脐带间充质干细胞衰老研究进展

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摘要 人脐带间充质干细胞(human umbilical cord mesenchymal stem cells, hUC-MSCs)是一种来源于人脐带组织且具有自我更新、多向分化和免疫调节能力的多潜能干细胞。相比于其他干细胞, hUC-MSCs具有容易获取、分离和培养, 移植体内后免疫原性低、无明显致瘤性等优点, 已成为细胞治疗和再生医学中重要的干细胞来源之一。然而, hUC-MSCs在体外培养时容易趋向衰老、逐渐失去快速增殖和多向分化潜能, 从而限制其临床应用。该文主要就近年来在hUC-MSCs衰老机制以及延缓hUC-MSCs衰老的相应策略等方面的研究进展, 作一简要概述和讨论。

关键词 脐带间充质干细胞; 干细胞; 细胞衰老; 延缓衰老

Research Progress on the Senescence of Umbilical Cord Mesenchymal Stem Cells

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Abstract hUC-MSCs (human umbilical cord mesenchymal stem cells) are a class of multipotent stem cells derived from human umbilical cord with the capacity of self-renewal, multi-directional differentiation and immune regulation. Compared with other stem cells, hUC-MSCs are advantageous in easy acquisition, isolation, cultivation, and low immunogenicity and tumorigenicity after *in vivo* implantation. Accordingly, hUC-MSCs have become one of the most important stem cell sources in cell therapy and regenerative medicine. However, *in vitro* cultured hUC-MSCs are prone to undergo senescence, gradually lose rapid proliferation and multipotency, thereby limiting their clinical application. Here this review focused on the recent progress on the mechanisms underlying the senescence of hUC-MSCs and the corresponding strategies to delay the senescence of hUC-MSCs for a brief summarization and discussion.

Keywords umbilical cord mesenchymal stem cells; stem cells; cellular senescence; delay senescence

1 脐带间充质干细胞

人脐带间充质干细胞(human umbilical cord mesenchymal stem cells, hUC-MSCs)是从脐带内表皮层和华通氏胶(Wharton's jelly)中分离出来的, 具有自我更新、多向分化和免疫调节功能的多潜能干细胞[1], 并且具有表达间充质细胞特异性细胞表面标志物的特征。国际细胞治疗协会间充质和组织干细胞委员会对MSCs做出了规范标准要求[2]: (1) MSCs需在培养皿表面贴壁生长; (2) 高表达(≥95%) CD73、CD90和CD105, 低表达(≤2%) CD34、CD45和人类白细胞抗原(human leukocyte antigen, HLA); (3) 可在体外特定条件下诱导分化为成脂细胞、成骨细胞

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和成软骨细胞。同时满足以上条件才符合MSCs的国际研究标准。

相比于其他类型的干细胞, hUC-MSCs是从新生儿组织中分离出来的, 具有无痛采集以及更加容易获取、分离、培养、扩增和纯化的优点[3]。此外, hUC-MSCs更接近胎儿期, 可以在没有分化的情况下快速扩增和自我更新[4], 并且没有伦理问题以及肿瘤细胞、病毒和其他致病生物的传播[5]。hUC-MSCs可以通过非侵入性手段收集, 并且由于其细胞表面低表达主要组织相容性复合体I/II(major histocompatibility complex I/II, MHC I/II)蛋白而具有低免疫原性。已有研究表明, hUC-MSCs在体内应用不会导致过敏和肿瘤发生[6]。此外, hUC-MSCs可以作为重要的免疫调节细胞, 在移植后的神经系统中介导保护性免疫[7], 并且能被宿主免疫系统良好耐受[1-8]。hUC-MSCs不仅可以不断自我更新, 还可以在特定条件下分化成一种或多种类型的细胞, 如成骨细胞、软骨细胞和脂肪细胞, 进而对损伤的人体组织和器官进行修复[9]。hUC-MSCs细胞具有较高的迁移以及归巢活性[10], 有利于发挥其自身免疫调节功能。除了定向分化为损伤细胞外, hUC-MSCs还可以通过自分泌或旁分泌途径分泌多种生物活性因子, 包括生长因子、细胞因子、趋化因子和代谢产物[4], 或通过胞外囊泡, 如外泌体和微囊泡分泌生物活性因子对局部微环境或信号作出反应, 从而修复损伤组织。与传统治疗相比, hUC-MSCs可以显著改善疾病的各种临床症状[11]。

目前, hUC-MSCs可以用于治疗各种疾病, 主要是由于其具有的独特功能对hUC-MSCs的治疗应用至关重要。(1) 定向分化: hUC-MSCs可以定向分化为特定细胞促进组织再生并改善组织功能[9]。(2) 免疫调节: hUC-MSCs可以抑制免疫细胞(如T细胞、B细胞和Tfh细胞)的增殖; 且可以诱导巨噬细胞从促炎表型转化为抗炎表型; 并通过分泌白介素-10(interleukin-10, IL-10)和白介素-4(interleukin- 4, IL-4)来减轻炎症。这些免疫反应改变都有助于组织修复[12]。(3) 旁分泌效应: hUC-MSCs通过分泌可溶性分子, 如角质形成细胞生长因子(keratinocyte growth factor, KGF)、肝细胞生长因子(hepatocyte growth factor, HGF)和其他细胞因子促进组织再生[13]。(4) 抗炎作用: hUC-MSCs可以抑制炎症因子白介素-1β(interleukin-1β, IL-1β)、肿瘤坏死因子-α(tumor necrosis factor-α, TNF-α)和白介素-8(interleukin-8, IL-8)的分泌, 减少炎症和氧化应激, 从而抑制细胞凋亡[14]。(5) 抗纤维化活性: hUC-MSCs可以通过刺激纤维化相关的细胞凋亡以及HGF和其他分子的分泌发挥抗纤维化活性, 也可以通过调节相关信号通路和促进血管重塑来发挥功能[15]。综上, hUC-MSCs已成为细胞治疗和再生医学中重要的干细胞来源。

2 脐带间充质干细胞的临床应用

hUC-MSCs移植通过定向分化和分泌活性因子, 被广泛应用于多种类型疾病的治疗(表1)。根据疾病的类型主要分为以下几个方面。

hUC-MSCs在调节先天和适应性免疫系统方面具有独特优势, 并为改善炎症提供了耐受性环境。hUC-MSCs通过分泌新型免疫抑制因子壳多糖酶3样蛋白1(chitinase-3-like protein 1, CHI3L1), 抑制T细胞增殖并调节Th17分化, 进而减轻急性移植物抗宿主病(acute graft versus-host disease, AGVH)症状[16]。hUC-MSCs外泌体(hUC-MSCs exosomes, hUC-MSCs Exo)同样在系统性红斑狼疮(systemic lupus erythematosus, SLE)中发挥抗炎和免疫调节作用[17]。转录因子Sox9(SRY-related HMG-box gene 9)和转化生长因子β1(transforming growth factor-β1, TGF-β1)的强表达可以促进hUC-MSCs的成软骨形成, 并降低炎症反应程度以及疼痛标志物的表达水平, 从而有效地治疗椎间盘相关疾病[18]。综上, hUC-MSCs通过定向分化以及分泌活性因子对免疫系统发挥调节作用。

研究也证实了hUC-MSCs在治疗神经系统疾病中的显著疗效。MSCs具有多向分化潜能, 可被诱导分化为星形胶质细胞和小胶质细胞, 通过减少炎症因子的释放, 改善神经炎症反应发挥促进组织修复和保护神经元的功能[19-20]。除了具有神经元分化潜能外, hUC-MSCs还可以通过旁分泌机制直接保护神经组织[21], 进而在神经系统疾病的恢复中发挥重要作用。在阿尔茨海默病(Alzheimer's disease, AD)小鼠模型中, hUC-MSCs分泌的HGF通过下调过度磷酸化的tau蛋白, 改善神经原纤维缠结和逆转脊柱损失, 并通过激活cMet-AKT-GSK3β信号通路促进AD海马神经元的突触可塑性, 从而恢复AD小鼠的学习和记忆能力[22]。研究表明, hUC-MSCs已成功应用于创伤性脑损伤后遗症患者, 使其神经功能得到显著改善[23]。

表1 hUC-MSCs及hUC-MSCs Exo的临床应用及治疗机制

Table 1 Clinical applications and therapeutic mechanisms of hUC-MSCs and hUC-MSC Exo

应用类型 Application type	临床应用 Clinical application	潜在治疗机制 Potential mechanisms of action	参考文献 References
hUC-MSCs	Heart failure	Differentiation into cardiomyocytes and improvement of vascular function through paracrine effects and anti-fibrosis	[27]
hUC-MSCs	Intervertebral disc related diseases	Differentiation into chondrocytes	[18]
hUC-MSCs	Alzheimer's disease	Differentiation into neurons and reversal of microglia phenotype	[22]
hUC-MSCs	Hypoxic ischemic encephalopathy	Differentiation into neurons and secretion of nerve growth factor	[7]
hUC-MSCs	Liver fibrosis	Differentiation into hepatocytes and secretion of HGF to restore the function of hepatocytes	[28-29]
hUC-MSCs	Spinal cord injury	Differentiation into nerve cells and suppression of inflammatory responses	[30]
hUC-MSCs	Chronic kidney disease	Differentiation into renal cells and secretion of HGF to restore cellular autophagy	[31]
hUC-MSCs	Acute graft-versus-host disease	Suppression of immunity	[16]
hUC-MSCs Exo	Colorectal cancer	Induction of anti-tumor response	[32]
hUC-MSCs Exo	Systemic lupus erythematosus	Suppression of immunity	[17]
hUC-MSCs Exo	Acute liver failure	Suppression of immunity	[25]
hUC-MSCs Exo	Autoimmune dry eye	Suppression of immunity	[33]
hUC-MSCs Exo	Chronic obstructive pulmonary disease	Suppression of the inflammatory response	[34]
hUC-MSCs Exo	Acute kidney injury	Suppression of p38/MAPK and promotion of cell proliferation	[35]
hUC-MSCs Exo	Nerve injury-induced pain	Suppression of immunity	[36]
hUC-MSCs Exo	Spinal cord injury	Suppression of immunity	[37]
hUC-MSCs Exo	Skin photodamage and aging	Suppression of oxidative stress/promotion of autophagy	[38]

hUC-MSCs对肝损伤疾病也有显著的治疗作用。hUC-MSCs通过促进肝细胞再生和抑制肝细胞凋亡来修复受损的肝组织[24]。在肝损伤小鼠模型中，富含miR-455-3p的hUC-MSCs Exo可以抑制巨噬细胞的活化，降低炎症因子水平，改善肝损伤和维持全身稳态[25]。hUC-MSCs还可以通过抑制Notch信号转导和逆转Stat1/Stat3(signal transducer and activator of transcription 1/3)通路的失衡来改善患有肝衰竭或肝损伤大鼠的肝功能和纤维化，并促进肝修复[26]。

hUC-MSCs还被广泛应用于多种其他疾病的治疗。在II型糖尿病(type II diabetes mellitus, T2DM)小鼠中，hUC-MSCs通过激活PI3K/AKT(phosphatidylinositol 3 kinase/protein kinase B)信号通路有效降低血糖水平，为治疗糖尿病提供一种新的策略[39]。此外，hUC-MSCs通过分泌HGF逆转终末氧化蛋白产物(advanced oxidation protein products, AOPP)对HK-2细胞自噬的抑制作用，发挥治疗慢性肾脏病(chronic kidney disease, CKD)的功能[31]。目前，研究人员正在利用hUC-MSCs和hUC-MSCs Exo对某些疾病，如神经系统缺陷[40]、肝脏疾病[41]和免疫系统疾病[42]等进行临床治疗的研究，其中一些已完成I期或II期临床试验[43](表2)。因此，基于hUC-MSCs的干细胞治疗可能会成为未来涉及多种疾病的一种常用治疗策略。

3 脐带间充质干细胞衰老

3.1 脐带间充质干细胞的衰老

目前，自体或异体hUC-MSCs临床试验项目已经在中国、美国和韩国等多个国家获得国家药品监督管理部门批准，正处于临床试验各个阶段。hUC-MSCs作为临床细胞治疗的重要来源，在调节免疫功能和抗纤维化等多个方面发挥重要作用。然而hUC-MSCs作为理想供体细胞的同时，研究也发现其在医学治疗上的局限性。hUC-MSCs在体外扩增时易衰老，导致其逐渐失去快速增殖和多向分化的能力[54]。因此，细胞衰老是制约hUC-MSCs在再生和组织工程中大规模体外扩增应用的主要因素之一。

细胞衰老是细胞对各种各样的应激源作出应答后的一种复杂的细胞状态，包括功能和复制能力的变化，最终会导致细胞增殖停止、组织缺乏恢复

表2 hUC-MSCs及hUC-MSCs Exo的临床试验

Table 2 Clinical trials of hUC-MSCs and hUC-MSCs Exo

疾病类型 Disease type	临床试验阶段 Phases of clinical trials	潜在治疗机制 Potential mechanisms of action	参考文献 References
Acute-on-chronic liver failure	I/II	Differentiation into hepatocytes and secretion of HGF	[41]
Hereditary spinocerebellar ataxia	I/II	Differentiation into neurons and secretion of cytokines	[40]
Acute respiratory distress syndrome in COVID-19	I/II	Immune regulation and tissue repair	[44]
Multiple sclerosis	I	Differentiation into neurons and secretion of cytokines	[45]
Psoriasis	I	Immune regulation	[46]
Autism spectrum disorder	I	Immune regulation and suppression of the inflammatory response	[47]
T2MD	I/II	Immune regulation and suppression of the inflammatory response	[48]
Rheumatoid arthritis	I/II	Suppression of immunity	[49]
Immune non-responders with AIDS	II	Immune regulation	[50]
Poor healing after uterine injury	I	Immune regulation and suppression of the inflammatory response	[51]
Peripheral arterial disease	I	Suppression of immunity	[52]
Cerebral palsy	I	Differentiation into neurons and secretion of cytokines	[53]

能力以及机体对某些疾病的易感性增加等[55]。研究表明, hUC-MSCs的衰老具有区别于常规细胞衰老的特性。随着细胞的不断传代, 衰老hUC-MSCs的成脂和成骨多向分化潜能显著减弱, 且间充质干细胞表面抗原CD73、CD90和CD105表达丧失[56]。此外, hUC-MSCs的衰老特征也具有常规细胞衰老的共性。体外长期培养的hUC-MSCs伴随增殖能力的降低和衰老相关β-半乳糖苷酶(senescence-associated β-galactosidase, SA-β-gal)活性的增加等典型细胞衰老特征[57]。细胞衰老不仅表现出细胞周期停滞的现象, 还表现在通过自分泌或旁分泌途径分泌细胞因子来影响临近细胞和组织微环境[58], 这种现象被称为衰老相关分泌表型(senescence-associated secretory phenotype, SASP)。典型的SASP分泌因子包括生长激素、细胞因子、血管生成因子和细胞外基质金属蛋白酶, 如白介素-6(interleukin-6, IL-6)、IL-8和纤溶酶原激活物抑制子-1(plasminogen activator inhibitor-1, PAI-1)[59]。一方面, SASP通过招募免疫细胞来清除衰老细胞, 因而具有短期促进组织修复和再生的有益功能。另一方面, SASP通过分泌促炎细胞因子诱导慢性炎症, 分泌促衰老细胞因子诱导周围正常细胞衰老, 最终促进衰老进程和衰老相关疾病的发展[60]。

3.2 脐带间充质干细胞衰老的机制

3.2.1 *脐带间充质干细胞衰老的影响因素* hUC-MSCs衰老具有常规细胞衰老的一些共性, 其类型主要包括电离辐射、紫外照射和氧化应激等压力诱导DNA损伤造成的压力诱导早熟型衰老(stress-induced premature senescence, SIPS), 以及端粒缩短导致细胞生长状态停滞的复制型衰老(replicative senescence, RS)。

当机体产生的活性氧(reactive oxygen species, ROS)与抗氧化物质失调时, 所处的状态就被称为氧化应激。随着hUC-MSCs传代次数的增加, 机体内ROS数量不断积累将导致氧化应激的产生, 进而对DNA造成损伤, 致使DNA甲基化和去甲基化平衡失调, 从而抑制或沉默相关基因的表达[61]。ROS在信号转导过程中还可以对细胞中的蛋白进行可逆或者不可逆的修饰, 导致细胞生长、分化停滞以及死亡[62]。在氧化应激过程中有两类关键蛋白, 热休克蛋白(heat shock proteins, HSPs)[63]和核因子红细胞2-相关因子2(nuclear factor rythroid 2-related factor 2, NRF2)参与调控细胞的衰老[64]。在细胞衰老过程中, HSPs可以发挥分子伴侣的作用, 帮助受损蛋白质正确折叠以获得正常功能, 或是介导受损蛋白的泛素化降解和自噬过程, 以达到清除受损蛋白的目的。一方面, NRFs通过调控还原蛋白酶的合成, 发挥抗氧化作用进而抑制ROS的过量产生; 另一方面, NRFs对相关抗氧化信号通路的调节可以减少ROS对细胞的损伤。当HSPs和NRF2功能受损时, ROS增多, 细胞中的蛋白质聚集体无法被正常清除, 影响细胞增殖和分化, 进而导致细胞衰老[65]。

线粒体不仅是能量的供应者，而且在细胞衰老和衰老相关疾病中发挥着重要作用[66]。已知ROS升高是触发细胞衰老的机制之一，线粒体作为ROS的主要来源，其融合和分裂的动态异常会导致ROS的生成，进而导致细胞损伤和细胞衰老[67]。衰老的MSCs表现出线粒体融合增加和线粒体分裂减少，促进了线粒体中ROS的产生，并加剧了早期MSCs的衰老。因此，线粒体的动态异常也会导致MSCs分化命运的改变，并促进MSCs衰老[68-69]，有文献表明来自妊娠期糖尿病孕妇的hUC-MSCs表现出早熟型衰老特征，可能与线粒体功能异常相关[70]。环境因素引起能量代谢的改变以及过量ROS副产物的产生，可能通过改变线粒体组分导致线粒体功能障碍，导致ROS持续释放的恶性循环[67]，从而促进细胞衰老和相关疾病的发生[71]。

研究证实在hUC-MSCs的长期培养过程中，细胞形态变长、变大，增殖变慢，细胞最终在培养过程中衰老。该过程往往伴随促增殖基因表达水平减少、端粒酶活性逐渐降低以及端粒长度逐渐缩短。端粒缩短通常会触发DNA损伤，进而引起hUC-MSCs基因组不稳定，最终导致hUC-MSCs进入衰老和生长停滞状态[57]。研究表明，与年轻小鼠(2月龄)相比，从衰老小鼠(20~24月龄)中获得的MSCs显示出端粒酶表达能力受损和端粒长度缩短，并且细胞增殖率和分化能力受损[72]，即使在MSCs生长早期时，端粒酶敲除的MSCs也会丧失其分化能力[73]，而端粒酶在MSCs中过表达能够增强其增殖和分化能力[74]。

3.2.2 脐带间充质干细胞衰老的相关信号通路 衰老细胞的生长停滞通常是通过将细胞周期滞留于G_1期阻断DNA复制启动来实现的[75]。细胞周期由几个关键因子，包括细胞周期蛋白、细胞周期蛋白依赖性激酶(cyclin-dependent kinase, CDK)、细胞周期蛋白依赖性激酶抑制因子以及视网膜母细胞瘤肿瘤抑制蛋白(retinoblastoma protein, RB)共同调节，衰老信号通常通过降低CDK活性或增加CDK抑制因子水平来促进细胞衰老[76]。

在hUC-MSCs衰老过程中，ROS的上调会导致p38/丝裂原活化蛋白激酶(mitoge nactivated protein kinase, MAPK)信号通路被异常激活，进而上调p53、p21和p16等细胞周期负调控因子的表达(图1)。p21和p16的核积累通过抑制细胞周期蛋白-CDK复合物活性从而促进RB持续激活，激活的RB通过隔离E2F家族成员来抑制细胞周期进程[77]。研究表明，p21、p16和p27等细胞周期负调控因子通过抑制CDK活性导致hUC-MSCs早熟型衰老的发生[70]。来自妊娠期糖尿病孕妇的hUC-MSCs由于线粒体损伤使得ROS水平升高进而导致早熟型衰老的产生，并且与来自正常孕妇的hUC-MSCs相比，来自妊娠期糖尿病孕妇的hUC-MSCs中*p16*和*p27*转录水平显著升高。除此之外，在来自妊娠期糖尿病孕妇的hUC-MSCs中也检测到p53以及p16蛋白在传代第7代至第10代时水平也相对升高[70]。

PI3K/AKT信号通路也是细胞衰老过程中的一条关键信号通路。高龄妇女和卵巢早衰患者卵巢功能下降，严重降低了卵巢储备能力和生育能力，伴随着PI3K/AKT信号通路中某些关键蛋白活性的降低。利用透明质酸(hyaluronic acid, HA)纳米支架移植hUC-MSCs后，通过激活PI3K/AKT信号通路挽救了卵泡在体内和体外的生存[78]，为卵巢早衰患者和高龄妇女带来了新的希望。此外，成纤维细胞生长因子2(fibroblast growth factor-2, FGF-2)也能够通过激活PI3K/AKT信号通路抑制MSCs衰老并促进其增殖，从而维持MSCs的干性[79]。

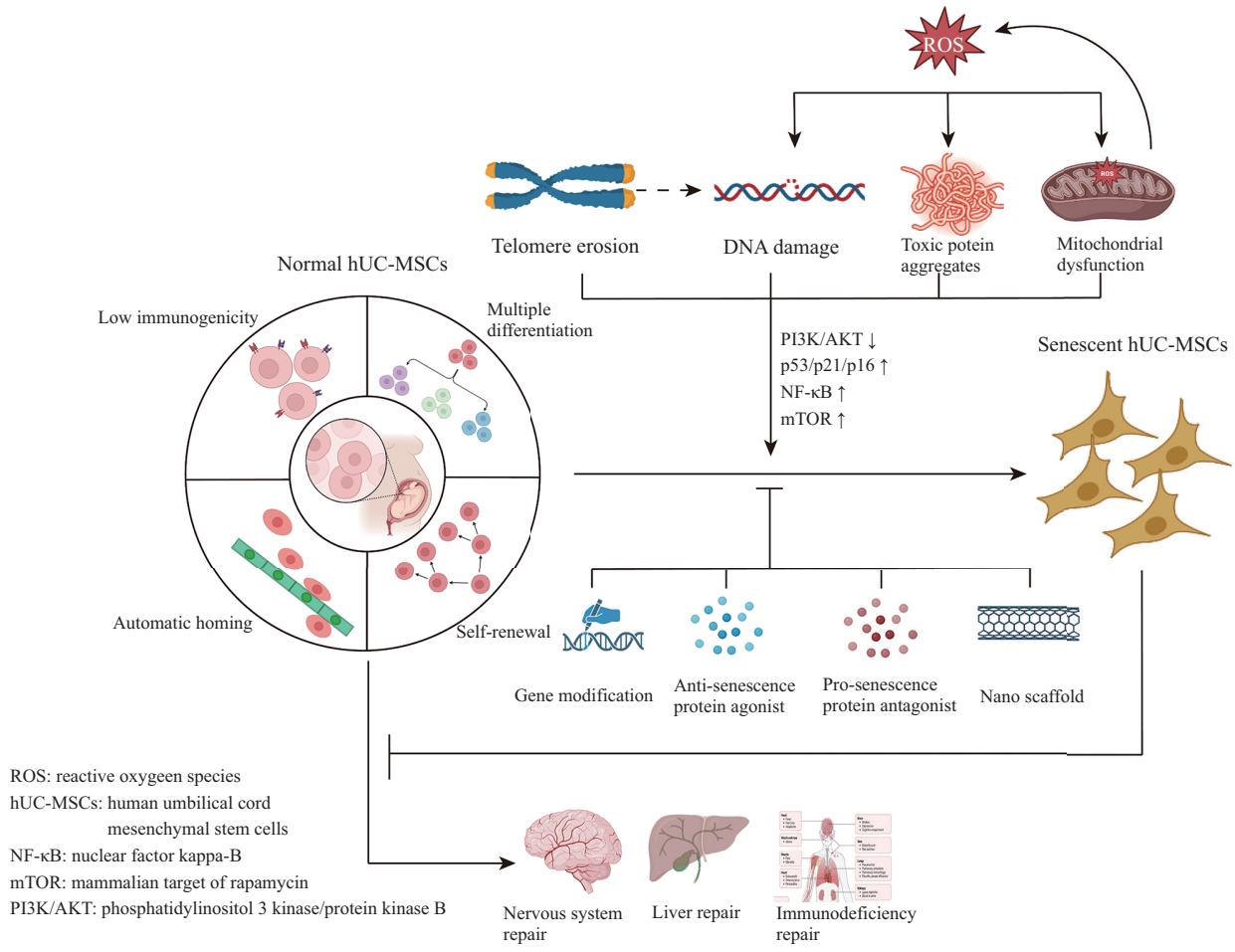
在氧化应激和DNA损伤条件下，内源或外源压力还可以激活细胞核因子-κB(nuclear factor kappa-B, NF-κB)相关的信号通路，进而激活免疫和炎症反应，通过SASP释放炎症因子和各种衰老相关因子，影响邻近细胞和组织微环境，以自分泌或者旁分泌方式加强衰老信号的转导[80]。晚期MSCs可以分泌SASP相关的白介素-1α(interleukin-1α, IL-1α)和IL-8，IL-1α和IL-8的旁分泌作用通过NF-κB依赖的方式诱导早期MSCs的细胞衰老，因此在早期MSCs中使用NF-κB抑制剂可以阻断由晚期MSCs的旁分泌SASP引起的早期MSCs衰老[81]。

促进细胞合成代谢以及抑制分解代谢自噬途径的雷帕霉素靶蛋白(mammalian target of rapamycin, mTOR)同样会诱导细胞衰老。mTOR抑制自噬会导致有毒细胞废物积累以及ROS水平的升高，从而导致细胞衰老的发生[82]。mTOR复合体1(mTOR complex 1, mTORC1)是自噬的主要调节因子，在营养丰富的条件下，mTORC1可以通过介导ULK1(Unc-51-like kinase-1)(Ser637和Ser757)和Atg13(autophagy-related protein 13)(Ser258)特定位点磷酸化而使自噬调节复合物失活，从而抑制自

噬小体的生物发生, 最终导致细胞衰老[83]。文献表明, 抗坏血酸能够通过抑制mTOR信号阻碍MSCs衰老[84]。mTOR1同样可以受到腺苷单磷酸激活激酶(AMP-activated kinase, AMPK)信号通路的影响。AMPK可以通过负向调控mTORC1来正向调控自噬, 从而发挥延缓细胞衰老作用。已有研究表明, FGF-21通过AMPK信号通路介导线粒体动力学来调节MSCs的衰老[85]。

4 延缓脐带间充质干细胞衰老的方法

在hUC-MSCs治疗急性肝衰竭的应用过程中, 传代培养第5代hUC-MSCs比第10代细胞在归巢至肝脏和增强肝细胞增殖以及抑制凋亡方面更有效[86], 并且衰老细胞的消除可以促进干细胞增殖并延缓衰老特征的出现[87]。与上述观察结果一致, 第10代和第15代的hUC-MSCs在体外传代过程中出现复制型衰老, 表现为增殖和迁移能力下降, 细胞衰老加速, 氧化应激水平和SASP释放量增加[88]。反复多次传代将诱导hUC-MSCs衰老, 并不同程度地影响其增殖能力、分化潜能、分泌活性和治疗效果[89]。因此, 找到有效策略来延缓长期培养诱导的hUC-SMCs衰老, 并增强其归巢能力以提高临床表现显得非常重要。目前主要有以下几种方法来延缓hUC-MSCs衰老(图1)。



hUC-MSCs是一种具有自我更新、多向分化和自动归巢能力以及低免疫原性的干细胞, 端粒缩短、活性氧诱导的DNA损伤、毒性蛋白质聚集体和线粒体功能障碍可通过调控PI3K/AKT、p53/p21/p16、NF-κB和mTOR等信号通路诱导hUC-MSCs衰老。基因修饰、抗衰老蛋白质激动剂、促衰老蛋白质抑制剂以及纳米支架可延缓hUC-MSCs衰老, 从而恢复其组织修复的功能。↑: 激活; ↓: 抑制。

hUC-MSCs are stem cells with low immunogenicity and the capacities of self-renewal, multiple differentiation and automatic homing. Telomere erosion, reactive oxygen species-induced DNA damage, toxic protein aggregates and mitochondrial dysfunction can lead to senescence of hUC-MSCs via modulation of PI3K/AKT, p53/p21/p16, NF-κB and mTOR signalings. Gene modification, anti-senescence protein agonists, pro-senescence protein inhibitors and nano scaffolds can delay the senescence of hUC-MSCs, thereby restoring their function of tissue repair. ↑: activation; ↓: suppression.

图1 人脐带间充质干细胞的衰老机制及其延缓衰老的方法

Fig.1 The mechanisms underlying the senescence of hUC-MSCs and the methods to delay their senescence

4.1 基因修饰

在基因层面，逆转或延缓hUC-MSCs细胞衰老并保持其体外增殖能力的方法有两种：细胞重编程和microRNA处理[90-91]。细胞重编程包含两种类型：通过重新编码细胞内基因重置表观遗传的完全重编程和通过DNA甲基化和组蛋白修饰使表观遗传年轻化的部分重编程[92-93]。目前，细胞重编程可通过直接调控Oct4、Sox2、Klf4和c-Myc四种转录因子改善细胞的衰老特征，延长早衰小鼠的寿命[92]，或者通过小分子药物间接调控转录因子将细胞恢复到原始状态。microRNA通过多维靶点来延缓hUC-MSCs衰老[94]。例如，miR-106a、miR-106b、miR-93、miR-25和miR-155等多种microRNA可以通过抑制p51、p21和p16等相关蛋白的表达以及SASP来延缓细胞衰老。在暴露于氧气–葡萄糖条件下，miR-25-3p寡核苷酸模拟物可以降低脂肪MSCs的死亡数量，并且延缓细胞衰老速度[95]。然而，以上延缓hUC-MSCs衰老的方法都存在一定的临床应用限制。首先，调节hUC-MSCs衰老途径的关键因子也是正常生物学功能的重要生理调节因子，通过干预完全抑制或激活这些途径可能会产生其他负面影响。其次，单种microRNA延缓hUC-MSCs衰老的效果不明显，需将几种衰老相关的microRNA联合使用才能发挥治疗功能[94]。

4.2 抗衰老蛋白质激动剂

目前一些研究基于具有抗衰老功能的蛋白质开发了激动剂，以应用于延缓hUC-MSCs衰老。SIRT3(sirtuin 3)是一种NAD^+依赖性蛋白去乙酰化酶，定位于线粒体基质，是线粒体功能的关键调节因子，可减少线粒体ROS产生，降低氧化应激水平。SIRT3的激动剂亚精胺(spermidine, SPD)可以通过上调SIRT3蛋白表达水平，抑制p53、p21以及SA-β-gal的表达，发挥延缓hUC-MSCs的复制型衰老以及早熟型衰老的作用[96]。作为自噬诱导剂[97]，SPD也可能通过激活细胞自噬来延缓hUC-MSC衰老。NRF2是细胞抗氧化反应的核心因子，能清除细胞中的ROS，减少SASP释放，延缓细胞衰老进程。研究证明，使用NRF2的激动剂TBHQ后，NRF2通过下调ROS水平延缓了骨髓间充质干细胞(bone marrow mesenchymal stromal cells, BM-MSCs)的衰老[98]，TBHQ可能在hUC-MSCs中也发挥同样的抗衰老作用。端粒酶活性降低导致的端粒缩短也是BM-MSCs衰老的关键诱因，使用端粒酶激动剂AGS-499和AGS-500处理BM-MSCs可以增加端粒酶的表达水平，恢复端粒长度，从而抵抗氧化应激诱导的细胞衰老[99]。

4.3 促衰老蛋白质抑制剂

除了抗衰老蛋白质的激动剂外，促衰老蛋白质的抑制剂同样能够发挥延缓hUC-MSCs衰老的功能。雷帕霉素(rapamycin, RAPA)已经被证实具有延缓细胞衰老和促进MSCs的血管生成的功能，主要机制可能是通过抑制mTOR信号通路的激活，降低细胞内的ROS水平，从而缓解DNA损伤对细胞产生的伤害。RAPA不仅能维持MSCs克隆能力，使其维持早期细胞形态，还能增强其成骨分化及免疫调节能力[100]。因此，利用RAPA延缓hUC-MSCs衰老，可以促进hUC-MSCs的临床应用。研究表明，SASP会引发周围正常细胞的衰老，晚期MSCs可以分泌IL-1α和IL-8等相关炎症因子，通过激活早期MSCs的NF-κB信号通路介导细胞衰老。使用NF-κB的抑制剂JSH-23可阻断晚期MSCs的SASP旁分泌对早期MSCs衰老程序的启动，保护年轻细胞免受衰老细胞的影响[81]。

4.4 纳米支架

最近研究发现，由纳米羟基磷灰石/壳聚糖/聚乙丙交酯(nHA/CS/PLGA)为原材料构建的纳米支架可以为延缓hUC-MSCs的复制型衰老提供合适的微环境。hUC-MSCs在体外长期传代后会表现出形态增大、增殖减慢和分化减少的衰老表型。但在模拟天然组织基质的nHA/CS/PLGA支架上的培养在很大程度上阻止了hUC-MSCs在第27代的细胞衰老，表明在长期体外扩增过程中，通过改变培养微环境，可以保持hUC-MSCs的干性并延缓衰老[101]。研究表明，细胞培养基质的硬度也会影响hUC-MSCs长期培养过程中的衰老程度。与塑料凝胶上培养的细胞相比，使用较软的聚丙烯酰胺凝胶培养的hUC-MSCs增殖活力高，细胞形态、细胞硬度以及牵引力等机械性能正常，并且具有较高的成脂分化潜能[102]，这种新型细胞扩增系统将在hUC-MSCs的研究和临床治疗过程中发挥重要作用。

5 结语与展望

hUC-MSCs是一种来源于人脐带组织的多潜能干细胞，具有自我更新、多向分化和自动归巢等特性，且对比于其他干细胞具有来源丰富、易于培养以及免疫原性和致瘤性低等优势，已被应用于治疗

多种疾病且具有良好的治疗效果。但hUC-MSCs体外扩增次数的增多以及环境对细胞产生的氧化应激, 可能会导致细胞呈现衰老状态, 传代后期细胞形态变长、变大, 增殖变慢, 增殖相关基因减少, 细胞逐渐失去增殖能力和治疗特性, 从而限制其临床应用。因此, 很多研究致力于探究hUC-MSCs衰老的分子机制, 以期找到相应策略来延缓hUC-MSCs衰老。目前虽然已存在多种可恢复hUC-MSCs增殖活力和延缓hUC-MSCs衰老的方法, 但这些方法或多或少存在一定的临床应用限制, 可能会对细胞正常的生物学功能产生负面影响或者需要多种治疗方法联合使用才能达到治疗效果。同时, hUC-MSCs的衰老机制尚未被完全阐明, 体外衰老状态逆转的细胞其临床应用的安全性以及治疗效果也有待验证, 这些问题都需要通过进一步的研究来解决。

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