

间充质干细胞来源的外泌体 miR-3614-5p 通过抑制铁死亡改善模型大鼠先兆子痫进展的机制研究

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摘要: **目的** 研究间充质干细胞来源外泌体微小核糖核酸-3614-5p (miR-3614-5p) 对模型大鼠先兆子痫 (preeclampsia, PE) 进展的调节作用及相关机制。**方法** 将36只SD大鼠(24只雌性和12只雄性)以雌雄比例2:1合笼饲养自然受孕。24只妊娠大鼠随机分为假手术组(sham组)、PE模型组(PE组)和外泌体miR-3614-5p组(PE+exo组), 每组8只。PE组通过皮下注射100 mg/kg的NG-硝基-L-精氨酸甲酯建立大鼠PE模型; PE+exo组构建PE模型, 同时第14天腹腔注射160 μ g/ml的外泌体悬液(0.5 ml/只/天), 连续6天, 实验持续21天; sham组则给予等量生理盐水。在妊娠第0, 7, 14和21天测量血压和尿蛋白浓度。RT-qPCR检测miR-3614-5p水平及B细胞淋巴瘤-2(Bcl-2)、Bcl相关X蛋白(Bax)的mRNA水平; ELISA检测Caspase-3活性、活性氧(ROS)水平及丙二醛(MDA)、谷胱甘肽(GSH)和亚铁离子(Fe^{2+})含量; Western blot检测谷胱甘肽过氧化物酶4(GPX4)和溶质载体家族7成员11(SLC7A11)蛋白水平。**结果** 与sham组大鼠相比, PE组大鼠的胎盘组织(0.43 ± 0.05 vs 1.01 ± 0.07)和外周血(0.51 ± 0.07 vs 1.01 ± 0.12)中miR-3614-5p表达显著下调, 差异具有统计学意义($t=19.070, 10.180$, 均 $P<0.01$)。与上清液相比, 源自MSCs的外泌体中miR-3614-5p显著富集。与sham组相比, PE组大鼠第21天的舒张压(175.43 ± 6.02 mmHg vs 113.26 ± 5.11 mmHg)、收缩压(123.57 ± 5.63 mmHg vs 82.63 ± 5.26 mmHg)及尿蛋白含量(175.48 ± 13.21 mg/ml vs 67.65 ± 5.76 mg/ml)显著升高($t=22.606, 16.440, 23.168$, 均 $P<0.01$); 与PE组相比, PE+exo组舒张压(124.57 ± 5.33 mmHg vs 175.43 ± 6.02 mmHg)、收缩压(89.76 ± 3.88 mmHg vs 123.57 ± 5.63 mmHg)及尿蛋白含量(97.69 ± 7.23 mg/ml vs 175.48 ± 13.21 mg/ml)显著降低, 差异具有统计学意义($t=18.493, 13.577, 16.713$, 均 $P<0.01$)。与sham组相比, PE组大鼠胎盘组织中Caspase-3活性($238.56\% \pm 13.22\%$ vs $100.12\% \pm 5.93\%$)、Bax水平(3.18 ± 0.71 vs 1.01 ± 0.11)、ROS水平($387.65\% \pm 25.98\%$ vs $100.51\% \pm 5.89\%$)、MDA含量(33.21 ± 3.17 nmol/mg vs 14.83 ± 2.69 nmol/mg)和 Fe^{2+} 浓度(38.77 ± 6.53 nmol/ml vs 17.51 ± 3.15 nmol/ml)显著升高, 而Bcl-2水平(0.47 ± 0.08 vs 1.01 ± 0.12)、GSH含量(4.12 ± 1.22 nmol/mg vs 9.76 ± 0.93 nmol/mg)、GPX4蛋白(0.48 ± 0.06 vs 1.01 ± 0.24)和SLC7A11蛋白(0.51 ± 0.11 vs 1.01 ± 0.11)水平则显著降低($t=6.459\sim 32.863$, 均 $P<0.01$); 与PE组相比, PE+exo组胎盘组织中Caspase-3活性($117.35\% \pm 8.67\%$ vs $238.56\% \pm 13.22\%$)、Bax水平(1.13 ± 0.45 vs 3.18 ± 0.71)、ROS水平($128.73\% \pm 14.37\%$ vs $387.65\% \pm 25.98\%$)、MDA含量(18.13 ± 3.89 nmol/mg vs 33.21 ± 3.17 nmol/mg)和 Fe^{2+} 浓度(19.05 ± 3.45 nmol/ml vs 38.77 ± 6.53 nmol/ml)显著降低, 而Bcl-2水平(1.04 ± 0.11 vs 0.47 ± 0.08)、GSH含量(7.86 ± 1.07 nmol/mg vs 4.12 ± 1.22 nmol/mg)、GPX4蛋白(0.98 ± 0.14 vs 0.48 ± 0.06)和SLC7A11蛋白(1.11 ± 0.09 vs 0.51 ± 0.11)水平则显著升高, 差异具有统计学意义($t=6.093\sim 29.633$, 均 $P<0.01$)。**结论** miR-3614-5p在PE模型大鼠的胎盘组织和外周血中显著下调。MSCs来源的外泌体miR-3614-5p通过抑制铁死亡改善大鼠的PE进展。MSCs来源的外泌体miR-3614-5p可能是PE治疗的一个新的潜在的生物标志物。

关键词: 先兆子痫; 外泌体; 微小核糖核酸-3614-5p; 铁死亡

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Mechanism Study of Mesenchymal Stem Cell Derived Exosome miR-3614-5p to Improve the Progression of Preeclampsia in Model Rats by Inhibiting Iron Death

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Abstract: Objective To investigate the regulatory effects of exosome microRNA-3614-5p (miR-3614-5p) derived from mesenchymal stem cells on the progression of preeclampsia (PE) in model rats and its related mechanisms. **Methods** Thirty-six SD rats (24 females and 12 males) were housed in cages at a female-to-male ratio of 2:1 for natural conception. Twenty-four pregnant rats were randomly divided into sham group (sham group), PE model group (PE group) and exosome

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miR-3614-5p group (PE+exo group), with 8 rats in each group. The PE model was established by subcutaneous injection of 100 mg/kg NG-nitro-L-arginine methyl ester in PE group. PE model was constructed in PE+exo group. Meanwhile, 160 μ g/ml exosome suspension (0.5 ml/individual/day) was intraperitoneally injected on the 14th day for 6 consecutive days, and the experiment lasted for 21 days. Sham group was given an equal amount of normal saline. Blood pressure and urinary protein concentration were measured on days 0, 7, 14 and 21 of pregnancy. The levels of miR-3614-5p, B lymphoblastoma-2 (Bcl-2) and Bcl-associated X protein (Bax) mRNA were detected by RT-qPCR. The activity of Caspase-3, the levels of reactive oxygen species (ROS) and the content of malondialdehyde (MDA), glutathione (GSH) and ferrous ion (Fe^{2+}) were detected by ELISA. Western blot was used to analyze the protein levels of the iron death-related protein glutathione peroxidase 4 (GPX4) and solute carrier family 7 member 11 (SLC7A11). **Results** Compared with the sham group, the expression of miR-3614-5p in the placental tissues (0.43 ± 0.05 vs 1.01 ± 0.07) and peripheral blood (0.51 ± 0.07 vs 1.01 ± 0.12) of rats in the PE group was down-regulated, with significant differences ($t=19.070, 10.180$, all $P<0.01$). Compared with supernatant liquid phase, miR-3614-5p in exosomes derived from MSCs was enriched. Compared with sham group, the diastolic blood pressure (175.43 ± 6.02 mmHg vs 113.26 ± 5.11 mmHg), systolic blood pressure (123.57 ± 5.63 mmHg vs 82.63 ± 5.26 mmHg) and urinary protein content (175.48 ± 13.21 mg/ml vs 67.65 ± 5.76 mg/ml) of rats in PE group were increased on the 21st day with statistical significance between groups ($t=22.606, 16.440, 23.168$, all $P<0.01$). Compared with PE group, diastolic blood pressure (124.57 ± 5.33 mmHg vs 175.43 ± 6.02 mmHg), systolic blood pressure (89.76 ± 3.88 mmHg vs 123.57 ± 5.63 mmHg) and urinary protein content (97.69 ± 7.23 mg/ml vs 175.48 ± 13.21 mg/ml) in PE+exo group were decreased, and the differences between groups were significant ($t=18.493, 13.557, 16.713$, all $P<0.01$). Compared with sham group, Caspase-3 activity ($238.56\% \pm 13.22\%$ vs $100.12\% \pm 5.93\%$), Bax level (3.18 ± 0.71 vs 1.01 ± 0.11), ROS level ($387.65\% \pm 25.98\%$ vs $100.51\% \pm 5.89\%$), MDA content (33.21 ± 3.17 nmol/mg vs 14.83 ± 2.69 nmol/mg) and Fe^{2+} concentration (38.77 ± 6.53 nmol/ml vs 17.51 ± 3.15 nmol/ml) in placenta tissue of PE group were increased, while Bcl-2 level (0.47 ± 0.08 vs 1.01 ± 0.12), GSH content (4.12 ± 1.22 nmol/mg vs 9.76 ± 0.93 nmol/mg), GPX4 protein (0.48 ± 0.06 vs 1.01 ± 0.24) and SLC7A11 protein (0.51 ± 0.11 vs 1.01 ± 0.11) levels were decreased ($t=6.459\sim 32.863$, all $P<0.01$); Caspase-3 activity ($117.35\% \pm 8.67\%$ vs $238.56\% \pm 13.22\%$), Bax level (1.13 ± 0.45 vs 3.18 ± 0.71), ROS level ($128.73\% \pm 14.37\%$ vs $387.65\% \pm 25.98\%$), MDA content (18.13 ± 3.89 nmol/mg vs 33.21 ± 3.17 nmol/mg) and Fe^{2+} concentration (19.05 ± 3.45 nmol/ml vs 38.77 ± 6.53 nmol/ml) in placental tissues of PE+exo group were decreased, while Bcl-2 level (1.04 ± 0.11 vs 0.47 ± 0.08), GSH content (7.86 ± 1.07 nmol/mg vs 4.12 ± 1.22 nmol/mg), GPX4 protein (0.98 ± 0.14 vs 0.48 ± 0.06) and SLC7A11 protein (1.11 ± 0.09 vs 0.51 ± 0.11) levels were increased compared with PE group, with significant differences between groups ($t=6.093\sim 29.633$, all $P<0.01$). **Conclusion** In the placental tissues and peripheral blood of PE rats, miR-3614-5p was down-regulated. Exosomes overexpressing miR-3614-5p derived from MSCs suppressed PE progression in rats by inhibiting ferroptosis. These results suggested that exosomes miR-3614-5p derived from MSCs may be a novel potential biomarker for PE treatment.

Keywords: preeclampsia; exosomes; microRNA-3614-5p; ferroptosis

先兆子痫 (preeclampsia, PE) 是一种妊娠疾病, 其特征在于妊娠第 20 周后发生的临床症状, 如高血压和尿蛋白。PE 影响 7%~10% 的孕妇, 表现出较高的孕产妇和围产期死亡率^[1-2]。分娩是治疗该疾病的唯一有效方法, 因此治疗 PE 主要集中在血压和其他临床表现的管理上^[3-5]。外泌体为直径约 30~150 nm 的一种细胞外囊泡, 含有信使 RNA (messenger RNA, mRNA)、微小 RNA (microRNA, miRNA) 等多种 RNA^[6-8]。miRNAs 是一种非编码 RNA, 在转录后调控基因表达^[9]。最近研究表明, miRNA 在 PE 的病理生理学中发挥重要作用^[10-11]。此外, PE 患者的胎盘或血液中某些 miRNA 水平异常表达可用于评估 PE 的发生或严重程度^[11], 例如 miR-210^[12]。越来越多的研究检测了循环外泌体在 PE 中的重要性, 但这些 miRNA 影响 PE 的发

生和发展机制尚不完全清楚。因此本研究旨在探讨脐带血间充质干细胞来源的外泌体 miR-3614-5p 在 PE 大鼠中的调节作用及其调节机制, 为阐明 PE 发病机制和早期诊断生物标志物的筛选奠定一定的基础。

1 材料和方法

1.1 动物来源 选择 36 只 (24 只雌性和 12 只雄性) 8 周龄的 SD 大鼠, 均在 12 h 的光/暗循环控制环境下饲养, 恒温约 25 $^{\circ}\text{C}$, 相对湿度约 55%, 自由进食进水。所有动物护理和实验程序均经我院伦理委员会批准 (批准号: HNGA-2022102), 并符合美国国立卫生研究院批准的《实验动物的护理和使用指南》。

1.2 试剂与仪器 反转录试剂盒 (TaKaRa 公司); 山羊抗兔、兔抗鼠 IgG (碧云天生物有限公

司); TRIzol 试剂 (Life Technologies, Carlsbad, CA); ExoQuick exosome 提取试剂盒 (System Biosciences, 美国); exoRNeasy 血清/血浆试剂盒 (Qiagen, Hilden, 德国); Prime Script RT 试剂盒 (Takara, 大连, 中国); miScript SYBR Green 荧光定量 PCR 试剂盒 (QIAGEN, 德国); RIPA 裂解缓冲液和 BCA 蛋白测定试剂盒 (Beyotime Institute of Biotechnology, 上海); ECL 化学发光试剂盒 (Thermo Scientific, 美国); 还原性谷胱甘肽 (glutathione, GSH)、丙二醛 (malondialdehyde, MDA) 和活性氧 (reactive oxygen species, ROS) 的 ELISA 检测试剂盒 (南京建成工程研究所有限公司, 中国); β -actin 抗体 (ab8227), 谷胱甘肽过氧化物酶 4 (Glutathione peroxidase 4, GPx4, ab125066) 抗体、溶质载体家族 7 成员 11 (Solute Carrier Family 7 Member 11, SLC7A11, ab275411) 抗体、山羊抗兔 IgG (ab6721) 抗体及铁含量分析试剂盒 (ab83366) (英国 Abcam 公司)。MK3 型酶标仪 (美国 Thermo Fisher Scientific 公司); Multizoom AZ100 型光学生物显微镜 [尼康仪器 (上海) 有限公司]; Centrifuge5804R 型高速冷冻离心机 (德国 Eppendorf 公司); Tanno 5200 型化学发光凝胶成像仪 (上海天能科技有限公司); Spectra-Maxi3x 型多功能酶标仪 (美国 MD 公司)。

1.3 方法

1.3.1 PE 大鼠模型构建和外泌体治疗: 将 36 只 SD 大鼠 (24 只雌性和 12 只雄性) 以雌雄比例 2:1 合笼饲养, 在雌鼠阴道口发现阴栓的时间确定为怀孕的第一天。将妊娠大鼠随机分为假手术组 (sham, $n=8$), PE 模型组 ($n=8$) 和外泌体 miR-3614-5p 组 (PE+exo, $n=8$)。如前所述^[3], 通过连续皮下给药 100 mg/kg/天的 NG-硝基-L-精氨酸甲酯在妊娠大鼠中诱导 PE; PE+exo 组大鼠构建 PE, 同时在第 14 天腹腔注射蛋白浓度为 160 μ g/ml 的外泌体悬液, 0.5 ml/只大鼠/天, 共 6 天; 假手术组大鼠使用相同体积的生理盐水。在妊娠第 0, 7, 14 和 21 天测量血压和尿蛋白浓度。第 21 天, 每只大鼠腹腔注射 250 mg/kg 戊巴比妥钠, 通过没有心跳和眨眼反射以及 2~3min 分钟没有自主呼吸来确认安乐死成功。剖宫产后采集子宫胎盘组织和脐带血。

1.3.2 脐带血外泌体的分离和鉴定: 通过使用 exoRNeasy 血清/血浆试剂盒提取来自脐带血的外泌体。根据制造商的说明, 获得在缓冲液 XE 中稀释的约 100 ml 外泌体, 通过 Western blot 检测外泌体表面标记蛋白 CD63, CD9 和 CD81 的表达对其进行鉴定。

1.3.3 RT-qPCR 分析: 根据制造商的说明, 使用

Trizol 试剂从大鼠胎盘组织中提取总 RNA。使用 Prime Script RT 试剂盒进行逆转录, 在 ABI 7500 实时 PCR 系统上, 使用 SYBR Premix Ex Taq II 试剂盒在以下条件下进行实时 PCR 分析: 95 $^{\circ}$ C 1 min, 95 $^{\circ}$ C 20 s, 56 $^{\circ}$ C 10 s 和 72 $^{\circ}$ C 15 s, 35 个循环。PCR 条件如下: 将 2 μ l cDNA 与 0.4 μ l Taq 聚合酶, 0.8 μ l 上下游引物和 6 μ l ddH₂O 添加到 10 μ l 2 \times SYBR 绿色 PCR 主混合液中, 最终体积为 20 μ l。引物序列如下: miR-3614-5p-F: 5'-AAC AAG CCA CTT GGA TCT GAA GG-3', miR-3614-5p-R: 5'-CAG TGC AGG GTC CGA GGT-3', U6-F: 5'-CCA GUU UAC CUA ACG CAA UTT-3', U6-R: 5'-TTC ACG AAT TTG CGT GTC AT-3', Bax-F: 5'-CGG GGT TAT CTC TTG GGC-3', Bax-R: 5'-GTG AGA GCC CCG CTG AAC-3', Bcl-2-F: 5'-TTA TCC AGC AGC TTT TCG G-3', Bcl-2-R: 5'-GGC GGC AGA TGA ATT ACA-3', GAPDH-F: 5'-ACC AGG AAA TGA GCT TGA CA-3', GAPDH-R: 5'-GAC CAC AGT CCA TGC CAT C-3'。U6 和 GAPDH 作为内参, 并通过 $2^{-\Delta\Delta Ct}$ 方法计算相对表达水平。

1.3.4 Western blot 分析: 用 RIPA 裂解缓冲液裂解组织样本。然后通过 BCA 蛋白测定试剂盒测量蛋白质浓度。将等量的蛋白质在 10g/dl SDS-PAGE 上进行分离, 并转移到 PVDF 膜上, 室温下用 5g/dl 脱脂乳封闭 2 h, 与以下初级抗体孵育: Anti- β -actin (1:1 000, Abcam, ab8227), Anti-GPx4 (1:1 000, Abcam, ab125066), Anti-SLC7A11 (1:1 000, Abcam, ab275411), 4 $^{\circ}$ C 孵育过夜。次日将膜与辣根过氧化物酶缀合的山羊抗兔 IgG (1:1000, Abcam, ab6721) 在室温下孵育 1h。 β -actin 作为内参, 通过 ECL 化学发光试剂盒对条带进行可视化, 并通过化学发光成像系统进行拍照。

1.3.5 Fe²⁺, MDA, ROS, GSH 水平和 Caspase-3 活性检测: 按照 ELISA 试剂盒使用说明书, 分别检测组织匀浆液中的 Fe²⁺, MDA, ROS 和 GSH 水平。

1.4 统计学分析 所有统计结果均采用 SPSS 22.0 软件进行分析。计量资料表示为平均值 \pm 标准差 ($\bar{x} \pm s$)。数据满足正态分布且方差齐性, 两组之间采用 Student's *t* 检验进行比较分析, 多组之间采用单因素方差分析 (one-way ANOVA) 进行比较。 $P < 0.05$ 为差异具有统计学意义。

2 结果

2.1 miR-3614-5p 在 PE 大鼠胎盘组织和外周血中的表达 RT-qPCR 检测结果显示, 与 sham 组相比, PE 组大鼠胎盘组织中 miR-3614-5p (0.43 ± 0.05 vs 1.01 ± 0.071) 表达显著下调, 差异具有统计学意义 ($t=19.070$, $P<0.01$)。同时 PE 组大鼠外周血

中 miR-3614-5p (0.51 ± 0.07 vs 1.01 ± 0.12) 表达水平亦较 sham 组显著降低, 差异具有统计学意义 ($t=10.180, P<0.01$)。

2.2 MSCs 来源的外泌体的鉴定 Western blot 检测可见外泌体阳性特异性表面标志物 CD63, CD9 和 CD81, 由此说明外泌体已成功从 MSCs 中进行分离, 见图 1。RT-qPCR 检测发现, 与上清液相比, miR-3614-5p (13.12 ± 1.24 vs 1.02 ± 0.03) 在外泌体中显著富集 ($t=-16.897, P<0.01$) ; miR-3614-5p 抑制剂显著降低了外泌体中 miR-3614-5p 表达水平。以上表明, MSCs 能有效地将 miR-3614-5p 包装成外泌体。

2.3 PE 大鼠的舒张压、收缩压、尿蛋白结果 见表 1。与 sham 组相比, PE 组大鼠的收缩压和舒张压及尿蛋白显著升高 ($t=22.606, 16.440, 23.168$, 均 $P<0.01$) ; 与 PE 组比较, PE+exo 组收缩压、舒张压显著降低, 差异具有统计学意义 ($t=18.493, 13.577, 16.713$, 均 $P<0.01$)。

表 1 外泌体 miR-3614-5p 对 PE 大鼠舒张压、收缩压、尿蛋白的影响 ($\bar{x} \pm s$)

项 目	sham 组	PE 组	PE+exo 组	<i>F</i>	<i>P</i>
舒张压 (mmHg)	113.26 ± 5.11	175.43 ± 6.02	124.57 ± 5.33	289.981	<0.001
收缩压 (mmHg)	82.63 ± 5.26	123.57 ± 5.63	89.76 ± 3.88	154.264	<0.001
尿蛋白 (mg/ml)	67.65 ± 5.76	175.48 ± 13.21	97.69 ± 7.23	285.911	<0.001

表 2 外泌体 miR-3614-5p 对 PE 大鼠 Caspase-3 活性, Bax 和 Bcl-2 蛋白的影响 ($\bar{x} \pm s$)

项 目	sham 组	PE 组	PE+exo 组	<i>F</i>	<i>P</i>
Caspase-3 活性 (%)	100.12 ± 5.93	238.56 ± 13.22	117.35 ± 8.67	479.188	<0.001
Bax 蛋白	1.01 ± 0.11	3.18 ± 0.71	1.13 ± 0.45	49.677	<0.01
Bcl-2 蛋白	1.01 ± 0.12	0.47 ± 0.08	1.04 ± 0.11	75.064	<0.01

表 3 外泌体 miR-3614-5p 对 PE 大鼠 ROS,MDA,Fe²⁺,GSH,GPX4T 和 SLC7A11 的影响 ($\bar{x} \pm s$)

项 目	sham 组	PE 组	PE+exo 组	<i>F</i>	<i>P</i>
ROS (%)	100.51 ± 5.89	387.65 ± 25.98	128.73 ± 14.37	656.161	<0.001
MAD(nmol/mg)	14.83 ± 2.69	33.21 ± 3.17	18.13 ± 3.89	71.089	<0.01
Fe ²⁺ (nmol/ml)	17.51 ± 3.15	38.77 ± 6.53	19.05 ± 3.45	52.321	<0.01
GSH(nmol/mg)	9.76 ± 0.93	4.12 ± 1.22	7.86 ± 1.07	56.494	<0.01
GPX4 蛋白	1.01 ± 0.24	0.48 ± 0.06	0.98 ± 0.14	26.327	<0.01
SLC7A11 蛋白	1.01 ± 0.11	0.51 ± 0.11	1.11 ± 0.09	76.780	<0.01

2.5 外泌体 miR-3614-5p 抑制 PE 大鼠中细胞铁死亡的发生 见表 3、图 2。与 sham 组相比, PE 组大鼠 ROS 水平、MDA 含量和 Fe²⁺ 含量显著升高, GSH 含量及 GPX4 和 SLC7A11 蛋白水平显著降低, 差异具有统计学意义 ($t=32.863, 11.183, 9.173, 10.446, 6.459, 9.637$, 均 $P<0.01$)。与 PE 组相比, PE+exo 组大鼠 ROS 水平、MDA 含量和 Fe²⁺ 含量显著降低, GSH 含量及 GPX4,SLC7A11 蛋白水平显著升高, 差异具有统计学意义 ($t=29.633, 9.175, 8.508, 6.927, 6.093, 11.565$, 均 $P<0.01$)。以上

2.4 外泌体 miR-3614-5p 抑制 PE 大鼠的细胞凋亡 见表 2。与 sham 组相比, PE 组大鼠 Caspase-3 活性和 Bax 表达水平显著升高, Bcl-2 表达水平显著降低, 差异具有统计学意义 ($t=28.402, 8.867, 10.313$, 均 $P<0.01$) ; 与 PE 组相比, PE+exo 组大鼠 Caspase-3 活性和 Bax 表达水平显著降低, Bcl-2 表达水平升高, 差异具有统计学意义 ($t=24.867, 8.377, 10.886$, 均 $P<0.01$)。以上结果提示外泌体 miR-3614-5p 抑制 PE 大鼠的细胞凋亡。

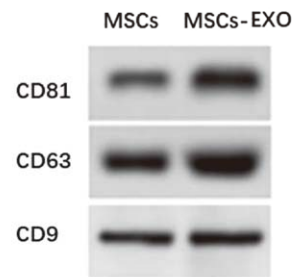


图 1 MSCs 和外泌体中 CD81,CD63 和 CD9 蛋白表达

结果表明, 外泌体 miR-3614-5p 通过抑制细胞铁死亡缓解 PE 大鼠的疾病进展。

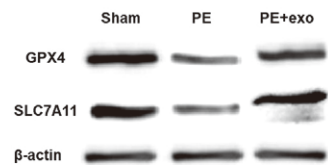


图 2 外泌体 miR-3614-5p 对各组大鼠细胞铁死亡标志蛋白 GPX4 和 SLC7A11 的影响

3 讨论

PE是一种严重的妊娠障碍,对公共卫生造成了重大负担^[3]。PE以蛋白尿和高血压或其他不利条件为特征,并与广泛的母体内皮功能障碍有关。PE的发病机制始于受精卵浸润蜕膜,导致着床失败(例如,由于上皮外滋养层细胞浸润干扰和螺旋动脉重构异常)。此后,大量的血清因子从发育不全的胎盘释放到血液中,形成以血管紊乱引起的多器官功能障碍为特征的PE^[4]。最近研究报道,外泌体通过包裹miRNA/DNA和膜结合蛋白以自分泌和旁分泌的方式影响细胞功能^[8,14]。血液外泌体的miRNA谱已被证明对PE的病理生理评估和疾病的预测具有一定价值^[15-16]。这些外泌体可能通过抑制滋养层细胞凋亡和促进细胞侵袭来修复PE的病理生理改变^[16]。有报道显示,干细胞外泌体通过抑制胎盘细胞凋亡和促进胎盘组织血管生成,以剂量依赖的方式改善了PE大鼠胎盘组织的形态^[17]。BAI等研究^[18]显示,人胎盘外泌体主要靶向单核细胞,并通过重编程循环单核细胞诱导母体全身免疫耐受。GU等^[19]报道显示,胎盘外泌体通过抑制人脐静脉内皮细胞体外增殖、成管和迁移,增加细胞通透性和凋亡破坏了内皮细胞的生物学功能,损害血管发育,导致疾病的发生。YAN等^[20]研究显示,人脐带间充质干细胞释放的外泌体通过驱动滋养层细胞生物功能和血管生成,阻碍缺氧诱导的滋养层细胞炎症,从而抑制PE进展。本研究发现,脐带血间充质干细胞来源的外泌体miR-3614-5p减少PE大鼠的细胞凋亡相关指标改变,抑制铁死亡发生,抑制大鼠的舒张压和收缩压升高,并减少了大鼠的尿蛋白含量,从而缓解了PE大鼠的疾病进展,这一发现与已有报道显示的外泌体抑制PE进展相一致。

与成人血液相比,脐带血具有更明显的特征,因此,研究探索脐带血成分变化可以帮助医生更好地了解胎儿的状态^[21]。据报道,miRNA表达的改变可能在PE的发病机制中发挥重要的作用^[10]。血清中的大多数miRNA都可以在外泌体中检测到,并且外泌体通过传递miRNA等生物信息充当细胞间通讯器^[7]。PAN等人^[22]研究报告,PE来源的血清外泌体中有157种差异表达的成熟miRNA,其中96种miRNA被上调,61种miRNA被下调。JIANG等^[20]研究发现,人脐带间充质干细胞释放的外泌体(huc-MSCs-EXO)中miR-140-5p显著富集,huc-MSCs-EXO通过转移miR-140-5p抑制卵泡抑素样3(follistatin like protein 3, FSTL3)表达,从而抑制滋养层细胞的炎性死亡,抑制PE进展。ZOU等研究^[23]显示,miR-134, miR-31-5p, miR-655, miR-412, miR-539, miR-409和miR-496在

PE外周血分离的外泌体中表达水平显著低于健康对照组,尤其是miR-31-5p,此外还发现miR-31-5p与PE患者临床常规诊断指标呈负相关。本研究发现,PE大鼠胎盘组织中miR-3614-5p显著下调,miR-3614-5p在外泌体中显著富集,推测与文献报道的外泌体功能相同,其可能通过传递miRNA(miR-3614-5p)在PE进展中发挥作用。

铁死亡是一种特殊类型的程序性细胞死亡,由磷脂过氧化物的铁依赖性积累引起^[24]。既往研究表明,PE与高铁状态和铁死亡密切相关^[25]。GSH, GPx和MDA是评价脂质过氧化程度的重要指标,可以帮助判断PE大鼠是否发生铁死亡。胎盘滋养细胞具有不同的细胞内铁调节模式和对铁死亡的独特敏感性^[26-27]。DING等^[27]通过生物信息学途径在PE患者中鉴定发现了30个与嗜铁相关的基因,通过GO和KEGG通路富集分析发现铁死亡与PE进展密切相关。有报道显示PE患者的母血和脐带血中MDA和GPx的水平较高^[28]。GPx4是唯一能够阻断ROS介导的脂质过氧化的GPx家族成员,已被用作铁死亡的重要标志物^[29]。相关研究也表明,PE患者胎盘中GPx4和SLC7A11蛋白水平低于对照组^[30]。GPx4和SLC7A11的减少表明阻止脂质过氧化的效率降低^[31]。EL-KHALIK等^[32]研究报道也显示,PE患者胎盘组织中Fe²⁺和MDA水平等促铁死亡介质较对照组显著上调,胎盘GSH水平及HO-1, GPx4活性等抗铁死亡调节因子显著下调。此外,SLC7A11也被发现显著下调,SLC7A11介导的信号通路的活化诱导了铁死亡,促进了PE进展。本研究结果与上述研究结果一致,促铁死亡相关因子显著表达,抗铁死亡因子表达显著下调,表明外泌体miR-3614-5p可以抑制PE大鼠的铁死亡,缓解PE进展。本研究基于体内实验证实外泌体miR-3614-5p干预对PE大鼠细胞凋亡和铁死亡具有抑制作用,但是体内是一个极其复杂的环境,影响因素众多,涉及多个基因参与和信号通路调节,以及生物学过程,因此,PE的发病机制的探究还有待进一步的研究进行完善。

综上所述,PE大鼠胎盘组织中miR-3614-5p表达下调,脐带血来源的外泌体miR-3614-5p通过抑制铁死亡,减少细胞凋亡,可抑制PE进展。为进一步探究PE的发病机制提供了一定的理论依据,也为PE治疗生物标志物的筛选提供了新的思路。

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