评述

间充质干细胞作用机制的研究进展

黄庆雷¹, 沈丽^{1,2*}, 邓钺^{1*}

1. 北京弘润天源基因生物技术有限公司, 北京 100191;

2. 北京大学医学部细胞生物学系, 北京 100191

* 联系人, E-mail: shenli@bjmu.edu.cn; yuedeng@cellonis.com

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摘要 间充质干细胞(mesenchymal stem cells, MSCs)是一类具有自我更新和多向分化潜能的成体干细胞。取决于局部微环境的刺激, MSCs可产生大量生物活性物质, 具有造血支持、提供营养、激活内源性干/祖细胞、组织损伤修复、免疫调节、促进血管新生、抗细胞凋亡、抗氧化、抗纤维化以及归巢等多方面的作用。临床试验结果表明, MSCs在许多疾病治疗中都表现出很好的效果, 特别是自身免疫性疾病、组织损伤性疾病和退行性疾病等。然而, MSCs在疾病治疗中的作用机制尚不明确, 本文重点介绍了目前研究发现的MSCs作用机制, 这些机制主要包括转分化和细胞融合、旁分泌作用、细胞与细胞接触依赖、胞外囊泡和线粒体转移以及表观遗传学调控等。此外, 还讨论了能够增强MSCs临床治疗效果的方法。

关键词 间充质干细胞, 作用机制, 细胞接触依赖, 线粒体转移, 表观遗传学调控

间充质干细胞(mesenchymal stem cells, MSCs)来源于胚胎发育早期的中胚层, 是一种异质性的细胞群体。机体内的MSCs包含自胚胎发育早期及之后不同发育阶段的干细胞及分化后代。人体几乎所有的组织都含有MSCs, 多种组织和器官中MSCs含量丰富, 如骨髓、脂肪、牙髓、脐带和胎盘等, 不同来源的MSCs蛋白质表达谱系不同, 其特性也略有差异, 但都满足国际细胞治疗协会制定的MSCs鉴定最低标准, 普遍具有自我更新能力、多向分化潜能和免疫调节等功能^[1,2]。

MSCs表达低水平的人类白细胞抗原 I (human leucocyte antigen, HLA-I) 类分子, 免疫原性较弱。MSCs不表达HLA-II类分子和B7-1, B7-2, CD40,

CD40L等共刺激分子, 这些分子是效应性T细胞激活所必需的。共刺激分子的缺失, 使得T细胞因缺少第二信号而不能激活, 导致辅助性T细胞(helper T cell, Th细胞)的无反应性, MSCs因此具有免疫豁免的特性, 异体移植也不会引起严重的免疫排斥反应^[3]。MSCs能够主动迁移到组织损伤的部位, 参与免疫调节和组织损伤修复。这些特性赋予了MSCs在组织工程和再生医学领域广阔的应用前景, 使其成为细胞治疗领域最具临床应用价值的干细胞。

根据www.ClinicalTrials.gov网站统计的数据表明, 截至2018年10月, 全球已注册705项基于MSCs的临床试验, 80%以上的临床试验处于临床I期和II期, 15%左右处于临床III期阶段^[4]。已完成的试验结果表明,

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MSCs用于多种疾病的治疗是安全和有效的, 这些疾病类型包括移植物抗宿主病、脊髓损伤、自身免疫性疾病、心血管疾病、骨及软骨损伤修复、克罗恩病、糖尿病及其并发症等^[5]. 目前, 全球已有11种间充质干细胞药物获批用于上述疾病的临床治疗^[6]. 然而, MSCs在疾病治疗过程中发挥作用的机制尚不明确, 仍需深入的研究和探索. 迄今为止, 文献中报道的作用机制包括MSCs的转分化和细胞融合、旁分泌作用, 细胞与细胞间接接触依赖, 胞外囊泡和线粒体转移以及表观遗传学调控等.

1 多向分化潜能

MSCs具有分化成多种细胞类型的潜能, 在特定信号和局部微环境的刺激下通过调控基因的差异性表达, 不仅可以分化为中胚层来源的细胞类型, 包括心肌细胞^[7], 血管内皮细胞^[8], 成脂、成骨和成软骨等细胞^[9], 也可以转分化为消化道上皮细胞、肺细胞、肠道上皮细胞^[10]、肌肉细胞等内胚层来源的细胞^[9], 还可以转分化为表皮细胞^[11]和神经细胞^[12]等外胚层来

源的细胞(图1)^[13]. 多条信号通路参与MSCs的细胞分化进程, 其中Wnt和TGF- β 超家族这两条主要的信号通路在细胞分化进程中发挥关键作用, 激活MSCs表面的Wnt信号通路受体会导致下游信号转导, 调节MSCs的增殖和分化等细胞进程^[14,15]. 不同组织来源的MSCs都具有很强的多向分化能力, 其中羊膜来源的MSCs亚群具有类似胚胎干细胞分化潜能特性, 表达Oct-4, Nanog, SOX-2, SSEA-4, REX-1等多种全能性标志基因的转录因子^[16]. MSCs具有三个胚层多向分化的能力, 可广泛应用于药物筛选、组织工程和细胞治疗等领域.

MSCs作用机制研究的早期阶段集中在其作为成体干细胞的自我更新、分化潜能和组织再生能力等方面, 并提出宿主细胞替换的作用机制^[17]. MSCs细胞替换的作用机制包括两个方面, 细胞转分化(transdifferentiation)和细胞融合(cell fusion). 细胞转分化是指在趋化因子的介导下, MSCs迁移到组织损伤部位并跨胚层分化为组织特异性细胞和连接细胞, 替代受损细胞从而起到组织再生和修复的作用^[18]. 细胞融合是指MSCs与宿主细胞发生融合, 导致宿主细胞的细胞核发生重编程, 融合细胞表达MSCs特异性基因, 从而避

图 1 MSCs的多向分化潜能. MSCs具有分化成三胚层多种类型细胞的潜能, 既可以分化成中胚层的脂肪细胞、骨细胞、软骨细胞和组织间连接细胞, 也可以转分化成为内胚层的肌肉细胞、肺细胞和肠道上皮细胞, 还可以转分化成为外胚层的表皮细胞和神经细胞^[13](体内MSCs的转分化仍存争议)

Figure 1 The multipotentiality of MSCs. MSCs have the ability to differentiate into multiple cell type of three embryonic germ layers. They can differentiate into cells of the mesodermal lineage, such as adipocytes, osteocytes, chondrocytes, and connective tissue cells. They may also transdifferentiate into cells of the endodermal lineage, such as muscle, lung, and gut epithelial cells, or transdifferentiate into cells of the ectodermal lineage, such as neurons and epithelial cells^[13] (The transdifferentiation potential of MSCs *in vivo* is still controversial)

免损伤的宿主细胞发生凋亡^[19]。过去十几年的动物实验和临床试验结果表明, 无论自体还是异体MSCs移植, 仅有少量细胞能够在组织损伤修复过程中长期定植并存活下来^[20], 表明宿主细胞替换虽然是MSCs在组织损伤修复中的一种作用方式, 但不是MSCs作为细胞疗法发挥作用的主要机制。MSCs参与组织损伤修复还涉及组织内源性干/祖细胞的激活、免疫调节和炎症反应的应答等一系列复杂的生物学进程^[4,21,22]。宿主细胞替换很可能是MSCs移植后对损伤组织的一种应激保护机制。

2 生物活性物质

MSCs旁分泌作用可以产生大量的可溶性细胞因子、趋化因子和生长因子等促进组织损伤的修复, 通常被认为是MSCs参与组织损伤修复的主要机制。此外, MSCs还通过产生膜结合的小分子蛋白发挥作用, 如整合素家族蛋白和黏附分子等^[23], 包括旁分泌因子和膜结合的小分子蛋白在内的大量生物活性物质协同作用形成一个综合的复杂调控网络, 可激活靶细胞内多条信号通路^[24]。MSCs移植后可迅速迁移到组织损伤部位, 通过旁分泌作用激活组织内源特异性干/祖细胞的增殖和分化, 对于组织损伤的修复进程发挥重要作用^[25,26]。研究发现, MSC产生的生物活性物质参与

免疫调节、血管新生、抗细胞凋亡、抗氧化、抗纤维化和趋化作用等多个生物学进程(图2)。一种因子可参与多个生物学进程的调控, 如血管内皮细胞生长因子(vascular endothelial growth factor, VEGF)既参与血管新生, 也参与到抗细胞凋亡的进程。

2.1 免疫调节相关因子

MSCs可产生多种免疫调节因子, 无论对自体还是同种异体的免疫细胞, 无论对固有免疫细胞还是获得性免疫细胞都能发挥免疫调节作用。迄今为止, 发现的免疫调节因子主要有白细胞介素6(IL-6)、肝细胞生长因子(hepatocyte growth factor, HGF)、吲哚胺2,3双加氧酶(indoleamine-2,3-dioxygenase, IDO)、血红素氧合酶1(heme oxygenase-1, HO-1)、转化生长因子 β 1(transforming growth factor-beta1, TGF- β 1)、一氧化氮(nitric oxide, NO)、人类白细胞抗原I类分子G5(human leukocyte antigen class I molecule G5, HLA-G5)、前列腺素E2(prostaglandin E2, PGE2)和白细胞介素10(IL-10)等^[27]。

不同种属之间MSCs发挥免疫调节作用的因子存在明显的差异, 如人和小鼠的MSCs通过不同的细胞因子发挥对淋巴细胞的抑制作用。人源MSCs通过产生大量的IDO(仅少量的NO)在抑制淋巴细胞活性和增强调节性T细胞的功能方面发挥主要作用; 而鼠源MSCs则

图2 MSCs产生的生物活性物质^[27]

Figure 2 Bioactive substances produced by MSCs^[27]

是通过一氧化氮合酶(inducible nitric oxide synthase, iNOS)产生大量NO在抑制淋巴细胞活性方面发挥关键作用^[28]. MSCs产生的多种免疫调节因子之间可通过协同或拮抗发挥作用, 不仅参与免疫反应的调控, 而且还参与炎症反应的应答和组织损伤修复等多个生物学进程^[29,30].

2.2 血管新生相关因子

间充质干细胞移植后, 可有效改善动物模型的心肌梗死和冠状动脉性心脏病^[31,32], MSCs发挥作用的机制是诱导血管新生(angiogenesis)^[33]和动脉生成(arteriogenesis)^[34]. 血管新生主要在原有毛细血管或微静脉基础上通过血管内皮细胞的增殖和迁移, 从先前存在的血管处以芽生或非芽生(或称套叠)的形式生成新的毛细血管网. 动脉生成是指新形成或原有的血管沟重塑形成更大并肌组织化的小动脉和侧副管^[35]. 血管生成受血管新生诱导因子和抑制因子紧密调控, 称之为“血管新生开关”. 只有当组织微环境中血管新生诱导因子和促进血管新生的黏附分子受体的浓度达到一定水平, 才会逐渐启动血管新生的进程.

MSCs治疗缺血性疾病时, 局部缺血组织中的MSCs可以起到周细胞(pericytes)和血管周围细胞(perivascular cell)类似的功能, 刺激血管内皮细胞增殖和分化形成管状结构, 然后逐渐形成血管网络^[36]. Wang等人^[37]对缺血模型的研究发现, 低氧诱导因子1(hypoxia-inducible factor 1, HIF-1)信号通路的激活可以启动血管新生的进程. MSCs在血管新生过程中主要通过分泌细胞因子来起作用, MSCs产生的VEGF、碱性成纤维细胞生长因子(basic fibroblast growth factor, bFGF)、HGF、单核细胞趋化蛋白1(monocyte chemotactic protein 1, MCP1)、基质细胞衍生因子1(stromal cell-derived factor 1, SDF1)等对于血管网络的重塑发挥关键作用^[38,39]. 此外, MSCs产生的促进血管新生的诱导因子还包括胎盘生长因子(placental growth factor, PIGF)、NO、胰岛素样生长因子(insulin-like growth factor, IGF)等^[40-43].

MSCs旁分泌作用可产生大量的细胞因子、趋化因子和生长因子, 可激活组织内源特异性干/祖细胞的增殖和分化, 还参与免疫调节、促进血管新生、抗凋亡、抗氧化、抗纤维化和趋化作用等多个生物学进程.

2.3 抗凋亡相关因子

MSCs既可以产生抑制细胞凋亡的蛋白, 也可以通过旁分泌作用影响细胞凋亡的信号通路. MSCs产生的抑制细胞凋亡蛋白包括B细胞淋巴瘤因子2(B cell lymphoma 2, Bcl-2)、存活素(survivin)和蛋白激酶B(protein kinase B, PKB也称为Akt)^[44,45]. MSCs产生的抗凋亡相关细胞因子包括VEGF, IL-10, HGF, 斯钙素1(stanniocalcin-1, STC1)和粒细胞-巨噬细胞集落刺激因子(granulocyte-macrophage colony stimulating factor, GM-CSF)等.

Bcl-2蛋白家族分为两类, 抑制细胞凋亡的蛋白(Bcl-2和Bcl-xL)或促进细胞凋亡的蛋白(Bax, Bak和Bad). Bcl-2和Bax蛋白均可在细胞周期检验点的上游发挥作用, 通过激活caspase-3调节细胞凋亡的进程^[46]. 当Bcl-2蛋白表达占优势时, 可抑制caspase活化受体的释放, 此时细胞不易对促细胞凋亡信号进行应答; 相反, 当Bax蛋白表达占优势时, 可促进细胞程序性凋亡^[47]. survivin是目前发现最强的细胞凋亡抑制因子, 大鼠局部缺血模型通过静脉回输MSCs后, 体内的survivin蛋白表达水平明显增加, 从而起到抑制缺血部位细胞凋亡的作用^[45]. Akt信号通路在细胞凋亡和存活中发挥重要作用, Akt1基因编码丝氨酸/苏氨酸蛋白激酶, 可通过磷脂酰肌醇3激酶(phosphoinositide 3-kinase, PI3K)依赖的机制被胞外信号激活, 调控细胞增殖和生长. 对大鼠脑损伤模型的研究发现, 移植MSCs后, Akt蛋白表达量增加^[44].

急性肾损伤模型的研究中发现, VEGF基因修饰的MSCs通过促进微循环, 可显著抑制细胞凋亡并促进正常细胞的增殖^[48]. MSCs产生的肝细胞生长因子可上调抗凋亡基因**bcl-2**的表达, 下调促凋亡基因**bax**和**TNF- α** 的表达^[49]. 肝损伤模型的研究中发现, 抗炎因子IL-10参与抑制细胞凋亡, 促进肝细胞的增殖^[50]. 大鼠脑缺血模型静脉注射MSCs后, 除Bcl-2和Akt蛋白表达量增加外, 伴随着粒细胞-巨噬细胞集落刺激因子表达量的显著增加^[44]. Ohkouchi等人^[51]研究发现, MSCs产生的STC1可减少由活性氧介导的细胞凋亡.

2.4 抗氧化相关因子

当受到生理或病理等内外环境因素刺激时, 机体的氧化和抗氧化系统之间的稳态和平衡被破坏, 从而

导致细胞内活性氧分子(reactive oxygen species, ROS)的大量产生和积累,造成氧化应激(oxidative stress, OS)。氧化应激造成的过多活性氧分子会刺激细胞引发脂质过氧化反应,使细胞的结构以及功能遭到破坏,从而导致多种疾病的发生,如高血压、糖尿病、心血管疾病、组织纤维化和癌症等^[52-54]。在动物实验不同疾病模型的研究中已发现多种抗氧化相关的因子,如超氧化物歧化酶(superoxide dismutase, SOD)、核因子E2 P45相关因子2(nuclear factor-erythroid 2 p45 subunit-related factor 2, Nrf2)、STC-1和HO-1等。

在由四氯化碳诱导的肝损伤模型研究中发现, MSCs移植后可显著增加损伤肝脏组织内SOD的表达水平^[55]。暴露于受损肝脏细胞后, MSCs大量分泌SOD可有效抑制ROS的产生,降低肝脏组织内的氧化应激水平^[55]。MSCs通过分泌STC-1调节线粒体呼吸链可减少由活性氧介导的细胞凋亡,从而促进细胞存活^[51]。在由脂多糖诱导的急性肺损伤模型的研究中, MSCs产生的HO-1在抗氧化方面发挥重要作用,可有效抑制脂质过氧化反应,减少丙二醛的产生^[56]。在辐射诱导的肝损伤模型的研究中, MSCs产生的Nrf2和SOD在减少损伤肝脏细胞产生活性氧分子方面发挥主要作用^[50]。

2.5 抗纤维化相关因子

纤维化是指各种原因造成组织损伤后的愈合反应,表现为组织内细胞外基质过度沉积、组织顺应性和再生能力下降,导致组织功能障碍。组织纤维化包括心肌纤维化、肝纤维化、肾纤维化和肺纤维化等,是许多疾病致残和致死的重要原因。纤维化发生的机制尚不明确,在纤维化的局部微环境,成纤维细胞被激活导致细胞外基质组分特别是I型胶原蛋白的聚集,组织肌肉修复相关一系列基因的转录水平下调,细胞因子和生长因子的表达水平下降,从而抑制了组织的再生能力^[57]。因此,细胞外基质组分的降解对于降低组织纤维化的程度和促进肌肉内源性再生起到关键作用^[58,59]。

研究发现, MSCs的抗纤维化作用与免疫调节,促进血管新生和抗炎等协同发挥作用, MSCs对多种组织纤维化疾病模型都有很好的治疗效果。在肺损伤模型的研究中发现, MSCs移植后可通过分泌STC-1减轻肺纤维化的严重程度^[60]。STC-1通过多种方式抑制组

织纤维化,可减少成纤维细胞胶原蛋白的分泌量,也可通过解偶联内质网上线粒体呼吸链来降低活性氧水平,还可以抑制上皮细胞产生TGF- β 1^[60]。在肾损伤模型的研究中发现, MSCs移植后可减轻TGF- β 1诱导的肌成纤维细胞化和胶原沉积,增加基质金属蛋白酶(matrix metalloprotenases, MMP)的表达水平,发挥其对胶原的降解作用^[61]。MSCs移植后细胞外基质的降解,不是MSCs直接对细胞外基质进行重塑的结果,而是通过旁分泌作用抑制成纤维细胞的增殖,促进局部微环境其他细胞产生基质金属蛋白酶发挥对细胞外基质的降解^[62]。研究还发现, MSCs介导细胞外基质降解的过程可能需要MSCs分泌HGF和HO-1的参与^[63,64]。

2.6 趋化作用相关因子

动物实验研究中发现,无论是静脉回输移植还是局部组织注射, MSCs具有快速迁移到机体炎症发生部位并滞留在炎症部位发挥抗炎和免疫调节的作用^[65-67]。炎症反应包含组织创伤和其他慢性炎症反应,如移植抗宿主病和肿瘤等。自体或外源性MSCs,在机体面临缺血、缺氧或组织损伤等因素的刺激下,能定向趋化性迁移,越过血管内皮细胞至靶向组织并定植存活的过程称为“归巢”。

目前已发现多种趋化因子和生长因子以及多条信号通路参与MSCs的“归巢”进程。基质细胞衍生因子1(stromal cell-derived factor 1, SDF1又称为CXCL12)是研究最多的一种趋化因子配体,通过与MSCs表达的特异性受体CXCR4相互作用,介导MSCs剂量依赖的趋化效应^[68]。单独过量表达CXCR4的变体蛋白,也可以显著增强MSCs的迁移能力^[69]。多种动物模型的研究表明, SDF1/CXCR4轴在募集MSCs迁移到组织损伤部位的过程中发挥主要作用^[70]。此外, MSCs还表达c-Met, CCR1和CD44等多种趋化因子受体,它们相应的配体分别为HGF, MIP-1(alpha)和透明质酸(hyaluronic acid, HA)^[71-73]。通常情况下,趋化因子受体能够感受外部环境趋化因子的浓度差异,并引导细胞到达趋化因子浓度较高的部位。此外,研究发现PI3K、蛋白激酶B、细胞外信号调节激酶(extracellular signal related kinase, ERK)、p38等多条信号通路参与MSCs的定向趋化作用^[70,74]。

3 免疫调节作用

MSCs的免疫调节作用具有很强的可塑性,局部微环境刺激因子的种类和数量不同,静息状态的MSCs既可以被激活变为促炎的MSC1型,也可以被激活转变为抗炎的MSC2型^[75,76].在大量促炎因子(如IFN- γ , TNF- α 等)刺激下,或TLR3激活后,静息状态的MSCs会被激活转变为抗炎MSC2型,产生大量的NO/IDO等免疫调节因子以及趋化因子,通过抑制效应性T细胞的功能,诱导调节性T细胞(Treg)产生等机制发挥免疫抑制作用.而在低水平的促炎因子(如少量IFN- γ , TNF- α 等),或TLR4激活后,或高水平的抗炎因子(如TGF- β , IL-10等)刺激下,静息状态的MSCs会转变为促炎MSC1型.促炎MSC1仅产生少量免疫调节因子,通过释放趋化因子募集淋巴细胞到达炎症发生部位,可促进效应性T细胞的增殖、激活和分化,抑制调节性T细胞的产生(图3A)^[77].

MSCs发挥免疫调节作用,主要通过两种方式:一种是旁分泌细胞因子,包括IDO(人)或NO(小鼠),PGE2, IL-6, IL-10和TGF- β 等;另外一种为细胞与细胞间直接接触依赖的机制. MSCs无论对固有免疫细胞包括自然杀伤细胞、中性粒细胞、巨噬细胞、肥大细胞和树突状细胞等,还是对获得性免疫细胞包括T细胞和B细胞等,都有较强的免疫调节作用.

3.1 固有免疫细胞

固有免疫(innate immunity)是机体在种系发育和进化过程中形成的天然免疫防御功能,具有反应快速、非特异识别、无细胞增殖、无免疫记忆和维持时间短等特点,也称为非特异性免疫,是机体抵御病原体入侵的第一道屏障^[78]. MSCs通过旁分泌作用参与调控的固有免疫细胞类型包括自然杀伤细胞(natural killer, NK)、中性粒细胞、巨噬细胞和树突状细胞等(图3B).

(1) NK细胞. NK细胞是固有免疫系统具有重要功能的效应细胞,其表面表达多种激活受体和抑制受体,这两类受体的平衡调控NK细胞的杀伤活性^[79]. NK细胞通过产生细胞因子和直接对靶细胞的杀伤作用,在抗病毒反应、抗肿瘤和免疫监视等生物学进程中发挥重要作用^[80].然而, MSCs表达一系列可以激活NK细胞的配体,如ULBP-3(UL16 binding protein 3),

nectin-2, CD54和CD155等,表达此类配体的MSCs易被活化的NK细胞识别并杀伤^[78].

大量研究表明, MSCs可抑制NK细胞的活性,包括抑制NK细胞的增殖,抑制NK细胞因子的分泌,抑制NK细胞对靶细胞的杀伤能力. MSCs抑制NK细胞的活性是剂量依赖的,只有维持较高的MSC/NK细胞比例,才表现出显著的抑制效果^[81]. MSCs主要通过两种方式发挥对NK细胞的抑制作用:旁分泌作用产生大量生物活性物质,如IDO, PGE2, TGF- β 1, activin-A和HLA-G5等;细胞直接接触依赖的机制在MSCs发挥对NK细胞活性的抑制方面也起到重要的作用^[82].

旁分泌作用产生的免疫抑制因子可下调NK细胞表面激活受体NKG2D, NKp30和NKp44的表达量以及颗粒酶B的释放水平^[81-84]. MSCs通过旁分泌作用抑制NK细胞活性的进程中, IDO和PGE2发挥主要作用,而且通过协同作用增强了对NK细胞的抑制效果.阻断两者任何一种因子的产生, MSCs对NK细胞活性的抑制作用都会明显减弱^[83]. Selmani等人^[84]研究发现, MSCs分泌可溶性的HLA-G5除可抑制NK细胞的活性外,还可抑制活化的NK细胞对MSCs的细胞裂解作用. Transwell实验表明, MSCs除通过旁分泌作用抑制NK细胞的活性外,也可通过细胞与细胞间直接接触依赖的机制发挥作用^[81]. Lu等人^[85]研究发现,表达TLR4的MSCs可通过直接接触的方式抑制NK细胞表面NKG2D和CD69等激活受体的表达,从而起到抑制NK细胞杀伤活性的作用^[86].

(2) 中性粒细胞. 中性粒细胞是急性炎症反应过程中发挥重要作用的细胞之一,组织损伤后数分钟内就可以从血液中被募集到损伤部位^[87]. 中性粒细胞清除病原体的作用机制包括吞噬作用、脱颗粒作用释放超氧化物歧化酶和溶酶体酶以及中性粒细胞的细胞外基质形成诱捕网的方式^[87]. 大部分研究发现, MSCs对中性粒细胞的调节起有利作用. 即使MSCs/中性粒细胞的比例处于很低的水平, MSCs仍可通过分泌IL-6显著抑制中性粒细胞的凋亡^[88]. MSCs还可以通过分泌IL-8和巨噬细胞迁移抑制因子(macrophage migration inhibitory factor, MIF)募集中性粒细胞迁移到炎症部位,增强中性粒细胞清除病原体的能力^[89]. 然而在组织损伤模型中, MSCs产生的胞外囊泡能够抑制中性粒细胞进入组织损伤部位^[90], MSCs还可以抑制损伤部位的中性粒细胞活化,通过细胞间黏附分子-1(Inter-

图 3 MSCs的免疫调节作用。A: 间充质干细胞免疫调节的可塑性。静息状态的MSCs既可以被激活变为促炎的MSC1型,也可以被激活转变为抗炎的MSC2型。在低水平的促炎因子(如少量IFN- γ , TNF- α 等)刺激下,静息状态的MSCs会转变为促炎MSC1型,仅产生少量免疫调节因子,可促进效应性T细胞的增殖、激活和分化,抑制Treg细胞的产生。在高水平的促炎因子刺激下,静息状态的MSCs会被激活转变为抗炎MSC2型,产生大量的免疫调节因子,通过抑制效应性T细胞的功能,诱导Treg细胞产生等机制发挥免疫抑制作用; B: 间充质干细胞对免疫细胞的调节作用。MSCs可靶向多种免疫细胞,既可以对NK细胞、中性粒细胞、巨噬细胞和DC细胞等固有免疫细胞进行免疫调节,也可以对T细胞和B细胞等获得性免疫细胞进行免疫调节

Figure 3 The immunomodulatory capacity of MSCs. A: Plasticity of mesenchymal stem cells in immunomodulation. The resting MSCs can either convert into pro-inflammatory MSC1 phenotype or convert into anti-inflammatory MSC2 phenotype, depending on different inflammation status. Under weak inflammation conditions (such as low levels of IFN- γ and TNF- α), the resting MSCs convert into immune enhancing MSC1 phenotype. MSC1 secrete low levels of immune suppressive factors, which in favor of the proliferation, activation and differentiation of effective T cells, however suppress the development of Treg cells. Under vigorous inflammation conditions, the resting MSCs convert into anti-inflammatory MSC2 phenotype. MSC2 produce large amounts of immune suppressive factors, to inhibit immune responses through direct suppression of effective T cells, or induce production of Treg cells. B: Broad immunomodulatory capacity of MSCs. MSCs can regulate both the innate immune cells (such as NK cells, neutrophils, macrophages, and DCs) and the adaptive immune cells (such as T cells and B cells)

cellular adhesion molecule 1, ICAM-1)介导的胞吞作用内吞中性粒细胞,避免中性粒细胞无限制地激活,对组织损伤部位产生过度伤害^[91]。

(3) 巨噬细胞。巨噬细胞是固有免疫系统中一种重要的细胞类型,生物学特性具有很强的可塑性。根据

其表型和分泌细胞因子的差异分为两种不同的功能亚群,经典活化(classically activated)的巨噬细胞M1型和旁路活化(alternatively activated)的巨噬细胞M2型。在不同的炎症介质的刺激下,单核细胞既可以被Th1细胞产生的细胞因子刺激,发育成促进炎症应答中起作

用的M1型巨噬细胞;也可以在Th2细胞产生的细胞因子刺激下,发育为具有抗炎作用的M2型巨噬细胞^[92].通常情况下,M1型巨噬细胞可产生大量的促炎因子和趋化因子,表现出显著的抗微生物感染能力;M2型巨噬细胞具有抗炎和免疫调节作用,通过产生大量IL-10和营养因子促进组织损伤修复^[93].

体外细胞实验研究表明,将巨噬细胞与MSCs共培养,将会诱导M2型巨噬细胞的产生并分泌高水平的IL-10,仅表达少量TNF- α 等促炎因子,M2型巨噬细胞低表达CD86和MHCII类共刺激分子^[94,95].体内动物实验研究表明,MSCs能够募集巨噬细胞到组织损伤部位,促进损伤组织的修复和免疫平衡的维持^[96].MSCs对巨噬细胞的免疫调节是通过旁分泌和细胞接触依赖两种方式共同发挥作用, MSCs旁分泌作用产生的PGE₂,可与巨噬细胞表面的EP2和EP4受体相结合,以及通过细胞接触依赖的机制激活巨噬细胞内的信号通路,改变下游基因的表达情况,从而使巨噬细胞向M2型发生转变^[97].

(4) 树突状细胞. DCs作为机体内功能最强大的抗原递呈细胞(antigen presenting cell, APC)参与体内多种生理和病理进程,在肿瘤免疫、感染免疫、移植免疫和自身免疫性疾病的发生和发展进程中发挥重要作用^[98]. MSCs不仅抑制成熟树突状细胞的抗原递呈能力,也可以抑制未成熟树突状细胞(imature dendritic cells, imDCs)的分化和成熟,还可以抑制DCs前体细胞CD34⁺造血祖细胞的分化进程.

体内实验研究表明, MSCs抑制分化成熟的DCs细胞表达HLA-DR分子、CD1 α 以及共刺激分子CD80和CD86,抑制其产生IL-12,从而将分化成熟的DCs转变为imDCs^[99]. MSCs通过旁分泌和细胞间接触依赖机制可激活Jagged1, Jagged2或IL-10-SOCS3等信号通路,也可以将分化成熟的DCs转变为调节性树突状细胞(regulatory dendritic cells)^[100~102]. MSCs对成熟DCs细胞发挥免疫抑制作用,导致DCs细胞与迁移相关的蛋白CCR7和CD49d β 1的表达水平下降,其迁移能力下降.此外,被抑制的DCs细胞抗原递呈能力和分泌炎症因子的能力下降,导致激活T细胞的效率降低^[103,104].研究发现, MSCs旁分泌作用产生的PGE₂与DCs细胞表面的EP4受体相结合,激活PI3K信号通路,在MSCs发挥对成熟DCs细胞活性抑制过程中起到主要作用^[105].

MSCs对imDCs分化和成熟的抑制作用,很大程度上是通过MSCs与CD34⁺造血祖细胞直接接触依赖的方式实现的^[106]. MSCs与树突状细胞的前体细胞即CD34⁺造血祖细胞直接接触后,激活造血祖细胞内Notch信号通路,导致下游*Hes1*基因的过量表达,CD34⁺造血祖细胞只能分化为未成熟的树突状细胞(图4D)^[106].此外, MSCs可以显著抑制CD14⁺单核细胞向树突状细胞方向的分化^[99].

3.2 获得性免疫细胞

(1) T细胞. 基于T细胞介导的免疫应答有三个主要阶段:第一阶段是抗原的识别和抗原递呈;第二阶段是T细胞激活、增殖和分化;第三是效应阶段. MSCs对T细胞发挥免疫抑制作用,不仅能通过与抗原递呈细胞相互作用参与第一阶段的调控,而且可通过抑制T细胞的增殖,影响T细胞的激活和改变T细胞亚群分化的进程,参与到T细胞免疫应答第二阶段的调控.

初始T细胞(naive Tcell)的活化需要T细胞受体信号和共刺激信号两条信号通路同时激活.根据微环境细胞因子的种类和刺激的强度不同, CD4⁺T细胞被激活后可分化为辅助性T细胞1(Th1), Th2, Th9, Th17和Treg亚群^[107~109]; CD8⁺T被激活后可分化为细胞毒性T细胞,分泌颗粒酶、穿孔素和多种细胞因子对感染细胞进行杀伤^[110].效应T细胞是初始T细胞接受抗原刺激后,经过增殖和分化形成的细胞,具有释放淋巴细胞因子的功能. MSCs旁分泌产生的TGF- β 和HGF等细胞因子可抑制效应T细胞的增殖,导致效应T细胞内的细胞周期蛋白D2(cyclin D2)表达的下调和p27^{kip1}表达的上调,最终导致细胞周期被阻滞在G1期^[111,112].

MSCs还可以通过产生IDO抑制T细胞的增殖,诱导活化T细胞的凋亡. MSCs在 α -TNF和 γ -IFN等促炎因子的刺激下,细胞膜表面会产生大量的IDO^[113].IDO是一种含铁的血红素单体膜结合蛋白,是肝脏外唯一可以催化色氨酸代谢的限速酶.色氨酸是细胞维持活化和增殖所必需的氨基酸,同时也是构成蛋白质不可缺少的重要成分,色氨酸的缺乏可使细胞功能受限.IDO可催化色氨酸转变为L-犬尿酸和吡啶甲酸.T细胞对色氨酸的浓度特别敏感,微环境中色氨酸处于较低浓度时,T细胞增殖会被阻滞在G1期,不能正常增殖,造成T细胞的缺乏,而且T细胞的增殖一旦被抑制很难被再次激活.色氨酸的代谢产物(L-犬尿酸和吡啶甲酸)不仅

图 4 细胞与细胞间接接触依赖的机制。A: MSCs可通过细胞接触依赖的机制, 激活FAS-FASL信号通路进而诱导炎症性T细胞的凋亡; B: MSCs表面的CCL20与Th17表面的CCR6相互作用, 募集Th17到MSCs周围。通过细胞间接接触依赖的机制, MSCs可诱导分化成熟的Th17细胞转变为调节性T细胞; C: MSCs与B细胞通过PD-1/PD-L1相互作用, 激活B细胞程序性死亡受体信号通路, 可抑制B细胞的增殖。此外, 还可通过细胞接触依赖的机制, 将B细胞诱导成调节性B细胞; D: MSCs与树突状细胞的前体细胞即CD34⁺造血祖细胞直接接触后, 通过激活细胞内的信号通路, 导致CD34⁺造血祖细胞只能分化为未成熟的树突状细胞

Figure 4 Mechanisms of direct cell-cell contact. A: Through direct cell-cell contact, MSCs can induce transient apoptosis of inflammatory T cells via the FAS ligand (FASL)-dependent FAS pathway. B: MSCs can recruit Th17 cells via the interaction between CCL20 and CCR6, and induce a T cell regulatory phenotype upon cell-cell contact. C: Through direct interaction between PD-1 and PD-L1, MSCs can inhibit B cell proliferation and induce the conversion of B cells to regulatory B cells that secrete IL-10. D: MSCs can license CD34⁺ hemopoietic progenitor cells to differentiate into immature DCs through direct cell-cell interaction and afterwards the activation of intracellular signaling pathway

可直接抑制T细胞增殖, 还可诱导其凋亡^[114]。IDO介导的色氨酸耗竭机制仅对活化的T细胞产生抑制作用, 对静息状态的T细胞无明显的影响。

MSCs旁分泌的细胞因子除可抑制T细胞的增殖并诱导其凋亡外, 还能抑制T细胞的激活并改变T细胞亚群的分化进程。MSCs抑制辅助性T细胞产生IFN- γ 和IL-17等细胞因子, 但促进辅助性T细胞大量分泌IL-10, 当局部微环境IL-10浓度达到一定程度时会分泌可溶性的HLA-G5, 从而抑制初始T细胞向Th1和Th17方向

分化, 并诱导调节性T细胞的产生^[84,115]。Zhou等人^[116]的研究发现, 脂肪来源的MSCs发挥对T细胞的免疫抑制作用, 一定程度上是通过抑制T细胞NF- κ B(nuclear factor-kappaB, NF- κ B)的磷酸化来实现的。

MSCs与T细胞的直接接触在对T细胞的免疫调节中也发挥重要作用。Akiyama等人^[117]的研究发现, MSCs可以通过细胞接触依赖的机制, 激活FAS-FASL信号通路进而诱导炎症性T细胞(inflammatory T cells)的凋亡(图4A)。在诱导炎症性T细胞凋亡的进程中, MSCs通过分泌单核细胞趋化蛋白-1(monocyte chemotactic protein-1, MCP-1)募集其他炎症性T细胞到MSCs的周围。坏死T细胞的凋亡小体会刺激巨噬细胞分泌大量的TGF- β , 诱导初始T细胞向Treg的方向分化, 进一步增强机体的免疫耐受和免疫抑制能力^[117]。Ghannam等人^[118]研究发现, MSCs细胞表面表达CCL20和CD54分别与Th17细胞表面的CCR6和CD11a/CD18相互作用, 可募集Th17到MSCs周围。MSCs通过细胞接触依赖的机制, 上调环氧合酶-2(cyclooxygenase-2, COX-2)的表达, 直接对Th17细胞产生抑制作用, 可抑制分化成熟的Th17细胞产生IL-17和IL-22等炎症因子, 此过程需要MSCs分泌的PGE2与T细胞上的受体EP4结合^[119]。通过细胞间直接接触, MSCs诱导分化成熟的Th17细胞表观遗传学修饰发生改变, 导致基因表达谱系发生改变, 最终Th17转变为Treg(图4B)^[118]。MSCs移植后, 对机体Th17/Treg细胞平衡的调节是MSCs移植发挥免疫调节的一个重要作用位点^[120]。

(2) B细胞。B细胞是获得性免疫系统中又一重要的效应细胞, 是体内唯一能产生免疫球蛋白的细胞, 也是一类重要的专职抗原递呈细胞。B细胞的细胞膜上表达表面受体和表面抗原, 它们参与抗原识别、免疫细胞间以及免疫细胞与免疫分子间的相互作用。初始B细胞(naive B)在抗原刺激及Th细胞的辅助下增殖和分化成熟, 最终被激活为能产生抗体的浆细胞和记忆细胞, 再次免疫应答时能迅速对病原体做出应答^[121,122]。根据是否表达CD5, 可将B细胞分为B1(CD5⁺)细胞和B2(CD5⁻)细胞两个亚群, B2细胞即通常参与体液免疫应答的B细胞。近年来发现一类调节性B细胞亚群(regulatory B cells, Bregs), 不同种属的表型分别为CD19⁺CD24^{high}CD38^{high}(人)^[123]和CD1d^{high}CD5⁺CD19⁺(小鼠)^[124], 它们能够产生IL-10并发挥免疫调节功能。

MSCs能够通过旁分泌作用抑制B细胞的增殖、分化和激活等细胞进程. 体外实验结果表明, MSCs与B细胞共培养, 可抑制B细胞内丝氨酸/苏氨酸激酶和p38丝裂原活化的蛋白激酶的磷酸化水平^[125], 然而可促进ERK1/2(extracellular response kinase1/2, ERK1/2)的磷酸化修饰水平^[126], 并将B细胞正常的细胞周期阻断在G0/G1期, 通过下调B淋巴细胞诱导成熟蛋白-1(B lymphocyte-induced maturation protein-1, Blimp-1)的表达水平抑制B细胞终末分化, 抑制B细胞分化为浆细胞, 抑制浆细胞抗体的分泌, 但不诱导B细胞的凋亡^[125,127]. 研究发现, MSCs发挥对B细胞的抑制作用, 包括抑制B细胞的增殖、抑制浆细胞的分化成熟和抑制抗体分泌等都依赖MSCs与T细胞的直接接触^[128].

除旁分泌作用之外, MSCs还可通过细胞接触依赖的机制发挥对B细胞的免疫调节作用. Schena等人^[129]的研究发现, 高浓度 γ -干扰素激活后, MSCs可通过细胞与细胞间的直接接触, 激活程序性死亡受体(programmed death, PD)-1/PD-L1信号通路抑制B细胞的增殖^[129]. Franquesa等人^[123]研究发现, MSCs可通过细胞接触依赖的机制, 将B细胞诱导成免疫表型为CD19⁺CD24^{high}CD38^{high}的调节性B细胞, 可产生大量的IL-10进一步抑制其他细胞的免疫应答(图4C).

4 细胞间物质传输

胞外囊泡(extracellular vesicles, EVs)是一类重要的细胞间传输介质, 在细胞与细胞之间物质传输和信号交流方面发挥至关重要的作用. MSCs产生的胞外囊泡其内包裹的生物活性物质具有免疫调节作用, 还可对多种组织损伤进行修复^[130]. 此外, MSCs与靶细胞之间还可通过形成隧道纳米管(tunneling nanotubes, TNTs)、主动转运线粒体和内质网等细胞器以及其他胞质成分. 间充质干细胞通过胞外囊泡和隧道纳米管进行细胞与细胞间的物质传输通常认为是旁分泌机制的不同作用方式.

4.1 胞外囊泡

MSCs能够产生和释放多种类型的囊泡, 统称为胞外囊泡, 根据其大小、来源途径和组成成分的不同, 可分为外泌体(exosomes)、微泡(microvesicles)和凋亡小

体(apoptotic bodies)三种类型^[131]. 胞外囊泡是大多数细胞在自然条件下都可以产生的膜性囊泡, 在细胞间的信息通讯和物质传导中发挥着重要的作用. 外泌体来自于晚期的内涵体, 直径介于30~130 nm, 膜性囊泡内包裹着信使RNA(messenger RNA, mRNA)、微小RNA(microRNA, miRNA)、脂质体和蛋白质等大量生物活性物质(图5A)^[130]. 微泡也称为脱落的囊泡, 直径介于100~1000 nm, 从细胞质膜上脱落释放产生, 其产生过程与胞质内钙离子内流和细胞膜骨架的重构有关, 其内富含磷脂酰丝氨酸、整合素、选择素和CD40配体等物质(图5A)^[130,132]. 凋亡小体是凋亡细胞的细胞膜以出芽(ectocytosis)方式形成, 直径介于50~4000 nm, 其大小和形态具有高度的异质性, 富含膜相关的组蛋白和多种细胞器^[130,133].

研究表明, 外泌体和微泡是MSCs产生的最重要的胞外囊泡, 除大小和组成成分不同外, 它们的来源途径也存在差异. 外泌体是由细胞内的多泡体(multivesicular bodies, MVBs)膜与细胞膜融合后, 通过胞吐作用的方式释放到细胞外环境中形成. 微泡是细胞受到生物信号(白介素、肿瘤坏死因子、内毒素、凋亡蛋白因子)或理化刺激(低氧环境、氧化应激或胞质内钙离子浓度增加)时, 细胞骨架发生重塑, 以出芽的方式从细胞质膜上脱落产生^[134]. 外泌体和微泡的外膜脂质双分子层都来源于细胞质膜, 膜上含有MSCs来源的大量膜蛋白、抗原、多种酶类和受体等信号分子^[135]. 外泌体和微泡作用于靶细胞的方式也基本相同, 主要通过三种方式: (i) 识别靶细胞表面的特异性受体, 通过受体-配体相互作用激活靶细胞内的信号传导^[136]; (ii) 可直接与受体细胞的质膜相互融合, 将细胞膜表面受体分子和脂质体传递给受体细胞^[137]; (iii) 通过内吞作用被靶细胞摄取, 将其内包裹的生物活性物质传输给靶细胞^[133]. 外泌体和微泡通过将囊泡内包裹的蛋白质、RNA和脂质体等生物活性物质传递给受体细胞, 进而调控受体细胞的各种生理和病理进程^[138,139].

近10多年以来, 胞外囊泡特别是外泌体受到了广泛的关注和研究. 研究发现, 哺乳动物体内绝大多数的细胞类型均可产生外泌体, 如淋巴细胞、肥大细胞、树突状细胞、内皮细胞、神经细胞和肿瘤细胞等, 其中MSCs被认为是产生外泌体能力最强的细胞^[140]. 外泌体发挥生物学功能与其内含物的种类和数量密不可分, 细胞所处微环境不同以及刺激方式不同,

图 5 MSCs介导的细胞间物质传输。A: 蛋白质和RNAs等物质通过外泌体和微泡等胞外囊泡从MSCs向靶细胞传输; B: MSCs通过隧道纳米管向损伤细胞传输线粒体等细胞器, 以及钙离子、镁离子、RNAs、蛋白质和多肽等小分子物质

Figure 5 MSC-mediated transfer of cellular components. A: MSCs can mediate the transfer of proteins and RNAs to target cells by extracellular vesicles such as exosomes or microvesicles. B: MSC-mediated transfer of organelles (such as mitochondria), ions (such as calcium and magnesium), and small molecules (such as RNAs, proteins, and peptides) to damaged cells through tunneling nanotubes

都会导致MSCs产生不同的外泌体, 而不同的外泌体具有不同的活化状态和信号特征^[141]。根据外泌体数据库ExoCarta最新的数据显示, 截至2018年10月, 不同组织细胞所产生的外泌体共含有1116种脂质体、3408种mRNA、2838种miRNA以及41860种蛋白质。外泌体中含有的mRNAs可转录成为cDNA, 并在受体细胞内翻译成为具有生物活性的蛋白质^[142]。

Toh等人^[143]的研究表明, 外泌体主要通过传输大量的蛋白质对靶细胞发挥调节作用。除蛋白质外, 外泌体内包裹的RNA也具有一定的调控作用, 如微小RNA(miRNA)是一类长度约为20~24个核苷酸的非编码RNA, 可调控众多编码蛋白的基因表达, 主要通过靶基因3'UTR区域完全或不完美配对, 降解靶基因的mRNA或抑制其翻译等方式参与调控所在细胞的凋亡、增殖和分化等各种细胞进程^[144]。MSCs来源的外泌体在多种疾病治疗的临床试验中均显示出与MSCs移植类似的治疗作用, 其疗效具有剂量依赖性^[145,146]。外泌体代替MSCs进行疾病治疗, 可避免移植的MSCs不能通过肺部毛细血管而被截留的问题, 更加安全有效^[133]。外泌体能越过血脑屏障, 在治疗脑部疾病方面具有无可比拟的优势^[133]。

4.2 线粒体转运

线粒体是真核细胞中非常重要的细胞器, 作为细胞内的“能量动力工厂”, 通过氧化磷酸化产生ATP供应细胞生存所需要的能量, 调节氧自由基的产生、细胞色素C释放介导的细胞凋亡以及在钙离子稳态维持等多种生物学功能中发挥关键作用^[147]。线粒体内含有环状的DNA(mitochondrial DNA, mtDNA), 具有独立的转录和翻译系统, 是一种高度动态平衡的半自主性细胞器^[147]。线粒体功能异常会引起活性氧产生增多以及细胞凋亡途径的激活, 导致许多疾病的发生和发展。

隧道纳米管是近10多年来新发现的一种通讯连接方式, MSCs和靶细胞间可形成隧道纳米管, 线粒体以及mtDNA可通过隧道纳米管从MSCs定向转运至靶细胞^[148]。隧道纳米管是一种介导细胞讯息传递的线状膜性管道, 在哺乳动物细胞中普遍存在, 由细胞膜和纤维形肌动蛋白F-actin为主的细胞骨架成分组成^[149]。隧道纳米管的长度和直径有着较大的波动范围, 长度介于几个 μm 到100 μm 之间, 能通过细胞外空间直接连接远距离的细胞, 直径变化从十几个nm到1000 nm以上, 因此有研究报道将其分为较细(直径<0.7 μm)和较粗(直径 $\geq$ 0.7 μm)两种类型^[149]。Onfelt等人^[149]在较粗隧道纳米管的组成成分中发现了微管蛋白(tubulin), 同时还观察到线粒体的双向转运现象。细胞间的隧道纳米管连接是瞬时的, 一直处于形成和断裂的持续变化之中, 其形成和消失基本只相隔几分钟^[150]。隧道纳米管不仅能够直接交换 Ca^{2+} , Mg^{2+} , RNAs, 多肽/蛋白质等胞质小分子物质, 还能够远距离运输线粒体、内质网、高尔基体和溶酶体等细胞器成分(图5B)^[149,151,152]。隧道纳米管的形成可能反映了细胞在应激或损伤状态下表现出的一种自我防御和营救机制。

线粒体转移仅发生在靶细胞无功能性线粒体时, 或细胞处于应激、损伤等状况。线粒体转移涉及多种方式, 除主要通过隧道纳米管转运外, 还可通过间隙连接、胞外囊泡以及细胞融合后选择性丢失供体细胞核等方式进行输送^[153]。此外, 有研究报道, 细胞外基质中游离线粒体也可以被受体细胞通过肌动蛋白依赖的内化作用(internalization)直接吸收^[154]。多条信号传导途径参与激发隧道纳米管的形成, 并促使线粒体从供体细胞转移到受体细胞, 这些信号通路包括Rho GTPase^[155], Fas配体^[156], P53/AKT/PI3K^[152], M-se/

Ral/exocyst等通路^[157]。多种应激信号,如受损的线粒体、线粒体DNA和线粒体产物的释放以及活性氧分子水平的升高,以及受损细胞微环境都能激发供体细胞隧道纳米管的突起和形成。钙敏感性衔接蛋白Miro1对线粒体在隧道纳米管内的转移具有加速与引导作用^[158]。MSCs通过隧道纳米管的成功转移线粒体的同时伴随着受体细胞内损伤线粒体的清除,损伤的线粒体依赖溶酶体通过自噬作用而降解^[159]。MSCs可与多种组织细胞之间形成隧道纳米管的通讯连接,在相互连接的细胞间形成了复杂的网络结构,推动深层次的细胞交流。形成隧道纳米管进行线粒体等细胞器的传输是干细胞进行组织损伤修复的一大重要机制^[160]。

5 表观遗传学调控

表观遗传学调控是指在不改变DNA序列的情况下,由染色质变化导致基因功能发生可遗传的改变,主要包括4个方面:DNA甲基化、组蛋白修饰、染色质重塑和非编码RNA的调控^[161]。DNA甲基化主要是在染色质水平通过影响转录因子与DNA的接触对基因转录活性进行调节,通常基因中甲基化程度高的区域是失活的,而未被甲基化的区域是有活性的^[162]。DNA甲基化和去甲基化修饰在基因组印记、X染色体失活、转座子转录和特定类型细胞的基因表达等方面发挥重要作用^[163]。真核生物DNA分子缠绕在组蛋白上形成核小体,组蛋白的修饰可影响DNA和组蛋白的亲合性,从而改变染色质的疏松和凝集状态,间接影响基因的表达情况^[164]。组蛋白的修饰位点较多,组蛋白翻译后修饰的类型有十多种,主要包括乙酰化与去乙酰化、磷酸化与去磷酸化、甲基化与去甲基化,此外还有泛素化、腺苷酸化、ADP核糖基化、SUMO化修饰(sumoylation)、生物素化、去精氨酸化(deimination)和脯氨酸同分异构化等,不同类型的组蛋白修饰组合构成了“组蛋白密码”^[164,165]。染色质重塑主要是指染色质重塑因子复合物利用ATP水解产生的能量,驱动核小体的装配、拆解或重新排列等方式调控染色质结构,从而调节转录相关因子与DNA的接近程度^[166]。大量证据表明,非编码RNA在调控基因表达过程中发挥重要作用,可调控数以千计基因的表达。非编码RNA根据大小和功能主要分为3个类型:siRNAs, miRNAs

和LncRNAs^[167,168]。

哺乳动物体内所有细胞均来自于最初的受精卵,个体每个细胞内都含有相同的遗传信息,但由于表观遗传学调控导致基因表达模式的不同,同一受精卵分裂产生的细胞朝不同方向分化,成为形态各异和功能不同的细胞,最终构成各种组织和器官^[169]。表观遗传学调控在MSCs干性的维持、细胞分化、细胞衰老、细胞转分化以及细胞稳态的维持等方面发挥关键作用^[170]。MSCs的多谱系分化能力,正是由于MSCs受表观遗传学调控后细胞内特定基因差异性表达的结果^[171]。表观遗传学调控发生在MSCs特定基因的转录调节区,主要通过对DNA的修饰(DNA的甲基化)和染色质结构的改变(组蛋白修饰和染色质重塑)来实现^[171]。非编码RNA的调控也是表观遗传学调控的重要方式之一,可影响MSCs的增殖、分化和衰老等细胞进程^[172,173]。

MSCs表观遗传学调控相关的研究大部分集中在MSCs的细胞分化和转分化领域^[174]。MSCs细胞内DNA和组蛋白上发生的可逆化学修饰,能改变MSCs的分化潜能,并决定细胞最终的分化命运^[175]。Saidi等人^[176]研究发现, MSCs通过对DNA成脂相关基因的去甲基化,以及组蛋白上H3K4和H3K27两个位点的甲基化修饰,导致细胞大量表达成脂分化相关基因的转录因子,而抑制其他基因的转录活性,开启MSCs向成脂细胞分化的进程。Jing等人^[177]研究发现, Wnt信号通路相关基因启动子区域组蛋白H3K9位点的乙酰化,可促进骨髓来源的MSCs向成骨细胞的分化。Dong等人^[178]研究发现,组蛋白的乙酰化修饰可促进骨髓来源的MSCs向肝细胞的分化。Wu等人^[179]研究发现,染色质重塑也参与MSCs细胞谱系分化的调节,染色质的动态改变与MSCs向成骨细胞分化相关基因的转录活性密切相关。非编码RNA也参与到MSCs分化进程的调控中, Xiao等人^[180]研究发现, ADINR(adipogenic differentiation induced noncoding RNA)可特异性地上调成脂细胞分化关键的转录调节因子C/EBP α 的表达水平,从而促进MSCs向脂肪细胞的分化。

表观遗传学调控涉及MSCs自身特性和功能发生改变的领域还有待进一步的研究,目前仅有少量文献报道。Wang等人^[181]研究发现, p300是一种组蛋白乙酰基转移酶,可上调骨髓来源MSCs中NANOG和SOX2等全能性基因的表达,对于MSCs干性(stemness)的维持

起到重要作用. Shen等人^[182]研究发现, 组蛋白去甲基化酶JMJD6(histone demethylase Jumonji C domain-containing protein 6, JMJD6)通过对*PDE1C*基因的增强子区域的组蛋白H4R3me2a位点的脱甲基化, 导致PDE1C蛋白表达水平增加, 可增强脂肪来源MSCs的增殖能力和细胞迁移能力. 非编码RNA的调控不仅可影响MSCs的多向分化潜能, 而且也能调节MSCs的衰老和衰老进程. Ghasemzadeh等人^[183]研究发现, miR302a可以上调Parp1蛋白的表达水平, miR34a能显著抑制Parp1蛋白的表达, 因为Parp1蛋白是干细胞全能性重要决定因子之一, 所以非编码RNA可以间接调控MSCs的全能性. Vono等人^[173]研究发现, 衰老血管内皮细胞产生的miR-31通过胞外囊泡被MSCs摄取后, 可诱导MSCs出现衰老细胞的表型. Kim等人^[184]研究发现, 表观遗传学修饰药物处理后可显著提高MSCs分泌IL-10和IDO的水平, 增强其免疫抑制能力, 对类风湿性关节炎患者Th17细胞的功能起到很好的抑制作用.

6 展望

深入研究MSCs的作用机制, 可充分利用和发挥MSCs生物学特性方面的优势, 为疾病治疗提供新的策略. 例如, MSCs来源的外泌体除本身可以用于疾病治疗外, 还可作为天然的纳米载体用于药物传递系统, 将其运载的药物传递至靶细胞^[185]. 干细胞产生隧道纳

米管传输线粒体为疾病治疗提供了新的策略, 可直接分离线粒体用于挽救受损细胞. 了解MSCs自我更新以及细胞分化进程中的表观遗传学调控机制, 可精确操纵MSCs的定向分化, 增强MSCs的治疗效果, 无论对于MSCs在组织工程技术领域的应用, 还是MSCs作为再生医学药物的临床试验, 都具有重要的指导意义.

大量临床实验结果表明, 因疾病不同, 所需的最佳剂量、细胞用药的途径以及使用细胞的频率等都有所差异. 普遍认为, 制约MSCs长期疗效的限制因素主要是移植后MSCs在体内滞留时间和存活时间较短^[186]. 研究者通过探索不同的方法致力于增强MSCs内在的生物学活性以提高治疗效果, 通常从以下4个方面改善疗效: (i) 移植足够的细胞剂量, 通常静脉回输最佳剂量为 $1.0 \times 10^6 \sim 2.0 \times 10^6$ 个MSCs/kg体重^[187]. (ii) 增强MSCs归巢到组织损伤部位的能力. (iii) 抑制移植后细胞凋亡进程, 延长MSCs的存活时间^[188]. (iv) 增强MSCs的旁分泌作用, 如低氧或 γ -IFN预处理MSCs可提高MSCs产生生物活性物质的能力^[189,190]. 通常基因修饰MSCs可显著增强操作基因的功能, 如过量表达*CXCR4*基因, 可增强修饰MSCs的迁移能力^[69]; 过量表达*Bcl-2*基因, 可增强修饰MSCs的抗凋亡能力, 修饰细胞能够产生更多的血管内皮细胞生长因子, 显著提高损伤心肌细胞的存活能力^[191]; 过量表达*Miro1*基因, 可增强修饰MSCs的线粒体转移能力^[158]. 然而, 过量表达特定基因可能会带来未知的风险, 临床试验前需充分考虑其安全性.

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Progress in research on functional mechanisms of mesenchymal stem cells

HUANG QingLei¹, SHEN Li^{1,2} & DENG Yue¹

1 Beijing Cellonis Biotechnologies Co., Ltd, Beijing 100191, China;

2 Department of Cell Biology, Peking University Health Science Center, Beijing 100191, China

Mesenchymal stem cells (MSCs) are a type of adult stem cells that are characterized by self-renewal and multipotent differentiation. MSCs can produce numerous bioactive molecules, depending on the stimulation of local environment. They can also facilitate hematopoiesis, produce trophic factors, activate endogenous stem or progenitor cells, promote tissue repair, and regulate the immune system. In addition, they can enhance angiogenesis, antiapoptosis, antioxidation, and antifibrosis, and migrate to injury sites. Advanced clinical trials show outstanding therapeutic effects of MSCs, especially in autoimmune-related diseases, injured tissues, and aging-related degenerative diseases. However, the in-depth functional mechanisms of MSCs remain unclear. In this review, we highlight the following functional mechanisms of MSCs: transdifferentiation and cell fusion, paracrine activity, cell-cell contact dependence, microvesicle secretion, mitochondrial transfer, and epigenetic regulation. In addition, we discuss methods to enhance the effectiveness of MSCs in clinical applications.

mesenchymal stem cells, functional mechanisms, cell-cell contact dependence, mitochondrial transfer, epigenetic regulation

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