·综述·

骨髓间充质干细胞治疗局部骨质疏松

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摘要: 骨质疏松症(Osteoporosis)是一种全身性的骨代谢障碍性疾病,以骨量减少,骨微结构退化,骨脆性增加,易发生骨折为特征的全身性疾病。随着社会逐渐进入老龄化,骨质疏松症也日趋成为影响健康的主要疾病,对骨质疏松治疗方面的研究就显得更加重要了。对骨质疏松症的更进一步研究发现骨髓间充质干细胞(BMSC)的衰老也是骨质疏松的发病机制之一。骨髓间充质干细胞(BMSC)是多能的干细胞,即能够自我更新并分化成多种细胞类型,包括脂肪细胞、成骨细胞、软骨细胞。BMSC 的植入能够有效增加局部骨量,提高骨密度,改善局部骨质疏松情况,可纠正骨代谢失衡,减少骨量丢失,增加成骨,为局部骨质疏松的治疗提供了一种新方法。

关键词: 骨质疏松; 骨髓间充质干细胞; 干细胞移植

Research progress of the treatment of regional osteoporosis with BMSCs transplantation

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Abstract: Osteoporosis is a systemic disorder of bone metabolism, characterized by bone loss, bone microstructure degradation, increased bone fragility, and increased risks of bone fractures. With the aging of the population, osteoporosis increasingly becomes the main disease affecting health. So the study of osteoporosis treatment becomes more important. Further research of osteoporosis reveals that the senescence of bone marrow stem cells (BMSCs) is also a possible pathogenesis of osteoporosis. BMSCs are pluripotent stem cells, which are capable of self-renewal and differentiation into various cell types, including fat cells, osteoblasts, and cartilage cells. Implantation of BMSCs can effectively increase the local bone mass, increase the bone mineral density, and improve the local situation of osteoporosis. Hence, it can correct bone metabolic imbalance, reduce bone loss, and increase osteogenesis, which can provide a new method for the treatment of regional osteoporosis.

Key words: Osteoporosis; Bone marrow mesenchymal stem cells; Stem cell transplantation

骨质疏松症是一个系统性的骨骼疾病,它的特点是骨密度(BMD)降低和骨折风险增加,诊断骨质疏松症已经根据评估的骨骼密度,定义为减少 T 分数低于 -2.5[1]。根据世界卫生组织报道世界范围内由骨质疏松引起的髋关节骨折有 170 万人,预计到 2050 年将达到 600 万[2]。据估计,到 2025 年仅髋关节骨折会引起每年 700000 人死亡,260 万例残疾[3,4]。骨质疏松症的治疗和骨折的康复治疗导致巨大的经济损失。髋部骨折和椎体的压缩性骨折治疗成本都非常高[5]。

1 骨质疏松的病因

骨质疏松的危险因素有人种、老龄、女性绝经、吸烟、过量饮酒或咖啡饮料、制动、缺乏阳光和运动、有影响骨代谢的疾病或服用影响骨代谢的药物,其中最重要的因素是老龄和雌激素缺乏。随年龄的增长和机体的衰老,BMSCs 的数量以及与成骨有关的激素和生长因子如雌激素、胰岛素样生长因子(insulin-like growth factor、IGF-Ⅰ)、钙调节激素和转换生长因子-β(transforminggrowth factor, TGF-β])、成纤维细胞生长因子(fibroblast growth factor, FGF)、血小板源性生长因子(pletlet-derived growth factor, PDGF)等在骨组织中的含量均出现减少[6],这些改变是造成老年性骨形成功能减退的重要原因。雌激素在骨代谢过程中同

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样发挥着至关重要的作用。雌激素减少通过影响成骨细胞和破骨细胞导致骨量减少[7,8]。据估计,30%的女性绝经期后 10 至 15 年有骨质疏松症[9]。此外,据报道,白介素 1、白介素 6,肿瘤坏死因子和其他细胞因子,直接或间接导致骨质疏松症,这些细胞因子的产生是受雌激素调控的[10,11]。

2 骨质疏松症发病机制的细胞水平研究

成骨细胞和破骨细胞是成骨过程中最重要的两种细胞。成骨细胞形成新骨基质在骨重塑过程中可以平衡破骨细胞介导的骨吸收,维持体内骨系统的平衡[12]。

骨质疏松的发病机理是由于成骨细胞的成骨作用不能够平衡破骨细胞介导的骨吸收,导致骨量逐渐减少,骨密度减低。几项研究已经强调了假设成骨细胞的活动减少导致骨质流失的发病机制[13,14]。成骨细胞来源于骨髓间充质干细胞(BMSCs)[15]。李良等通过将 3 月龄和 6 月龄的去卵巢骨质疏松大鼠的骨髓间充质干细胞分别提取出来,进行成骨活性比较,发现去卵巢骨质疏松大鼠 BMSC 增殖能力明显下降,向成骨细胞分化能力的降低和向脂肪细胞分化能力的增强,而 6 月龄的去卵巢骨质疏松大鼠这一变化更加明显[16]。汤亭亭等取 3 种年龄层次的大鼠(1 月龄、9 月龄、24 月龄),抽取骨髓进行体外培养,计算纤维细胞集落形成单位(colony forming unit-fibroblastic, CFU-F)的数量,结果发现骨髓中 BMSCs 的数量随增龄而减少[17]。Shih Sheng Jiang 及其同事通过研究 14 个年龄从 36 到 74 岁的 BMMSC 的捐助者的基因,表明 HEXA, HEXB, CTSK, SULF1, ADAMTS5, SPP1, COL8A2, GPNMB, TNFAIP6, RPL29 这些基因参与骨质疏松的发病机制,并发现 BMSC 随着年龄增长有衰老的表现[18]。提示 BMSC 的衰老也可能是骨质疏松的机制之一。

3 骨质疏松的常规治疗

根据骨质疏松症治疗指南,下列是治疗骨质疏松的常用药物:钙、雌激素、合成代谢雄激素类固醇、降血钙素,活性维生素 D_3,依普黄酮、维生素 K_2,双膦酸盐产品[19],然而大多数这类药物,目前对于骨质疏松的治疗还是侧重于抑制破骨细胞的骨吸收作用,而在增加成骨细胞活性方面还没有明显进展。这些药物治疗也会产生一系列副作用,例如双膦酸盐类药物相关的下颌骨骨坏死(BRONJ)是一个众所周知的副作用[20]。最明显的特征就是骨外露,伴随的疼痛和肿胀超过 6~8 周也无好转[21,22]。长期应用雌激素替代治疗会增加乳腺癌的发病率[23,24]。因此临床上急切需要一种既安全又有效的治疗方法。

4 干细胞移植治疗骨质疏松

BMSCs 具有干细胞的共性,即具有自我复制和多向分化能力,体外不同诱导条件下,能够向成骨细胞、成软骨细胞、肌细胞、脂肪细胞及基质细胞等不同的细胞系分化[25]。因此临床上在用干细胞治疗关节软骨损伤[26-28],血友病或心肌梗死等方面取得一定进展[29]。骨髓间充质干细胞还有易于分离、增殖,具有多向分化潜能等优点,使其成为免疫调节、组织修复和细胞治疗的首选应用材料[30-32]。

4.1 干细胞的移植

干细胞移植一般选择组织工程学的方法。组织工程是一个新兴的跨学科领域。它应用生命科学原理和工程学开发生物替代品,恢复、维护和改善功能受损/或失去的组织[33,34]。组织工程目前占领了再生医学领域前沿位置。可喜的是新兴的另一类细胞组织工程学,能够完全恢复结构和功能缺陷[34]。干细胞的组织工程骨包括三个主要步骤。首先,干细胞提取、分离和扩增。其次,干细胞种植在合适的支架上。最后,重新植入体内完成重建。支架能够为细胞的生长提供了一个良好的微环境,所以支架的选择通常是至关重要的。支架的选择一般基于以下五点:(1)良好的组织相容性:材料应对移植细胞和周围组织无免疫原性,不引起排斥反应[35]。(2)良好的生物降解性:材料在完成支架作用后应能降解,降解率应与组织生长率相适应,降解时间应能根据组织生长特性人为调控,在新组织形成以前提供机械负载力[36]。(3)良好的细胞界面:材料应有良好的表面活性,利于细胞的粘附生长,更重要的是应能激活细胞特异基因的表达,维持正常的细胞表型[37]。(4)三维立体多空结构:生物材料应可加工成三维立体结构,孔隙率达 90% 以上,要有较高的面积体积比,利于细胞粘附生长,血管神经长入,营养成分渗入和代谢产物排出;孔隙的直径应该在 300~1200 μm,只有这样才能充分支持细胞迁移、增殖、分化和生长因子的转移。小的孔径不充分,而大的孔径又会降低支架的机械功能[38]。(5)可塑性和机械强度:生物材料可制成所需形状,并有一定的机械强度,为新生组织提供支持,在植入宿主体内后可保持一定时间,直至新生组织形成并具有一定外

形后再降解。在 Yasuhiro Okamoto 将 BMSC 植入切除卵巢的骨质疏松大鼠的股骨段,发现与空白对照组相比,骨密度有明显提高[39]。Weng 等人将 BMSCs 与藻酸钙凝胶混合并注入切除卵巢的骨质疏松家兔的股骨远端。8 周后发现,与单纯注射藻酸钙凝胶相比,BMSCs-藻酸钙凝胶注射后局部组织有更多的骨质形成[40]。许多激素和生长因子也在成骨分化过程中很重要,例如甲状旁腺激素(PTH)瘦素,雌激素,糖皮质激素,前列腺素 E2(PGE2),骨化三醇,骨形成蛋白(BMP-2),转移生长因子(TGFβ)成纤维生长因子(FGF-2),类胰岛素生长因子(IGF-1)。因此通过转染的方法使得干细胞产生的细胞外基质中存在促进成骨的生长因子就显得尤为重要[41]。唐尤超等将 hBMP2 基因修饰的 MSCs 复合珊瑚羟基磷灰石支架植入骨质疏松大鼠下颌骨缺损部位,4 周后可在珊瑚羟基磷灰石边缘发现新生骨质,8 周后可见相互连接的板状成熟骨基质,与对照组相比,实验组大鼠新生骨量明显提高[42]。

4.2 干细胞治疗骨质疏松的风险

第一,肿瘤发生的危险。干细胞与肿瘤细胞有很多相似的功能如无限增殖,有很强的抗凋亡能力[43,44]。因此干细胞可能被认为是潜在的候选人为恶性转化。此外,类似的生长调节剂和控制机制同时参与癌细胞和干细胞的维修[43]。这或许就是为什么肿瘤形成常被视为安全使用干细胞医药产品的一个主要障碍。最近,一个 13 岁男性共济失调毛细血管扩张症患者被诊断为供体派生的多灶性脑瘤,在接收神经干细胞移植 4 年之后。有报道骨肉瘤源自间质干细胞[45]。此外,干细胞移植后卡波西肉瘤[46]、皮肤癌[47]和口腔鳞状细胞癌[48]的发生率都有明显升高。除了是细胞本身形成肿瘤,干细胞可能影响增长或扩散现有的肿瘤细胞[49]。MSC 可以提供创建一个宽松的基质环境利于肿瘤生长或 MSC 可以减少免疫排斥反应从而允许肿瘤细胞继续生长。第二,是与免疫反应有关的风险,特别是对于同种异体干细胞移植。第三,人类病原体的传播风险[50]。

5 展望

随着人类寿命的延长和社会老龄化进展,我国正快速进入老龄化社会,目前我国 > 60 岁的老年人约 1.73 亿,各种老年病的威胁日益严重。据统计,2006 年全国 > 50 岁人群中骨质疏松症发病率女性为 20.7%,男性为 14.4%,全国约有 6944 万骨质疏松患者,约有 2 亿 1 千万人存在低骨量。MSCs 与骨质疏松发病机制以及 MSCs 移植治疗骨质疏松的研究成果为我们提供了一种新的思路和方法。虽然这方面的研究尚处于探索阶段,干细胞治疗也会产生多种风险,但是随着各种技术不断提高,材料科学的继续发展,干细胞移植治疗骨质疏松将有可能成为一种疗效确实、安全可靠的治疗方法。

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