间充质干细胞靶向治疗早发性卵巢功能不全的现状及前景

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摘要 早发性卵巢功能不全(premature ovarian insufficiency, POI)既往又称卵巢早衰(premature ovarian failure, POF), 是一种复杂的内分泌疾病, 常见于40岁以下的女性。其特点是卵巢功能在40岁前停止, 对女性生理及心理均有较大影响, 最大的影响是导致不孕。目前可用于治疗POI的方法有限, 而且往往效果不佳。干细胞移植是近年来国内外学者研究较多的一种新的治疗POI的方法, 特别是对于有生育需求的POI女性来说, 干细胞疗法是最终的治疗选择。迄今为止, 用于POI治疗的最成熟的干细胞是间充质干细胞(mesenchymal stem cells, MSCs), 其有效性已得到临床前和临床研究的证实, 组织工程和干细胞治疗策略已成为恢复卵巢功能和改善POI女性生活质量的有前途的方法。该综述旨在全面综述POI的研究现状, 重点阐述不同来源的MSCs用于治疗POI的临床应用进展及发展前景, 包括骨髓来源、脐带来源、脂肪来源及经血来源的MSCs通过微创、靶向治疗后的效果, 以及不同来源的MSCs对卵巢功能恢复的比较。此外, 该文还讨论了模拟自然卵巢微环境并支持卵巢细胞、卵泡生长和成熟的生物材料及支架的开发, 且强调了与组织工程和基于干细胞的POI治疗相关的挑战和伦理考虑, 并提出了解决这些问题的潜在方案。

关键词 早发性卵巢功能不全; 卵巢储备功能; 卵巢早衰; 间充质干细胞; 靶向治疗; 组织工程; 生物材料

The Current Status and Prospects of Mesenchymal Stem Cells in Targeted Therapy for Premature Ovarian Insufficiency

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Abstract POI (premature ovarian insufficiency), also previously known as POF (premature ovarian failure), is a complex endocrine disease, which is common in women under 40 years old. Its characteristic is that the ovarian function stops before the age of 40, which has a greater impact on the female's physiology and psychology, and the biggest influence is to lead to infertility. At present, the methods that can be used to treat POI are limited, and the effect is often not good. Stem cell transplantation is a new method for the treatment of POI studied by scholars at home and abroad in recent years. Especially for female with POI in need of reprodution, stem cell therapy is the ultimate choice. To date, the most mature stem cell therapy for POI therapy is MSCs (mesenchymal stem cells), and its efficacy has been confirmed by preclinical and clinical studies. Tissue engineering and stem cell therapy strategies have become promising methods to restore ovarian function and improve the quality of life of female

收稿日期: 2024-05-31 接受日期: 2024-07-23

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Received: May 31, 2024 Accepted: July 23, 2024

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with POI. This review aims to comprehensively review the research status of POI, focusing on the progress and development prospect of MSCs from different sources for clinical application to treat POI, which includes efficacy of MSCs derived from bone marrow, umbilical cord, adipose and menstrual blood after minimally invasive targeted therapy, and the comparison of ovarian function recovery between different sources of MSCs. Additionally, this paper discusses the development of biomaterials and scaffolds that mimic the natural ovarian microenvironment and support the growth and maturation of ovarian cells and follicles. Furthermore, the review highlights the challenges and ethical considerations associated with tissue engineering and stem cell-based therapies for POI, and proposes potential solutions to address these issues.

Keywords premature ovarian insufficiency; ovarian reserve function; premature ovarian failure; mesenchymal stem cells; targeted therapy; tissue engineering; biomaterials

早发性卵巢功能不全(premature ovarian insufficiency, POI)既往又称卵巢早衰(premature ovarian failure, POF)[1], 是最常见的女性生殖衰老性疾病, 但同时又是一种复杂的内分泌疾病。常见于40岁以下女性[2], 发病率仅为0.9%~3.0%, 可因年龄、种族及地区经济水平而异[3]。20岁女性发生POI的可能性为0.01%, 30岁为0.1%, 40岁为1%, POI以卵巢功能障碍为特征, 导致40岁前卵巢功能过早终止[4]。与POI相关的另两个疾病状态为卵巢储备功能减退(diminished ovarian reserve, DOR)和卵巢早衰(premature ovarian failure, POF)[5], DOR是指由于卵母细胞的数量减少和(或)质量下降, 导致卵巢功能减退所引起的生育力下降, 无40岁以下的年龄限制, 常伴有抗苗勒氏管激素(anti-Müllerian hormone, AMH)水平降低、促卵泡激素(follicle-stimulating hormone, FSH)水平升高及卵巢的窦卵泡计数(antral follicle count, AFC)减少; 而POF的特征是女性40岁以前出现闭经、FSH>40 U/L和雌激素水平降低, 并伴有不同程度的低雌激素症状, 是POI的终末阶段。据估计, POI或早绝经影响了10%的女性人口, 尽管这些女性年龄很小, 但仍会有怀孕问题[6]。本综述旨在全面综述POI的研究现状, 重点阐述不同来源的间充质干细胞(mesenchymal stem cells, MSCs)用于治疗POI的临床应用进展及发展前景, 包括骨髓来源、脐带来源、脂肪来源及经血来源的MSCs通过微创、靶向移植治疗后的效果, 以及不同来源的MSCs对卵巢功能恢复的比较。

1 POI的病因

POI的病因通常包括遗传缺陷、X染色体紊乱、脆性X基因前突变、常染色体异常和感染因素[1], 还涉及心理、免疫、酶代谢、环境等因素。POI的遗传病因具有高度异质性, 可能涉及各种遗传缺陷的相互作用, 染色体异常、基因多态性和单基因突变已被认为是POI的病因[7]。与POI有关的X染色体缺陷表明, 该染色体对正常卵巢功能至关重要, 因为这些缺陷会导致POI的发生[2]。而有女性接种HPV疫苗后发生POF的报道, 这可能是一个少见病因, 其中90%的病例病因不明[8]。近年来, 医源性导致POI的常见原因是用于治疗恶性或良性疾病的放疗和化疗, 放射治疗后发生POI的风险取决于放射治疗的区域(盆、腹腔或全身)以及剂量和年龄, 而化疗的性腺毒性作用主要取决于药物和剂量, 也与年龄有关[3]。其他如腹腔镜手术、卵巢打孔术、卵巢良性包块及卵巢巧克力囊肿的手术等都有可能导致POI[3,9-10]。据文献报道并统计, 约30%的POI患者存在自身免疫功能异常, 常伴发自身免疫性疾病或其他内分泌疾病, 自身免疫性POI患者存在明显免疫功能失衡[11]。其他多种因素, 包括卵子功能异常、卵泡闭锁加速和卵泡功能降低也可导致POI的发生。事实上, 在50%的不明原因不孕患者中已经检测到具有多个靶点的抗卵巢抗体, 并且有研究报道了自身抗体的存在增加了自身免疫性疾病患者发生POI的风险[12]。然而, 大多数POI是特发性的[12-13], 这就需要进一步探讨并制定解决这一问题的新策略。

2 POI的临床特征

POI常见临床特点为继发性闭经, 伴有不孕和低雌激素。除了情绪不稳定、心情烦躁、月经失调、性欲减退等相关症状外, 短期内还会出现潮热、盗汗、阴道干涩、性交不适或疼痛、皮肤失去弹性、子宫和乳房变小、睡眠障碍、焦虑、认知能力下降

及抑郁等围绝经期症状; 远期并发症包括心血管疾病风险增加、骨骼健康受损、神经认知障碍[7,10]以及严重的社会心理后遗症, 甚至增加过早死亡的风险, 影响女性的寿命, 这对女性的身心健康都有较大的影响。当然, POI对生育功能最大的影响是导致不孕[14]。

3 POI的诊断

诊断指标通常包括雌激素水平低、FSH>25 U/L、卵巢储备明显减少(AMH低下)。因此, 迄今为止提出的最合适的诊断标准是欧洲人类生殖和胚胎学学会(European Society of Human Reproduction and Embryology, ESHRE) POI指南制定小组的指南, 即至少4个月的少/闭经和间隔>4周的两次FSH水平升高>25 U/L[3]。腹腔镜检查显示POI患者缺乏卵泡的发育, 卵巢皮质皱缩, 体积缩小, 质地变硬。

4 POI卵巢的组织学特征

卵巢组织学根据POI表现型而变化。组织学可发现两种类型的POI: 一种卵巢较小, 没有卵泡; 另一种卵巢大小正常, 部分卵泡成熟。然而, 大多数窦卵泡在组织学上是异常的, 如卵泡闭锁或从部分脱落到颗粒细胞完全缺失。在光学显微镜下, 卵巢内的髓质和外部的皮质区没有明确的分界, 并融合在一起, 纤维成分和细胞的分布不均匀, 而在光镜和透射电子显微镜下, 可见卵巢内致密的结缔组织和少量白化小体[15]。研究表明, POI患者AMH的可检出性可能与其卵巢中存在≥15个卵泡显著相关[16]。尽管各组病例数在统计学上不够充分, 但有≥15个卵泡女性的平均血清AMH水平为2.16 ng/mL, 而无卵泡和≤5个卵泡女性分别为0.42 ng/mL和0.33 ng/mL。因此, 尽管超声检查中看不到卵泡, 但通过评估血清AMH水平, 可筛选出最有可能生长卵泡的POI患者[16]。

研究揭示了生殖衰老和其他器官衰老之间相互关联的复杂性和偶然的不确定性[17]。最近, 研究都阐述了炎症性衰老和POI之间的密切联系[18-19], POI患者卵巢活检样本显示, 卵巢中存在淋巴细胞浸润和免疫反应[20]。在生殖领域, 预防卵巢衰老、提高卵巢功能的治疗方法以及由炎症引起的卵巢功能恶化等重要研究课题, 是当前的研究热点[4]。

5 POI的治疗现状

POI在西医方面曾应用或尝试的治疗方法有性激素补充治疗、脱氢表雄酮治疗、免疫抑制治疗、干细胞移植、基因治疗、辅助生殖技术等, 而中医治疗包括针灸治疗、中药膏方、中药汤剂等。目前临床上常用的主要以激素替代治疗(hormone replacement therapy, HRT)、免疫调节、中医中药治疗, 虽可缓解部分临床症状, 但难以从根本上修复卵巢的结构和功能[3]。HRT虽然是目前临床治疗POI的主要方法, 但长期使用HRT会增加血栓性疾病、乳腺癌、卵巢癌和子宫内膜癌的风险。因此, 在开始治疗之前, 有必要告知HRT在自然绝经前不会增加患乳腺癌的风险, 但禁用于乳腺癌幸存者[1,10,14]。为了找到安全有效的POI治疗方案, 许多科学家已经探索了创新的技术和新的治疗策略[4,14,21], 主要包括体外激活(*in vitro* activation of primordial follicles, IVA)、生物材料策略、干细胞和外泌体疗法、线粒体激活和卵巢内注射富血小板血浆(platelet-rich plasma, PRP)[22], 还有卵巢组织移植、人工卵巢构建、人工配子和线粒体替代疗法等, 但受到成本高、实用性差和伦理学等方面的限制[23]。

干细胞移植是近年来国内外学者研究较多的一种新的治疗POI的方法, 特别是对于有生育要求的POI女性来说, 干细胞疗法是最后的治疗选择。迄今为止, 用于POI治疗的最成熟的干细胞是间充质干细胞(mesenchymal stem cells, MSCs)[1,24]。MSCs治疗生殖系统疾病的有效性已得到临床前和临床研究的证实, 为POI导致的不孕不育和改善女性生殖健康带来了巨大希望, 也为全球POI患者带来福音[1,13,24-25]。

6 MSCs治疗POI的机制

MSCs是成熟的多能干细胞, 具有高分化潜能, 可从骨髓、脂肪、脐带、羊水、胎盘和皮肤等各种组织来源中分离出来。在体外培养扩增能力强, 并具有低免疫原性、低致瘤性、无伦理问题、免疫调节功能、在趋化作用下可促进伤口愈合等诸多优点。多项关于MSCs治疗POI的基础研究已经阐述了其良好的治疗效果, 也深入探讨了MSCs移植的“归巢效应”, 此外, MSCs移植还具有促进颗粒细胞(granule cells, GCs)增殖、抑制GCs凋亡、促进血管生成并分泌多种细胞因子、调节卵巢微环境、抗炎及免疫调节、抗组织纤维化、促进卵巢再生修复和改善子宫内膜容受性等作用机制[4,13,24,26-27](图1)。临床研究表明, 自体MSCs移植可以恢复月经, 缓解更年期症

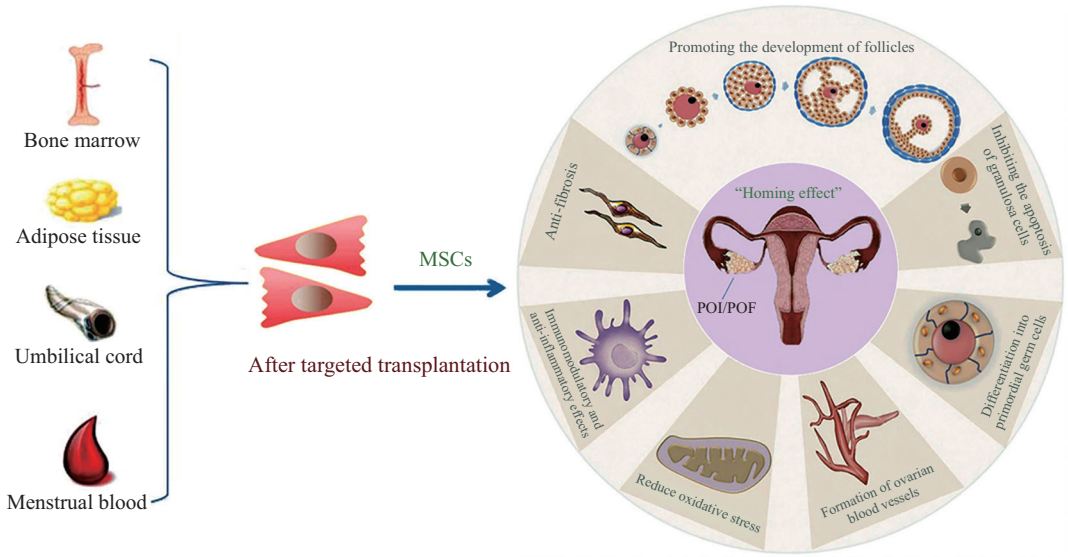

图1　4种不同来源的间充质干细胞(MSCs)临床治疗POI/POF的作用机制(根据参考文献[13]修改)

Fig.1　The mechanisms of treating POI/POF with four kinds of MSCs from different sources (modified from the reference [13])

状，改善卵巢功能，帮助患者受孕[4,13]，MSCs的迁移和分泌特性增强了它们的治疗活性[24]，有利于POI患者卵巢功能的恢复。其他研究报道，在接受MSCs治疗的和一部分接受MSCs骨髓移植的因化疗和全放射治疗所导致的POF，这些女性的卵巢功能和妊娠都得到了改善[28]。

最近的研究发现，人体多种组织来源的MSCs都可释放一定水平的前列腺素E2(prostaglandin E2, PGE2)，而PGE2对MSCs的生物学活性与功能也存在明显影响[29-30]。PGE2通过与其受体结合，不但参与多种免疫细胞和炎症反应调节，影响血流量和分泌功能，还能影响MSCs及生殖细胞的功能和发育。PGE2等一些炎症介质也影响脂肪、骨髓和脐带MSCs免疫调节的可塑性[31]，研究结果表明，PGE2可以促进MSCs的迁移，EP2介导的FAK和ERK1/2激活对于PGE2诱导的MSCs迁移至关重要，这表明EP2受体和FAK/ERK通路的激活可能是一种有希望的策略，可以提升MSCs的归巢效率，从而增强MSCs移植的治疗潜力[32]。

7　MSCs靶向移植治疗POI的几种方案及比较

几乎所有临床前研究都表明，从不同来源获得的干细胞几乎都有望治疗POI，最早进行的几项人体临床试验都是通过骨髓间充质干细胞(bone marrow mesenchymal stem cells, BMMSCs)进行的[9,33-35]。目前，用于临床应用治疗女性POI的人源MSCs主要有BMMSCs、脐带间充质干细胞(umbilical cord mesenchymal stem cells, UCMSCs)、ADMSCs及经血间充质干细胞(mesenchymal stem cells derived from menstrual blood, MenSCs)(图1)，其他类型的MSCs仅限于动物模型或实验室研究阶段[6,36-37]。

7.1　自体骨髓干细胞(autologous bone marrow mesenchymal stem cells, ABMMSCs)靶向治疗

自2016年开始，EDESSY等[33]、SHREYA等[34]、GABR等[35]及TANDULWADKAR等[38]研究团队通过腹腔镜卵巢靶向移植及一侧卵巢动脉介入等方式将ABMMSCs或结合富血小板血浆(platelet-rich plasma, PRP)移植到卵巢，治疗POI/POF，在月经恢复、妊娠结局等方面都取得良好效果(表1)。

7.2　自体脂肪间充质干细胞(adipose-derived mesenchymal stem cells, ADMSCs)靶向治疗

2021年，MASHAYEKHI等[37]首次在9例女性中进行ADMSCs卵巢内移植治疗特发性POF，并评估其安全性、可行性和有效性。经阴道超声引导，进行卵巢靶向移植。但其中有2例因卵巢体积缩小、组织硬，难以固定和定位，改用腹腔镜卵巢靶向移植(表1)。研究表明，临床应用ADMSCs治疗1年以上是安全的、可行的、有效的(FSH下降，月经恢复)，双侧卵巢靶向移植效果可能更好。

7.3　自体MenSCs靶向治疗

ZAFARDOUST等[39]于2018年启动了一项大有

表1 4种不同来源的间充质干细胞(MSCs)临床治疗POI/POF的几种方案及结局

Table 1 Several strategies and outcomes of treating POI/POF with four kinds of MSCs from different sources

应用研究 Application study	病例数/年龄 Number of cases /age	MSCs来源 Source for MSCs	MSCs移植方式 MSCs transplantation pathway	月经恢复/妊娠结局 Menstrual recovery/pregnancy outcome	参考文献 References
EDESSY, et al. (2016)	10 patients with POF (26-33 years old)	ABMMSCs	Laparoscopically injected into the ovaries	Results showing return of menses (after 3 months) in two patients and one ongoing pregnancy, with one live birth (after 11 months and delivered a healthy full term baby)	[33]
SHREYA, et al. (2018)	A 45-year-old POI patient	ABMMSCs	Laparoscopic instillation of ABMDSC in ovaries	After 8 weeks autologous bone marrow-derived SC therapy(ABMDSCT), pregnancy and delivery of a healthy 2.7 kg female baby through assisted reproduction	[34]
GABR, et al. (2016)	30 patients with POF (18-40 years old)	ABMMSCs	Direct laparoscopic infusion into the ovarian stroma and atheterism into the ovarian artery of one side	86.7% POF patients improved hormone profile 4 weeks after treatment. 60% showed ovulation; three patients underwent IVF; one spontaneous regnancy	[35]
ZAFARDOUST, et al. (2023)	90 patients with poor ovarian responder (POR) (25-45 years old)	MenSCs	Under transvaginal ultrasonography injected into bilateral ovary	A total of 18 out of 80 individuals (22.5%) became pregnant spontaneously in the cell-treated group, as compared to 6 out of 81 individuals (7.4%) in the control group (P=0.005)	[36]
MASHAYEKHI, et al. (2021)	9 patients with POF (20-39 years old)	ADMSCs	Under transvaginal ultrasonography/laparoscopic instillation of ADMSCs in ovaries	Two POF patients had a return of menstruation second months after the intervention,which continued for 3, 5, 7 and 8 months. Two others reported menstruation resumption at 1 month after the intervention	[37]
TANDULWADKAR, et al. (2016)	2 patients with POI (26 and 33 years old)	ABMMSCs	Laparoscopic intraovarian instillation of autologous bone marrow-derived stem cells (ABMDSCs) with PRP	A 26-year-old woman successfully conceived naturally after 22-month post-intraovarian ABMDSC therapy and delivered a healthy baby of 2.7 Kgs; another 33-year-old woman conceived spontaneously after 15-months post-intraovarian ABMDSC mixed with PRP therapy	[38]
ZAFARDOUST, et al. (2020)	15 patients with poor ovarian responder (POR)/ age = 35(4) (interquartile range)	MenSCs	Under transvaginal ultrasonography injected into left ovary	Four of 15 patients with POR got naturally pregnant during 3 months after treatment. Altogether, 7 women had clinical pregnancy that resulted in 5 live births	[39]
YAN, et al. (2020)	61 patients with POI (a median age of 30)	UCMSCs	UCMSCs were transplanted to the patients by ultrasound-guided transvaginal injection	Four successful clinical deliveries were obtained from POI patients after transplantation, and 4 babies were developed normally. Three patients showed follicle development in the injection ovary, 2 patients resumed normal menstruation	[51]

前景的I/II期临床试验, 试验注册号(trial registration number, TRN): IRCT20180619040147N2。通过左侧卵巢内注射自体MenSCs可以改善卵巢低反应(poor ovarian response, POR)女性的妊娠率和活产率, POR及POI同属卵巢功能减退, 也是导致女性不孕的主要原因。结果MenScs组的卵母细胞受精率和胚胎数均高于胞浆内单精子注射组(ICSI组)(P=0.04和P=0.008)。2023年ZAFARDOUST等[36]又进一步进行大样本双侧卵巢靶向治疗, 他们将180名POR的女性分为接受双侧MenSCs卵巢内注射组和未注射组各90人(表1)。结果发现MenSCs疗法表现出良好的耐受性, 没有引起任何安全问题。这说明MenSCs疗

法使接受ICSI/IVF的POR女性产生更多数量的成熟卵母细胞和胚胎。因MenSCs来源于子宫内膜, 由于解剖位置的关系, 移植MenSCs被合理地认为对患有生殖功能障碍的女性具有更好的治疗效果[40]。因此, 在MenSCs中表达的同源和特异性受体可能引导干细胞在生殖器官(子宫和卵巢)停留更长时间, 这从时间和空间的角度为改善受损卵巢提供了最佳的机会[41-42], 但细胞数量和输注途径对MenSCs移植的治疗效果也起着至关重要的作用。有人认为化疗导致的POF, 应用MenSCs治疗是独特且有前途的替代方案[43]。虽然MenSCs有易于获取、无伦理问题、无致瘤性等优点, 但从经血中获取的样本受到真菌和细菌污染的可能性更大[36]。因此, MenSCs的来源和质量是MenSCs移植对POI治疗效果的重要保证, 这与供体的年龄和身体状况密切相关。研究表明, 从中年供体分离的MenSCs长期传代培养潜力不如年轻供体, 且随着供体年龄的增长, 参与细胞生长发育的基因表达水平显著下调[44]。如果女性处于病理状态下, 其体内分离出的MenSCs的特征和功能可能会受到影响, 因此, 子宫内膜异位症及自身免疫性疾病的患者准备进行自体MenSCs移植之前均应慎重选择[43]。

7.4 UCMSCs靶向治疗

来源于胎儿附属物特别是UCMSCs更易获取, 可以从脐带的不同部分或整根脐带中分离出来, 并具有低抗原性、无致瘤性、恢复受损组织的特性[45]。因此, UCMSCs正在成为治疗环节可接受的BMMSCs替代品。例如用于异体或自体使用的非侵入性收集方法, 以及减少伦理冲突可能性的安全采集模式, 这都是因为hUCMSCs不会对母亲或孩子造成危险或损害。90%的人类脐带可以快速被分离, 并能产生足够的数量, 是治疗用MSCs的可靠来源, 因为它们可以被冷冻和解冻, 克隆繁殖, 改变以产生外源蛋白, 并在培养基中培养。与BMMSCs相比, 其多能性, 低畸胎瘤发生率(因为hUCMSCs不致瘤并存在于间质组织中)和较低的免疫能力和较高的免疫抑制能力, 似乎有助于作为POF的一种潜在治疗选择[46]。实验证明, 在脐带与胎盘交界部位提取的MSCs具有更重要的特性, 可脱离母体发挥强大的繁殖再生能力[47]。此外, UCMSCs对T细胞的增殖表现出抑制作用, 其迁移率也明显高于BMMSCs[48], 不但未发现UCMSCs移植与肿瘤发生风险之间存在相关性[1], 而且研究表明, 当UCMSCs用作治疗时, 华通氏胶干细胞(human Wharton's jelly stem cell, hWJSC)裂解物被证明可抑制肿瘤的发展, 通过增强促凋亡*Bax*和降低/下调抗凋亡*Bcl-2*和*SURVIVIN*基因表达对乳腺癌、骨肉瘤和卵巢癌细胞显示肿瘤抑制作用[49], hUCMSCs还可作为其他多能干细胞或胚胎干细胞的营养层[50]。迄今为止, UCMSCs治疗POI的临床研究报道日渐增多, 具有更为广阔的研究及临床应用前景[46]。

2020年, YAN等[51]在一项应用UCMSCs移植治疗61例POI患者的研究中, 发现每个发育阶段的卵泡, 包括窦卵泡(antral follicle count, AFC)、优势卵泡(dominant follicles, DFC)和成熟卵泡(mature follicles, MFC)数量都显著增加。结果显示, 第1、2、3次手术后获得成熟卵泡的患者分别为31.1%(19/61)、11.8%(6/50)和13.3%(4/30), 患者卵泡发育有明显改善的趋势, 包括AFC上升, DFC和MFC数量增加, 以及AMH水平升高, 成功地恢复了61名妇女的卵巢功能, 没有引起严重的不良事件, 结果表明UCMSCs在POI疾病的临床治疗中具有广阔的应用前景。研究发现, 闭经时间小于1年的POI患者似乎更容易在治疗后获得成熟卵泡, 卵巢状况较好的患者(术前有窦卵泡)更有可能通过UCMSCs靶向注射获得更好的效果[51]。根据ClinicalTrials.gov的数据, 目前全球有11项临床研究正在应用MSCs治疗POI/POF, 其中5项应用UCMSCs移植治疗POI/POF患者的临床试验仍在进行中[46]。

几十年来, 妇科医生们一直坚持的原始卵泡池固定理论, 被最近的研究所颠覆, 证实了卵巢干细胞的存在[10]。2012年, VOGLER等[52]通过腹腔镜从8位POF患者的卵巢中收集假定的干细胞: 先搔刮卵巢表面, 取卵巢组织, 冲洗卵巢表面。培养第5天时发现有卵母细胞样细胞, 结果8例患者中有7例卵巢上皮细胞培养成功, 证实了卵巢干细胞在POF患者中的存在。因此, 通过以往基础研究并经临床试验, 应用MSCs治疗POF取得了重大进展。

7.5 MSCs靶向移植的方式及比较

目前应用MSCs治疗POI的主要几种方式有: (1) 腹腔镜卵巢靶向治疗; (2) 通过介入技术靶向移植进卵巢组织内或卵巢动脉; (3) 经阴道超声引导的卵巢靶向等直接卵巢输注/注射等方式[13](图2)。MSCs通过刺激休眠卵泡, 可以部分或大部分逆转卵巢的衰老过程。虽然这几种移植技术也存在包

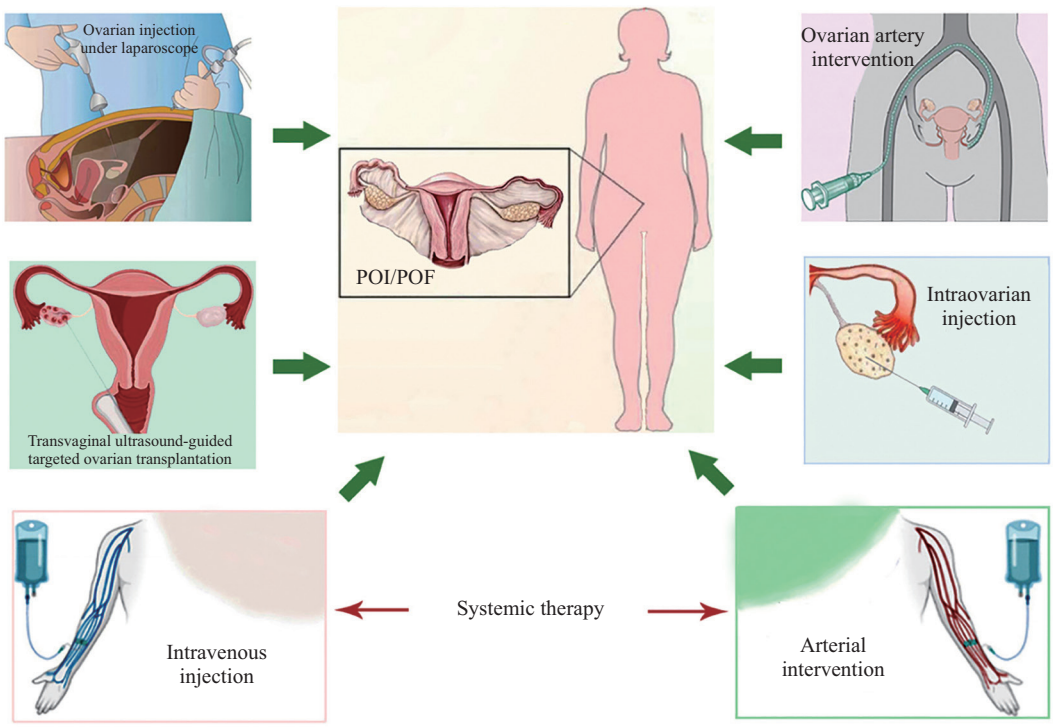

图2 间充质干细胞(MSCs)靶向移植治疗POI/POF的几种方式

Fig.2 Several approaches for targeted transplantation of MSCs in the treatment of POI/POF

括移植的细胞数量、移植时间的选择、疗效的长短、注射速度、移植频率和给药技术等相关问题, 但最近临床研究的所有参与者都接受了UCMSCs的卵巢内注射, 没有一例发生与治疗相关的严重副作用或并发症[46]。因此, 治疗POI大多选择的是hUCMSCs、BMMSCs, 少数选择ADMSCs及MenSCs, 所用MSCs的数量为1.0×10^7~9.0×10^7[6]。

IGBOELI等[53]报道了2例POF患者, 通过腹腔镜用5 mm注射器(22号针头)分别行ABMMSCs移植术。助手用无损伤抓钳固定卵巢, 医生在5分钟时间内向卵巢中心缓慢注射细胞浓缩液4 mL, 使卵巢逐渐膨胀。注射结束时, 将针头在原位停留5分钟, 以避免细胞从卵巢内溢出。术后移植侧卵巢的体积增大, 而注射生理盐水的另一侧卵巢体积没有变化。移植后1周及1、3、6和9个月的随访中, 血清雌二醇(estradiol, E2)水平持续上升, FSH、LH均有所改善, 令人惊喜的是这2名患者在移植后的7个月内月经来潮。

虽然POI对女性的心理及生理健康有重要影响, 但对女性生育力的影响更为重要。因此, 许多临床研究主要针对与妊娠相关的问题, 并分析了POI的主要评价指标及结局, 如卵巢大小、血清激素水平如AMH、LH、FSH和E2; 每月AFC和DFC的数量; 出生和妊娠的婴儿数量; 获得的卵母细胞数量、成熟卵母细胞数量、优质胚胎数量、临床妊娠百分比、流产或ICSI/IVF周期活产, 以及诸如体温、皮疹、阴道出血、头痛、传染病、肝功能异常、肿瘤和肾功能异常也进行了研究[51]。由于缺乏MSCs分离、培养、鉴定、制备和给药方法的标准及系统化方案, 导致治疗场合中的结果不一致。因此, 在临床应用中制定MSCs的制备标准非常必要, 因为根据细胞来源、特定疾病和预期用途的不同, 实际治疗过程也各不相同[46]。

综上所述, 通过各种方法、从多种来源获得的MSCs在POI的治疗中表现出相同的治疗效果[13,24]。即使在年长的POF患者中, MSCs也具有恢复患者生殖功能的临床转化和实际功能的潜力[46], 可从根本上恢复卵巢功能, 这是因为它们易于获取并可分化为大多数组织。

8 MSCs靶向治疗在临床应用中的难点

虽然MSCs移植治疗POI的临床应用有了显著进展, 但由于移植区域微环境的干扰, 特别是缺氧或缺血损伤的组织对MSCs的临床应用造成了严重阻

碍[54]。正常水平的活性氧(reactive oxygen species, ROS)在调节卵泡生长、血管生成和性激素合成方面发挥着重要作用, ROS的减少可以保护卵巢线粒体的结构和功能, 增加抗氧化和抗凋亡酶的水平, 抑制卵巢的老化和氧化损伤[55]。因此, MSCs移植后, 受损卵巢的血管生成和间质损伤的修复得到了显著改善, 在一定程度上归因于调节了ROS的产生[43]。然而, ROS和抗氧化剂之间的平衡被打破就会造成氧化应激(oxidative stress, OS)的严重后果, 表现为卵泡、卵母细胞发育受损, POI和生殖功能下降[56-57]。最近的研究表明, POI的发生和进展可能受到OS的显著影响[4]。SOBINOFF等[58]认为, 吸烟可通过OS诱导窦卵泡卵母细胞凋亡, 从而导致卵泡缺陷。所以, 虽然MSCs移植会受到多种环境因素的影响, 但最重要的因素之一就是OS, 它会导致移植部位移植的MSCs损耗, 影响治疗效果[1,59]。铁死亡也是影响MSCs移植疗效的因素之一[60], 而抑制MSCs的铁死亡有望提高MSCs治疗的疗效, 是一种有前途的新治疗策略[61]。

静脉输注是干细胞移植治疗的主要途径之一[62], 但SABERI等[63]证明, 移植到靶器官的细胞, 超过80%会发生凋亡, 凋亡及坏死大多发生于移植后的4天内。PARK等[64]指出, 细胞移植后, 在POF患者卵巢中的细胞数量逐渐减少, 4周后几乎消失。其他研究也表明, 移植的干细胞在体内的存活率很低[55], 可能影响疗效, 从而增加治疗时间和剂量, 这表明提高细胞存活率在POI干细胞治疗中的重要性[65]。由于卵巢是位于盆腔深部体积较小的器官, 通过静脉输注移植的MSCs的归巢率可能很低, 到达靶器官的干细胞可能只有一小部分[66-67], 细胞归巢效率低可能会降低疗效, 增加成本, 甚至导致副作用[65], 这使得MSCs移植的临床应用受到一些限制[68]。当然, 直接把MSCs注射到卵巢中可以克服这个问题, 但需要经验丰富的手术专家, 否则可能增加损伤和感染的风险[69]。已经注意到, 注射到卵巢中的MSCs倾向于在间质组织中沉积, 这可能会损害颗粒细胞或卵母细胞的有益功能[67]。但对于外源性BMMSCs来说, 移植在体内后的短暂停留时间和较低的存活率反而降低了肿瘤发生的风险[70], 之前的研究也表明, BMMSCs在移植后至少6个月是安全的, 没有发现明显的致瘤性[71]。虽然BMMSCs因治疗包括POF在内的多种疾病而受到全球极高关注, 但临床试验中还存在严重缺陷, 如采集时的相关不适、与衰老相关的移植功效下降以及BMMSCs等免疫排斥替代品(例如其他来源的MSCs[72])的潜力。

BMMSCs和ADMSCs已经被用于POI治疗的临床研究, 但人源BMMSCs含量极微, 骨髓单核细胞中占比不足千分到百分之一, 并存在获取困难、数量低、患者承受痛苦及风险大等现实问题。此外, 这两种MSCs自我更新的能力都受到供体年龄和侵入性采集方法(如抽脂和骨髓抽吸)的限制, 因此也影响了这两种MSCs使用的成功率[4,13], 也就是随着供体年龄的增长, 供体MSCs的数量和功能也相应下降。MSCs的衰老还可能与端粒缩短、DNA损伤、表观遗传学和免疫学特征有关[73-74], 而不同的研究也发现POF患者中端粒长度缩短、端粒酶活性降低[75], 这意味着可能通过重新激活端粒酶实现卵巢年轻化的一个美好未来[9,76]。既然成人自体来源MSCs在临床应用领域存在诸多不足, 应用胎盘附属物来源的MSCs可能更具优势, 特别是在年龄大的POI患者中的应用。

9 临床应用的改进及未来前景

迄今为止, 很多研究表明, 对比POI的其他治疗方法, 干细胞治疗具有理想的效果[1,8,10,13,24-25,46]。但如何将向全身移植的干细胞高效地靶向损伤组织, 如何降低移植后MSCs的损失率、提高移植效率也是研究的一项重点课题。目前, 缺乏适当生态位的细胞移植一直是影响干细胞治疗效果的主要问题, 研究人员已经尝试使用生物材料为干细胞创造一个有利的微环境[65]。生物材料具有促进细胞相互作用、优异的稳定性和生物降解性、良好的被动和主动靶向性等优点, 在包括再生医学在内的各种应用中显示出巨大的潜力[77]。因此, 生物材料与MSCs的结合是治疗POI的一种很有前途的先进方法。一项关于组织工程的研究显示, 胶原蛋白支架复合MSCs移植后可以增加干细胞的存活时间, 提高其附着力和增殖力, 限制其向外转移, 附着在胶原蛋白支架上的MSCs仍具有生物活性, 具有长期的治疗作用[78]。SU等[79]利用胶原蛋白支架进行ADMSCs移植, 增加了MSCs在卵巢中的滞留时间, 有助于卵巢功能的长期恢复。将UCMSCs移植到胶原支架上, 可以激活长期不孕症POF患者休眠卵巢中的卵泡[1]。目前, MSCs治疗POI的主要途径是把

细胞直接移植到卵巢组织内, 但这可能导致针刺造成卵巢损伤, SHIN等[80]采用透明质酸凝胶支架皮内移植MSCs的方法有效地避免了这一缺点, 虽然只是基于动物模型, 但有效地延长了移植细胞的存活率, 并显著恢复了卵巢功能。此外, MAO等[81]证明了使用微流体装置将生物材料包裹到藻酸盐中可以在静脉注射后显著增加MSCs在体内的存活率, 细胞团形成和随后用聚赖氨酸交联的组合导致注射的MSCs半衰期增加了一个数量级以上。因此, 微凝胶封装技术能够维持MSCs的存活并提高整体免疫调节能力, 总体上可适用改善MSCs的治疗。

大量研究已经证明, 在移植前对干细胞进行适当的热休克、缺氧、氧化应激或细胞因子预处理可以提高细胞的功能和存活率, 并获得更好的效果[55,79,82]。热休克预处理(heat shock pretreatment, HSP)是移植前后保护细胞的有效方法, HSP可以抑制细胞凋亡, 提高BMMSCs在化疗环境中的存活率[83-84], 对POI有较好的治疗效果。HSP还可以增强MSCs的免疫调节能力[85], 这可能会增强BMMSCs的治疗潜力。研究人员已经证实, 在许多模型中, 42 °C左右的热休克持续数小时可以延长干细胞存活并提高其治疗效果[86]。其他预处理如适当暴露于缺氧或OS状态下可以使细胞通过多种途径适应缺血或氧化环境, 这有利于细胞在受损器官中的存活[87]。PEYVANDI等[88]发现, MSCs移植前用去铁胺(deferoxamine, DFO)预处理明显促进了干细胞向靶区归巢, 从而提高了细胞治疗效率。应用低强度脉冲超声(low-intensity pulsed ultrasound, LIPUS)在细胞移植前预处理MSCs, 可能为改善全身移植的MSCs向靶组织归巢提供一种新颖、方便、安全的技术[89]。人羊膜来源MSCs经LIPUS预处理后, 可以促进多种因子的分泌, 从而改善受损卵巢的微环境[89]。值得关注的是, 预处理后的MSCs对化疗诱导的微环境表现出更强的耐受性, 体内治疗效果更好[65]。虽然早期应用BMMSCs移植治疗POI的报道较多, 但低迁移率和存活率往往限制了其治疗潜力。研究表明, 褪黑素(melatonin)、左旋肉碱(L-carnitine)、芹菜素(apigenin)预处理可显著促进BMMSCs的归巢和提高其存活率[63,90-92]。WANG等[93]发现人脐带血富血小板血浆可以促进hUCMSCs增殖并减少细胞凋亡。还有研究发现, BMMSCs和内皮祖细胞的共移植可以促进股骨头坏死部位的血管生成[94], 由此我们可以进一步探索, 也许BMMSCs与内皮祖细胞共移植能够以同样的方式显著改善POI患者的卵巢功能。由于MSCs的治疗效果高度依赖于退化和受损区域MSCs的移植、迁移和活力, 而OS是体内和体外促进细胞死亡的主要因素之一。通过螯合铁离子或添加自由基捕获抗氧化剂(例如DFO或维生素E)来减少OS已被证明可以延长人类MSCs的寿命, 同时保留其分化潜力[95]。未来, 基因修饰的BMMSCs移植可能是增强其治疗效果的一种有前景的方法[65,96]。

使用干细胞和生物材料治疗POI具有积极的前景, 最近的研究为POI的未来治疗提供了新的靶点。近年来, 基于诱导多能干细胞(induced pluripotent stem cells, iPSCs)的方法可以克服目前供体来源MSCs生产过程的主要限制, 似乎很有发展潜力, 因为它可以从单次献血中产生几乎无限数量的MSCs[97]。尽管iPSCs与许多潜在风险有关, 例如遗传和表观遗传异常, 以及由于*c-Myc*等癌基因的过度表达而增加患癌症的风险, 但应用iPSCs恢复卵巢功能仍然有很大希望[98]。2016年的研究表明, 人iPSCs可用于创建卵巢颗粒样细胞(ovarian granulosa like cells, OGLCs)[98]。2020年, YAMASHIRO等[99]描述了一种详细的方案, 从体细胞产生iPSCs, 然后其分化为生殖细胞, 随后发育为卵母细胞。在生产标准化和应用安全性得到确定后, iPSCs技术最终将用于治疗POI和不孕症, 以及许多其他严重的临床疾病[4]。

随着对干细胞作用机制研究的不断加深, 也逐渐开展其临床应用, 但必须考虑细胞的功能潜力和微生物安全性, 并确保培养的细胞不发生转化, 建立一个专业系统来检测MSCs的生产质量, 这极具挑战性。此外, 细胞质量的巨大差异来自不同的供体和组织。因此, 利用侵入性较小的分离技术获得更可靠、更有效的MSCs已成为治疗选择[1]。有研究又提出POF无细胞治疗(cell-free therapy)的概念, 其优点是在受损的卵巢内仍然可以重新激活卵泡[65], 值得进一步探索和研究。总之, 在今后应用干细胞和生物材料治疗POI的临床应用中, 非侵入、无创伤的获取细胞方式, 适当的手术入路, 最佳的干细胞数量和材料制备方法以及细胞质量的标准化检测流程等方面的研究应得到更多的关注。

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