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心肌梗塞后再生和修复新途径：心外膜

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摘　要

心血管疾病在全球发病率居高不下，给人类健康带来严重威胁。心肌梗死(myocardial infarction, MI)后心肌组织无法再生和修复，最终导致心功能障碍。心外膜及心外膜祖细胞不仅对胚胎心脏发育形成起重要调控作用，而且在心肌损伤及修复中扮演重要角色。有研究表明在成人心脏中，损伤会激活心外膜，并观察到胚胎样反应。因此心外膜被认为是一类心肌再生与修复的新来源和新途径，本文结合心外膜起源、心外膜细胞调控信号、心外膜在心肌再生中作用等最新研究情况，评述国内外相关研究进展，讨论心外膜的作用和调控机制，展望未来研究方向。

关键词

心外膜，修复，再生

New Pathways for Regeneration and Repair after Myocardial Infarction: Epicardium

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Abstract

The incidence of cardiovascular diseases remains high in the world, which poses a serious threat to human health. Failure of myocardial tissue to regenerate and repair after myocardial infarction (MI) ultimately leads to heart dysfunction and heart failure. Epicardium and epicardium progenitor cells not only play an important role in the development and formation of embryonic heart, but also play an important role in myocardial injury and repair. Studies have shown that in adult

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hearts, damage activates the epicardium and embryonic-like responses are observed. Therefore, the epicardium is considered as a new source and approach for myocardial regeneration and repair. In this review, combined with the latest research on the origin of the epicardium, the regulatory signals of epicardium and epicardium progenitor cells, and the role of the epicardium in myocardial regeneration, we summarized relevant research progress at home and abroad, discussed the role and regulatory mechanism of the epicardium, and looked forward to future research directions.

Keywords

Epicardium, Repair, Regeneration

1. 引言

心血管疾病(CVD)给全球带来巨大的经济负担，严重威胁我国居民生命健康，具有高发病率、高死亡率。据《中国心血管病报告 2021》调查显示，推算 CVD 现患人数 3.3 亿，冠心病 1139 万，CVD 的死亡率仍居首位[1]。MI 是冠心病最常见的后果之一，MI 后多达 10 亿个心肌细胞因缺血而死亡[2]，继而出现心肌间质瘢痕修复。传统研究认为，人类心肌细胞是永久细胞，一旦受损无法进行自发再生。如何修复及再生受损心肌一直是人们重点关注及研究的课题。在近 20 年的研究中，尽管提出刺激成熟心肌细胞去分化和增殖、基因疗法、注射干细胞或组织工程贴片递送等心脏再生疗法[3]，但尚未完全成功向临床转化应用。各种类型的干细胞，如心脏祖细胞[4]、间充质干细胞[5]、诱导多能干细胞[6]等，已被研究作为心肌梗死后促进心肌修复的候选细胞。通过调节转录因子[7]、刺激 microRNA 途径[8]、递送 siRNA [9]、激活生长因子信号[10]可以促进心肌细胞增殖。基于明胶的支架，包括明胶及其衍生物甲基丙烯酸明胶，由于其模拟天然细胞外基质的能力，在心脏组织工程中引起了极大的关注[11]。将心脏祖细胞、生物活性分子或药物与生物支架相结合，在心脏组织修复和再生方面显示出巨大的前景。

心脏实际上不是一个终末分化的器官，新的心肌细胞是在成年后产生的，这意味着心脏本身可能含有心血管祖细胞的来源，或者现有的心肌细胞增殖低下[12]。将人类成人纺锤形心外膜来源细胞(EPDCs)注射到梗死的心肌中，可以保留心脏功能，并减少梗死后早期和晚期病理重塑[13]。在小鼠心肌梗死模型中，覆盖损伤部位的心外膜最初完全消失，并在损伤后三日内从幸存的心外膜重建[14]。大量证据支持心外膜在心脏修复和再生中发挥作用的假设，部分通过上皮到间充质转化(EMT)提供分化为心脏细胞类型的心外膜祖细胞，部分释放有助于心脏修复的旁分泌因子。心外膜可以通过不同方式再生修复受损的心脏，因此，心外膜被提议作为治疗心血管疾病的潜在靶点。本文就人类心外膜来源、发育及再生修复作用的研究进展作一综述。

2. 心外膜起源和发育、标记物

在大多数物种中，心外膜由单个细胞层构成，而在人类心脏中，心外膜是由覆盖在结缔组织的多层间皮细胞组成[15]。心外膜提供旁分泌信号，从妊娠中期开始促进发育中的心脏的生长，心外膜衍生的细胞充当多种心脏细胞类型的祖细胞。在心脏发育过程中，心外膜作为祖细胞池、冠状血管形成和心肌生长的来源中心[16] [17]。严格来说，PEO 不是一个器官，仅仅是胚胎心脏组织发育过程中的一个临时性

结构组织；其为包含 Tbx18 [18]、WT1 [19]、Tcf21 [20]、CFU-Fs 等多种心外膜祖细胞标记基因的祖细胞池[21]。最早提出心外膜与心肌起源不同的是由 Kurkiewicz 于 1909 年首次报道，他发现在鸡胚中心外膜可能来源于原始绒毛状心外膜，即我们目前称之为前体心外膜(PE) [16]。早期认为心外膜来源有多种假说，目前较公认的观点为：心外膜主要来源于前体心外膜器官(PEO)。

在小鼠胚龄(E) 9.5 天左右，PEO 逐渐迁移覆盖于心肌表面，约 E11.5 天左右形成较完整的心外膜。随后，部分 PE 在经历 EMT 后，以 EPDCs 的形式迁移到心肌中[22]。EMT 受到不同转录因子、转化生长因子-β (TGF-β) [23]、血小板衍生因子(PDGF) [24]、成纤维细胞生长因子(FGF) [25]等生长因子和基因 Nfact1 等调节。研究表明当 Nfatc1 表达缺失时，EPDCs 向心肌的迁移受损[26]。

一旦 EPDCs 迁移到心肌中，它们将开始分化成冠状血管平滑肌细胞、心脏成纤维细胞、内皮细胞，以及可能的心肌细胞亚群[27]。EPDCs 衍生的谱系受到有关基因调控，如 Wilm’s tumour 1 (Wt1)、Tcf21、Tbx18 等基因调控[28]。大多数基于 Wt1 和 Tcf21 谱系阳性心外膜细胞会分化为成纤维细胞和血管平滑肌细胞[20] [29]。研究表明，在 Tcf21 表达缺失的情况下，心外膜细胞保留其前体心外膜状态[30]。表达 Tbx18 的心外膜祖细胞可以产生心肌成纤维细胞和冠状动脉平滑肌细胞[18]。此外，PDGFRA 表达促进心外膜来源的心脏成纤维细胞形成[31]，PDGFRB 促进心外膜来源的冠状动脉血管平滑肌细胞形成[24]。

除了对心脏发育的直接细胞贡献外，心外膜还是旁分泌信号的来源，视黄酸介导的信号转导诱导心外膜细胞中 FGF-9 和 FGF-2 的表达，这是促进心肌生长的两种有丝分裂信号[32] [33]。心外膜层 EPDCs 还可以产生趋化因子，如 C-X-C 基序趋化因子配体 12 (CXCL12)，调节冠状动脉血管的形成[34]。

3. 心外膜再生修复机制

出生后心外膜保持静止状态，然而，当心脏损伤时，该上皮层会再激活，从而引发胚胎样反应。随后，心外膜细胞经历 EMT 并迁移到心肌组织中，有助于损伤后修复。在心肌梗死后的最初几天内，心外膜细胞胚胎基因表达上调，包括 Wt1、Raldh2 和 Tbx18 [35] [36]；EPDCs 中几种 EMT 相关转录因子上调，包括 Smad1、Snail、Slug 和 Twist [14] [35]。

3.1. 心外膜胚胎基因的激活

最近的体内研究表明，内源性心外膜在心肌梗死后被重新激活，从而重新表达胚胎标志物。在 Zhou 等人研究中，胚胎心外膜标记基因 Wt1、Tbx18 和 Raldh2 在心肌梗死后动态上调[35]。与胎儿 EPDCs 不同，心肌梗死后成人 EPDCs 主要保留在心脏表面[35]。

心肌梗死后，心外膜层细胞大量扩增，大部分细胞表达心外膜标志物 Wt1。Wt1 对心外膜组织祖细胞的形成很重要，主要形成心肌成纤维细胞和冠状动脉血管平滑肌细胞，其次是内皮细胞和心肌细胞[37]。高水平的 Wt1 可以使 EPDCs 能够迁移到心脏的受损区域[38]。Thymosin Beta 4 (Tβ4)诱导心外膜细胞中 Wt1 的再表达，并促进 EPDCs 迁移和血管生成[38]。

研究发现 Wnt 信号与心外膜细胞的激活及 EMT 过程有关。Wnt1 最初在心外膜中表达，随后由受损区域的心脏成纤维细胞表达[39]。一旦 Wnt 与其受体结合，就会激活 β-catenin，而 β-catenin 又会激活 Tcf/Lef，从而导致 Cyclin D1 的表达，从而引起心外膜细胞的增殖和活化[40]。

参与心外膜活化的另一个因素是神经调节蛋白 1 (Nrg1)。在未受伤的斑马鱼中，Nrg1 表达的再激活诱导心肌细胞去分化、心外膜激活、血管生成的增加[41]。

3.2. 激活 EMT 过程

EMT 是上皮细胞失去细胞极性和细胞间粘附并获得迁移和侵袭特性成为间充质干细胞的过程。

PDGF 及其受体 PDGFR 被证明在 EMT 过程中起重要作用。心外膜细胞上表达 PDGFR 基因可以促

进血管平滑肌细胞(VSMC)和心肌成纤维细胞的形成。PDGF受体信号转导是心外膜EMT所必需的，PDGF受体的缺失导致EMT过程障碍并无法形成任何心外膜衍生物[31]。Sox9在PDGF受体缺陷型心外膜细胞中减少，Sox9的过表达恢复了心外膜迁移和EMT基因表达[31]。

先前的体外研究已确定TGF-β是小鼠和鸡胚胎PEO和胚胎心外膜EMT的关键调节因子[23]。鸡胚胎心外膜细胞的TGF-β调节EMT依赖于TGF-β I型受体和激活素受体样激酶(ALK) 5的活性[23]。可溶性血管细胞粘附分子-1 (VCAM-1)能够抑制TGF-β诱导的EMT [42]。在人类成人心外膜细胞的体外模型中，心外膜细胞自发地进行EMT，EMT前后的心外膜细胞均表达心外膜标志物Wt1，添加TGF-β可诱导上皮特征丧失，并启动人类成人心外膜细胞间充质分化的开始。在TGF-β刺激下，检测到平滑肌细胞标志物α-平滑肌肌动蛋白(α-SMA)的表达增加，EMT基因Snail1的表达上调[43]。

NF-κB激活是TGF-β2/PDGFBB诱导的心脏上皮到间充质转化的重要步骤[44]。此外，NF-κB还参与在TGF-βR3刺激下心外膜来源细胞迁移过程中的胶原蛋白侵袭[45] [46]。抑制NF-κB作用会阻碍心脏再生，导致心肌细胞增殖减少和心外膜反应减少[47]。

透明质酸(HA)和透明质酸介导的受体(HMMR)被发现与斑马鱼心脏再生过程中的心外膜细胞EMT高度相关。斑马鱼部分心室切除后，研究者检测到透明质酸合酶(Has)表达增加。抑制HA的产生以及HMMR的消耗阻碍了心脏再生。在心肌梗死的大鼠模型中，HA和HMMR在损伤后的最初几天内均上调并定位在梗死区域，这表明该途径也可能在哺乳动物的心脏修复中发挥重要作用[48]。

3.3. 旁分泌过程参与心脏的修复

EPDCs旁分泌因子促进心肌梗死后冠状动脉血管的存活和生长。在发育过程中，受到心外膜分泌VEGFA、FGF等促血管生成因子的刺激，冠状动脉在心外膜下形成[32] [49]。心肌梗死可重新激活心外膜胎儿样反应，心肌梗死后EPDCs分泌的因子在体外和体内均具有强效的促血管生成活性，包括VEGFA和FGF2，并表明这两个因子有助于EPDCs的血管生成活性[35]。

3.4. 心包液外泌体 Clusterin 促进 EMT

Foglio等人发现在心肌梗死小鼠三天的心脏中鉴定出心包液(PF)中的Clusterin可以诱导心外膜EMT。Clusterin在体内对急性MI后的心肌细胞具有保护作用，并且是TGF-β诱导的重要介质[50]。

4. 问题与展望

心肌再生和修复具有广泛且重要的临床应用价值。利用干细胞进行细胞移植治疗被认为是心肌再生最有潜力的手段。胚胎干细胞、诱导多能性干细胞、心脏祖细胞的深入研究给哺乳动物心肌再生的研究带来希望。心外膜及心外膜来源的祖细胞可能是一种在心肌再生和修复中的新途径和来源。未来的研究热点除了进行深入揭示心外膜祖细胞及其调控机制外；从心外膜途径进行心肌损伤和修复的研究，可能具有重要的临床价值。因为，在人类心脏组织，通过心包穿刺等较简单、微创的临床手段可能让通过心外膜干预来进行心肌再生治疗成为现实。随着心外膜及心脏祖细胞的调控机制深入研究，可能为治疗性心肌再生与修复、甚至阐明各种先天性心脏病及获得性心脏疾病的发病机理找到新突破口和途径。

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