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人脐带间充质干细胞培养上清对米非司酮处理的人子宫内膜基质细胞存活、凋亡和子宫内膜容受性的影响

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[摘要] **目的:** 探讨人脐带间充质干细胞培养上清(hUCMSCs-Sup)对米非司酮(Ms)处理的人子宫内膜基质细胞(hEndoSCs)增殖、凋亡和子宫内膜容受性的影响,并阐明其可能的作用机制。**方法:** 体外培养hEndoSCs,分为对照组和40、60、80及100 $\mu\text{mol}\cdot\text{L}^{-1}$ Ms组,MTT法检测各组细胞存活率。hEndoSCs分为对照组、40 $\mu\text{mol}\cdot\text{L}^{-1}$ Ms组和60 $\mu\text{mol}\cdot\text{L}^{-1}$ Ms组,流式细胞术检测各组细胞凋亡率,Western blotting法检测各组细胞中凋亡相关蛋白B细胞淋巴瘤2(Bcl-2)和Bcl-2相关X蛋白(Bax)蛋白表达水平并计算Bcl-2/Bax比值。hUCMSCs-Sup作用后,hEndoSCs分为对照组、Ms组、Ms+hUCMSCs-Sup组和Ms+hUCMSCs-Sup+3-甲基腺嘌呤(3-MA)组,MTT法检测各组细胞存活率,流式细胞术检测各组细胞凋亡率,Western blotting法检测各组细胞中微管相关蛋白1轻链3B-II(LC3B-II)和微管相关蛋白1轻链3B-I(LC3B-I)蛋白表达水平并计算LC3B-II/LC3B-I比值,实时荧光定量PCR(RT-qPCR)法检测各组细胞中子宫内膜容受性标志分子mRNA表达水平。**结果:** 与对照组比较,40、60、80和100 $\mu\text{mol}\cdot\text{L}^{-1}$ Ms组细胞存活率明显降低($P<0.05$),且具有时间和剂量依赖性。与对照组比较,40和60 $\mu\text{mol}\cdot\text{L}^{-1}$ Ms组细胞凋亡率明显升高($P<0.05$),细胞中Bcl-2/Bax比值明显降低($P<0.05$)。hUCMSCs-Sup作用后,与对照组比较,Ms组细胞存活率和细胞中LC3B-II/LC3B-I比值明显降低($P<0.05$),细胞凋亡率明显升高($P<0.05$),细胞中同源框基因A10(HOXA10)、白血病抑制因子(LIF)和整合素亚基 $\beta 3$ (ITGB3)mRNA表达水平明显降低($P<0.05$);与Ms组比较,Ms+hUCMSCs-Sup组细胞存活率和细胞中LC3B-II/LC3B-I比值明显升高($P<0.05$),细胞凋亡率明显降低($P<0.05$),细胞中HOXA10、LIF和ITGB3 mRNA表达水平表达明显升高($P<0.05$);与Ms+hUCMSCs-Sup组比较,Ms+hUCMSCs-Sup+3-MA组细胞存活率和细胞中LC3B-II/LC3B-I比值明显降低($P<0.05$)。**结论:** hUCMSCs-Sup可提高Ms处理后hEndoSCs的存活率,降低其凋亡率,提高子宫内膜容受性,其机制可能与hUCMSCs-Sup激活hEndoSCs自噬有关。

[关键词] 脐带间充质干细胞;人子宫内膜基质细胞;细胞凋亡;细胞自噬;子宫内膜容受性

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Effect of culture supernatant of human umbilical cord mesenchymal stem cells on survival, apoptosis and endometrium receptivity of human endometrial stromal cells after treated with mifepristone

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ABSTRACT Objective: To discuss the effect of human umbilical cord mesenchymal stem cells culture supernatant (hUCMSCs-Sup) on the proliferation, apoptosis, and endometrium receptivity of the human endometrial stromal cells (hEndoSCs) treated with mifepristone (Ms), and to clarify the possible mechanism. **Methods:** The hEndoSCs were cultured *in vitro* and divided into control group and 40, 60, 80, and 100 $\mu\text{mol}\cdot\text{L}^{-1}$ Ms groups. The survival rates of the cells in various groups were detected by MTT assay. The hEndoSCs were divided into control group, 40 $\mu\text{mol}\cdot\text{L}^{-1}$ Ms group, and 60 $\mu\text{mol}\cdot\text{L}^{-1}$ Ms group. The apoptotic rates of the cells in various groups were detected by flow cytometry; the expression levels of apoptosis-related protein B-cell lymphoma-2 (Bcl-2) and Bcl-2-associated X protein (Bax) proteins in the cells in various groups were detected by Western blotting method, and the ratio of Bcl-2/Bax was calculated. After treated with hUCMSCs-Sup, the hEndoSCs were divided into control group, Ms group, Ms+hUCMSCs-Sup group, and Ms+hUCMSCs-Sup+3-methyladenine (3-MA) group. The survival rates of the cells in various groups were detected by MTT assay; the apoptotic rates of the cells in various groups were detected by flow cytometry; the expression levels of microtubule-associated protein 1 light chain 3B- II (LC3B- II) and microtubule-associated protein 1 light chain 3B-I (LC3B- I) proteins in the cells in various groups were detected by Western blotting method, and the ratio of LC3B- II /LC3B- I was calculated; the expression levels of endometrium receptivity marker molecules mRNA in the cells in various groups were detected by real-time fluorescence quantitative PCR (RT-qPCR) method. **Results:** Compared with control group, the survival rates of the cells in 40, 60, 80, and 100 $\mu\text{mol}\cdot\text{L}^{-1}$ Ms groups were significantly decreased ($P<0.05$) in a time-dependent and dose-dependent manner. Compared with control group, the apoptotic rates of the cells in 40 and 60 $\mu\text{mol}\cdot\text{L}^{-1}$ Ms groups were significantly increased ($P<0.05$), and the ratios of Bcl-2/Bax were significantly decreased ($P<0.05$). After treated with hUCMSCs-Sup, compared with control group, the survival rate of the cells and ratio of LC3B- II /LC3B- I in the cells in Ms group were significantly decreased ($P<0.05$), the apoptotic rate was significantly increased ($P<0.05$), and the expression levels of homeobox A10 (HOXA10), leukemia inhibitory factor (LIF), and integrin subunit beta 3 (ITGB3) mRNA in the cells were significantly decreased ($P<0.05$); compared with Ms group, the survival rate of the cells and ratio of LC3B- II /LC3B- I in the cells in Ms+hUCMSCs-Sup group were significantly increased ($P<0.05$), the apoptotic rate was significantly decreased ($P<0.05$), and the expression levels of HOXA10, LIF, and ITGB3 mRNA in the cells were significantly increased ($P<0.05$); compared with Ms+hUCMSCs-Sup group, the survival rate of the cells and ratio of LC3B- II /LC3B- I in the cells in Ms+hUCMSCs-Sup+3-MA group were significantly decreased ($P<0.05$). **Conclusion:** hUCMSCs-Sup can increase the survival rate and decrease the apoptotic rate of the hEndoSCs after treated with Ms, and increase the endometrium receptivity, and its mechanism may be associated with the activation of autophagy of the hEndoSCs by hUCMSCs-Sup.

KEYWORDS Umbilical cord mesenchymal stem cell; Human endometrial stromal cell; Apoptosis; Autophagy; Endometrium receptivity

子宫壁分为外层(浆膜)、中层(肌层)和内层(黏膜),内层即子宫内膜。子宫内膜分为功能层和基底层。功能层受卵巢性激素调节发生周期性变化。靠近肌层处的基底层主要由子宫内膜基质细胞(human endometrial stromal cells, hEndoSCs)组成,不受性激素影响,不发生周期性变化^[1-2]。hEndoSCs负责子宫内膜蜕膜化、血管重建和免疫细胞招募,在修复子宫内膜损伤中起着关键作用^[3]。子宫内膜损伤可导致hEndoSCs凋亡,细胞活力降低,从而导致子宫内膜萎缩和子宫内膜稳态失衡^[4]。内膜损伤后的修复异常导致的宫腔黏连(intrauterine adhesions, IUA)和薄型子宫内膜,是试管助孕患者胚胎着床失败和不孕的重要原因^[5]。干细胞疗法作为一种新型的细胞疗法,可以通过抑制炎症、增强细胞增殖和血管重塑等途径发挥修复组织损伤、抗炎、促血管生成及免疫调节等作用^[2]。人脐带间充质干细胞(human umbilical cord mesenchymal stem cells, hUCMSCs)来源丰富,易于获取,免疫原性低下,增殖能力强,无伦理性争议,是组织修复和器官重建的理想种子细胞来源,广泛应用于再生医学领域^[6-7]。研究^[8-13]显示:hUCMSCs能够改善支气管及肺发育不良和肝功能,治疗成人重度再生障碍性贫血、类风湿性关节炎、间质性膀胱炎和糖尿病等,也可以修复受损子宫内膜,提高内膜容受性。hUCMSCs分泌物作为其主要功能物质,修复作用已被证实,但其相关修复机制尚不清楚。本研究拟采用米非司酮(mifepristone, Ms)处理hEndoSCs,观察hUCMSCs培养上清(hUCMSCs culture supernatant, hUCMSCs-sup)对hEndoSCs增殖、凋亡和子宫内膜容受性标志分子表达的影响,并阐明其可能的作用机制。

1 材料与方法

1.1 细胞和主要试剂 hUCMSC和hEndoSCs由吉林省人民医院中心实验室提供。DMEM/F12基础培养基(SH30023.01)购自美国HyClone公司,胎牛血清(FB15015)购自美国Clark生物公司,磷酸盐缓冲液(phosphate buffer saline, PBS)(FG701-01)、青-链霉素双抗(FG101)、Trypsin-EDTA(#FG301-01)、细胞凋亡检测试剂盒(#FA101-02)、RNA提取试剂盒和逆转录试剂盒均购自北京全式金生物技术有限公司,Ms(M8046)和自噬抑制剂3-甲基腺嘌呤

(3-methyladenine, 3-MA)(M9281)购自美国Sigma公司,MTT试剂(M8180)购自北京索莱宝科技有限公司,实时荧光定量PCR(real-time fluorescence quantitative PCR, RT-qPCR)反应体系SYBR Green试剂盒购自美国Bio-Rad公司,GAPDH抗体(sc-137179)购自上海圣克鲁斯生物技术有限公司,B细胞淋巴瘤2(B-cell lymphoma-2, Bcl-2)(#3498)、Bcl-2相关X蛋白(Bcl-2-associated X protein, Bax)(#5023)和微管相关蛋白1轻链3B(microtubule-associated protein 1 light chain 3 beta, LC3B)(#3868)抗体均购自美国Cell Signaling Technology公司。

1.2 hUCMSCs的分离和培养 hUCMSCs提取自吉林省人民医院产科(产妇已知情同意)提供的新生儿脐带组织,由吉林省人民医院中心实验室分离鉴定并传代保存。hUCMSCs用含10%胎牛血清和1%双抗的DMEM/F12培养基培养,待细胞生长融合至70%~80%时传代。第3~6代细胞融合至约80%时换液培养48h,收集细胞上清液用于后续实验。

1.3 hEndoSCs的分离和培养 hEndoSCs提取自吉林省人民医院生殖医学中心提供的子宫内膜刺激患者(患者已知情同意)的新鲜子宫内膜组织,由吉林省人民医院中心实验室分离鉴定并传代保存。hEndoSCs采用含10%胎牛血清和1%双抗的DMEM/F12基础培养基,于5%CO₂、37℃条件下培养,待细胞铺满平皿,消化传代后用于后续实验。

1.4 MTT法检测各组细胞存活率 将消化后的hEndoSCs悬液接种于96孔细胞培养板中(边缘孔用100μL 0.9%NaCl填充),每孔 8×10^3 个细胞和100μL完全培养基,5%CO₂、37℃条件下培养24h。hEndoSCs分为对照组和不同浓度Ms组,弃掉原培养液,分别加入含0、40、60、80和100μmol·L⁻¹Ms的DMEM/F12培养基,每组设6个复孔,分别作用24和48h,加入20μL MTT(5g·L⁻¹),4~6h后移除培养液,加入100μL DMSO置于振荡器上震荡10min,使结晶体充分溶解,采用酶联免疫检测仪于492nm波长处检测各组细胞的吸光度(A)值,计算各组细胞存活率。细胞存活率=(实验组A值-空白组A值)/(对照组A值-空白组A值)×100%。hEndoSCs分为对照组、Ms组、Ms+hUCMSCs-Sup组和Ms+

hUCMSCs-Sup+3-MA组,除对照组处其余各组加入含 $60\ \mu\text{mol}\cdot\text{L}^{-1}$ Ms的培养基作用24 h,后2组将培养液分别替换为含50% (体积比) hUCMSCs-Sup和hUCMSCs-Sup+4 mmol $\cdot\text{L}^{-1}$ 3-MA的培养液,每组设6个复孔,培养48 h后同前处理,计算各组细胞存活率。

1.5 流式细胞术检测各组细胞凋亡率 将hEndoSCs均匀铺于6孔细胞培养板中,每孔加入 2×10^5 个细胞和2 mL完全培养基,5% CO_2 、37 $^\circ\text{C}$ 条件下培养24 h。hEndoSCs分为对照组、40 $\mu\text{mol}\cdot\text{L}^{-1}$ Ms组和60 $\mu\text{mol}\cdot\text{L}^{-1}$ Ms组,弃掉原培养液,分别加入含0、40和60 $\mu\text{mol}\cdot\text{L}^{-1}$ Ms的培养基,作用24 h后用0.25%胰酶消化收集于1.5 mL EP管中,离心机提前预冷至4 $^\circ\text{C}$,1 000 $\text{r}\cdot\text{min}^{-1}$ 离心5 min,弃掉上清,PBS缓冲液洗涤3次,加入100 μL 结合缓冲液悬液制备单细胞混悬液。加入5 μL Annexin V-FITC和5 μL PI染液混匀。室温下避光反应10 min,采用流式细胞仪检测各组细胞凋亡率。hEndoSCs分为对照组、Ms组和Ms+hUCMSCs-Sup组,分别加入含0和60 $\mu\text{mol}\cdot\text{L}^{-1}$ Ms的培养基,作用24 h后,Ms+hUCMSCs-Sup组培养液替换为含50% (体积比) hUCMSCs-Sup的培养液,培养48 h后同前处理,采用流式细胞仪检测各组细胞凋亡率。实验重复3次。

1.6 Western blotting法检测各组细胞中Bcl-2、Bax和LC3B蛋白表达水平 将hEndoSCs均匀铺于6孔细胞培养板中,每孔加入 2×10^5 个细胞和2 mL完全培养基,5% CO_2 、37 $^\circ\text{C}$ 条件下培养24 h。hEndoSCs分为对照组、40 $\mu\text{mol}\cdot\text{L}^{-1}$ Ms组和60 $\mu\text{mol}\cdot\text{L}^{-1}$ Ms组,处理方法见“1.5”,采用Western blotting法检测各组细胞中Bcl-2和Bax蛋白表达水平,计算Bcl-2/Bax比值。hEndoSCs分为对照组、Ms组、Ms+hUCMSCs-Sup组和Ms+hUCMSCs-Sup+3-MA组,处理方法见“1.4”,采用Western blotting法检测各组细胞中LC3B蛋白表达水平,计算LC3B-III/LC3B-I比值。

收集各组细胞,提取各组细胞总蛋白,采用BCA法测定总蛋白含量。每孔分别取15 μg 蛋白上样,将蛋白转移至PVDF膜上,5%脱脂奶粉封闭后,加入适量稀释GAPDH(1:1 000)、Bax(1:1 000)、Bcl-2(1:1 000)和LC3B(1:1 000)抗体4 $^\circ\text{C}$ 孵育过夜,加入二抗(1:500),室温下振荡孵育2 h,采用ECL发光液发光,充分反应后,

采用Image J软件分析蛋白条带灰度值,计算目的蛋白表达水平,实验重复3次。目的蛋白表达水平=目的蛋白条带灰度值/GAPDH蛋白条带灰度值。Bcl-2/Bax比值=Bcl-2蛋白表达水平/Bax蛋白表达水平;LC3B-II/LC3B-I比值=LC3B-II蛋白表达水平/LC3B-I蛋白表达水平。

1.7 RT-qPCR法检测各组细胞中子宫内膜容受性标志分子mRNA表达水平 将hEndoSCs均匀铺于6孔细胞培养板中,分为对照组、Ms组和Ms+hUCMSCs-Sup组,每孔加入 2×10^5 个细胞和2 mL完全培养基,5% CO_2 、37 $^\circ\text{C}$ 条件下培养24 h;弃掉原培养液,分别加入含0和60 $\mu\text{mol}\cdot\text{L}^{-1}$ Ms的培养基,作用24 h后,Ms+hUCMSCs-Sup组细胞更换为含50% (体积比) hUCMSCs-Sup的培养液继续培养。培养48 h后采用TRIzol试剂提取细胞总RNA,根据A(260 nm)值对样品总RNA进行初步定量,逆转录后形成cDNA,采用RT-qPCR法检测各组细胞中子宫内膜容受性标志分子mRNA表达水平。反应条件:94 $^\circ\text{C}$ 预变性5 min;94 $^\circ\text{C}$ 变性1 min,55 $^\circ\text{C}$ 退火30 s,72 $^\circ\text{C}$ 延伸1 min,循环40次;72 $^\circ\text{C}$ 终延10 min。采用GAPDH进行归一化处理。采用 $2^{-\Delta\Delta\text{Ct}}$ 法计算目的基因mRNA表达水平。引物序列:同源框基因A10(homeobox A10, HOXA10), F 5'-AAGCTGAGCTCATCGTTTCC-3', R 5'-G-CACAGGAAAGTCTTGCTAAGG-3';白血病抑制因子(leukemia inhibitory factor, LIF), F 5'-A-TGAACCAGATCAGGAGCC-3', R 5'-CATAG-CTTGTCAGGTTGTTG-3';整合素亚基 β 3(integrin subunit beta 3, ITGB3), F 5'-TCAG-GCGTCTGTAGAGGCTT-3', R 5'-ATGCACA-TCCTTCGATAAAGACTG-3';GAPDH, F 5'-T-CAGGCGTCTGTAGAGGCTT-3', R 5'-ATG-CACATCCTTCGATAAAGACTG-3'。

1.8 统计学分析 采用SPSS 20.0统计软件进行统计学分析。各组细胞存活率和凋亡率,各组细胞中Bcl-2/Bax和LC3B-II/LC3B-I比值及HOXA10、LIF和ITGB3 mRNA表达水平均符合正态分布,以 $\bar{x}\pm s$ 表示,多组间样本均数比较采用单因素方差分析,组间样本均数两两比较采用LSD- t 检验。以 $P<0.05$ 为差异有统计学意义。

2 结果

2.1 不同浓度Ms作用后各组细胞存活率 与对照组比较,40、60、80和100 $\mu\text{mol}\cdot\text{L}^{-1}$ Ms组细胞存

活率逐渐降低 ($P < 0.05$)。随作用时间的延长, 相同浓度 Ms 组细胞存活率逐渐降低, 与 0 h 比较, 作用 24 和 48 h 时同浓度 Ms 组细胞存活率明显降低 ($P < 0.05$); 与 24 h 比较, 作用 48 h 时同浓度 MS 组细胞存活率明显降低 ($P < 0.05$)。见表 1。

表 1 不同浓度 Ms 作用后各组细胞存活率

Tab. 1 Survival rates of cells in various groups after treated with different concentrations of Ms ($n=6, \bar{x} \pm s, \eta/\%$)

Group	Survival rate of cells		
	(t/h) 0	24	48
Control	100.00±1.36	100.00±1.59	100.00±5.23
Ms($\mu\text{mol}\cdot\text{L}^{-1}$)			
40	100.00±3.36	94.20±2.18* Δ	69.30±1.42* Δ #
60	100.00±4.27	69.73±2.09* Δ	33.90±1.88* Δ #
80	100.00±3.58	55.97±1.13* Δ	22.11±2.43* Δ #
100	100.00±6.20	54.22±1.02* Δ	16.70±1.22* Δ #

* $P < 0.05$ compared with control group; $\Delta P < 0.05$ compared with 0 h; # $P < 0.05$ compared with 24 h.

2.2 不同浓度 Ms 作用后各组细胞凋亡率和细胞中 Bcl-2/Bax 比值 与对照组比较, 40 和 60 $\mu\text{mol}\cdot\text{L}^{-1}$ Ms 组细胞凋亡率明显升高 ($P < 0.05$), 细胞中 Bcl-2/Bax 比值明显降低 ($P < 0.05$)。见图 1、图 2 和表 2。

2.3 hUCMSCs-Sup 作用后各组细胞存活率和凋亡率 与对照组比较, Ms 组细胞存活率明显降低 ($P < 0.05$), 细胞凋亡率明显升高 ($P < 0.05$); 与 Ms 组比较, Ms+hUCMSCs-Sup 组细胞存活率明显升高 ($P < 0.05$), 细胞凋亡率明显降低 ($P < 0.05$)。见表 3 和图 3。

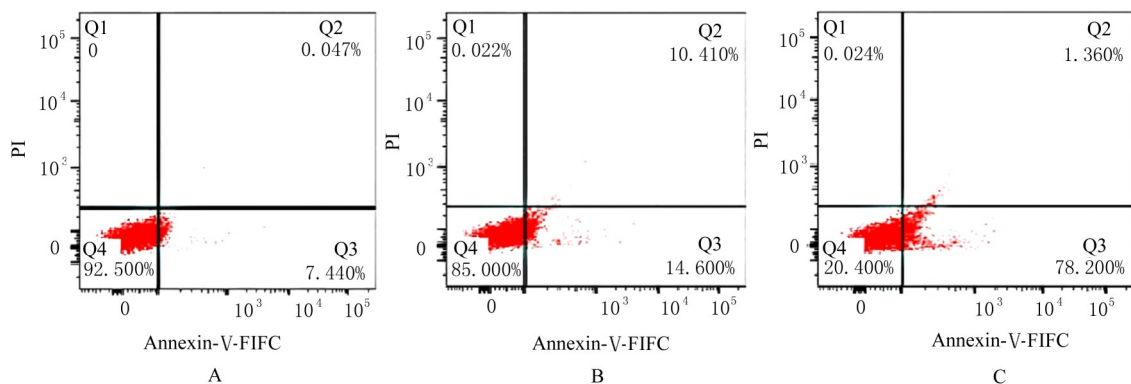
2.4 hUCMSCs-Sup 作用后各组细胞存活率和细胞中 LC3B-II/LC3B-I 比值 与对照组比较, Ms 组细胞存活率和细胞中 LC3B-II/LC3B-I 比值明显降低 ($P < 0.05$); 与 Ms 组比较, Ms+hUCMSCs-Sup 组细胞存活率和细胞中 LC3B-II/LC3B-I 比值明显升高 ($P < 0.05$); 与 Ms+hUCMSCs-Sup 组比较, Ms+hUCMSCs-Sup+3-MA 组细胞存活率和细胞中 LC3B-II/LC3B-I 比值明显降低 ($P < 0.05$)。见图 4 和表 4。

2.5 hUCMSCs-Sup 作用后各组细胞中 HOXA10、LIF 和 ITGB3 mRNA 表达水平 与对照组比较, Ms 组细胞中 HOXA10、LIF 和 ITGB3 mRNA 表达水平明显降低 ($P < 0.05$), Ms+hUCMSCs-Sup 组细胞中 HOXA10、LIF 和 ITGB3 mRNA 表达水平表达明显升高 ($P < 0.05$); 与 Ms 组比较, Ms+hUCMSCs-Sup 组细胞中 HOXA10、LIF 和 ITGB3 mRNA 表达水平表达明显升高 ($P < 0.05$)。见表 5。

3 讨论

在辅助生殖技术中, 子宫内膜损伤是不孕患者胚胎着床失败和妊娠丢失的主要原因^[14]。与正常子宫内膜比较, 受损子宫内膜中相关基因表达水平、形态和功能特征完全改变, 细胞凋亡率明显升高^[15]。研究^[16]表明: hUCMSCs 及其衍生物可以影响细胞增殖和分化等进程, 具有改善宫腔黏连和子宫内膜损伤的潜力。

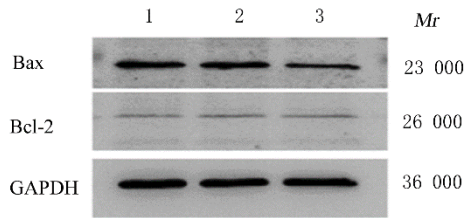
本研究结果显示: Ms 可下调 hEndoSCs 存活率, 且具有时间和剂量依赖性, 并通过调节 Bcl-2/Bax 通路诱导细胞凋亡。hUCMSCs 可修复受



A: Control group; B: 40 $\mu\text{mol}\cdot\text{L}^{-1}$ Ms group; C: 60 $\mu\text{mol}\cdot\text{L}^{-1}$ Ms group.

图 1 流式细胞术检测不同浓度 Ms 作用后各组细胞凋亡率

Fig. 1 Apoptotic rates of cells in various groups after treated with different concentrations of Ms detected by flow cytometry



Lane 1: Control group; Lane 2: $40 \mu\text{mol}\cdot\text{L}^{-1}$ Ms group; Lane 3: $60 \mu\text{mol}\cdot\text{L}^{-1}$ Ms group.

图2 Western blotting法检测不同浓度Ms作用后各组细胞中Bax和Bcl-2蛋白表达电泳图

Fig. 2 Electrophoregram of expressions of Bax and Bcl-2 proteins in cells after treated with different concentrations of Ms detected by Western blotting method

表2 不同浓度Ms作用后各组细胞凋亡率和细胞中Bcl-2/Bax比值

Tab. 2 Apoptotic rates of cells and ratios of Bcl-2/Bax in cells in various groups ($n=3, \bar{x}\pm s$)

Group	Apoptotic rate($\eta/\%$)	Ratio of Bcl-2/Bax
Control	7.44 ± 0.38	3.02 ± 5.02
Ms($\mu\text{mol}\cdot\text{L}^{-1}$)		
40	$14.61\pm 1.11^*$	$2.27\pm 0.04^*$
60	$20.40\pm 1.82^*$	$1.21\pm 0.14^*$

* $P<0.05$ compared with control group.

损组织,减少细胞凋亡发生^[15],最终通过自身分化及旁分泌作用对损伤子宫内膜进行修复^[17]。hUCMSCs-Sup中含有的外泌体^[18]和细胞因子等具有促细胞增殖和抗凋亡作用。hUCMSCs来源外泌体能够明显促进血管内皮细胞增殖和迁移,抑制内皮细胞凋亡^[19-20]。骨髓间充质干细胞条件培养液

表3 各组细胞存活率和凋亡率

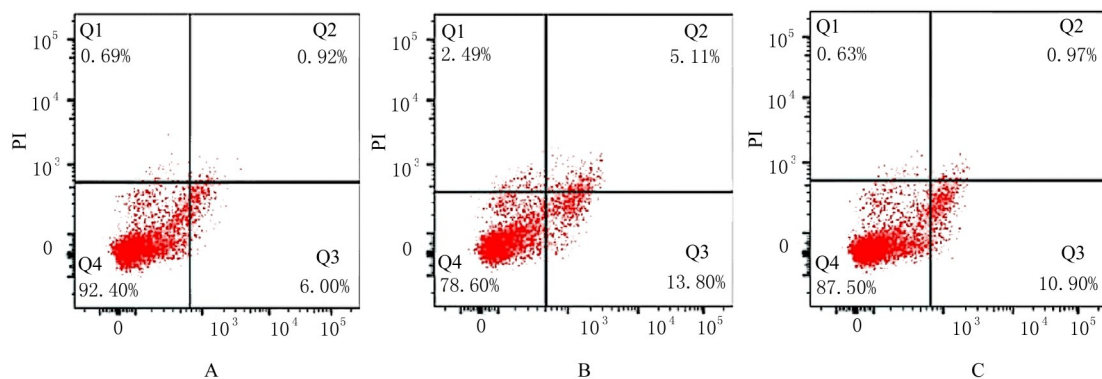
Tab. 3 Survival rates and apoptotic rates of cells in various groups ($n=3, \bar{x}\pm s, \eta/\%$)

Group	Survival rate	Apoptotic rate
Control	100.00 ± 3.41	6.60 ± 1.25
Ms	$68.83\pm 1.47^*$	$20.43\pm 0.71^*$
Ms+hUCMSCs-Sup	$85.00\pm 1.41^{\Delta}$	$11.30\pm 0.61^{\Delta}$

* $P<0.05$ compared with control group; $\Delta P<0.05$ compared with Ms group.

能促进多发性骨髓瘤细胞的增殖,抑制细胞凋亡,降低Bcl-2和Bruton酪氨酸激酶(Bruton's tyrosine kinase, BTK) mRNA及蛋白表达水平^[21]。本研究结果显示:加入hUCMSCs-Sup后,Ms处理的hEndoSCs存活率明显升高,细胞凋亡率明显降低,推测hUCMSCs-Sup可能通过促细胞增殖和抗凋亡作用恢复受损的子宫内膜。

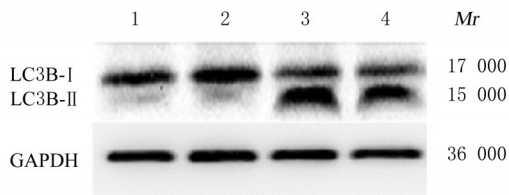
基础自噬对于维持子宫内膜稳态和介导子宫内膜特异性功能至关重要,包括月经周期、胚胎着床和蜕膜化,并参与早期妊娠^[22]。自噬水平在子宫内膜增殖期保持较低水平,但在分泌期明显增加,LC3-II和裂解的含半胱氨酸的天冬氨酸蛋白水解酶3(cleaved cysteinyl aspartate specific proteinase-3, cleaved caspase-3)的表达水平也明显升高。此外,自噬水平紊乱可导致子宫内膜异位症、子宫内膜增生、子宫内膜癌、子宫腺肌症和平滑肌瘤等子宫内膜病变及流产^[23-24]。研究^[25]表明:干细胞可以提高宫腔黏连小鼠子宫内膜的自噬水平,促进血管生成和细胞增殖,提高妊娠率。本研究结果显示:加入hUCMSCs-Sup后hEndoSCs中LC3B-II /



A: Control group; B: Ms group; C: Ms+hUCMSCs-Sup group.

图3 流式细胞术检测各组细胞凋亡率

Fig. 3 Apoptotic rates of cells in various groups detected by flow cytometry



Lane 1: Control group; Lane 2: Ms group; Lane 3: Ms+hUCMSCs-Sup group; Lane 4: Ms+hUCMSCs-Sup+3-MA group.

图4 Western blotting法检测各组细胞中LC3B蛋白表达电泳图

Fig. 4 Electrophoregram of expressions of LC3B protein in cells in various groups detected by Western blotting method

表4 各组细胞存活率和细胞中LC3B-II/LC3B-I比值

Tab. 4 Survival rates of cells and ratios of LC3B-II/LC3B-I in cells in various groups ($n=3, \bar{x} \pm s$)

Group	Survival rate ($\eta/\%$)	Ratio of LC3B-II / LC3B-I
Control	100.00 \pm 1.29	0.39 \pm 0.10
Ms	67.12 \pm 1.85*	0.19 \pm 0.02*
Ms+hUCMSCs-Sup	84.67 \pm 2.32 Δ	1.32 \pm 0.57 Δ
Ms+hUCMSCs-Sup+3-MA	35.12 \pm 1.67 $\#$	1.03 \pm 0.25 $\#$

* $P<0.05$ compared with control group; $\Delta P<0.05$ compared with Ms group; $\#P<0.05$ compared with Ms+hUCMSCs-Sup group.

表5 各组细胞中HOXA10、LIF和ITGB3 mRNA表达水平

Tab. 5 Expression levels of HOXA10, LIF, and ITGB3 mRNA in cells in various groups ($n=3, \bar{x} \pm s$)

Group	HOXA10	LIF	ITGB3
Control	1.00 \pm 0.33	1.00 \pm 0.27	1.00 \pm 0.72
Ms	0.58 \pm 0.12*	0.44 \pm 0.70*	0.63 \pm 0.02*
Ms+hUCMSCs-Sup	2.40 \pm 0.14 Δ	1.93 \pm 0.07 Δ	2.16 \pm 0.16 Δ

* $P<0.05$ compared with control group; $\Delta P<0.05$ compared with Ms group.

LC3B-I 比值明显上调, 提示hUCMSCs-Sup可激活细胞自噬; 加入自噬抑制剂3-MA阻断hUCMSCs-Sup激活自噬后, hUCMSCs-Sup上调hEndoSCs存活率的作用受到抑制, 推测hUC-MSCs-Sup可通过激活hEndoSCs自噬发挥促细胞增殖和抗细胞凋亡作用。

目前普遍认为, ITGB3、HOXA10和LIF是评估子宫内膜容受性的重要指标。ITGB3、HOXA10和LIF在胚胎“种植窗”期子宫内膜组织中表达水平明显升高, 在反复妊娠失败患者内膜组

织中低表达, 且与内膜厚度呈正相关关系^[26]。ITGB3^[27]是重要的细胞黏附分子, 主要介导细胞之间的黏附和信号转导, 并促进胚泡植入和胎盘滋养层的形成。HOXA10是同源盒基因家族中的一员, 表达于子宫内膜上皮的间质和肌层, 对子宫内膜的发育具有时空表达的特异性。LIF属于白细胞介素6 (interleukin-6, IL-6) 超家族^[28], LIF受体的存在与胞饮突的形成有关, 加入干细胞分泌制剂后其表达水平可升高。研究^[29]表明: Ms可明显影响子宫内膜容受性标志物的表达, 降低妊娠率。间充质干细胞移植可减少子宫内膜纤维化面积, 提高容受性标志物ITGB3和LIF的表达, 增强大鼠的生育能力^[30]。干细胞分泌制剂也可以有效地提高子宫内膜容受性^[31]。本研究结果显示: 加入hUCMSCs-Sup后可恢复Ms下调的hEndoSCs中容受性相关标志物的表达, 提示hUCMSCs-Sup具有改善子宫内膜容受性的能力。

综上所述, hUCMSCs-Sup可提高Ms处理的hEndoSCs存活率, 降低细胞凋亡率, 提高子宫内膜容受性, 其作用机制可能与hUCMSCs-Sup激活hEndoSCs细胞自噬有关。

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所有作者声明不存在利益冲突。

作者贡献声明:

武梦雪参与研究设计和论文撰写, 陈士玲和刘艳参与研究数据的获取和分析, 林秀英和付建华参与论文修改和审校, 米旭光和方艳秋参与研究设计。

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