陈晓, 博士, 浙江大学教授, 博士生导师, 浙江大学–爱丁堡大学联合学院副院长, 国家优秀青年基金获得者, 浙江省杰出青年基金获得者, 浙江大学干细胞与再生医学研究中心执行主任。担任中国生物医学工程学会组织工程与再生医学分会青年工作组秘书长, 中华医学会骨科学分会第十一届委员会青年委员会基础学组副组长, 中华医学会骨科学分会第十一届委员会转化与创新学组青年委员(副组长), 国际矫形与创伤外科学会中国区常委。实验室长期专注于运动系统干细胞及再生研究, 根据组织特异性在肌腱的种子细胞和支架中的基础应用研究进行了一系列的研究创新。

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生长因子在肌腱再生中的应用

丁楠浩　范春梅　陈晓*

(浙江大学医学院附属第二医院骨外科与李达三叶耀珍干细胞与再生医学研究中心,
浙江省组织工程与再生医学技术重点实验室, 浙江大学医学院, 杭州 310058)

摘要　肌腱损伤是一个全球性的常见健康问题, 肌腱的特性使得肌腱的愈合变得十分困难, 损伤部位愈合后常常被疤痕组织替代, 造成肌腱生物力学性质的损害。近年来, 许多证据表明生长因子能够促进肌腱的再生愈合, 这为治疗肌腱损伤开辟了新的方向。该文主要总结了近年来用于治疗肌腱损伤的相关生长因子及其对肌腱损伤的治疗作用和递送方法。阐明了未来可能通过生长因子、干细胞和生物支架的联合应用的组织工程的方法实现肌腱疤痕的消除及肌腱个体化再生。

关键词　再生医学; 肌腱再生; 生长因子; 组织工程

Application of Growth Factors in Tendon Tissue Regeneration

DING Nanhao, FAN Chunmei, CHEN Xiao*

(*Dr. Li Dak Sum-Yip Yio Chin Center for Stem Cells and Regenerative Medicine and Department of Orthopedic Surgery of the Second Affiliated Hospital, Key Laboratory of Tissue Engineering and Regenerative Medicine of Zhejiang Province, Zhejiang University School of Medicine, Hangzhou 310058, China*)

Abstract　Tendon injury is a common global health problem. The properties of tendons make their natural healing difficult and scar tissues often replace the natural tissues in the injured site after healing, which results in biomechanical damage of tendon. In recent years, many evidences suggest that growth factors play an important role in the regeneration and healing of tendons, which develops a new direction for the treatment of tendon injury. In this review, the major growth factors have been applied to treat tendon injuries so far are summarized with their effects and delivery strategies. It is indicated that the combination of growth factors, stem cells and biological scaffolds may be used in tendon tissue engineering to achieve the elimination of tendon scar and the individualized re-

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*通讯作者。Tel: 13656642830, E-mail: chenxiao-610@zju.edu.cn
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*Corresponding author. Tel: +86-13656642830, E-mail: chenxiao-610@zju.edu.cn

generation of tendons as well.

Keywords regenerative medicine; tendon regeneration; growth factors; tissue engineering

肌腱(tendon)是一种少细胞、乏血管的特殊结缔组织, 主要由丰富的细胞外基质(extracellular matrix, ECM)组成。肌腱ECM包括胶原蛋白、弹性蛋白、蛋白聚糖、糖蛋白和其他大分子[1-4]。肌腱是人体运动系统的关键组成部分, 能够在肌肉和骨骼之间传递机械力, 具有良好的生物力学性质。肌腱本身的优秀生物力学性质的基础来自于肌腱中I型胶原纤维组成的高度组织化的细胞外基质(ECM)及其有序性[2,5]。

由于人们在日常活动中过度地使用肌腱, 肌腱损伤已经成为当今社会常见的运动系统疾病。肌腱损伤包括急性与慢性两种类型, 急性损伤主要包括肌腱断裂, 慢性损伤以长期反复超负荷轻微损伤累积出现肌腱退化和剧烈疼痛为特征[6-8]。根据流行病学调查结果, 年龄、过度运动是诱发肌腱损伤最主要的因素。肌腱损伤发生率随着年龄的增长而增加, 80岁以上人群中肩部肌腱损伤发病率超过50%[9]。高强度运动损伤常引起肌腱出现明显退行性病变, 长期累积最终会导致肌腱断裂, 因而跟腱断裂高发于30~49岁的男性[10-11]。除了年龄、运动损伤之外, 机体代谢水平的异常改变如高胆固醇血症等也会导致机体更易出现肌腱病变的倾向[10,12]。

无论是急性还是慢性损伤, 由于肌腱的内在再生能力很弱, 当ECM合成和降解之间失去平衡时, 伤口修复通常会形成由成纤维细胞和杂乱的ECM(主要是胶原蛋白)构成的无功能组织(通常被称为疤痕组织)且无法恢复天然基质结构, 从而导致疤痕形成[13-15]。此外, 在修复过程中, 随着炎症的刺激, 肌腱细胞还会高表达成纤维细胞的活化标志物导致肌腱纤维化发展[16]。与天然肌腱相比, 疤痕在代谢、组织学结构、生物力学性质等方面存在较大的差异, 这使得肌腱强度显著降低, 即使经过长期愈合也无法恢复到正常肌腱的水平, 容易出现肌腱黏连、萎缩和再次断裂等并发症[8,17-21]。

1 肌腱疤痕的产生

由于再生能力较弱, 肌腱损伤通常通过纤维化过程愈合, 从而产生损害愈合肌腱功能的疤痕组织, 这个过程中可分为三个重叠的阶段[22]。

1.1 炎症阶段

常见于损伤早期。在此阶段, 损伤部位出现明显的炎症反应, 有大量白细胞浸润, 巨噬细胞大量聚集吞噬坏死碎片, 肌腱细胞也被募集到受伤区域并被刺激增殖, 该阶段是影响肌腱功能再生的关键阶段[2,23]。过度的炎症反应所导致的最终结局是肌腱疤痕的增生。

1.2 增殖阶段

在炎症阶段后期, 肌腱损伤部位细胞进行大量的生物合成活动。巨噬细胞在此阶段主要发挥修复作用: 通过释放生长因子与细胞因子, 直接激活驻留于肌腱损伤部位的肌腱细胞, 使得肌腱细胞分化为肌成纤维细胞, 成为合成III型胶原蛋白的主要来源[24]。之后, 肌成纤维细胞和巨噬细胞开始分泌并沉积主要由III型胶原蛋白构成的ECM, 此时的ECM在组织修复过程中起着重要的结构支撑和调节作用, 并最终在后续阶段从III型胶原蛋白部分转变为I型胶原蛋白[5,25-27]。一般而言, 在增殖阶段之后, 肌成纤维细胞通常会发生凋亡或恢复到静止的成纤维细胞状态[28]。但若炎症持续存在, 则肌成纤维细胞将持续保持活化状态, 导致胶原纤维产生并沉积的速度大于组织重塑所能适应的速度, 从而导致组织纤维化。

1.3 重塑阶段

在组织修复后期, I型胶原蛋白合成开始占主导地位, 逐步改善ECM的有序性。此外, 成纤维细胞密度和合成活性逐渐降低, 使得ECM沉积减缓以及III型与I型胶原纤维比率逐渐正常化[20]。这一阶段可能持续长达数年, 但在正常生理状况下绝大部分损伤肌腱在此阶段无法完全恢复到原始肌腱的结构。相对于天然肌腱, 重塑后的肌腱III型与I型胶原蛋白的比例增大, III型胶原蛋白形成更小的原纤维(fibril), 结构也更为紊乱, 这也是肌腱疤痕生物力学性能显著下降且易于再次损伤的主要原因[25,29,30]。

2 生长因子对肌腱疤痕生成的影响

众所周知, 成人肌腱愈合的特点是疤痕形成, 修复组织结构杂乱, 机械性能降低[31]。肌腱愈合反应的基本机制包括生长因子、细胞因子表达和细胞系的募集[6]。研究表明, 炎症在肌腱疤痕的形成中发

挥了极为重要的作用。即炎症是肌腱疾病过程中疤痕形成的主要诱因, 也是驱动肌腱修复的免疫反应的重要因素[32]。控制生长因子、细胞因子表达以控制炎症强度, 对于减少疤痕的形成有重要的意义。

2.1 细胞因子

在肌腱断裂愈合的过程中, 细胞因子释放的主要来源是肌腱细胞以及募集的炎性细胞如中性粒细胞和巨噬细胞。

在肌腱断裂愈合过程中的细胞因子可以分为促炎症细胞因子与抗炎症细胞因子。其中肿瘤坏死因子α(tumor necrosis factor alpha, TNFα)、白细胞介素-1β(interleukin-1β, IL-1β)、IL-6等是典型的促炎症细胞因子, IL-10、IL-4是抗炎症细胞因子[33]。肌腱损伤后, 迁移的白细胞释放外源性细胞因子TNFα、IL-1β, 激活肌腱细胞并分泌细胞因子。TNFα刺激肌腱细胞释放促炎和抗炎症细胞因子, 包括IL-1β、TNFα、IL-6和IL-10等[34]。此外, IL-1β能够诱导基质金属蛋白酶(matrix metalloproteinase, MMP)等的形成促使ECM的降解, 减少疤痕的产生, 这在重塑阶段发挥重要的作用[19,35,36]。在肌腱细胞中, IL-6在TNFα和IL-1β的作用下的表达高度上调, 释放的IL-6诱导激活JAK/STAT-3信号通路, 并由此上调血管内皮生长因子(vascular endothelial growth factor, VEGF)以促进血管增殖, 促进肌腱的愈合[36-38]。

2.2 生长因子

生长因子是机体参与调控细胞生长和分化的信号分子, 大量不同生长因子所组成的复杂系统, 在肌腱愈合过程中具有重要的调节作用(表1)。病理状态下机体生长因子多由损伤部位炎症促进巨噬细胞等免疫细胞释放[39]。有研究表明, 在肌腱损伤后, 多种生长因子包括胰岛素样生长因子-1(insulin-like growth factor-1, IGF-1)、血小板衍生生长因子(platelet-derived growth factors, PDGF)、血管内皮生长因子(VEGF)、成纤维细胞生长因子-2(fibroblast growth factor, FGF-2)、转化生长因子-β(transforming growth factor, TGF-β)和骨形态发生蛋白(bone morphogenic protein, BMP)等显著上调, 并在愈合过程中发挥作用。其中, IGF-1能刺激损伤部位成纤维细胞的增殖和迁移, 并在重塑过程中促进ECM成分的再生, IGF-1的缺失导致修复反应较差[20]。PDGF主要参与愈合的早期阶段, 诱导IGF-1的合成并刺激DNA合成[40]。在愈合的后期, VEGF促进损伤区域的血管生长。FGF-2指导并刺激肌腱细胞发育和增殖, 也是血管生成的调节剂[41]。TGF-β家族的生长因子存在于肌腱愈合的所有阶段且具有重要的作用, 尤其是在炎症和增殖阶段, 具有刺激胶原蛋白的产生、调节纤连蛋白结合模式和蛋白酶, 以及介导炎症细胞迁移的作用, 是影响肌腱纤维化疤痕形

表1 肌腱修复中使用的主要生长因子

Table 1 Major growth factors used in tendon repairs

生长因子 Growth factors	递送策略 Delivery strategies	肌腱 Tendons	效果 Effects	参考文献 References
TGF-β1	Recombinant adenovirus vector transduction	Rat Achilles tendon	Decreased the deposition of type III collagen and promote type I collagen fiber bundles; Accelerate the restoration of mechanical strength	[78]
CDMP-2	Local injection	Rabbit patellar tendon	Improve biomechanical properties of the healing tendon	[67]
VEGF	Recombinant adenovirus vector transduction	Chicken flexor tendon	Increased tendon healing strength in the early stage of healing	[82]
	Gene-loaded nanoparticle-coated sutures			[80]
BMP-7	Gelatin hydrogel sheet	Rat rotator cuff	Improve the orientation of rotator cuff collagen fiber Improve biomechanical properties of the healing tendon	[83]
FGF-2	FGF-2-soaked gelatin hydrogel	Rat rotator cuff	Facilitate the formation of tendon-like tissue	[84]
PDGF-BB	Heparinized collagen sutures	Chicken flexor tendon	Improve biomechanical properties and the vascularity of the healing tendo	[85]

成的重要生长因子[42-44]。其中促纤维化因子TGF-β1能诱导成纤维细胞分化为肌成纤维细胞并维持肌成纤维细胞的存活。抗纤维化因子TGF-β3同样具有重要的作用, 有研究表明, 不同TGF-β同种型比例是导致肌腱纤维化的原因之一[45-46]。骨形态发生蛋白(BMP)是TGF-β超家族的一个亚群, 通过影响组织分化来影响肌腱愈合[30]。

3 利用生长因子诱导肌腱再生的方法与临床探索

成人肌腱疤痕愈合的特点是肌腱修复形成的疤痕无法达到正常肌腱的生物力学性能, 当下改善肌腱疤痕介导愈合的治疗干预是该领域的进步, 最终目标是实现无疤痕再生愈合。由于肌腱愈合修复机制依旧未能完全明了, 这也就限制了后续的临床应用研究。现有的临床与基础研究希望通过组织工程中干细胞、生长因子和生物支架材料等领域来改变肌腱修复微环境从而改善肌腱疤痕的产生[5,20,39,47]。其中在肌腱愈合修复过程中发挥重要作用的生长因子受到了重视, 许多研究尝试改变生长因子来促使肌腱的愈合。

3.1 调节机械负荷

适当的机械负荷能在肌腱再生中发挥积极作用, 在愈合过程中的肌腱已被证明对外部拉伸负荷有反应[48]。结构上, 当施加负荷时, 肌腱ECM中纤维和原纤维卷曲减少而更加对齐, 改善肌腱的组织结构[49]。代谢上, 机械负荷能够影响受损肌腱的免疫反应从而影响细胞因子的分泌[2]。临床上也有利用机械负荷实现肌腱更好的功能恢复的例子[50,51]。但也有文献指出, 机械负荷对细胞因子的作用存在两面性, 过度的机械负荷会加重肌腱损伤部位的炎症[29,52]。此外机械负荷的效果不仅与机械负荷本身大小相关, 也受到损伤肌腱类型与所处部位的影响[3]。由此可见, 机械负荷与肌腱愈合之间存在着复杂的关系, 有研究建立了一个机械负荷与肌腱愈合的多尺度计算模型, 这有助于未来指导通过机械负荷促进肌腱再生[53]。但当下距离实现利用机械负荷去改善肌腱再生情况还需要更多研究。

3.2 生长因子直接应用

就目前而言, 由于现有研究对生长因子的作用机制与相互作用研究仍然十分局限, 在这种情况下, 几乎很难确定使用生长因子促使肌腱分化的最佳方案: 生长因子的剂量、种类、治疗时间和生长因子间的相互作用都可能导致实验结果的显著改变。此外, 肌腱损伤部位的生长因子递送策略也依旧处于探索中。因此, 生长因子的直接使用在很大程度上仍处于实验阶段, 临床应用尚少且效果尚存在争议[54-59]。

3.2.1 生长因子选择 (1) PRP。根据现有的文献, 很多研究都聚焦于一种富含生长因子的生物制剂, 即富血小板血浆(platelet-rich plasma, PRP)。PRP是患者自身血液的衍生物, 其中含有超生理浓度的血小板。血小板激活后可以释放显著高于生理状况下血浆浓度的生长因子、细胞因子和一系列生物活性蛋白来促进损伤愈合[60]。许多研究揭示了PRP在促进肌腱愈合方面的积极作用, 它能显著改善肌腱–骨界面处的生物力学特性和组织学外观[17,61]。虽然在过去的几十年中, PRP已成为肌肉骨骼损伤的辅助治疗剂。然而, PRP的临床效果的验证一直存在着矛盾。有文献指出, 注射PRP对于肌腱功能的改善并没有预期的效果, 在近期的部分临床随机对照实验中明确指出PRP注射治疗急性跟腱断裂、肩袖撕裂并没有出现肌腱功能的改善[59,62-63]。这可能是由于当下PRP制备方法尚无统一标准及患者的PRP之间也存在个体差异造成的, 但毫无疑问, 这些差异严重阻碍了PRP的研究与临床应用。

(2) 其他生长因子。有研究提出利用重组人血小板衍生生长因子(recombinant human platelet-derived-growth factor-BB, rhPDGF-BB)或其他生长因子如TGF-β、VEGF等作为PRP的替代品, 以消除PRP成分差异对肌腱愈合的影响, 从而作为消除PRP临床治疗效果个体差异的一种选择[64,65]。这也是一种研究的方向, 但是生长因子之间通常存在协同作用, 单一的生长因子的应用效果存在着一定的局限性[66]。由于生长因子间相互作用的具体机制尚未完全明确, 未来利用多种生长因子进行治疗的方法还有待研究, 效果也有待考察。

3.2.2 生长因子递送策略 (1) 注射与浸渍缝合线。这是将生长因子递送至特定损伤部位最直接的方法。局部注射相对无创且简单。在实验室研究中, 已经有证据证明这种方法治疗肌腱损伤的有效性, 包括肌腱内注射IGF-1可加速跟腱损伤大鼠模型肌腱功能恢复, 注射软骨衍生形态发生蛋白-2(cartilage-derived morphogenic protein-2, CDMP-2)能改善兔手术修复模型中的早期肌腱愈合[30,67]。

使用浸渍缝合线则可以避免与局部注入相关的溢出损失的缺点且无需额外的手术步骤, 在动物模型上有着不错的疗效[68-69]。这些方法虽然已经在临床上有所应用, 但是依旧不算十分成熟, 存在着一定的限制和缺点。其主要缺点是生长因子在损伤部位维持时间较短, 且肌腱再生所需的机械环境难以维持。由于肌腱愈合多持续数月至数年, 这种短暂维持的生长因子可能无法带来可观的疗效。但近年来有研究显示纳米粒子涂层的缝合线能有效地将生长因子递送到组织并控制生长因子的持续释放[70]。显然, 这种缝合线将成为一种治疗肌腱损伤的重要临床治疗工具并具有广阔的研究前景。

(2) 支架(scaffold)。由于注射和浸渍缝合线应用生长因子存在的缺陷, 寻求新的更加合适的应用方式也就显得十分重要。为了保持肌腱再生所需的机械环境和维持生长因子作用的时间, 植入合适的生物支架是理想的方式。细胞和材料之间的相互作用是基于生物材料的组织再生的一个基本主题[71]。生物支架的设计基于两个关键点: 材料和结构[72]。在材料上, 理想支架应具有良好的细胞相容性、无免疫原性或细胞毒性、可吸收性。而在结构上, 支架应当模仿天然健康组织的结构以支持并引导肌腱生长。

生物支架基于生物来源可以来自自体移植、异种移植和同种异体移植。自体移植物有极佳的生物相容性, 但难以获得并可能导致供体部位损伤。相对而言, 异种移植物和同种异体移植物更容易获得, 但存在发生免疫排斥反应的风险。生物支架由于供体自身的特异性还具有不可预测的降解速率、机械性能不足和非特异性诱导能力, 对支架的效果产生不利影响[73]。相对地, 使用天然或合成生物材料制造的人工合成支架具有相对稳定的生物化学性质, 甚至可能做到定制其降解特性, 以控制其在生物环境下的降解速率, 具有标准化的可能性。但与生物来源支架相比, 合成支架更有可能使得患者出现免疫排斥反应, 且在结构上与天然健康组织存在着一定的差异。

此外, 相对于使用注射和浸渍缝合线应用生长因子的方法, 支架利用物理吸附、共价键和离子键、超临界流体技术等方式吸附固定生长因子, 能够提高生长因子在损伤部位维持的时间[74]。如富含血小板的纤维蛋白(platelet-rich fibrin, PRF)支架可以对细胞因子表现出锁定作用, 减缓细胞因子以及生长因子的释放[74-75]。近年来, 对于支架中生长因子受控释放, 现有研究已经取得了一定的进展。有研究者利用具有较好生物相容性的聚酯合成支架, 产生具有可调降解速率和生物活性剂释放曲线的混合材料, 这可在一定程度上控制生长因子等生物活性剂的释放[76]。这对于实现利用生长因子调控肌腱修复有着十分重要的意义。表2总结了部分用于制造肌腱修复支架的主要材料。

3.3 基因治疗

除了直接应用外, 生长因子还可以通过基因治疗传递到损伤部位。基因疗法将特定生长因子的特定基因传递给细胞以改变其合成和功能, 开始产生生长因子。相对于生长因子在局部的直接应用, 基因疗法能够更长时间地维持生长因子的作用, 且在原位产生生长因子、避免免疫原性等方面具有极大的优势[5]。基因疗法的效果在诸多研究中已得到证

表2 肌腱修复中使用的主要支架材料

Table 2 Major scaffold materials used in tendon repairs

支架种类 Scaffold types	支架材料 Scaffold materials	肌腱 Tendons	优点 Advantages	参考文献 References
Biologic scaffolds	Silk fibroin (SF)	Rabbit medial collateral ligament	Outstanding bioactivity, biocompatibility, biodegradability and biodegradability	[86-87]
	Gelatin	Rabbit Achilles tendon		[88]
	Hyaluronic acid	Equine flexor tendon		[89-90]
Synthetic scaffolds	Polydioxanone (PDO)	Human rotator cuff	High availability, reproducibility and mechanical properties	[91]
Composite scaffolds	PLGA nanofiber mats and HBDS	Dog flexor tendon	Depend on the properties of the used biologic and synthetic materials	[92]
	Polycaprolactone (PLC) chitosan (CS) and hyaluronic acid (HA)	Rabbit anterior cruciate ligament		[93]
	Polycaprolactone/chitosan (HAp-PCL/CS)	—		[94]

实, 如利用转基因骨髓间充质干细胞(bone marrow-derived stem cells, BMSCs)或者重组腺病毒等方式将*TGF-β1*基因递送至修复位点, *TGF-β1* BMSCs治疗的受伤跟腱能更为迅速地愈合并获得更好的生物力学性质[65,77-78]。但确定基因治疗的合适靶点、基因转移的最佳方法和时机、组织和宿主异质性、载体和输送系统以及安全性等许多问题依旧悬而未决[39,79]。因此, 要实现这一治疗方法, 还需要进一步研究的支持。值得注意的是, 近年来许多研究聚焦于使用纳米颗粒, 通过形成纳米颗粒–质粒复合物将生长因子基因转移至受损肌腱组织, 该方法具有非常出色的转染效率和治疗效果, 并且能规避病毒载体的一些安全性问题, 具有非常广阔的研究前景[80-81]。

4 总结

目前生长因子用于促进肌腱愈合的各种方法均尚未实现肌腱完全再生, 虽然生长因子的应用在一定程度上提高了肌腱愈合率和部分功能再生水平, 并在一定程度上提高了修复后的肌腱强度, 但其并未能显著改善损伤后的肌腱超微结构, 导致修复后的肌腱力学性能差, 易断裂率高[6,95]。面对这一困难, 生长因子联合基于分化能力突出的干细胞疗法可能能够成为实现肌腱再生的重要研究方向[14,96]。通过干细胞、支架和生长因子的联合应用, 结合分子生物学和材料科学的进展, 为受伤的肌腱建立适当的微环境, 可以更精确地恢复肌腱的生理学特性, 这对于实现肌腱再生具有重要意义, 可能是未来研究的方向。与此同时, 复杂的肌腱愈合机制还有很多疑问亟待阐明, 通过提高对肌腱愈合机制的理解, 对于实现成熟的肌腱修复和再生技术有着重要的意义。

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