管疾病的治疗靶点等。本文就外泌体的形成、组成和功能以及近几年外泌体在心血管疾病中的作用进行综述。

关键词

外泌体，心血管疾病，诊断，治疗

1. 引言

心血管疾病是全球人口的主要死因[1]。由于现代技术的发展，急性心肌梗死的早期存活率大大提高，但晚期患者因为失去收缩功能的心肌，心功能并不能得到改善，现有的药物治疗和介入策略都不能使梗死的心肌再生，远期的心力衰竭的疗效也十分有限[2]，因此该疾病仍然是世界上一项巨大的挑战[3]。多年来，研究者尝试多种策略试图在心血管疾病发生后寻找促进修复与再生的方法，干细胞移植为这类疾病的治疗带来了希望，使得细胞治疗逐渐成为一种可行的手段来治疗心血管疾病。该治疗方法可以追溯到 1990 年，当时使用的是骨骼肌成肌细胞在开胸手术期间首次移植到衰竭的心脏中[1]。从那时起，研究者关注的焦点逐渐转移到骨髓来源的细胞上，并且相关实验都取得阳性的结果。既往认为，分化特性是细胞移植后发挥治疗作用的主要机制；然而，新近的研究证实细胞移植到缺血的组织后并未发生分化成相应的心肌细胞或者内皮细胞等，而是通过分泌抗凋亡、促存活和抗炎因子等效果，进而改善缺血后的心功能[4]。细胞移植的分泌特性的研究是治疗心血管疾病极其重要的科学问题。因此，细胞分泌的胞外囊泡作为病理状态下的生物标记物和治疗剂的潜力得到世界上研究者的极大关注[5] [6]。

2. 外泌体

2.1. 外泌体的形成与功能

外泌体是一种细胞外脂质双层囊泡，直径范围在 30~150 nm 之间。它们是由于细胞膜向内部凹陷形成的一种囊泡，在于质膜融合时囊泡将其内容物释放到细胞外液中，此时的囊泡被称为外泌体[7]。类似的胞外囊泡还有微粒体和凋亡小体，它们与外泌体的区别在于直径、生成机制以及作用功能的不相同[8]。外泌体最早在 1987 年由 Johnstone 等在绵羊的网织红细胞发现并经过超速离心提取得到[9]。从很多细胞和大多数的体液中都可以分离出外泌体，如唾液、尿液和血液等[10] [11] [12]。尽管最初被认定为细胞碎片，但外泌体的多种功能已被证实受到多种信号途径的调节，外泌体广泛参与细胞的各种病理生理的调控，包括信号转导、氧化应激、抗原提呈和免疫应答等[13] [14]。近几年的研究中，外泌体在肿瘤、炎症

等方面研究较多、其对肿瘤细胞的生长的调控已经相继在肝癌、胰腺癌、前列腺癌等多个肿瘤模型中得到证实[15] [16] [17]；另外也有相关实验结果证实肿瘤细胞可以通过分泌外泌体作用于周边的非肿瘤细胞，促进周边血管的新生，使得局部微环境更加适用于肿瘤的存活[18]；也有研究报道肿瘤细胞通过分泌外泌体作用于周围的免疫细胞，降低免疫活性，从而逃避免疫攻击，适应局部生长环境并发生远处转移[19]。

2.2. 外泌体的组成与分离

外泌体的内容物非常丰富，其中有多种生物活性物质，包括蛋白质、核酸和脂质等，这些物质使得外泌体在多种生理和病理过程中发挥重要作用[20]。例如外泌体的 miRNA 介导促血管生成、增殖、抗细胞凋亡和抗炎的作用[21] [22] [23] [24] [25]。目前，研究者主要依据外泌体的物理、化学和生物学特性分离与鉴定外泌体。物理方法包括差速离心法、超速离心法和色谱法等；化学方法包括聚合物沉淀法；生物方法包括免疫亲和吸附法[26]。

3. 外泌体与心血管疾病

3.1. 外泌体与心血管疾病的生物标志物

生物标志物可作为正常生物功能、病理过程和对治疗干预的药理反应的一种相关指标。由于生物状态下的直接评估有时过于昂贵或有时属于侵入性评估，生物标志物在鉴定疾病状态和评估疾病的风险方面具有极大的临床应用。因此，生物标志物可以作为早期检测病理状态和随后的治疗的工具。由于有些外泌体是在特定的应激条件或损伤条件下形成，因此循环系统中的外泌体逐渐被认定是心血管疾病的生物标志物的候选者。特别地，当患有与血管损伤，炎症和促血栓形成状态相关的动脉粥样硬化患者，他们的检验结果表现出血浆相关外泌体水平的升高。一些研究表明，用于预测心血管疾病风险的 Framingham 风险评分与循环外泌体之间存在相关性[27] [28]。它们的形成和清除反映了细胞活化和损伤之间的微妙平衡、细胞存活和凋亡以及血管重塑和血管生成。近期相关实验已经证明 miRNA 在外泌体中的选择性包装和它们通过特定信号分子的功能转移在治疗疾病中具有重要作用[29] [30]。此外，外泌体有助于检测心肌的恢复、再生或保护的内源性过程[31]。来自体液的外泌体在这些特异性表达疾病模式中，表现出可以作为心血管疾病的分子标志和治疗靶标的一种可能性。

内皮细胞和血管平滑肌细胞之间的细胞间信号对于维持血管结构，血管功能和血压以及心肌损伤后功能性血管形成至关重要[32] [33]。当暴露于高的动脉粥样硬化保护剪切应力时，内皮细胞分泌富含 miRNA-143/145 的外泌体。一旦转移到平滑肌细胞，外泌体 miRNA-143/145 表达会下降[34]。因此，外泌体可以在临床上作为一种优秀的生物标志物进行诊断，因为它们反映了受损病变的实时微环境的状况。可以在没有侵入性活组织检查的情况下得到靶区域中细胞的遗传信息，仅通过采集血液或尿液样本就可以得到，这是外泌体一个显着的优点，特别是像人的心脏，这种只能用侵入的手段得到相关样本时，外泌体可以以一种直接的、有规律的方式收集，因此可以实现对患者疾病的进展跟踪。

相关研究发现，肌钙蛋白水平未达到心梗诊断水平或心梗症状发作时间小于 3 小时的急性冠脉综合征患者，血浆中 miR-1，miR-499 和 miR-21 水平升高，并且与肌钙蛋白水平相结合时诊断价值增加[35]；然而，循环 miRNA 生物标志物的使用受到 qPCR 定量所需时间的限制。对稳定性冠状动脉疾病患者的分析发现，循环外泌体中 miRNA-126 和 miRNA-199a 的增加与未来主要不良心血管事件的风险降低相关[36]。急性心肌梗死后 2 至 3 周收集的血清中 p53 反应性 miRNAs (miR-194，miR34a)增加，这些 miRNA 主要存在于外泌体中；具有这些 miRNA 水平升高的患者更有可能在以后发生缺血性心力衰竭[37]。这种分析是在冷冻一年以上然后解冻的血清样品上进行的，这可能会破坏脂质囊泡，因此我们无法确定

miRNA 是否在外泌体内；尽管如此，这些发现对于早期识别可能发生心力衰竭的患者以及可能的促成机制非常重要。从颈动脉粥样硬化患者的血浆中分离的外泌体含有相对于对照患者富含半乳糖凝集素-3 (Galectin-3)的外泌体；此外，Galectin-3 浓度升高与心血管疾病死亡风险增加相关[38]。因此需要进行更多的研究，像对外周血外泌体含量的分析为预测疾病进程和发生心力衰竭的风险提供了潜在的诊断方法。

3.2. 外泌体与心血管疾病的治疗

由于细胞分泌和转运的作用，外泌体作为治疗工具引起全世界的关注。它们是拥有自体来源的天然生物制剂，同时可以保持内容物的完整性和稳定性。并且外泌体表面的膜蛋白对于相应受体细胞表面具有一定的亲和力，因此它们可以通过 miRNA 来提供精准治疗，如选择相应的靶细胞并且操控其中相应的成分，这将是个体化治疗和基因治疗的有力工具[39] [40]。

间充质干细胞来源的外泌体已经在心脏再生医学领域研究多年，尤其是心肌缺血再灌注损伤方面[41] [42]。科学家发现携带内皮分化信号的外泌体影响新血管的形成，说明外泌体在治疗血管生成中的有效性。据报道，人脐带间充质干细胞来源的外泌体通过保护心肌细胞免于凋亡和促进细胞增殖和血管生成来促进缺血性损伤后的心脏修复，但没有外泌体的人脐带间充质干细胞几乎不能改善心功能[43] [44]。并且人脐带间充质干细胞来源的外泌体促进体内伤口愈合，作用机制是由于内皮细胞中的 Wnt4/β 连环蛋白激活所介导[45]。一些研究表明，间充质干细胞来源的外泌体也可以增加 ATP 水平，通过 PI3K/Akt 途径减少氧化应激，增强心肌细胞活力并防止心肌缺血再灌注后的不良重塑[46]。类似地，外泌体热休克蛋白 70 (HSP70)可以刺激 toll 样受体-4 (TLR-4)信号转导，导致 ERK1/2 和 p38MAPK 的活化以及随后的热休克蛋白 27 (HSP27)在心肌细胞中的磷酸化[47]。

相关研究报道，胚胎干细胞来源的外泌体在心肌梗死后可以促进内源性修复来增强心脏功能。尽管 iPSC 的分泌蛋白组的含量尚未完全阐明，但其在肺上皮伤口愈合模型中显示出改善肺泡上皮伤口修复的特性，同时还能减少肺纤维化[48]。一项研究表明，从 iPSC 培养基上清的外泌体对缺血心肌有保护作用。iPSC 来源的外泌体通过抑制 Caspase3/7 信号通路来保护 H9C2 细胞免受 H_2O_2 诱导的氧化应激[49]。最近的研究已经表明 iPSC 来源的外泌体有发挥保护作用的生物活性分子。BobisWozowicz 及其同事发现，这些分子主要是 mRNA，miRNA 和蛋白质，它们被传递到人心脏间充质基质细胞(cMSC)，通过影响受体细胞的转录组和蛋白质组谱来发挥保护作用。此外，来自 iPSC 的外泌体表现出有增强 cMSCs 的心脏和内皮分化潜能[50]。来自小鼠 iPSC 的外泌体显示含有一组对维持 iPSC 多能性至关重要的特异性多能转录因子[51]。尽管很多外泌体内容物的作用还未完全确定，这些结果还值得科学家进一步研究。

4. 小结与展望

直到 2010 年初，干细胞移植被认为是治疗缺血性心脏病的潜在治疗选择。尽管源自人多能干细胞的功能性心肌细胞改善了心力衰竭动物模型中的心脏形态和功能，但它们也引发了一些心律失常反应[52] [53]。在未纯化的多能衍生的心肌细胞移植后也观察到畸胎瘤的形成[54] [55]。iPSC 的衍生似乎部分地解决了与免疫排斥相关的问题。但依然需要利用多能干细胞的强大再生生物学，同时可以避免与细胞移植方面的问题[56]。更重要的是，干细胞的确切作用机制尚未阐明，未来关于旁分泌因子(如外泌体)的在细胞治疗领域将进一步深入研究。在干细胞分泌蛋白质组中，外泌体似乎是最合适的递送载体。不同细胞来源的外泌体具有其天然细胞类型的不同抗原蛋白[57]，这使得能够对受体细胞受体具有特异性结合特性，从而为所需细胞类型提供靶向递送。该方法可以利用由自体 iPSC 衍生物(例如 iCM)产生的外泌体在将来实现个性化医疗的现实。Alvarez-Erviti 等人发明一种基于外泌体的递送系统的生物技术方法，是首个基于外泌体的药物递送系统，其显示 siRNA 的有效体内递送[58]。另一项研究表明，外源 siRNA 成功

通过外泌体并传递给人单核血细胞。血浆外泌体有效地将 siRNA 递送到靶细胞中，导致 MAPK-1 的选择性基因沉默[59]。

越来越多的研究表明，外泌体从受损或患病的心脏中释放出来，通过调节一系列细胞类型之间的综合相互作用，在影响疾病进程中发挥重要作用[60] [61] [62] [63]。同时，由于大量的实验和临床细胞治疗研究，人们已经了解到外泌体的特性及其在心肌损伤，修复和再生中的作用。Sahoo 等人也对心梗后心脏修复中外泌体的作用进行了综合评述[31]。然而，仍处于起步阶段的心血管外泌体领域需要采取重要措施来解决几个尚未解决的问题和新的挑战。一些需要探索的重要问题可能是：心肌的不同区域(例如梗塞/边界区)是否会释放出定量和定性不同的外泌体？什么类型的细胞在心肌缺血性损伤后启动信号传导？不同细胞类型分泌的外泌体在组成和功能方面有何不同？如何调节心脏外泌体的产生？心脏外体信号如何与常规类型的细胞间通讯相互作用？心脏外泌体在缺血/再灌注损伤中还有哪些其他作用？心脏外泌体如何影响心肌梗死后的免疫反应？可以利用心脏外泌体治疗心血管疾病吗？有没有办法在体内/体外修饰心脏外泌体，以扩大其有益效果和/或减轻其有害影响？在外泌体的研究中存在若干实际挑战，例如，在生理条件下研究它们的困难，组织和液体中囊泡的动态释放和摄取以及纯化和定量的不确定的效率。尽管如此，研究外泌体作为心脏病的细胞外传播者将揭示细胞－细胞和器官－器官通讯的新机制，帮助识别新的生物标志物，并为心血管疾病的新疗法开发提供新的见解。总之，我们综述了外泌体在心血管疾病的临床诊断和治疗方面的重要价值，但目前针对心血管疾病患者血清外泌体的研究依然不多，希望在不久的未来，研究者通过深入研究外泌体和外泌体中的内容物，为心血管疾病提供可靠的早期诊断的生物标志物及相关特异性的治疗靶点。

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