

# 人羊膜来源干细胞的生物学特性及应用潜力\*

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**摘要** 干细胞移植是一种有潜力的替代治疗方案,虽然大量实验模型研究已经证实了干细胞移植能够修复受损或退化的组织并恢复其功能,但是在实际治疗中仍面临诸多困难,如免疫排斥、伦理障碍以及致瘤性等。围产期干细胞可以解决干细胞治疗所面临的问题,被认为是潜在的可靠干细胞来源。与其他干细胞相比,人羊膜来源干细胞(human amniotic stem cells, hAMSCs)具有显著优势。动物实验发现,hAMSCs 具有高分化潜能以及免疫调节活性,在妇科疾病、神经系统疾病、肾脏疾病、肺部疾病、皮肤疾病、糖尿病、癌症等多种疾病的治疗中表现出了巨大潜力。随着科学的进步以及临床的迫切需求,hAMSCs 的临床应用也逐渐突破了传统治疗的局限性。综述 hAMSCs 生物学研究进展及应用潜力,以期 hAMSCs 的实验研究和临床应用提供理论依据。

**关键词** 人羊膜来源干细胞 人羊膜上皮干细胞 人羊膜间充质干细胞 生物学特性

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生物材料、再生因子/细胞因子以及干细胞在内的新兴领域是再生医学的重要组成部分。干细胞的可靠来源是细胞替代疗法的关键因素。骨髓间充质干细胞、胚胎干细胞和诱导多能干细胞等均为干细胞移植的潜在细胞来源,虽然这些干细胞的生物学潜力已经得到证明,但皆因使用限制而未广泛应用于临床。目前研究证实,人类胚盘的不同部位含有多能干细胞,特别是胚胎胎膜最内层的羊膜部位。羊膜是透明、光滑、无血管的单层薄膜(约 100  $\mu\text{m}$ ),由上皮和间充质组成<sup>[1]</sup>,含丰富的人羊膜来源干细胞(human amnion-derived stem cells, hAMSCs),包括人羊膜上皮干细胞(human amniotic epithelial stem cells, hAECs)和人羊膜间充质干细胞(human amnion-derived mesenchymal stem cells, hAD-MSCs)<sup>[2]</sup>。hAECs 和 hAD-MSCs 作为羊膜的两种主要干细胞,能够分泌多种细胞因子及生长因子,并可产生细胞外基质<sup>[3]</sup>。hAD-MSCs 来源于纤维母细胞层,hAECs 则主要来源于羊膜最内层,直接与羊

水和胎儿接触<sup>[4]</sup>。与其他干细胞相比,hAMSCs 具有明显优势,如多分化潜能、无致瘤性、低/无免疫原性、高组织相容性、伦理可接受性以及易获得性等<sup>[5]</sup>,hAMSCs 强大的旁分泌效应使其成为治疗多种疾病的潜在细胞源<sup>[4,6]</sup>。本文总结了 hAMSCs 的生物学特性及应用潜力,旨在为 hAMSCs 的实验研究以及临床应用提供理论参考。

## 1 hAMSCs 的生物学特性

### 1.1 hAMSCs 的表面标记物

研究表明,hAECs 和 hAD-MSCs 均表达 CD44、CD73、CD90 以及 CD105 等间充质干细胞表面标记物,二者缺乏 CD34、CD45、CD80 和 CD86 等细胞表面标记物。根据细胞表面抗原的分布可以发现,hAECs 缺乏特异性表面标记物,基本属于同质细胞群<sup>[7]</sup>,hAECs 表达上皮细胞特异性表面标志物和间充质干细胞的表型<sup>[8]</sup>。hAD-MSCs 表达间充质干细胞的表面标记物,如 CD90、CD105 和 CD271 等,也表达 SSEA-3 和 SSEA-4 等胚胎干细胞的表面标志物,但不表达上皮细胞表面标志物<sup>[9]</sup>。hAECs 和 hAD-MSCs 的表面标记物如表 1 所示。

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表 1 hAMSCs 的表面标记物  
Table 1 Surface markers of hAMSCs

hAMSCs	上皮标志物	间充质干细胞标志物	造血标志物	参考文献
	阳性	阳性	阴性	
hAESC	Cytokeratin, E-cadherin	CD29, CD166, CD90	CD34, CD45	[10]
	CK19	CD29, CD44, CD73, CD90, CD105	CD31, CD34, CD45, CD49d	[11]
		CD73, CD29	CD34, CD45	[12]
	CK7, E-cadherin	CD29, CD73, CD105	CD34, CD45	[13]
	E-cadherin, CD49f, CK7, EpCAM	CD44, CD90, CD105, CD146, PDGFR- b, CD29	CD45	[14]
hAD-MSC	-	CD29, CD44, CD73, CD90, CD105	CD34, CD45	[15]
	-	CD44, CD90, CD73, CD105	CD34, CD45, CD14, CD19, HLA-DR	[16]
	-	CD29, CD73, CD90, CD105	CD34, CD45, CD133	[17]
	-	CD29, CD44, CD49, CD73, CD90, CD105	CD31, CD34, CD45	[11]
	-	CD105, CD117	CD34	[18]

1.2 hAMSCs 的分化潜能

hAMSCs 具有较为全面的分化潜能,当受到外源生长因子或化学物质等刺激时,hAMSCs 有能力向三个胚层不同组织来源的细胞分化<sup>[7]</sup>。hAMSCs 可以表达胚胎干细胞的典型表面标记物,表明 hAMSCs 在干细胞移植治疗中具有巨大潜力。研究发现,在 DNA 去甲基化剂 5-氮杂胞苷、转化生长因子-β (transforming growth factor β, TGF-β) 和碱性成纤维细胞生长因子 (basic fibroblast growth factor, bFGF) 共培养、胰岛素样生长因子-1 (insulin-like growth factor-1, IGF-1)、淫羊藿次苷 II、人血小板裂解物 和 5-氮胞苷联合、人血小板裂解物的诱导下,hAD-MSCs 可分别分化为骨骼肌细胞<sup>[19]</sup>、人前交叉韧带成纤维细胞<sup>[20]</sup>、胰岛分泌细胞<sup>[21]</sup>、多巴胺能神经元样细胞<sup>[22]</sup>、心肌细胞<sup>[23]</sup> 和 内皮细胞<sup>[24]</sup>。其中,多巴胺能神经元样细胞属于外胚层,骨骼肌细胞、人前交叉韧带成纤维细胞和心肌细胞属于中胚层,胰岛分泌细胞属于内胚层。

与 hAD-MSCs 类似,hAESC 亦具有高度的分化可塑性,可分化为多种细胞。例如,在曲古抑菌素 A 和烟酰胺联合、表皮细胞生长因子的刺激下,hAESC 可分别分化为功能性视网膜色素上皮样细胞<sup>[25]</sup> 和肝细胞样细胞<sup>[26]</sup>。Zhou 等<sup>[27]</sup> 研究证实,hAESC 可被诱导分化为角膜上皮细胞,从而重建角膜缘干细胞缺乏患者的角膜上皮。体外实验发现,hAESC 在促血管生成条件下培养可形成毛细血管样结构,并在体内分化为肝窦内皮细胞<sup>[28]</sup>,这为肝脏疾病的治疗提供了新的选择。

1.3 hAMSCs 的免疫调节活性

已有研究证明,hAMSCs 具有免疫豁免的优势,通过分泌细胞因子发挥成体干细胞免疫调节的作用。在先天免疫调节方面,hAD-MSCs 以剂量依赖性方式抑制巨噬细胞 M2 向 M1 表型的极化<sup>[29]</sup>,并通过下调肝巨噬细胞的自噬水平促进巨噬细胞 M2 表型的极化<sup>[30]</sup>。值得注意的是,Pampalone 等<sup>[31]</sup> 在体外研究 hAD-MSCs 对肝硬化难治性腹水患者腹水细胞成分的免疫刺激或免疫抑制作用时,发现 hAD-MSCs 对于 M2 表型的极化有促进作用,但不会显著减少与 M1 表型相关的成分。这表明 hAD-MSCs 不会影响巨噬细胞和自然杀伤 (natural killer, NK) 细胞的吞噬活性,从而有助于机体恢复生理状态。在调控适应性免疫方面,新鲜分离的 hAD-MSCs 在体外通过未知的可溶性因子抑制淋巴细胞的增殖,并推测前列腺素是发挥作用的关键因子<sup>[32]</sup>。在急性移植抗宿主病小鼠模型中,hAD-MSCs 能够抑制 CD3<sup>+</sup> CD4<sup>+</sup> T 和 CD3<sup>+</sup> CD8<sup>+</sup> T 细胞浸润,提升 Treg 细胞比例,有效调节 Treg 细胞和效应 T 细胞的平衡,并下调靶器官内炎症细胞因子水平<sup>[33]</sup>。此外,hAD-MSCs 通过抑制心脏淋巴细胞的增殖并上调白介素-10 (interleukin-10, IL-10) 和 TGF-β 的水平,提高小鼠同种异体心脏移植存活率<sup>[34]</sup>,从而有望成为器官移植理想的细胞来源。

根据 hAESC 表达的细胞表面标记物可知,hAESC 具有与间充质干细胞类似的低免疫原性和调节免疫系统的能力。与 hAD-MSCs 相同,hAESC 能够

抑制巨噬细胞趋化活性,促进 M2 表型巨噬细胞极化<sup>[35]</sup>,通过抑制 NK 受体表达来阻断 NK 细胞的毒性,并能下调单核细胞的活性<sup>[36]</sup>,进而影响先天免疫。研究发现,hAESC 以剂量依赖性方式显著抑制幼稚 T 细胞的活化,降低受刺激 CD4<sup>+</sup> T 细胞分泌 TGF- $\beta$ 1 的能力<sup>[37]</sup>,还可促进 T 细胞向 Treg 细胞的极化<sup>[38]</sup>,以此增强对适应性免疫的调节能力。此外,暴露于促炎细胞因子混合物的 hAESC 通过调节 JAK1/2-STAT1/3 和 NF- $\kappa$ B1 途径分泌抗炎和免疫调节因子,实现局部免疫保护<sup>[39]</sup>。hAD-MSCs 和 hAESC 对于先天免疫应答和适应性免疫均具有调节特性,但调节能力有所不同。例如,hAD-MSCs 和 hAESC 均能抑制单核细胞来源的树突状细胞的生成和成熟,但 hAD-MSCs 的抑制作用强于 hAESC<sup>[40]</sup>。hAMSCs 所具有的免疫调节能力,使其成为治疗慢性炎症性疾病和抑制同种异体移植等导致的相关性免疫反应的潜在选择。

#### 1.4 hAMSCs 的旁分泌作用

hAMSCs 的免疫调节活性需借助旁分泌作用来实现。hAD-MSCs 通过分泌生长因子、血管生成相关因子等多种细胞因子,如血管内皮生长因子(vascular endothelial growth factor, VEGF)、成纤维细胞生长因子 2(fibroblast growth factor 2, FGF-2)、肝细胞生长因子(hepatocyte growth factor, HGF)、血小板衍生生长因子(platelet derived growth factor, PDGF)、IGF-1 等,促进细胞分化、血管生成和组织再生<sup>[41-42]</sup>。hAD-MSCs 也可分泌 IL-6、IL-8、单核细胞趋化因子-1(monocyte chemotactic protein, MCP-1)、基质细胞衍生因子-1 $\alpha$ (stromal cell-derived factor 1 $\alpha$ , SDF-1 $\alpha$ )等多种趋化因子,增强肝细胞的分化<sup>[42]</sup>。大部分活细胞都有分泌外泌体的能力,外泌体作为旁分泌信号的重要载体,是细胞间传递信息的重要组成部分。研究发现,hAD-MSCs 衍生的外泌体通过下调 Zeste 基因增强子同源物 2(en enhancer of zeste homolog 2, EZH2)来抑制雷帕霉素靶蛋白(mechanistic target of rapamycin, mTOR)信号通路,增强缺氧条件下滋养层细胞的增殖和自噬能力,进而改善妊娠期滋养层功能障碍<sup>[43]</sup>。

与 hAD-MSCs 相似,hAESC 的旁分泌因子种类和数量较多。研究发现,hAESC 分泌多种神经营养因子、生长因子和神经元细胞黏附分子,如脑源性神经营养因子、纤毛神经营养因子、VEGF、bFGF、IGF 等,促进细胞迁移、多巴胺能神经元/神经突的生长和血管生成等<sup>[11, 44-46]</sup>,并产生抗炎因子 IL-1 受体拮抗剂抑制神经

炎症<sup>[44]</sup>。此外,hAESC 还表现出抗纤维化的特性。研究发现,hAESC 以旁分泌的方式抑制 pSMAD 2/3 和 TGF- $\beta$ /Smad3 信号通路的激活,并上调基质金属蛋白酶 2 和基质金属蛋白酶 9 的表达以增加细胞外基质的降解,从而抑制纤维化<sup>[47-48]</sup>。外泌体是 hAESC 进行信息传递的重要载体。Wei 等<sup>[49]</sup>研究证实,hAESC 的外泌体通过激活 PI3K-AKT-mTOR 通路以增强成纤维细胞功能,从而促进血管生成,这是一种有前景的糖尿病疮疡愈合新策略。此外,源于 hAESC 的外泌体能够有效治疗皮肤色素沉着<sup>[50]</sup>、促进皮肤伤口愈合并无瘢痕形成<sup>[51]</sup>和恢复化疗所诱导的卵巢功能早衰(premature ovarian failure, POF)<sup>[52]</sup>,这表明 hAESC 的外泌体具有限制损伤并修复受损组织的能力。因此,进一步深入研究外泌体的作用和机制将为再生医学的发展带来新的生机。

## 2 hAMSCs 相对于其他干细胞的优势

与其他干细胞相比,hAMSCs 具有如下优势:(1)来源丰富,易于获得,无伦理和道德争议。胎盘是胎儿出生后的医疗废弃物,对胎盘的使用不会对供体产生任何伤害。(2)高组织相容性和低免疫原性。已有研究表明,将 hAMSCs 植入免疫类型不匹配的动物体内,可以在宿主体内存活较长时间,且不会引起宿主免疫细胞的任何排斥反应<sup>[53]</sup>。hAMSCs 分泌低水平的经典人白细胞抗原(human leukocyte antigen, HLA)-I 类分子(HLA-A、HLA-B、HLA-C 以及  $\beta_2$ -微球蛋白)<sup>[54-56]</sup>。最为关键的是,hAMSCs 不表达 HLA-II 类抗原(HLA-DR, HLA-DQ, HLA-DP)和共刺激因子(CD40、CD80 等)<sup>[57]</sup>。hAMSCs 的高组织相容性是其异种移植后低免疫原性的重要基础。(3)不具备致癌性。目前已有研究证实了 hAMSCs 的非致癌性<sup>[58-59]</sup>,且能够抑制癌细胞的增殖,延缓肿瘤的生长速度<sup>[60]</sup>。端粒酶的缺乏可以有效解释 hAMSCs 的非致癌性。(4)多能性。在不同条件下 hAMSCs 可以向三个胚层的细胞分化。

此外,Rossi 等<sup>[32]</sup>研究发现,抗细胞增殖是 hAMSCs 所固有的能力,这与骨髓间充质干细胞需在刺激因素的培养下才具有抗增殖作用不同。与 hAMSCs 类似,人脐带来源间充质干细胞(human umbilical cord-derived mesenchymal stem cells, hUC-MSCs)具有较高分化能力、低免疫原性,且取材方便、不涉及伦理问题。hUC-MSCs 分泌大量的生长因子,如 VEGF、bFGF 等促进组织再生<sup>[61]</sup>并调节 TGF- $\beta$  的表达,以外泌体的形式下调 IL-6、

IL-1 $\beta$  和肿瘤坏死因子- $\alpha$  (tumor necrosis factor, TNF- $\alpha$ ), 抑制上皮间充质转化, 改善炎症反应和纤维化<sup>[62-63]</sup>。hUC-MSCs 通过恢复 Th1/Th2 的平衡、抑制 NK 表达以及上调 CD8<sup>+</sup>CD28<sup>+</sup>T 等途径, 发挥免疫调节作用<sup>[64-65]</sup>。

对 hAMSCs 和 hUC-MSCs 的表面标记物进行比较发现, hUC-MSCs 对于 CD90 的表达强于 hAMSCs, 在传代至第 4~6 代时, hUC-MSCs 表达 CD105 的能力强于 hAMSCs, 但传代至第 6 代或第 7 代时, hAMSCs 表达 CD105 的能力则强于 hUC-MSCs<sup>[66]</sup>。Asgari 等<sup>[67]</sup>研究发现, 相比于 hAD-MSCs, hUC-MSCs 具有更高的分化为雌性生殖细胞的潜力, 并保持了更原始的生殖细胞特征。此外, hAMSCs 和 hUC-MSCs 对于促进伤口愈合和血管新生, 抑制异源激活淋巴细胞增殖的能力无明显差别<sup>[68-69]</sup>。

### 3 hAMSCs 的应用潜力

hAMSCs 凭借其高分化潜能、无致瘤性、低/无免疫原性以及易获得性等优势, 成为干细胞移植治疗多种疾病的潜在细胞源。目前, 已有大量动物实验研究表明, hAMSCs 具有多途径治疗妇科疾病、神经系统疾病、肾脏疾病、肺部疾病、肝脏疾病、糖尿病和癌症等多种疾病的巨大潜力(表 2)。近年来, 随着技术的进步和研究的深入, hAMSCs 已逐步应用于临床治疗。

#### 3.1 hAMSCs 的临床前研究

**3.1.1 妇科疾病** POF 是指 40 岁之前出现卵巢功能下降甚至卵巢衰竭的现象, 其特点是促性腺激素水平升高和雌激素水平降低, 伴随潮热、出汗和性欲低下等一系列低雌激素症状<sup>[70]</sup>。研究证实, hAD-MSCs 能够抑制颗粒细胞(granulosa cell, GC)凋亡, 促进血管生成, 并激活 P13K/Akt 信号通路, 促进 SDF-2/CXCR4 轴介导的迁移和归巢, 将 hAD-MSCs 归巢至卵巢, 促进分泌 VEGF 和血管内皮生长因子受体 2(vascular endothelial growth factor receptor 2, VEGFR2), 进而提升卵巢功能<sup>[41, 71]</sup>。此外, hAESC 以旁分泌方式显著抑制化疗诱导的细胞凋亡并激活颗粒黄体细胞的 TGF- $\beta$ /Smad 信号通路, 增加卵巢中次级和成熟卵泡的数量, 促进受损卵巢的血管生成<sup>[48]</sup>。

宫腔粘连(intrauterine adhesion, IUA)多是由于宫腔手术创伤或感染导致子宫内膜基底层受损, 使得子宫壁间相互粘连, 宫腔闭塞, 从而导致月经量减少、闭经甚至不孕等<sup>[72]</sup>。研究发现, 在 IUA 大鼠模型中移植

hAESC 能够上调 VEGF、bFGF 和 IGF 等生长因子以及雌激素受体、IL-4 的水平, 下调 I 型胶原蛋白、金属蛋白酶组织抑制剂-1(tissue inhibitor of metalloproteinase 1, TIMP 1)、TGF- $\beta$ 、 $\alpha$ -平滑肌肌动蛋白( $\alpha$ -smooth muscle actin,  $\alpha$ -SMA)、血小板衍生生长因子(platelet derived growth factor, PDGF)以及 TNF- $\alpha$  和 IL-1 $\beta$  等促炎因子的表达, 抑制大鼠模型的纤维化进程, 促进增殖和血管生成, 这表明 hAESC 在子宫内微环境中以旁分泌形式发挥作用<sup>[45-46]</sup>。此外, Yu 等<sup>[73]</sup>发现激活 Notch 信号能够加速 hAD-MSCs 向子宫内上皮细胞的分化, 促进子宫内膜的修复和再生。

在卵巢功能低下和年龄相关性卵巢储备功能低下的动物模型中进行的研究表明, hAD-MSCs 移植能够有效增加抗苗勒氏管激素和雌二醇的水平, 增加卵泡数量并提升卵母细胞状态, 同时显著抑制 GC 凋亡, 明显改善卵巢功能, 这可能与调节丝裂原活化蛋白激酶(mitogen-activated protein kinase, MAPK)信号通路有关<sup>[74-75]</sup>。

**3.1.2 神经系统损伤** 阿尔茨海默病(Alzheimer's disease, AD)和中风是常见的神经系统疾患, 目前尚无令人满意的治疗方法。研究发现, 移植 hAESC 和 hAD-MSC 能够减轻淀粉样蛋白沉积, 上调抗氧化酶和丙二醛的水平, 同时提升氧化物歧化酶的活性, 改善 AD 模型小鼠的氧化应激水平和认知缺陷<sup>[76-77]</sup>。微阵列分析表明, 经马鞭草素处理的 hAESC 治疗 AD 的能力显著提升<sup>[78]</sup>。Nazarinia 等<sup>[79]</sup>研究发现, hAD-MSCs 可以缓解脑缺血(intracerebral hemorrhage, ICH)大鼠模型脑组织运动和感觉功能障碍并抑制细胞凋亡, 发挥神经保护作用, 该机制可能与恢复 mTOR 活性和抑制自噬有关。与仅用 hAD-MSCs 治疗的大鼠相比, 利用脑源性神经营养因子基因转染的 hAD-MSCs 对 ICH 大鼠行为功能障碍的缓解更为迅速有效<sup>[80]</sup>。Kuramoto 等<sup>[81]</sup>研究证实, hAD-MSCs 移植能够有效改善 ICH 诱导的小鼠神经行为障碍, 其潜在机制可能是减少 ICH 损伤区域巨噬细胞/小胶质细胞数量, 并抑制巨噬细胞/小神经胶质功能, 但不会对脾脏中 CD11b<sup>+</sup>CD45<sup>+</sup>巨噬细胞造成影响, 这表明 hAD-MSCs 给药不会改变全身的免疫反应状态。

脊髓损伤(spinal cord injury, SCI)模型大鼠的研究证实, hAD-MSCs 移植后能够迁移至脊髓损伤处, 显著减少了 ED1<sup>+</sup>巨噬细胞/小胶质细胞和 caspase-3<sup>+</sup>细胞的数量, 并下调 TNF- $\alpha$ 、IL-6 和 IL-1 $\beta$  等炎性细胞因子

的水平,促进神经保护所需营养因子分泌以及轴突再生和血管生成,从而加速脊髓损伤后的功能恢复和肠功能障碍的缓解<sup>[82-83]</sup>。值得注意的是,hAD-MSCs 仅在 SCI 慢性期可以显著抑制脊髓中巨噬细胞的浸润<sup>[82]</sup>。

此外,hAMSCs 对于围产期脑损伤和帕金森病(Parkinson's disease,PD)等神经系统疾病亦有良好疗效。Leaw 等<sup>[84]</sup>研究证实,hAESC 释放的细胞因子能够抑制 M1 表型巨噬细胞的活性并增强其吞噬能力,抑制细胞凋亡和星形胶质细胞的增生,促进小胶质细胞的增生。此外,对暴露于脂多糖的待产母羊应用 hAESC 可抑制产后胎儿的炎症反应并减轻相关脑损伤<sup>[85]</sup>。hAESC 在抑制炎症因子释放的同时,能够促进多种神经营养因子、生长因子和抗炎因子的分泌,以增强多巴胺能神经元的存活能力和轴突再生,保护 PD 模型小鼠受损的神经系统<sup>[44]</sup>。

**3.1.3 肾脏疾病** hAMSCs 在治疗肾衰竭、急性肾损伤等肾脏疾病领域显示出了良好的潜力。研究发现,hAD-MSCs 能够迁移至慢性肾病大鼠模型损伤的肾组织,上调生长因子水平,促进肾细胞再生,抑制上皮间充质转化,改善肾功能<sup>[16]</sup>。在肾衰竭小鼠中注射 hAD-MSCs 可以显著降低血清肌酐、尿素和尿素氮水平,抑制肾纤维化<sup>[86]</sup>。Ren 等<sup>[87]</sup>研究证实,hAESC 及其来源的外泌体可以减少细胞凋亡,防止管周毛细血管损失,调节肾脏局部免疫反应,促进巨噬细胞向 M2 表型极化以降低急性肾损伤小鼠的死亡率,并改善肾功能,该机制可能与 hAESC 衍生的外泌体减轻肾缺血再灌注损伤有关。

**3.1.4 肺部疾病** 肺纤维化(pulmonary fibrosis,PF)是由肺损伤或肺部疾病引起的慢性间质性肺病的终末期改变,是一种难治性的呼吸系统疾病,目前尚无有效的治疗手段<sup>[88]</sup>。研究表明,将 hAD-MSCs 注射到白烟吸入诱导的小鼠 PF 模型中可缓解小鼠的肺损伤和肺纤维化,降低 CT 评分,增加肺表面活性物质相关蛋白(pulmonary surfactant-associated protein,SP)-A、SP-C 和 SP-D 的表达,同时下调 IL-6、TNF- $\alpha$  和 TGF- $\beta$ 1 的水平,促进 IL-10 的表达,有效改善小鼠肺部的炎症反应<sup>[89]</sup>。在博来霉素诱导的小鼠 PF 模型中,hAD-MSCs 促进了巨噬细胞向 M2 表型极化,并降低巨噬细胞和树突状细胞的抗原呈递能力<sup>[90]</sup>。此外,hAD-MSCs 能够有效抑制肺部 B 细胞的募集、驻留和成熟及抗原呈递能力<sup>[90]</sup>。hAESC 衍生的外泌体通过调节炎症和纤维化途径发挥保护肺的作用<sup>[91]</sup>,与抗纤维化药物 Serelaxin 联用可

增强治疗效果<sup>[92]</sup>。

支气管肺发育不良是早产儿的常见并发症之一。Li 等<sup>[15]</sup>研究证实,气管输注 hAD-MSCs 能够有效降低促炎细胞因子释放,抑制炎症细胞浸润,上调酰基氨基酸水解酶水平,改善高氧诱导的新生大鼠肺损伤。

**3.1.5 肝脏疾病** 肝纤维化是多种慢性肝病共有的病理过程,主要表现为肝内结缔组织异常增生形成瘢痕组织<sup>[93]</sup>。hAESC 能够有效减轻非酒精性脂肪性肝炎小鼠模型的肝纤维化以及炎症反应,这与抑制星状细胞的活化以及下调肝脏中巨噬细胞数量有关,其可能的机制是 hAESC 抑制 pSMAD 2/3 信号通路的激活<sup>[47]</sup>。此外,hAESC 能够促进肝祖细胞向肝细胞分化,以维持肝细胞的增殖,并减少 F4/80<sup>+</sup> 巨噬细胞的数量,从而保护受损的肝组织<sup>[94]</sup>。

肝硬化是慢性肝脏疾病的终末期阶段,可合并多种并发症,危及患者生命。Pietrosi 等<sup>[95]</sup>研究发现,hAMSCs 通过旁分泌机制改善正弦细胞表型,显著下调细胞间黏附分子-1 和血管性血友病因子的水平,并促进血管分泌因子 Hgf 和 Wnt2 的表达,调节炎症反应状态,发挥抗炎作用,抑制氧化应激反应,从而改善肝硬化大鼠模型的肝脏微循环功能障碍并降低门静脉高压。hAESC 在体内外均能分化为功能性肝细胞样细胞,将其移植于肝硬化小鼠模型后显示出了治疗效果<sup>[58]</sup>。以上说明 hAMSCs 可作为干细胞治疗肝硬化的细胞来源。

**3.1.6 糖尿病及糖尿病创面** 糖尿病(diabetes mellitus,DM)体外研究发现,hAESC 能够有效保护缺氧诱导的胰岛  $\beta$  细胞损伤,促进胰岛血运重建,提高胰岛逆转高血糖的能力<sup>[96]</sup>。糖尿病创面由于持续的高血糖状态而难以愈合,并容易反复破溃,是目前临床治疗的难点之一。Shu 等<sup>[97]</sup>研究证实,hAESC 能够促进角化细胞和成纤维细胞的迁移和增殖,增加成纤维细胞的胶原合成和  $\alpha$ -平滑肌肌动蛋白的表达,并抑制高糖诱导的角质形成细胞和成纤维细胞内活性氧和  $\beta$ -半乳糖苷酶的表达。此外,hAESC 还能够抑制 RAGE/P21 信号通路,下调表皮和真皮中 P21<sup>+</sup> 细胞的水平,增加 PCNA<sup>+</sup> 细胞的数量<sup>[97]</sup>。hAESC 通过旁分泌效应可以促进 M2 表型巨噬细胞的极化,加速内皮细胞的迁移、增殖和血管形成,抑制促炎因子 IL-1 $\beta$ 、IL-6 和 TNF- $\alpha$  的表达,上调促愈合细胞因子 VEGF、TGF- $\beta$ 1 和 IGF-1 的水平,从而有利于糖尿病创面愈合<sup>[98]</sup>。

**3.1.7 癌症** 目前,癌症的有效防治依然是临床医学

的难点。研究表明,hAD-MSCs 可以阻滞细胞周期,增加细胞凋亡,抑制基质金属蛋白酶 7 信号诱导的上皮间充质转化,以此抑制胰腺导管腺癌细胞 PANC1 系的增殖,延缓肿瘤生长和侵袭的速度<sup>[60]</sup>。Liu 等<sup>[17]</sup>研究证实,hAD-MSCs 高表达的 Dickkopf-3、Dickkopf-1 和胰岛素样生长因子结合蛋白-3 分别通过抑制 Wnt/ $\beta$ -catenin 信号通路和 IGF-1R 介导的 PI3K/AKT 信号通路显著抑制 Hepg2 细胞增殖并促进其凋亡。将 hAECs 作为疫苗接种于结肠癌小鼠模型,发现小鼠系统性和脾脏细胞毒性 T 细胞数量增加,并诱导对肿瘤细胞的交叉保护细胞毒性反应,导致肿瘤体积显著减小,这为将 hAECs 作为一种有效的癌症预防疫苗提供了依据<sup>[99]</sup>。此外,Rolfo 等<sup>[100]</sup>发现 hAD-MSCs 不仅能够诱导细胞周期停滞,还可通过调节宿主微环境来抑制前列腺癌细胞的生长。

**3.1.8 皮肤疾病** 目前研究发现,hAMSCs 对于皮肤损伤的愈合亦有积极作用。hAD-MSCs 和 hAECs 均可通过上调含胶原三螺旋重复蛋白 1、赖氨酰氧化酶样蛋白 2 和重组人半乳糖凝集素 1 蛋白的水平,显著增强角质形成细胞的迁移和分化,从而促进伤口愈合,该调节机制可能与 c-Jun 氨基末端激酶 (c-Jun N-terminal kinase, JNK) 信号通路有关<sup>[101]</sup>。Li 等<sup>[102]</sup>研究表明,hAMSCs 治疗皮肤损伤与 GSK3 $\beta$ / $\beta$ -catenin 和 PI3K/AKT 信号通路的激活密切相关。此外,应用 hAMSCs 后再生创面中 I 型胶原束的水平明显降低,与之相反,III 型胶原束的水平增加,这表明 hAMSCs 可以在不延

迟伤口愈合的情况下发挥抗纤维化作用<sup>[103]</sup>。

**3.1.9 肠道疾病** 克罗恩病 (Crohn's disease, CD) 是一种多因素导致的与免疫相关的慢性进展性胃肠道炎症疾病<sup>[104]</sup>。结肠炎和放射性直肠炎大鼠模型实验表明,hAD-MSCs 移植能够显著抑制单核细胞/巨噬细胞和 T 细胞的浸润,并下调 CXC 趋化因子配体 1、C-C 基序趋化因子配体 2 以及 TNF- $\alpha$ 、IL-6 等促炎因子的水平<sup>[105-106]</sup>。此外,hAD-MSCs 对于 p53 的转录活性、Caspase-3/7 的活性以及 p21 的表达亦展现出了抑制作用<sup>[106]</sup>。

**3.1.10 其他疾病** 急性移植物抗宿主病是异基因造血干细胞移植的致命并发症,是移植后导致患者非复发死亡的主要因素<sup>[107]</sup>。Tago 等<sup>[108]</sup>研究发现,hAD-MSCs 移植能够抑制急性移植物抗宿主病小鼠模型的免疫活性,调节细胞因子和趋化因子的分泌,与人骨髓来源间充质干细胞相比,hAD-MSCs 抑制 T 细胞活化和增殖的效应更强。此外,hAMSCs 对于硬化性胆管炎和多发性硬化症亦有显著疗效。Sugiura 等<sup>[109]</sup>研究发现,静脉注射 hAD-MSCs 可以降低硬化性胆管炎大鼠模型的细胞角蛋白 19、基质金属蛋白酶-9、TNF- $\alpha$  和单核细胞趋化蛋白-1 的表达,进而抑制胆道增生、胆周纤维化和炎症反应。多发性硬化症小鼠模型研究证实,hAECs 能够抑制中枢神经系统 CD3<sup>+</sup> T 细胞和 F4/80<sup>+</sup> 单核细胞/巨噬细胞的浸润,上调 TGF- $\beta$  和前列腺素 E2 的水平,显著抑制脾细胞增殖,有效缓解疾病症状<sup>[110]</sup>。

表 2 hAMSCs 在各种疾病中的生物学效应

Table 2 Biological effects of hAMSCs in various diseases

疾病	生物学功能
妇科疾病:POF <sup>[41,48,71]</sup> 、IUA <sup>[45-46,73]</sup> 、卵巢功能不全 <sup>[74]</sup> 、年龄相关性卵巢储备功能低下 <sup>[75]</sup>	抑制 GC 凋亡;促进血管生成;促进子宫内膜增殖和修复;改善子宫内微环境;增加卵泡数量,提高卵母细胞质量
神经系统疾病:AD <sup>[76-78]</sup> 、中风 <sup>[79-81]</sup> 、SCI <sup>[82-83]</sup> 、围产期脑损伤 <sup>[84-85]</sup> 、PD <sup>[44]</sup>	促进轴突再生和血管生成;调节炎症因子的释放以抑制神经炎症;免疫调节脑小胶质细胞以改善炎症;抑制细胞凋亡和星形胶质细胞的增生;分泌神经营养因子以保护神经;改善氧化应激反应
肾脏疾病:慢性肾病 <sup>[16]</sup> 、肾衰竭 <sup>[86]</sup> 、急性肾损伤 <sup>[87]</sup>	调节肾脏局部免疫应答,降低 IL-6 表达;促进肾细胞再生;减轻肾缺血再灌注损伤
肺部疾病:PF <sup>[89-92]</sup> 、支气管肺发育不良 <sup>[15]</sup>	改善高氧诱导的新生儿肺损伤;促进肺损伤修复;抑制纤维化途径;通过抑制 B 细胞反应等途径来调节炎症反应
肝脏疾病:肝纤维化 <sup>[47,94]</sup> 、肝硬化 <sup>[58,95]</sup>	调节炎症反应;抑制星形胶质细胞的激活;抑制氧化应激
糖尿病及糖尿病创面:DM <sup>[96]</sup> 、糖尿病创面 <sup>[97-98]</sup>	抑制炎症反应;促进胰岛的血运重建并提升胰岛逆转高血糖的能力;促进内皮细胞的迁移、增殖和血管形成,促进创面再上皮化和肉芽组织形成;促进 M2 表型巨噬细胞的极化

(续表 2)

疾病	生物学功能
癌症:胰腺导管腺癌 <sup>[60]</sup> 、肝细胞癌 <sup>[17]</sup> 、结肠癌 <sup>[99]</sup> 、前列腺癌 <sup>[100]</sup>	通过促进细胞凋亡和抑制细胞增殖来延缓肿瘤的生长和侵袭;导致系统性和脾脏细胞毒性 T 细胞数量增加,并诱导交叉保护性细胞毒性反应;改善肿瘤微环境,抑制炎症反应
皮肤疾病:皮肤损伤 <sup>[101-103]</sup>	抑制细胞凋亡和促进皮肤细胞增殖;增强角质形成细胞的迁移和分化
肠道疾病:CD <sup>[105]</sup> 、放射性直肠炎 <sup>[106]</sup>	抑制单核细胞/巨噬细胞和 T 细胞的浸润;抑制促炎因子的释放
急性移植物抗宿主病 <sup>[108]</sup>	调节细胞因子和趋化因子的分泌
硬化性胆管炎 <sup>[109]</sup>	抑制胆道增生、胆周纤维化和炎症反应
多发性硬化症 <sup>[110]</sup>	抑制中枢神经系统 CD3 <sup>+</sup> T 细胞和 F4/80 <sup>+</sup> 单核细胞/巨噬细胞的浸润

3.2 hAMSCs 的临床应用

随着医疗技术的发展,hAMSCs 的临床应用也逐步突破了传统治疗的局限性。Qin 等<sup>[59]</sup>在临床治疗严重尿毒症出现钙化防御伴持续性皮肤缺血及腿部大面积恶臭溃疡的患者时,由于症状和体征持续恶化,传统疗法已难以发挥疗效,因此被批准应用 hAD-MSCs 进行治疗。通过静脉注射和局部肌肉注射将 hAD-MSCs 应用于患者腿部溃疡处,1 个月后皮肤活检显示真皮内有成熟的非钙化血管再生,随访 15 个月时发现患者的皮肤软组织再生,骨和矿物质代谢的血液标志物较前改善,20 个月后经过再上皮化的受损部位恢复了完整性。Lim 等<sup>[111]</sup>首次在临床将同种异体的 hAESC 静脉注射给支气管发育不良的早产儿,观察其即刻安全性,发现首个婴儿发生了短暂的心肺损害,通过改善给药方式以及降低细胞浓度( $2.5 \times 10^5$  hAESC/mL 盐水)和输注速率(30 min 内输注完毕),在随后的 5 名婴儿中未发生与细胞给药相关的不良事件,表明 hAESC 可以安全地输送到支气管发育不良的婴儿体内。该研究团队在两年内持续对 5 名婴儿进行随访,在此期间未观察到可归因于 hAESC 给药带来的长期不良事件,表明应用低剂量的 hAESC 具有长期安全性<sup>[112]</sup>。该研究团队注册了一项更广泛的多中心剂量递增临床试验 (ACTRN12618000920291),以进一步确认 hAESC 静脉注射治疗支气管肺发育不良的安全性和有效性。目前已有多项临床试验被注册用于评估 hAMSCs 治疗临床疾病的有效性和安全性,如 hAESC 静脉注射治疗代偿性肝硬化的 I 期研究 (ACTRN12616000437460)<sup>[113]</sup>;临床试验 (ACTRN12620000676910p) 被注册用于评估使用 hAESC 治疗新冠肺炎相关呼吸衰竭和多器官并发病的疗效。随着这些临床试验的开展,hAMSCs 的临床疗效和安全性也将备受关注,未来有望作为新型替代疗法应用于临床。

4 总结与展望

hAD-MSCs 和 hAESC 所具有的独特优势,使其成为临床应用的重要潜在选择。HAMSCs 的低免疫原性,可以保护其免受宿主免疫系统的清除。目前尚无明确证据表明 hAMSCs 具备致瘤性。胎盘是一次性组织,从胎盘羊膜中可以获取大量 hAMSCs,且避免了伦理问题。hAMSCs 具有高分化潜能,可以分化为心肌细胞、肝细胞、肾细胞、表皮细胞、神经细胞和功能性胰岛素分泌细胞。同时,hAMSCs 具有强大的旁分泌效应,其分泌的多种细胞因子和外泌体在多种疾病的治疗中显示出了巨大潜力。hAMSCs 的上述特性以及生物学效应应使其成为细胞治疗和再生医学范畴中极其重要的组成部分。此外,越来越多的动物实验验证了 hAMSCs 在不同疾病中的治疗效果以及安全性,如妇科疾病、肝脏疾病、肾脏疾病、神经系统疾病、癌症、糖尿病以及皮肤疾病等,这些结果将为 hAMSCs 的临床应用提供证据支持。

hAMSCs 的临床应用尚存挑战。目前,针对 hAMSCs 的研究主要集中于动物模型,其中以小鼠模型居多,小鼠体内微环境与人体有很大不同,因此无法将动物实验的结果直接转化于人体。但是,当细胞被注射到人体极其复杂的微环境中,可以预测到 hAD-MSCs 和 hAESC 在衍生条件培养基中的特征和生物学效应在人体内大概率会向可预测的方向发展。此外,hAMSCs 的诱导增殖和分化的过程极其复杂,诱导方法有待改进。基于以上原因,今后应进一步优化培养和分化方法,有针对性地选择外源诱导物,以及通过广泛的 hAD-MSCs 和 hAESC 治疗不同疾病的临床试验以促进其临床应用。在临床试验中应特别注意 hAMSCs 的安全性,密切观察过敏和排斥反应以及肿瘤形成等。值得期待的是,已有研究证实了 hAMSCs 的临床有效性

和短期安全性。随着技术的发展和研究的深入, hAMSCs 的治疗潜力也将得到更大程度地挖掘。

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## Biological Characteristics and Application Potential of Human Amnion-derived Stem Cells

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**Abstract** Stem cell transplantation has been regarded as an attractive alternative therapy in medical research and clinical trials. It is confirmed by numerous experimental model studies that stem cell transplantation can repair damaged or degraded tissues and restore their function. However, immune rejection, ethical barriers and tumorigenicity are still the difficulties in the practical applications of stem cell therapy. In recent years, perinatal stem cells have been paid increasing attention by researchers as a potential source of cells to solve the above-mentioned problems. Compared with other stem cells, human amniotic stem cells (hAMSCs) show significant advantages in these aspects. Animal experiments have found that hAMSCs have high differentiation potential and immunomodulatory activity, demonstrating great potential for the treatment of gynecological diseases, neurological diseases, kidney diseases, lung diseases, skin diseases, diabetes, cancer and other diseases. At present, with the progress of science and to meet urgent clinical needs, the clinical application of hAMSCs has gradually broken through the limitations of traditional treatment. Many clinical studies have been registered to study the effectiveness and safety of hAMSCs, and some studies have been completed as planned, which has important guiding significance for clinical practice. Therefore, this article reviews the biological research progress and application potential of hAMSCs, in order to provide theoretical basis for experimental research and clinical application of hAMSCs.

**Key words** Human amniotic-derived stem cell Human amniotic epithelial stem cells Human amnion-derived mesenchymal stem cells Biological characteristics