论著·基础医学

人脐带间充质干细胞移植通过改善线粒体功能与减轻氧化应激修复雄性小鼠放射性生殖损伤

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[摘要] **目的** 探究人脐带间充质干细胞(human umbilical cord mesenchymal stem cells,hUC-MSCs)对雄性小鼠放射性生殖损伤的改善作用及其潜在机制。**方法** 在获得空军军医大学西京医院妇产科健康足月妊娠孕妇知情同意并签署知情同意书后,于2024年12月在手术室无菌条件下采集1段6~8 cm长的健康足月新生儿的脐带组织,用于分离培养hUC-MSCs。将45只6~8周龄健康雄性C57BL/6小鼠[体质量(22±2)g]通过随机数字表法分为对照+尾静脉注射PBS组(Control+PBS,n=3)、对照+尾静脉注射hUC-MSCs组(Control+MSCs,n=6)、照射+尾静脉注射hUC-MSCs组(Rad+MSCs,n=6)、对照组(Control,n=10)、照射组(Rad,n=10)及照射+干细胞干预组(Rad+hUC-MSCs,n=10)。使用^{60}Co γ射线对Rad组、Rad+MSCs组和Rad+hUC-MSCs组小鼠进行全身单次照射,剂量率1.28 Gy/min,总剂量6 Gy。照射结束后,Rad+MSCs组和Rad+hUC-MSCs组小鼠立即经尾静脉单次注射hUC-MSCs悬液(2×10^6/200 μL)。采用多模式动物活体成像系统及共聚焦荧光显微镜观察小鼠睾丸组织中hUC-MSCs的分布情况。照射后12周,取材并剥离双侧睾丸组织进行称量,计算睾丸系数;显微镜下评估小鼠精子质量;采用苏木精-伊红(hematoxylin and eosin staining,HE)染色观察小鼠睾丸形态结构;通过流式细胞术评估小鼠睾丸组织活性氧(reactive oxygen species,ROS)及线粒体膜电位(mitochondrial membrane potential,MMP)水平;通过比色法检测睾丸组织中丙二醛(malondialdehyde,MDA)、总超氧化物歧化酶(total super oxide dismutase,T-SOD)和过氧化氢酶(catalase,CAT)含量;应用TUNEL染色检测睾丸细胞凋亡情况;采用Western blot检测睾丸组织中E3泛素蛋白连接酶帕金蛋白(E3 ubiquitin-protein ligase parkin,Parkin)、磷酸酶和张力蛋白同源激酶1(PTEN-induced putative kinase 1,PINK1)、苄氯素1(Beclin1)、线粒体动力蛋白样GTP酶视神经萎缩蛋白1(OPA1 mitochondrial dynamin-like GTPase,OPA1)、线粒体动力相关蛋白1(dynamin-related protein 1,Drp1)、Bcl-2相关X蛋白(Bcl2-associated x protein,Bax)、B淋巴细胞瘤-2(B cell lymphoma-2,Bcl-2)的表达情况。**结果** 照射后24、48 h,多模式动物活体成像系统观察发现,Control+PBS组小鼠睾丸组织未见荧光信号,Control+MSCs组与Rad+MSCs组小鼠睾丸组织中均可见荧光信号,进一步通过共聚焦荧光显微镜观察发现,在Control+MSCs组与Rad+MSCs组小鼠睾丸间质区域可见红色荧光,检测到荧光染料标记的hUC-MSCs信号。照射后12周,与Control组相比,Rad组小鼠精子数量(P<0.05)和活力(P<0.01)显著降低,畸形率显著升高(P<0.001);睾丸生精细胞出现空泡化且排列紊乱,生精小管直径和生精上皮厚度减小(P<0.001);睾丸组织ROS水平升高(P<0.05),MMP水平显著降低(P<0.001);MDA含量明显升高(P<0.001),T-SOD活力升高(P<0.05),CAT活力降低(P<0.05);睾丸组织中Parkin(P<0.01)、PINK1(P<0.05)、Beclin1(P<0.05)、OPA1(P<0.01)和Drp1(P<0.05)蛋白表

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达升高,TUNEL⁺细胞增多($P<0.001$),Bcl-2/Bax比值降低($P<0.01$)。与Rad组相比,Rad+hUC-MSCs组小鼠精子数量和精子活力显著升高($P<0.05$),精子畸形率显著降低($P<0.001$);睾丸生精细胞空泡化和排列紊乱情况得到改善,生精上皮厚度增加($P<0.01$);睾丸组织ROS水平降低($P<0.05$),MMP水平明显升高($P<0.01$);MDA含量明显减少($P<0.001$),T-SOD活力降低($P<0.05$),CAT活力升高($P<0.05$);睾丸组织中Parkin、PINK1、Beclin1、OPA1和Drp1蛋白表达降低($P<0.05$),TUNEL⁺细胞减少($P<0.001$),Bcl-2/Bax比值升高($P<0.05$)。**结论** hUC-MSCs可能通过改善线粒体损伤,减轻睾丸组织氧化应激损伤和细胞凋亡,从而改善放射性雄性生殖损伤。

[**关键词**] 辐射损伤;睾丸;间充质干细胞;线粒体;氧化应激
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Transplantation of human umbilical cord mesenchymal stem cells repairs radiation-induced reproductive injury in male mice *via* improving mitochondrial function and attenuating oxidative stress

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[**Abstract**] **Objective** To investigate the ameliorative effect of human umbilical cord mesenchymal stem cells (hUC-MSCs) on radiation-induced reproductive damage in male mice and its potential mechanisms. **Methods** After obtaining informed consent and signed consent forms from healthy full-term pregnant women in the Department of Obstetrics and Gynecology at Xijing Hospital of Air Force Medical University, umbilical cord tissue approximately from 6 to 8 cm in length from healthy full-term newborns was collected under sterile conditions in the operating room for isolating and culturing hUC-MSCs; 45 healthy male C57BL/6 mice aged from 6 to 8 weeks with a body weight of 22±2 g were divided into Control+PBS (n=3), Control+MSCs (n=6), Rad+MSCs (n=6), Control (n=10), Rad (n=10), and Rad+hUC-MSCs (n=10) groups using a random number table method; the Rad, Rad+MSCs, and Rad+hUC-MSCs groups were subjected to whole-body single-dose irradiation using ^{60}Co γ-ray at a dose rate of 1.28 Gy/min and a total dose of 6 Gy; immediately after irradiation, the Rad+MSCs and Rad+hUC-MSCs groups received a single tail vein injection of hUC-MSCs suspension (2×10^6 cells/200 μL); the distribution of hUC-MSCs in testicular tissue was observed using a multimodal *in vivo* imaging system and confocal fluorescence microscopy; at 12 weeks post-irradiation, bilateral testes were harvested and weighed to calculate the testicular coefficient; sperm quality was assessed under a microscope; testicular morphology and structure were evaluated using Hematoxylin and Eosin (HE) staining; reactive oxygen species (ROS) and mitochondrial membrane potential (MMP) levels in testicular tissue were measured by flow cytometry; malondialdehyde (MDA), total superoxide dismutase (T-SOD), and catalase (CAT) contents were detected by colorimetry; testicular cell apoptosis was assessed using TUNEL staining; and Western blot was used to detect the expression levels of Parkin RBR E3 ubiquitin-protein ligase (Parkin), PTEN-induced putative kinase 1 (PINK1), Beclin1, OPA1 mitochondrial dynamin-like GTPase (OPA1), dynamin-related protein 1 (Drp1), Bcl2-associated X protein (Bax), and B cell lymphoma-2 (Bcl-2). **Results** At 24 h and 48 h post-irradiation, multimodal *in vivo* imaging showed no fluorescent signals in the testicular tissue of the Control+PBS group, while fluorescent signals were observed in the Control+MSCs and Rad+MSCs groups; further confocal fluorescence microscopy revealed red fluorescence in the interstitial regions of testicular tissue in the Control+MSCs and Rad+MSCs groups, detecting fluorescence dye labeled MSCs signals; at 12 weeks post-irradiation, compared to the Control group, the Rad group showed significantly

decreased sperm count ($P<0.05$) and motility ($P<0.01$), significantly increased malformation rate ($P<0.001$), vacuolization and disordered arrangement of spermatogenic cells, reduced seminiferous tubule diameter and spermatogenic epithelium thickness ($P<0.001$), elevated testicular tissue ROS levels ($P<0.05$), significantly decreased MMP levels ($P<0.001$), increased MDA content ($P<0.001$), elevated T-SOD activity ($P<0.05$), decreased CAT activity ($P<0.05$), increased protein expression of Parkin ($P<0.01$), PINK1 ($P<0.05$), Beclin1 ($P<0.05$), OPA1 ($P<0.01$), and Drp1 ($P<0.05$), increased TUNEL-positive cells ($P<0.001$), and decreased Bcl-2/Bax ratio ($P<0.01$); compared to the Rad group, the Rad+hUC-MSCs group exhibited significantly increased sperm count and motility ($P<0.05$), significantly decreased malformation rate ($P<0.001$), improved vacuolization and arrangement of spermatogenic cells, increased spermatogenic epithelium thickness ($P<0.01$), reduced testicular tissue ROS levels ($P<0.05$), significantly increased MMP levels ($P<0.01$), decreased MDA content ($P<0.001$), reduced T-SOD activity ($P<0.05$), increased CAT activity ($P<0.05$), decreased protein expression of Parkin ($P<0.05$), PINK1 ($P<0.05$), Beclin1 ($P<0.05$), OPA1 ($P<0.05$), and Drp1 ($P<0.05$), reduced TUNEL-positive cells ($P<0.001$), and increased Bcl-2/Bax ratio ($P<0.05$). **Conclusion** hUC-MSCs may alleviate radiation-induced male reproductive damage by mitigating mitochondrial damage, reducing testicular tissue oxidative stress injury and cell apoptosis.

[**Key words**] radiation injuries; testis; mesenchymal stem cells; mitochondria; oxidative stress

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随着核技术在医学、工业及航天等领域的广泛应用,核事故风险持续存在,电离辐射(ionizing radiation, IR)对机体的潜在危害日益受到关注。睾丸是对辐射高度敏感的组织之一。有研究报道,男性暴露于核事故环境后,生殖系统功能可能最先受损,具体表现为生精功能障碍,严重时可能导致不育[1-2]。意外辐射暴露常导致男性生精功能障碍,表现为少精子症、弱精子症甚至无精子症,是造成男性不育的重要原因之一。

目前,针对放射性雄性生殖损伤的临床干预手段有限、疗效欠佳且常伴有副作用[3-4]。因此,探索高效、安全的治疗方法成为亟待解决的问题。人脐带间充质干细胞(human umbilical cord mesenchymal stem cells, hUC-MSCs)安全且易于获得,研究表明,hUC-MSCs在组织愈合和再生中发挥着关键作用,对多种放射性损伤都有较好的治疗作用[5-6]。既往研究显示,MSCs能够缓解化学因素诱导的雄性生殖损伤,改善睾丸病理变化,促进精子发生并恢复生育能力[7-9],但其对放射性雄性生殖损伤的治疗效果及机制尚缺乏报道。

精子发生是高度依赖能量代谢的细胞分化过程,精子数量减少与活力下降是男性不育的主要表现之一[10]。线粒体作为细胞能量代谢的核心细胞器,其功能完整性对生精细胞存活及精子质量具有决定性作用。PINK1/Parkin通路是调控线粒体自噬的关键信号途径,在生理条件下负责维持线粒体质量稳态。因此,本研究探讨了hUC-MSCs对放射性雄性生殖损伤的改善作用,并初步解析了其机制,以期为放射性雄性生殖损伤的治疗提供新思路和新方法。

1 材料与方法

1.1 实验动物、主要试剂以及仪器

45只6~8周龄C57BL/6雄性小鼠[体质量(22±2)g]购自空军军医大学实验动物中心。间充质干细胞无血清培养基购自中国友康厚德生物公司,亲脂性荧光染料购自美国MCE公司,精子细胞获能(biggers-whitten-whittingham,BWW)培养基购自中国雷根生物公司,线粒体膜电位检测试剂盒购自中国索莱宝公司,活性氧检测试剂盒购自中国碧云天公司,总超氧化物歧化酶(total super oxide dismutase,T-SOD)检测试剂盒、丙二醛(malondialdehyde, MDA)检测试剂盒、过氧化氢酶(catalase,CAT)检测试剂盒以及细胞凋亡检测试剂盒(terminal-deoxynucleoitidyl transferase-mediated nick end labeling,TUNEL)购自中国伊莱瑞特公司,二喹啉甲酸(bicinchoninic acid,BCA)法蛋白定量试剂盒购自中国米鼠生物公司,兔抗小鼠β-actin抗体购自美国Cell Signaling Technology公司,兔抗小鼠张力蛋白同源激酶1(PTEN-induced putative kinase 1,PINK1)、E3泛素蛋白连接酶帕金蛋白(E3 ubiquitin-protein ligase parkin,Parkin)、线粒体动力相关蛋白1(dynamin-related protein 1,Drp1)、磷酸酶和苄氯素1(Beclin1)抗体以及小鼠抗小鼠

线粒体动力蛋白样GTP酶视神经萎缩蛋白1(OPA1 mitochondrial dynamin-like GTPase,OPA1)、Bcl2相关X蛋白(Bcl2-associated X protein,Bax)、B淋巴细胞瘤-2(B cell lymphoma-2,Bcl-2)抗体购自中国武汉三鹰生物技术公司。Coulter-XL. MCL流式细胞仪购自美国贝克曼公司,AniView100DXA多模式动物活体成像仪购自中国博鹭腾公司,FV3000共聚焦荧光显微镜购自日本奥林巴斯公司,全自动精子质量分析仪购自中国赛司医疗公司,Universal Hood Ⅱ电泳成像系统购自意大利Bio-Rad公司。

1.2 实验分组及动物模型建立

45只C57BL/6小鼠在温度为(22±2)℃、湿度为(50±10)%及12 h/12 h明暗循环的饲养环境下自由摄食饮水。经1周适应性饲养后,通过随机数字表法将小鼠分为对照+尾静脉注射PBS组(Control+PBS,n=3)、对照+尾静脉注射hUC-MSCs组(Control+MSCs,n=6)、照射+尾静脉注射hUC-MSCs组(Rad+MSCs,n=6)、对照组(Control,n=10)、照射组(Rad,n=10)及照射+干细胞干预组(Rad+hUC-MSCs,n=10),分别用于验证hUC-MSCs在小鼠睾丸组织中的分布情况及建立动物模型。使用^{60}Co γ射线对Rad组、Rad+MSCs组和Rad+hUC-MSCs组小鼠进行全身单次照射,剂量率为1.28 Gy/min,总剂量为6 Gy。照射结束后,立即对Rad+MSCs组和Rad+hUC-MSCs组小鼠经尾静脉注射hUC-MSCs PBS悬液(第3~6代,2×10^6/200 μL)。所有动物实验由空军军医大学动物福利委员会批准(IACUC-20220123)。

1.3 hUC-MSCs的分离培养及鉴定

1.3.1 hUC-MSCs分离培养 本研究已经空军军医大学西京医院伦理委员会批准(KY20202097-F-2)。在获得医院妇产科健康足月妊娠孕妇知情同意并签署知情同意书后,于2024年12月在手术室无菌条件下采集1段6~8 cm长的健康足月新生儿的脐带组织,置于含1×10^5 U/L青-链霉素的PBS溶液中暂存。随后,在超净台内分离华通氏胶,并剪碎为1 mm^{3}的组织块,均匀铺于25 cm^{2}培养瓶中。使用间充质干细胞无血清培养基进行原代培养,此后每2天更换1次培养液,并持续监测细胞生长情况。待细胞生长融合率>70%时,消化并过滤提纯细胞,继续传代培养备用。

1.3.2 hUC-MSCs表型鉴定 使用流式细胞仪对第3代hUC-MSCs表面特异性抗原进行鉴定。将细胞密度调整为1.0×10^6/mL后,分别设置同型对照管(加入FITC-IgG和PE-IgG)及样本检测管(分别加入CD105-PE/CD34-FITC、CD44-PE/CD45-FITC、CD29-PE/CD90-FITC抗体)。经4 ℃避光孵育30 min,洗涤后重悬细胞,随即上机检测CD45、CD34、CD90、CD105、CD44和CD29的表达水平。

1.4 hUC-MSCs在小鼠睾丸组织中的分布情况

取第3代hUC-MSCs,调整细胞密度为2×10^6/200 μL,采用亲脂性荧光染料对细胞进行荧光标记。于6 Gy γ射线照射结束后,立即给予Control+PBS组、Control+MSCs组和Rad+MSCs组小鼠尾静脉注射PBS或荧光染料标记的hUC-MSCs PBS悬液,然后于照射后24 h与48 h,使用多模式动物活体成像系统及共聚焦荧光显微镜观察小鼠睾丸组织中hUC-MSCs的分布情况。

1.5 睾丸系数计算和睾丸组织病理学检查

脏器系数是评价器官损伤的基本指标,睾丸系数按照公式得出:睾丸系数=双侧睾丸质量(g)/体质量(g)×100%。照射后12周,各组小鼠称量体质量并观察一般状况,小鼠麻醉后收集双侧睾丸,观察外观并称量睾丸质量,用于计算睾丸系数。每组7只小鼠睾丸冻存,其中5只小鼠单侧睾丸待制备单细胞悬液,其余睾丸均冻存。每组3只小鼠双侧睾丸组织经Bouin's液固定24 h,矢状面切开后,进行组织脱水、石蜡包埋,随后以5 μm厚度进行切片。切片经脱蜡、复水后,进行苏木精-伊红(hematoxylin and eosin staining, HE)染色。于每组切片样本中随机选取50个生精小管进行分析,使用Image J软件测量直径和生精上皮厚度。

1.6 精子质量分析

每组取6只小鼠新鲜双侧附睾尾,置于1 mL精子细胞BWW培养基中,将样本放入37 ℃培养箱中,以促进精子充分游离,过滤后得到精子悬液。取10 μL精子悬液,使用全自动精子质量分析仪检测精子活力;吸取部分精子悬液使用细胞计数板与Image J软件进行精子计数;另取部分精子悬液涂片,经4%多聚甲醛固定、HE染色后,于显微镜下观察精子形态。

1.7 睾丸组织线粒体膜电位和活性氧水平检测

每组取5只小鼠单侧新鲜睾丸组织,用于制备单细胞悬液,分别按照线粒体膜电位检测试剂盒说明书和活性氧检测试剂盒说明书进行操作,随后使用流式细胞仪分别检测各组小鼠睾丸组织线粒体膜电位和活性氧水平。

1.8 睾丸组织T-SOD活性、CAT活性和MDA含量检测

取冷冻睾丸组织制备组织匀浆,以4 ℃、12 000 r/min离心5 min,取上清保存待测,用BCA法检测蛋白浓度。按T-SOD、CAT和MDA试剂盒说明书检测上清中T-SOD活性、CAT活性和MDA含量。

1.9　TUNEL染色法

取各组小鼠睾丸组织的石蜡切片进行脱蜡、复水,并滴加蛋白酶K溶液后,滴加内源性过氧化物酶阻断剂,之后按照TUNEL原位细胞凋亡检测试剂盒说明书进行操作,光镜下观察并拍照。

1.10　Western blot实验

从小鼠睾丸组织中提取蛋白质样品,用BCA试剂盒检测蛋白浓度。每孔加25 μg蛋白样品,SDS-PAGE凝胶电泳分离后,湿转至聚二氟乙烯(polyvinylidene difluoride, PVDF)膜,随后使用5%脱脂牛奶室温封闭2 h,加入按比例稀释的一抗中(β-actin,1∶5 000;PINK1,1∶1 000;Parkin,1∶1 000;OPA1,1∶2 000;Drp1,1∶50 000;Beclin1,1∶10 000;Bax,1∶5 000;Bcl-2,1∶2 000),4 ℃孵育过夜,然后分别置于山羊抗兔二抗(1∶5 000)或山羊抗鼠二抗(1∶5 000)中,室温条件孵育2 h。使用超敏发光液,于Universal Hood Ⅱ电泳成像系统中进行曝光显影,并采用Image J软件对条带灰度值进行分析。

1.11　统计学分析

采用GraphPad Prism 9.0软件进行统计分析及图表绘制,计量数据以$\bar{x}\pm s$表示。多组间均值的比较使用单因素方差分析(one-way ANOVA),设定检验水准α=0.05。

2　结果

2.1　hUC-MSCs培养和鉴定结果

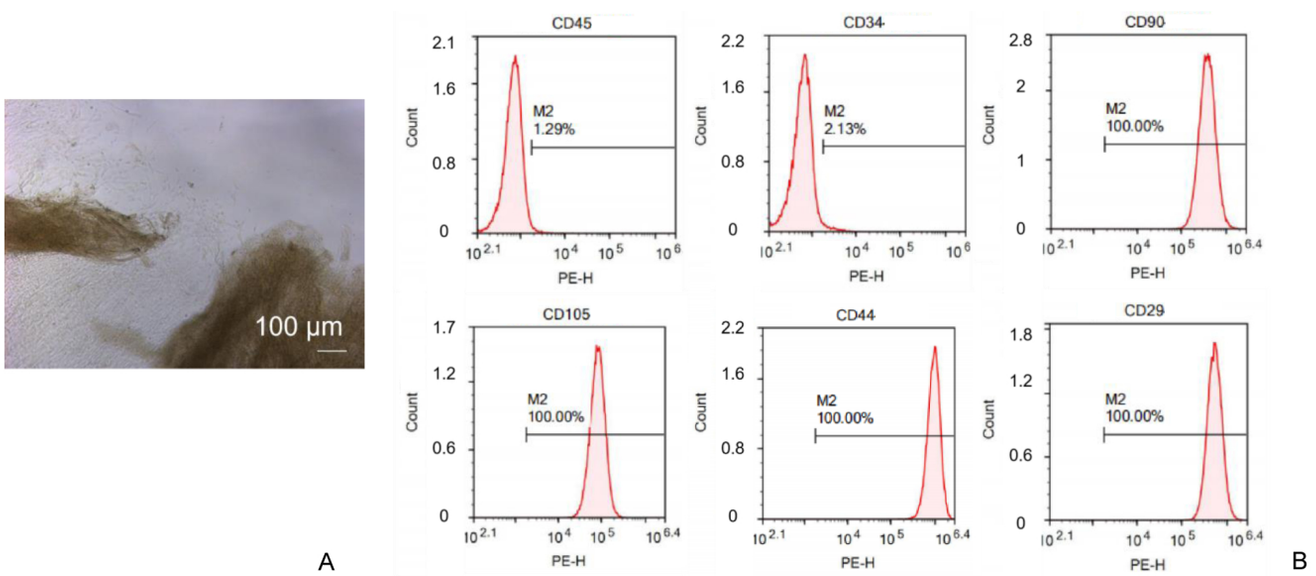
培养至第14天,可见华通氏胶组织块周围有细胞迁出并贴壁生长,融合度约达80%(图1A)。经传代后,细胞形态均一、稳定,呈梭形,符合hUC-MSCs的典型形态特征。使用流式细胞术进行hUC-MSCs免疫表型鉴定,结果显示,该细胞CD45、CD34阴性表达,CD90、CD105、CD44、CD29阳性表达(图1B)。提示培养获得的细胞为hUC-MSCs。

2.2　hUC-MSCs在小鼠睾丸组织中的分布情况

使用多模式动物活体成像系统观察hUC-MSCs在小鼠睾丸内的分布情况,结果显示,Control+PBS组小鼠睾丸组织未见荧光信号,Control+MSCs组与Rad+MSCs组小鼠睾丸组织中均可见荧光信号,提示经尾静脉注射的hUC-MSCs能够迁移至小鼠睾丸组织中(图2A)。进一步通过共聚焦荧光显微镜观察睾丸冰冻切片,结果显示,在Control+MSCs组与Rad+MSCs组小鼠睾丸间质区域可见红色荧光,检测到荧光染料标记的hUC-MSCs信号(图2B)。提示hUC-MSCs可进入小鼠睾丸组织中,并主要分布在睾丸间质区域。

2.3　hUC-MSCs可增加辐射损伤小鼠睾丸组织生精上皮厚度

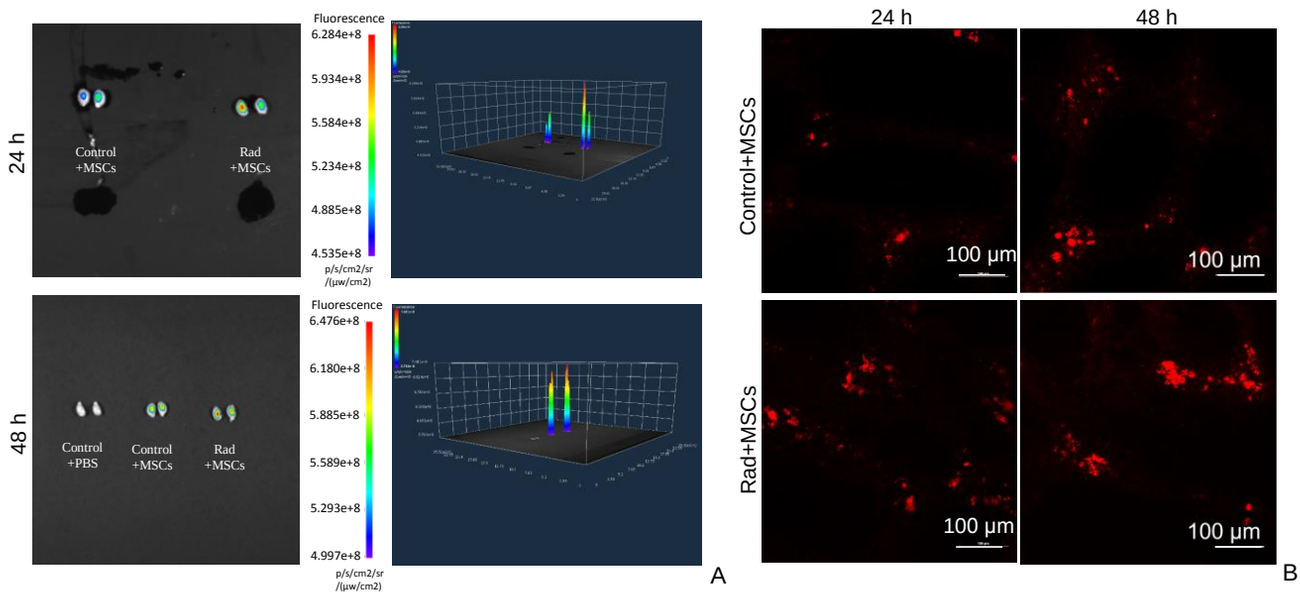
HE染色结果显示,小鼠在6 Gy单次照射12周后,与Control组相比,Rad组小鼠睾丸组织可见明显病理改变,具体表现为生精小管内生精细胞空泡化明显、细胞层次减少及排列紊乱,生精小管管腔中较成熟的长形精子细胞明显减少,管腔萎缩,各级生精细胞数量明显减少,仅靠近生精小管基底部有少数细胞



A: hUC-MSCs primary cells; B: Identification results of CD45, CD34, CD90, CD105, CD44, CD29 by flow cytometry

图1　hUC-MSCs形态及免疫表型鉴定

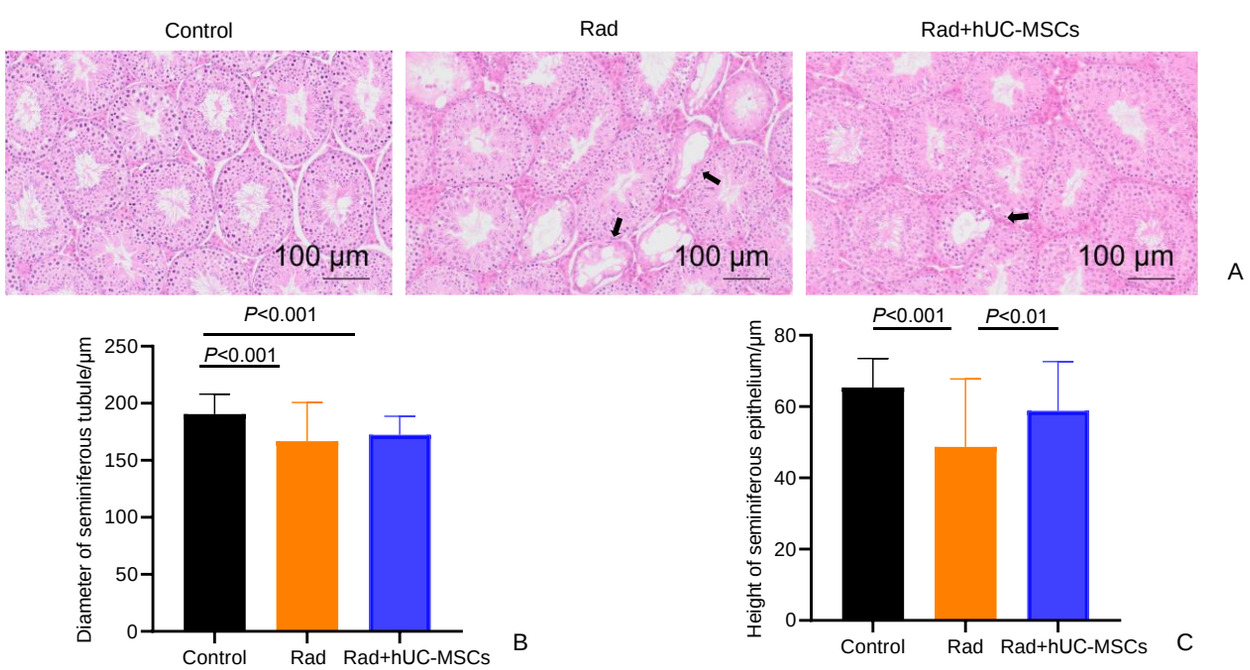
Figure 1　Morphological and immunophenotypic identification of hUC-MSCs



A: Fluorescence imaging and 3D gray value of testicular tissue; B: Distribution of hUC-MSCs in mouse testicular tissue

图 2 hUC-MSCs在小鼠睾丸组织中的分布情况

Figure 2 Distribution of hUC-MSCs in mouse testicular tissue



A: HE staining of testicular tissue, arrow indicating the pathological damage site of testis; B: Diameter of seminiferous tubule; C: Height of seminiferous epithelium

图 3 HE染色检测各组小鼠睾丸组织形态结构

Figure 3 HE staining results show the morphological structure of testicular tissue in each group

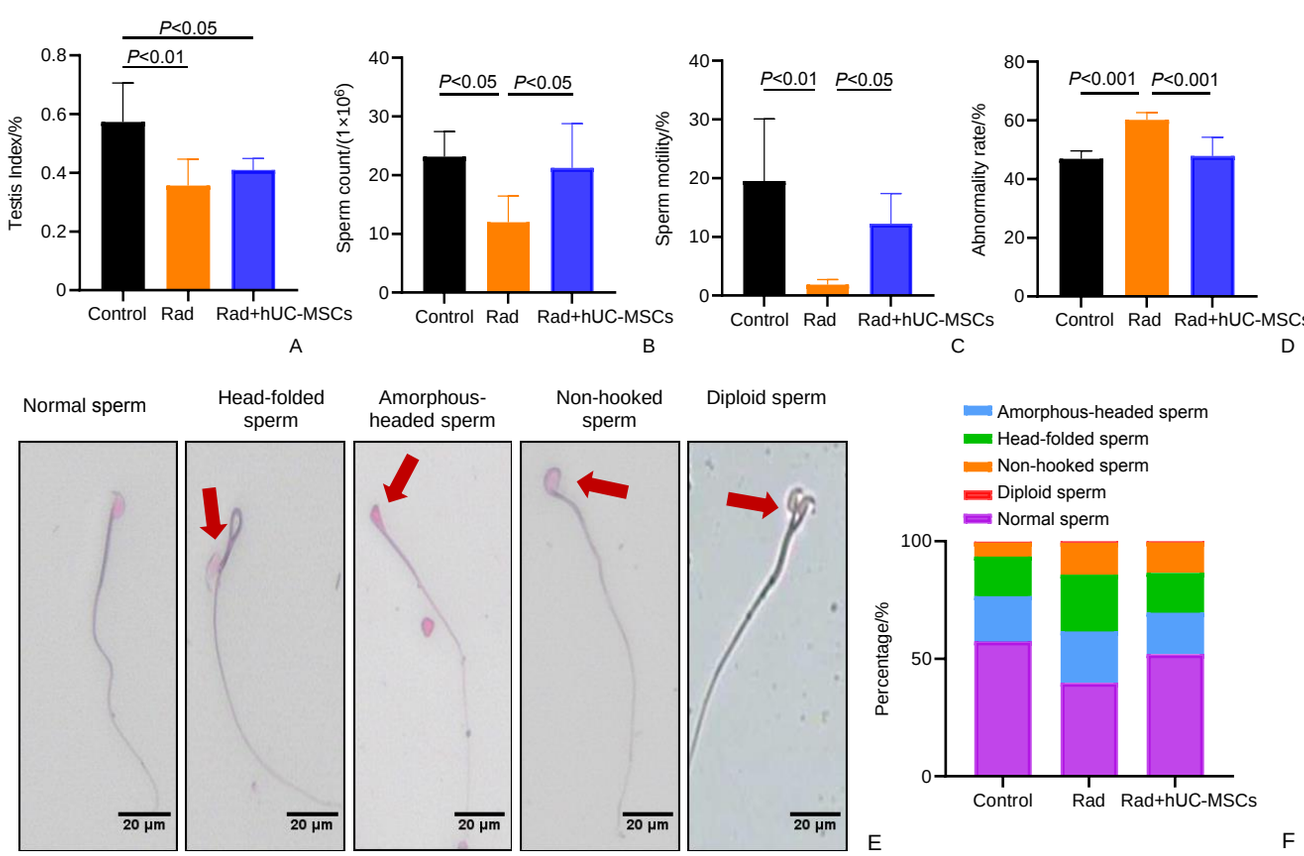
(图3A),且睾丸生精小管直径和生精上皮厚度均显著减小(P<0.001);与Rad组相比,Rad+hUC-MSCs组小鼠睾丸组织损伤部分恢复,生精上皮厚度显著增加(P<0.01,图3B、C),生精小管内生精细胞排列较整齐,空泡化程度减轻。提示hUC-MSCs治疗可改善6 Gy单次照射引起的小鼠睾丸组织结构损伤,对辐射所致生精小管形态异常及生精上皮损伤具有改善作用。

2.4　hUC-MSCs可改善辐射损伤小鼠精子质量

显微镜下评估小鼠精子质量,结果发现,照射后12周,与Control组相比,Rad组小鼠的睾丸系数(P<0.01)、精子数量(P<0.05)及活力(P<0.01)均明显降低;与Rad组相比,Rad+hUC-MSCs组小鼠精子数量及活力均明显升高(P<0.05,图4A~C)。观察并统计各组小鼠附睾尾中常见的精子形态及构成,结果发现,与Control组相比,Rad组小鼠的精子畸形率显著升高(P<0.001),畸形精子以体折叠和不定形为主;与Rad组相比,Rad+hUC-MSCs组小鼠精子畸形率显著下降(P<0.001,图4D~F)。提示hUC-MSCs干预可通过提升精子数量与活力、降低精子畸形率,有效缓解辐射致小鼠生殖功能损伤,改善6 Gy单次照射导致的小鼠精子质量损伤。

2.5　hUC-MSCs可减轻辐射所致小鼠睾丸细胞线粒体损伤

流式细胞术结果显示,与Control组相比,Rad组小鼠睾丸细胞线粒体膜电位显著降低(P<0.001),Rad+hUC-MSCs组小鼠睾丸细胞线粒体膜电位较Rad组明显升高(P<0.01,图5A、B);Western blot结果显示,与Control组相比,Rad组小鼠睾丸睾丸组织线粒体自噬相关蛋白PINK1(P<0.05)、Parkin(P<0.01)、Beclin1(P<0.05)表达显著升高,线粒体融合相关蛋白OPA1(P<0.01)和分裂相关蛋白Drp1(P<0.05)蛋白表达量升高;与Rad组相比,Rad+hUC-MSCs组小鼠睾丸组织线粒体自噬相关蛋白PINK1、Parkin、Beclin1表达降低(P<0.05),线粒体融合相关蛋白OPA1(P<0.01)和分裂相关蛋白Drp1(P<0.05)蛋白表达量降低(图5C~H)。提示hUC-MSCs干预可改善6 Gy单次照射导致的小鼠睾丸细胞线粒体损伤。



A: Testis index at 12 weeks after irradiation in Control group, Rad group and Rad+MSCs group; B: Sperm count at 12 weeks after irradiation in Control group, Rad group and Rad+MSCs group; C: Sperm motility at 12 weeks after irradiation in Control group, Rad group and Rad+MSCs group; D: Abnormality rate at 12 weeks after irradiation in Control group, Rad group and Rad+MSCs group; E: Common morphology of sperm in mouse epididymal tail, and arrow indicating sperm abnormality; F: Proportion of sperm morphology in mouse epididymal tail

图4　hUC-MSCs可改善辐射损伤小鼠精子质量

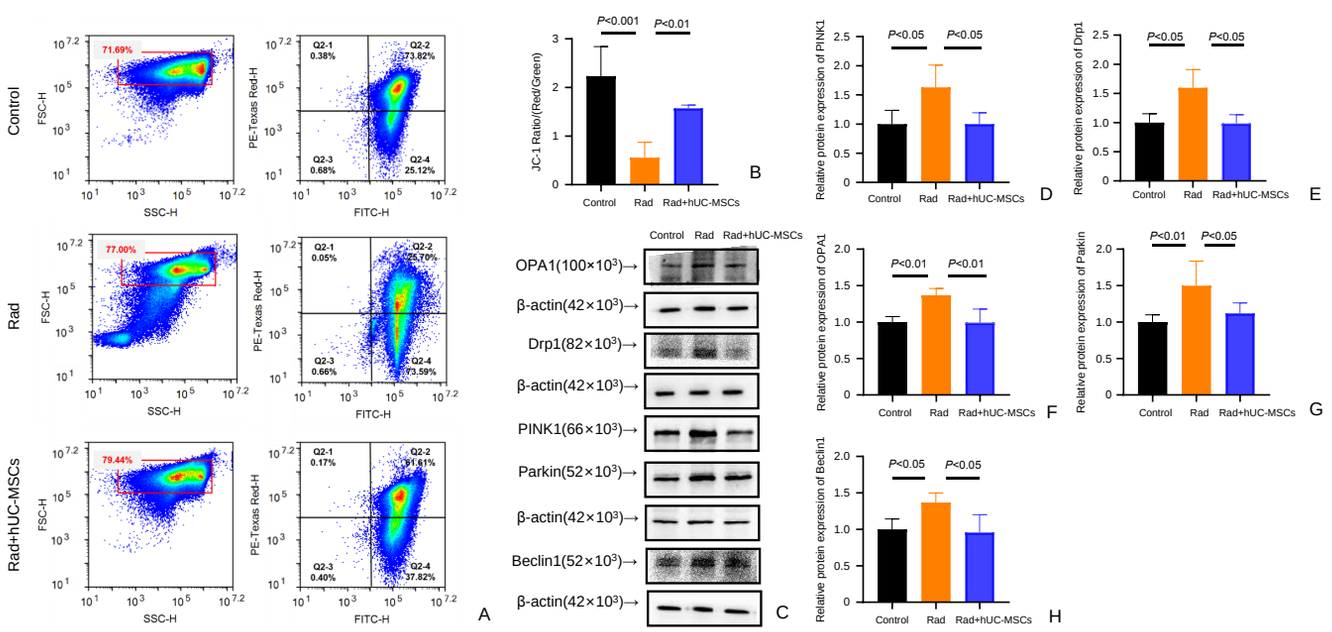
Figure 4　hUC-MSCs can improve the sperm quality of radiation injured mice

2.6　hUC-MSCs可降低辐射所致小鼠睾丸组织氧化应激水平

流式细胞术结果显示，与Control组相比，Rad组小鼠睾丸组织ROS水平升高（$P<0.05$），MDA含量显著升高（$P<0.001$），CAT活性降低（$P<0.05$），T-SOD活性升高（$P<0.05$）；Rad+hUC-MSCs组小鼠睾丸组织ROS水平较Rad组降低（$P<0.05$，图6A），MDA含量显著降低（$P<0.001$，图6B），CAT活性升高（$P<0.05$，图6C），T-SOD活性降低（$P<0.05$，图6D）。提示hUC-MSCs干预可改善6 Gy单次照射导致的小鼠睾丸组织氧化应激损伤。

2.7　hUC-MSCs可减轻辐射所致小鼠睾丸细胞凋亡

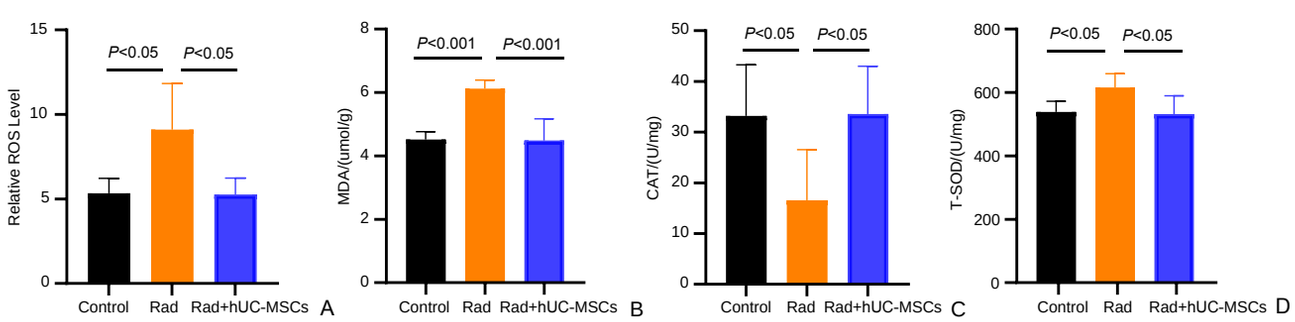
Western blot结果显示，Rad组小鼠睾丸组织Bcl-2/Bax较Control组明显降低（$P<0.01$），Rad+hUC-MSCs组小鼠睾丸组织Bcl-2/Bax较Rad组升高（$P<0.05$，图7A、B）；TUNEL染色结果显示，与Control组相比，Rad组小鼠睾丸组织中TUNEL阳性细胞明显增多（$P<0.001$），且这些凋亡细胞多分布于生精小管基底



A and B: Mitochondrial membrane potential and flow cytometry representative diagram of testicular cells; C~H: Western blotting detection of PINK1, Drp1, OPA1, Parkin and Beclin1 protein expression and gray value statistics in testicular tissue

图5　hUC-MSCs可减轻辐射所致小鼠睾丸细胞线粒体损伤

Figure 5　hUC-MSCs can alleviate radiation-induced mitochondrial damage in mouse testicular cells



A: ROS level in testicular tissue; B: MDA content in testicular tissue; C: CAT activity in testicular tissue; D: T-SOD activity in testicular tissue

图6　hUC-MSCs可降低辐射所致小鼠睾丸组织氧化应激水平

Figure 6　hUC-MSCs can reduce the level of oxidative stress in testicular tissue of mice induced by radiation

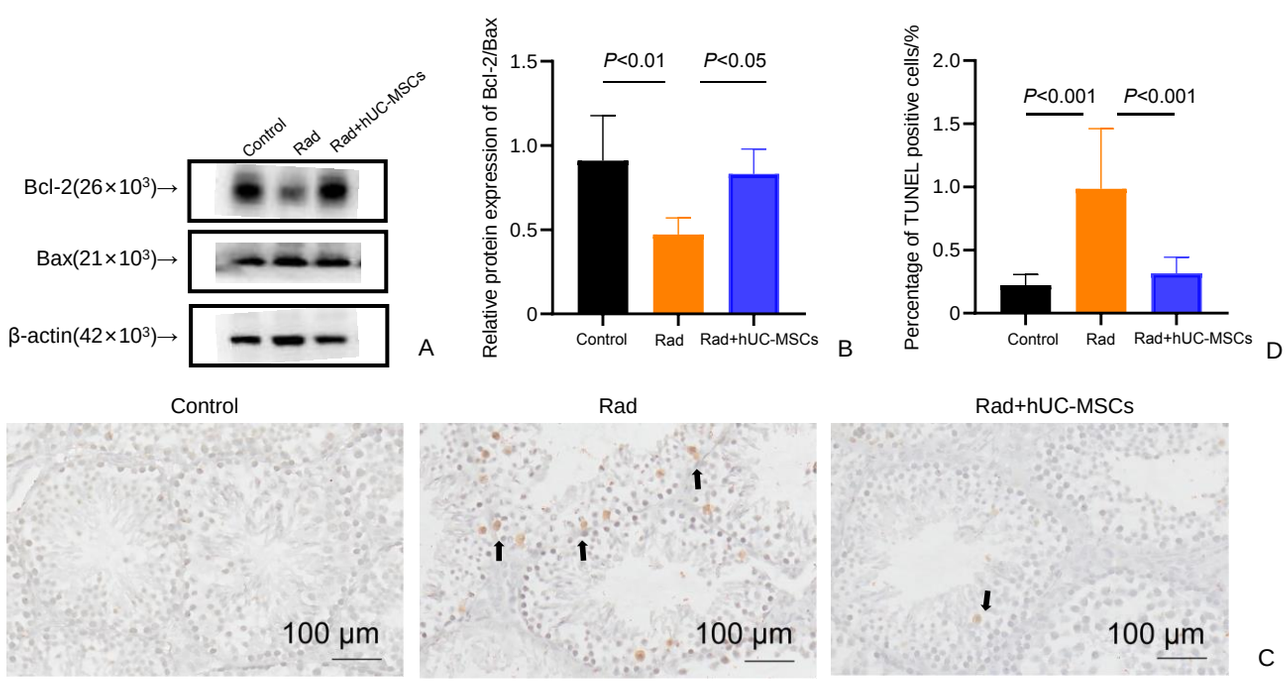
部,推测其主要为精原细胞和精母细胞;Rad+hUC-MSCs组小鼠睾丸组织中TUNEL阳性细胞较Rad组显著减少(P<0.001,图7C、D)。提示hUC-MSCs干预可减轻6 Gy单次照射导致的小鼠睾丸细胞凋亡。

3 讨论

放射治疗是临床肿瘤治疗的常见手段之一。睾丸作为对电离辐射高度敏感的器官,在接受电离辐射全身照射(total body irradiation, TBI)时极易受到损伤,TBI在医学中常用于骨髓移植前的预处理,据统计,绝大多数骨髓移植男性患者在接受12 Gy TBI预处理后发生永久不育[11]。动物实验表明,5~6 Gy的TBI即可引起小鼠生精障碍[12-13]。结合课题组前期预实验结果,本研究选用6 Gy作为放射性雄性生殖损伤动物模型的照射剂量。

3.1 hUC-MSCs可改善辐射损伤的小鼠精子质量

放射性雄性生殖损伤及其修复日益受到关注[14-15],关于放射性生殖损伤防治药物的研究近年来也有较多报道[16-17],如辅酶Q10等,但因效果有限限制了其应用。hUC-MSCs作为一种具有高度增殖与多向分化潜能的成体干细胞[18],在免疫调节、组织修复及抗凋亡等方面均发挥重要作用,有望成为治疗放射性生殖损伤的新途径。因此,本研究评估了hUC-MSCs对放射性雄性生殖损伤的促修复作用。精子发生是成熟精子细胞形成的复杂过程,对IR极为敏感[19]。精子数量、活力及形态是评估男性生育能力的关键指标。既往研究表明,IR暴露可导致精子质量下降,进而损害生育能力[20]。本研究首先从精子质量角度评估hUC-MSCs的修复效果,发现hUC-MSCs干预可提高精子数量与活力并降低畸形率。然而,hUC-MSCs对小鼠睾丸系数未见明显影响,因此我们进一步通过组织病理学分析评估其对精子发生过程的作用。结果与既往报道一致[21],hUC-MSCs干预改善了生精小管结构,表现为生精上皮厚度增加、生精细胞排列更为有序、数量增多,但生精小管直径未见显著变化,提示hUC-MSCs对辐射引起的精子发生障碍具有一定改善作用。接下来我们从氧化应激、凋亡、线粒体结构功能等方面探讨了hUC-MSCs改善放射性雄性生殖损伤的机制。



A and B: Western blotting representative bands and semiquantitative analysis of apoptosis related proteins in testis; C: Typical pictures of testicular tissue by TUNEL staining, and arrow indicating TUNEL positive cells; D: The quantitative analysis of TUNEL positive cells

图7 hUC-MSCs可减轻辐射所致小鼠睾丸细胞凋亡

Figure 7 hUC-MSCs can reduce the apoptosis of mouse testicular cells induced by radiation

3.2 hUC-MSCs发挥改善作用的机制

研究表明,MSCs的修复能力可能与其抗氧化应激和抗凋亡特性密切相关[22-23]。睾丸生殖细胞对辐射敏感,较低剂量的电离辐射即可引发精原细胞凋亡,影响生殖能力[24-25]。IR主要通过诱导水分子辐射分解,产生过量自由基,进而损伤DNA、蛋白质和脂质等生物大分子,引发精原细胞凋亡。因此,氧化应激和细胞凋亡在IR诱导的精子发生障碍中起关键作用[26]。MDA作为脂质过氧化的终产物,其水平可反映组织氧化损伤程度;SOD和CAT则是机体重要的抗氧化酶。本研究通过检测睾丸组织中ROS水平及MDA含量、T-SOD和CAT活性,探讨hUC-MSCs的改善作用机制。我们发现hUC-MSCs可降低ROS和MDA水平、上调CAT活性、下调T-SOD活性,表明其改善作用与增强机体抗氧化能力、缓解氧化应激损伤有关。

精子运动所需的能量主要由线粒体通过氧化磷酸化产生的ATP提供[27],既往研究已经证实,线粒体功能完整性与膜电位稳定性与精子活力密切相关[28]。因此,我们推测本研究中精子活力的降低可能与其线粒体损伤有关。线粒体持续的融合与分裂过程,对于维持其自身功能及细胞稳态至关重要[29]。本研究结果显示,与Control组相比,Rad组小鼠睾丸组织中线粒体融合蛋白OPA1与分裂蛋白Drp1表达均出现上调,提示电离辐射可致小鼠睾丸细胞线粒体动力学平衡失调,后者被认为是细胞应激及凋亡发生的重要诱因之一[30]。PINK1/Parkin通路是线粒体自噬的核心调控途径。当线粒体受损时,PINK1在线粒体外膜上稳定聚集,进而招募并激活胞质中的Parkin,启动线粒体自噬以清除功能障碍的线粒体[31]。线粒体功能障碍是辐射诱发细胞凋亡的重要机制之一[32-33]。在凋亡信号刺激下,促凋亡蛋白Bax转移至线粒体膜上形成通道,增加膜通透性,而抗凋亡蛋白Bcl-2可拮抗该过程,Bcl-2/Bax的比值变化直接影响细胞凋亡水平。本研究结果显示,hUC-MSCs干预可提高睾丸细胞线粒体膜电位,下调PINK1、Parkin和Beclin1蛋白表达,提升Bcl-2/Bax比值,并减少TUNEL阳性细胞数量,提示hUC-MSCs可能通过调控PINK1/Parkin通路介导的线粒体自噬与凋亡过程,减轻辐射对睾丸组织的损伤。

研究表明,线粒体损伤可引发细胞能量代谢紊乱并产生较多ROS,导致氧化应激水平升高,后者会进一步加剧线粒体功能障碍,并诱导细胞发生线粒体途径细胞凋亡[34-35]。此外,线粒体损伤也可诱发线粒体自噬,后者通过选择性清除受损线粒体,降低ROS水平,维持细胞内氧化还原稳态。结合文献报道,我们推测,hUC-MSCs可能通过减轻睾丸细胞线粒体损伤,降低氧化应激水平,从而抑制了生精细胞的过度凋亡。

关于hUC-MSCs在IR所致雄性生殖损伤中发挥改善作用的具体分子机制,仍有待深入阐明。在针对其他类型雄性生殖损伤模型的研究中,有研究者在显微镜下将hUC-MSCs注射到经白消安处理清除精子的小鼠生精小管管腔内,细胞示踪结果显示,hUC-MSCs能够长期存活并迁移至生精小管基底膜区域,同时表达生殖细胞标志物,提示其在生精小管微环境中可进一步分化为生殖细胞,促进生精小管结构修复[36-37]。另有研究报道,MSCs能够分泌多种对精子发生至关重要的因子,如胶质细胞源性神经营养因子(glial cell line-derived neurotrophic factor, GDNF)、成纤维细胞生长因子2(fibroblast growth factor 2, FGF2)、血管内皮生长因子(vascular endothelial growth factor, VEGF)以及色素上皮衍生因子(pigment epithelium-derived factor, PEDF)等,这些因子通过维持睾丸微环境稳定,间接调控精子发生过程[38]。本研究结果显示,hUC-MSCs可迁移至辐射损伤小鼠的睾丸组织中,但主要分布于睾丸间质。基于此,我们推测,在本实验条件下,hUC-MSCs对辐射所致雄性生殖损伤的改善作用,可能更多依赖于其旁分泌途径所释放的生物活性因子,间接促进精子发生过程。

综上所述,本研究表明,hUC-MSCs干预可有效改善电离辐射所致的雄性生殖损伤。其作用机制可能是:hUC-MSCs通过旁分泌效应调控PINK1/Parkin介导的线粒体自噬,改善辐射诱导的睾丸细胞线粒体功能和结构损伤,减轻睾丸组织氧化应激水平及凋亡,最终实现对雄性生殖功能损伤的改善。本研究为放射性雄性生殖损伤的修复提供了新思路和新方法。

利益冲突 本文所有作者声明不存在利益冲突

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