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缺氧处理的间充质干细胞外泌体荷载姜黄素联合用药 减轻小胶质细胞的炎症反应

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摘要 目的 研究缺氧处理的间充质干细胞(MSCs)外泌体(Exo)及其荷载姜黄素后对小胶质细胞炎症反应的影响,探讨缺氧处理对MSCs-Exo功能的增强作用。方法 收集培养在正常和缺氧条件下的人脐带间充质干细胞(hUC-MSCs)的上清液,使用超高速离心法分离Exo;通过透射电镜及Western blot鉴定后使用共孵育法荷载姜黄素。分别使用二甲亚砜(DMSO)、姜黄素、常氧外泌体、缺氧外泌体、荷载姜黄素的常氧外泌体、荷载姜黄素的缺氧外泌体处理脂多糖(LPS)诱导的小胶质细胞炎症模型,通过免疫荧光(IF)检测BV2细胞M1型标志物诱导型一氧化氮合酶(iNOS)的表达情况;Western blot和ELISA检测肿瘤坏死因子- α (TNF- α)、白细胞介素-1 β (IL-1 β)及IL-6在细胞及其培养液中的表达与分泌量。结果 常氧外泌体、缺氧外泌体、荷载姜黄素的常氧外泌体、荷载姜黄素的缺氧外泌体均呈直径30~150 nm的“茶托状”,且外泌体标志物CD9、CD81和TSG101的表达呈阳性。对BV2细胞炎症模型处理后,IF检测结果表明,与常氧外泌体组相比,缺氧外泌体处理可显著减少iNOS的表达;而与姜黄素组和荷载姜黄素的常氧外泌体组相比,荷载姜黄素的缺氧外泌体处理后iNOS的表达量显著减少。Western blot和ELISA检测结果表明,与常氧外泌体组相比,缺氧外泌体处理可显著减少炎症因子TNF- α 、IL-1 β 及IL-6的表达与分泌;而与姜黄素组和荷载姜黄素的常氧外泌体组相比,荷载姜黄素的缺氧外泌体处理后TNF- α 、IL-1 β 及IL-6的表达与分泌均显著减少。结论 缺氧处理能够增强人脐带间充质干细胞外泌体(hUC-MSCs-Exo)抑制小胶质细胞极化和分泌炎症因子的能力。此外,缺氧处理的间充质干细胞外泌体(Hypoxia-MSCs-Exo)可以作为药物递送载体,在荷载姜黄素后可以提高其溶解性、稳定性,有利于姜黄素被细胞摄取并与Hypoxia-MSCs-Exo发挥联合用药的治疗效果。

关键词 缺氧;间充质干细胞;外泌体;姜黄素;小胶质细胞;炎症

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小胶质细胞是中枢神经系统的天然免疫细胞,与多种神经炎症相关疾病的发病机制有关,通过调控小胶质细胞表型和功能,可以成为治疗相关疾病的有效方式^[1]。近年来,人脐带间充质干细胞外泌体(human umbilical cord mesenchymal stem cells exo-

acne vulgaris cohort when compared with the healthy control group ($P < 0.01$), while Cyanobacteria was significantly lower in the acne group ($P < 0.01$). At the genus level, the top five different bacterial taxa in both groups were *Bacteroides*, *Escherichia-Shigella*, *Klebsiella*, *Roseburia*, and *Parabacteroides*. Among them, *Bacteroides*, *Roseburia*, and *Parabacteroides* were more abundant in acne patients. Linear discriminant analysis identified five biomarkers all belonging to the *Bacteroidota* phylum in the acne and control groups. These biomarkers belong to the phylum *Bacteroidetes*. **Conclusion** There are significant differences in the composition of intestinal microbiota between acne patients and healthy people. Changes in the richness of specific bacterial genera may become new targets for the diagnosis and treatment of acne.

Key words acne vulgaris; gut microbiota; 16S rRNA gene; high-throughput sequencing; bacteroidota; bio-marker

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somes, hUC-MSCs-Exo)在调节小胶质细胞功能方面显示出巨大潜力。然而体外培养时, hUC-MSCs通常暴露于常氧环境,与自然生理条件下体内的氧浓度不同,这可能会影响其外泌体的生物学功能^[2]。有研究^[3]显示缺氧处理后, hUC-MSCs-Exo中含有更丰富的功能性生物分子,大大提升其活性和生物学功能。除此之外,由于外泌体具有低免疫原性、保护药物免受降解等独特的优势,可以作为优良的药物递送载体^[4]。因此,该研究旨在探讨缺氧处理hUC-MSCs后,其外泌体是否比常氧hUC-MSCs-Exo对小胶质细胞具有更强的调节作用;并进一步研究使用缺氧处理的间充质干细胞外泌体(Hypoxia-MSCs-Exo)作为递送载体荷载姜黄素后能否发挥联合用药的效果,增强对小胶质细胞炎症反应的调节作用。

1 材料与方 法

1.1 主要材料与仪器 人脐带取自贵州医科大学附属医院妇产科(经贵州医科大学附属医院人体伦理委员会批准,批件号:2022伦审第18号);小鼠BV2细胞系购自长沙丰晖生物科技有限公司;白细胞分化抗原9蛋白(CD9)抗体、CD81抗体、肿瘤易感基因101蛋白(TSG101)抗体以及姜黄素(curcumin)购自美国MedChemExpress公司;脂多糖(LPS)购自美国Sigma公司;BCA试剂盒购自上海碧云天生物科技有限公司;CD34-APC、CD73-FITC、CD90-FITC、CD105-PE和肿瘤坏死因子- α (tumour necrosis factor, TNF- α)、白细胞介素(interleukin, IL)-1 β 及IL-6的ELISA试剂盒购自苏州四正柏生物科技有限公司;TNF- α 、IL-1 β 和IL-6抗体及相应二抗购自武汉三鹰生物技术有限公司;ChemiDoc MP化学发光凝胶成像系统购自美国Bio-Rad公司;CP100NX超速离心机购自日本日立公司;Operetta CLS高内涵成像分析系统购自美国珀金埃尔默股份有限公司;BD LSRFortessa流式细胞仪购自美国BD公司;Nikon AXR共聚焦显微镜购自日本尼康公司。

1.2 方 法

1.2.1 hUC-MSCs分离与培养 无菌条件下将脐带剪成小段,剥离掉动脉和静脉后将华通胶剪碎成约1 mm³的组织块,转至培养皿中,加入少量含10% FBS的DMEM/F-12培养基,放入含5% CO₂的37℃细胞培养箱中培养。待细胞从组织块中爬出后,小心将组织块移出并换液。细胞汇合度达到80%时

进行传代培养。①常氧的hUC-MSCs培养:hUC-MSCs培养于含10% FBS、1%谷氨酰胺的DMEM/F-12培养基中,放入含有21% O₂、5% CO₂的37℃细胞培养箱中培养,细胞汇合度90%时收集上清液提取外泌体。②缺氧诱导的hUC-MSCs培养:hUC-MSCs培养于含10% FBS、1%谷氨酰胺的DMEM/F-12培养基中,放入含有1% O₂、5% CO₂的37℃细胞培养箱中培养,细胞汇合度90%时收集上清液提取外泌体。

1.2.2 hUC-MSCs诱导分化实验 根据成软骨诱导试剂盒说明书进行操作,取P4代hUC-MSCs,细胞计数后将3×10⁵个置于15 mL离心管中1 000 r/min低速离心8 min,使细胞形成微团。用成软骨诱导液+TGF- β 3在离心管中培养hUC-MSCs,每3~4 d换液,诱导5周,4%多聚甲醛固定,常规制作石蜡切片。切片经二甲苯脱蜡,梯度乙醇脱水,蒸馏水洗后进行阿利新蓝染色。根据成脂诱导试剂盒说明书进行操作,取P4代hUC-MSCs,细胞计数后以1×10⁵个/孔接种于6孔板中,常规培养基培养24 h后更换为脂肪诱导液A开始诱导,3 d后更换成脂诱导液B维持,24 h后再更换为成脂诱导液A诱导,循环诱导3周后进行油红O染色。根据成骨诱导试剂盒说明书进行操作,取P4代hUC-MSCs,细胞计数后分别以6×10⁴个/孔的密度接种于6孔培养板中,24 h后换用成骨诱导液,每3 d换液,诱导2周后进行茜素红染色。

1.2.3 流式细胞术鉴定hUC-MSCs表面标志物

取出培养的hUC-MSCs,用胰蛋白酶溶液消化后,1 000 r/min离心5 min,弃上清液。将细胞重悬于PBS中,调整细胞密度为1×10⁷个/mL,每管100 μ L,加入CD34-APC、CD73-FITC、CD90-FITC、CD105-PE抗体,同时设阴性对照,室温孵育30 min,在流式细胞仪中进行检测。

1.2.4 hUC-MSCs外泌体提取与鉴定 取状态良好的hUC-MSCs接种于175 cm²培养瓶中,置于常氧和低氧培养箱中培养,细胞密度达到80%时,更换为无外泌体血清培养基继续培养48 h,收集上清液立即置于4℃,1 655 r/min离心10 min;4 272 r/min离心20 min;9 554 r/min离心30 min去除死细胞、细胞碎片及大囊泡等,然后使用超速离心法经两次40 400 r/min离心70 min提取并纯化外泌体,通过透射电镜检测外泌体形态,通过BCA定量外泌体浓度后使用Western blot检测外泌体表面标志蛋白CD9、

CD81和TSG101的表达情况。

1.2.5 荷载姜黄素的常氧外泌体和荷载姜黄素的缺氧外泌体的制备与表征 将姜黄素(36.8 μg)分别与常氧外泌体(36.8 μg)和缺氧外泌体(36.8 μg)混合于1 mL PBS中,在黑暗室温环境下孵育15 min,将混合物通过超高速离心法以40 400 r/min离心70 min进行纯化;离心完成后收集沉淀,洗涤并重悬于PBS中,即为荷载姜黄素的常氧外泌体和荷载姜黄素的缺氧外泌体。使用多功能酶标仪在450 nm处测量经超高速离心后的PBS溶液中剩余姜黄素的吸光度(absorbance, A),通过标曲计算得出剩余姜黄素的含量,然后通过公式计算包封率:包封率=(总姜黄素量-游离姜黄素量)/总姜黄素量×100%。将单独姜黄素和外泌体姜黄素加入PBS中,于37 °C水浴锅中避光孵育,在30、60、90、120、150 min时,每个样品取100 μL,使用多功能酶标仪测定姜黄素浓度,从而计算得出稳定性。

1.2.6 BV2细胞培养和实验分组处理 将BV2细胞置于含有5% CO₂、37 °C恒温培养箱中,采用含10% FBS的H-DMEM培养基培养。实验分组:正常组(Con)、LPS组(LPS)、DMSO组(DMSO)、单独姜黄素组(Curcumin)、常氧外泌体组(Normoxia-Exo)、缺氧外泌体组(Hypoxia-Exo)、荷载姜黄素的常氧外泌体组(Normoxia-Exo-Cur)和荷载姜黄素的缺氧外泌体组(Hypoxia-Exo-Cur)。取对数生长期BV2细胞均匀接种于6孔板中,除Con组外的各组BV2细胞预先给予对应药物处理12 h后再给予终浓度1 μg/mL的LPS刺激12 h。其中姜黄素终浓度为10 μmol/L,常氧外泌体和缺氧外泌体用量均为10 μg,荷载姜黄素的常氧外泌体和荷载姜黄素的缺氧外泌体用量均包含终浓度为10 μmol/L的姜黄素以及10 μg的外泌体。

1.2.7 BV2细胞摄取hUC-MSCs-Exo 按照PKH26红色荧光标记试剂盒说明书步骤,对外泌体进行荧光标记。将红色荧光染料PKH26加入到外泌体悬液中,室温孵育5 min后用等体积的无外泌体血清进行封闭,PBS洗涤2次,去除未结合的染料。将10 μg标记后的外泌体加入到底部铺有细胞爬片的BV2细胞培养皿中,6 h后取出爬片,使用溶酶体绿色荧光探针(Lyso-Tracker Green)对BV2细胞的溶酶体进行染色并使用DAPI对BV2细胞的细胞核进行染色,激光共聚焦显微镜下拍照观察共定位情况;将PKH26标记的外泌体加入到BV2细胞培养基中,

同时加入Hoechst 33342对BV2细胞进行核染,将细胞培养板放入高内涵成像系统中培养1~6 h,实时拍照观察BV2细胞1~6 h摄取外泌体情况。

1.2.8 IF检测BV2细胞M1标志物iNOS的表达 取出不同处理组的BV2细胞爬片,用PBS清洗后,4%多聚甲醛固定30 min;5% Triton 室温破膜15 min;滤纸吸干多余液体,滴加5% BSA,室温封闭1 h;去封闭液,直接滴加iNOS(1:200)抗体后放入湿盒,4 °C孵育过夜,TBST清洗;滴加稀释好的荧光二抗,于湿盒中室温孵育1 h, TBST清洗;DAPI染核,避光孵育10 min, TBST清洗;封片固定,荧光显微镜下观察染色情况并拍照。

1.2.9 Western blot检测BV2细胞中TNF-α、IL-1β和IL-6的蛋白表达 收集不同处理组的BV2细胞提取总蛋白并定量,10%聚丙烯酰胺凝胶进行电泳,样品到达凝胶底部后终止电泳,并将蛋白转移(260 mA、1 h)至NC膜上。随后,室温下使用5%脱脂奶粉将膜封闭1 h,一抗TNF-α(1:1 000)、IL-1β(1:1 000)和IL-6(1:1 000)4 °C过夜,使用对应二抗室温孵育1 h。用通过化学发光成像系统曝光,并使用ImageJ软件分析灰度值。

1.2.10 ELISA检测BV2细胞上清液中TNF-α、IL-1β和IL-6的分泌量 收集不同处理组的BV2细胞上清液,按照ELISA试剂盒说明书操作步骤,分别检测TNF-α、IL-1β和IL-6的分泌水平。

1.3 统计学处理 数据使用ImageJ进行统计分析,统计图使用GraphPad Prism9.5版绘制;当方差齐时,用单因素方差(One-way ANOVA)分析进行多组数据间的比较,方差不齐时用非参数检验法进行分析,两样本比较时使用独立样本t检验, $P < 0.05$ 为差异有统计学意义。

2 结果

2.1 hUC-MSCs的分离与鉴定 为了分离出hUC-MSCs,将脐带华通氏胶接种于培养皿中,显微镜下观察,7 d后细胞从组织块边缘爬出,呈多角形,折光性良好;14 d后,细胞胞体变大并呈典型成纤维细胞样,大量细胞向外放射状生长,细胞排列整齐呈长梭形,并平铺于瓶底(图1A);为证明获得细胞为hUC-MSCs,流式细胞术分析其细胞表面标志物,结果表明细胞高表达hUC-MSCs阳性标记物CD73、CD90、CD105,同时低表达其阴性标记物CD34(图1B);通过分化实验进一步验证其发育潜能,成软骨

诱导后的细胞其阿利新蓝染色呈阳性,成脂诱导后,细胞油红O染色显现出位于细胞内部的脂滴,成骨诱导分化后细胞中可见与茜素红形成复合物而显现出的钙质沉积(图1C),证明分离的hUC-MSCs具有成骨、成脂、成软骨分化的潜能。

2.2 常氧外泌体和缺氧外泌体的分离与鉴定 为了获得常氧外泌体和缺氧外泌体,将hUC-MSCs置于常氧和缺氧培养箱中培养,显微镜下观察显示两组细胞均排列整齐呈长梭形,无明显形态学差异(图2A);使用超速离心法分别从常氧和缺氧培养的hUC-MSCs中分离出常氧外泌体和缺氧外泌体,透射电镜观察显示常氧外泌体和缺氧外泌体都呈直径30~150 nm的“茶托状”,符合典型的外泌体形态特征(图2B);为了比较两组方式处理对外泌体的产量影响,取等量细胞上清液提取外泌体后进行浓度检测,结果显示缺氧处理后外泌体的产量更高,同时为了比较两组外泌体的蛋白丰富度,通过Western blot检测等量常氧外泌体和缺氧外泌体的表面标志蛋白,结果显示与常氧外泌体相比,缺氧外泌体的CD9、CD81和TSG101蛋白的含量也更加丰富(图2C、2D)。

2.3 缺氧处理增强hUC-MSCs-Exo对BV2细胞的倾向性 为了分析两组外泌体被小胶质细胞所摄取的途径及摄取情况,PKH26标记外泌体后与BV2共孵育,并使用LysoTracker标记溶酶体,共聚焦显

微镜拍照结果显示常氧外泌体和缺氧外泌体在BV2细胞中与溶酶体高度共定位,说明两组外泌体均通过内吞途径进入细胞(图3A);通过高内涵细胞成像系统实时跟踪,结果显示与常氧外泌体相比,在1~6 h内缺氧外泌体在BV2细胞中的荧光信号都更加强烈,说明缺氧处理的hUC-MSCs-Exo更易被BV2细胞摄取(图3B、3C)。

2.4 缺氧处理增强hUC-MSCs-Exo对BV2细胞炎症反应的调节 为了对比常氧外泌体和缺氧外泌体对BV2细胞极化及炎症因子分泌的影响,通过IF检测BV2细胞iNOS的表达,结果显示与Control组相比,LPS诱导会导致小胶质细胞iNOS表达上调,而常氧外泌体和缺氧外泌体能够降低LPS导致的上调,其中缺氧外泌体效果更显著(图4A);同时,通过Western blot检测BV2细胞中炎症因子TNF- α 、IL-1 β 和IL-6的蛋白表达情况,结果显示与Con组相比,LPS组LPS诱导也会导致TNF- α 、IL-1 β 和IL-6蛋白表达上调,而常氧外泌体和缺氧外泌体同样能够降低LPS导致的上调,其中缺氧外泌体效果显著(图4B、4C);而通过ELISA检测BV2细胞上清液中炎症因子TNF- α 、IL-1 β 和IL-6的分泌情况,结果同样证实,经过LPS诱导的BV2细胞上清液中,促炎因子TNF- α 、IL-1 β 和IL-6的分泌量显著升高,而常氧外泌体和缺氧外泌体能够减少LPS导致的升高,其中缺氧外泌体效果更显著(图4D)。

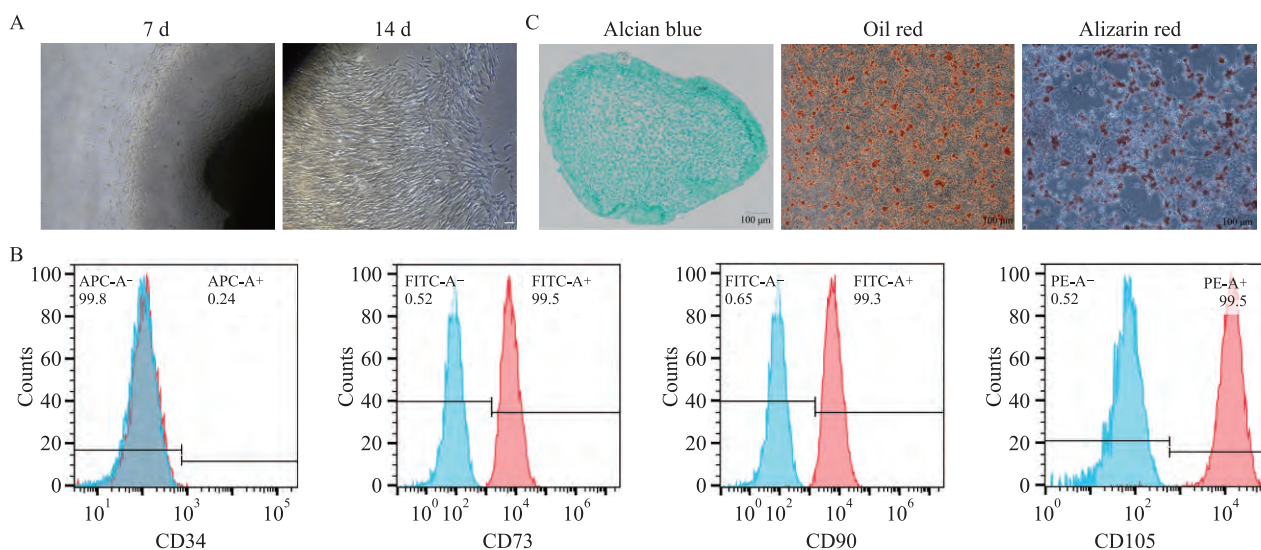


图1 hUC-MSCs分离与鉴定

Fig. 1 Isolation and identification of hUC-MSCs

A: The primary cells grown from the umbilical cord Wharton's jelly tissue block; The cells were then cultured to the fourth generation $\times 40$; B: Flow cytometry showed that hUC-MSCs highly expressed CD90, CD105, CD73, and lowly expressed negative molecule CD34; C: Identification of osteogenic, adipogenic and chondrogenic differentiation of hUC-MSCs $\times 40$.

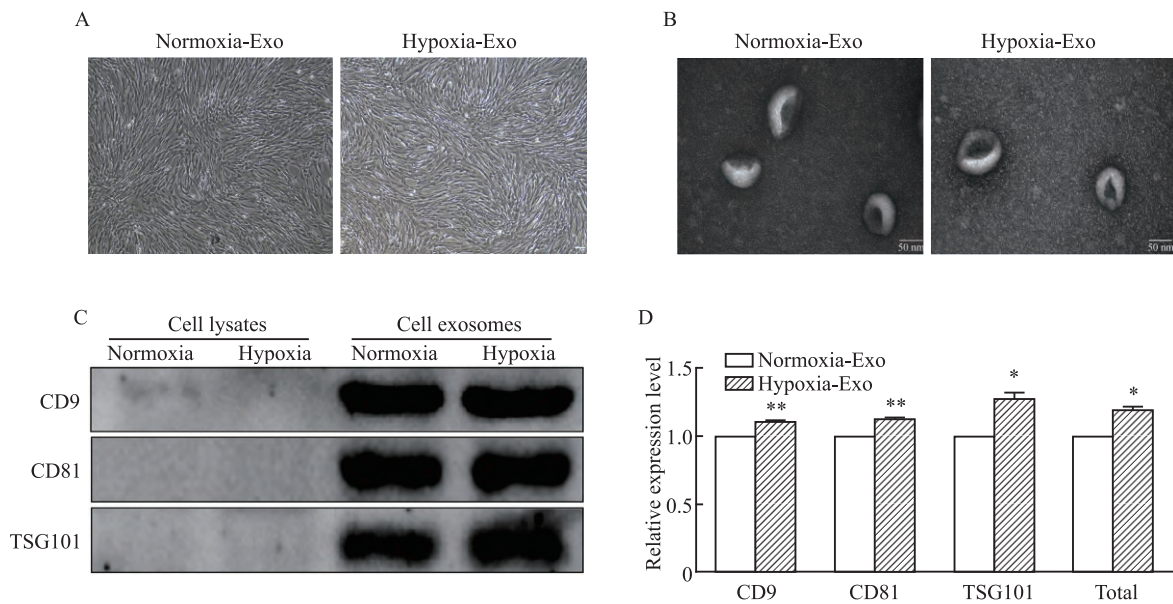


图2 hUC-MSCs-Exos提取与鉴定

Fig. 2 Extraction and identification of hUC-MSCs-Exos

A: The morphology of hUC-MSCs cultured under normoxia and hypoxia $\times 40$; B: Morphological characterization of Normoxia-Exo and Hypoxia-Exo by transmission electron microscopy $\times 80\ 000$; C: Identification of Normoxia-Exo and Hypoxia-Exo markers CD9, CD81, TSG101; D: Statistics of CD9, CD81, TSG101 and total protein; * $P < 0.05$, ** $P < 0.01$ vs Normoxia-Exo.

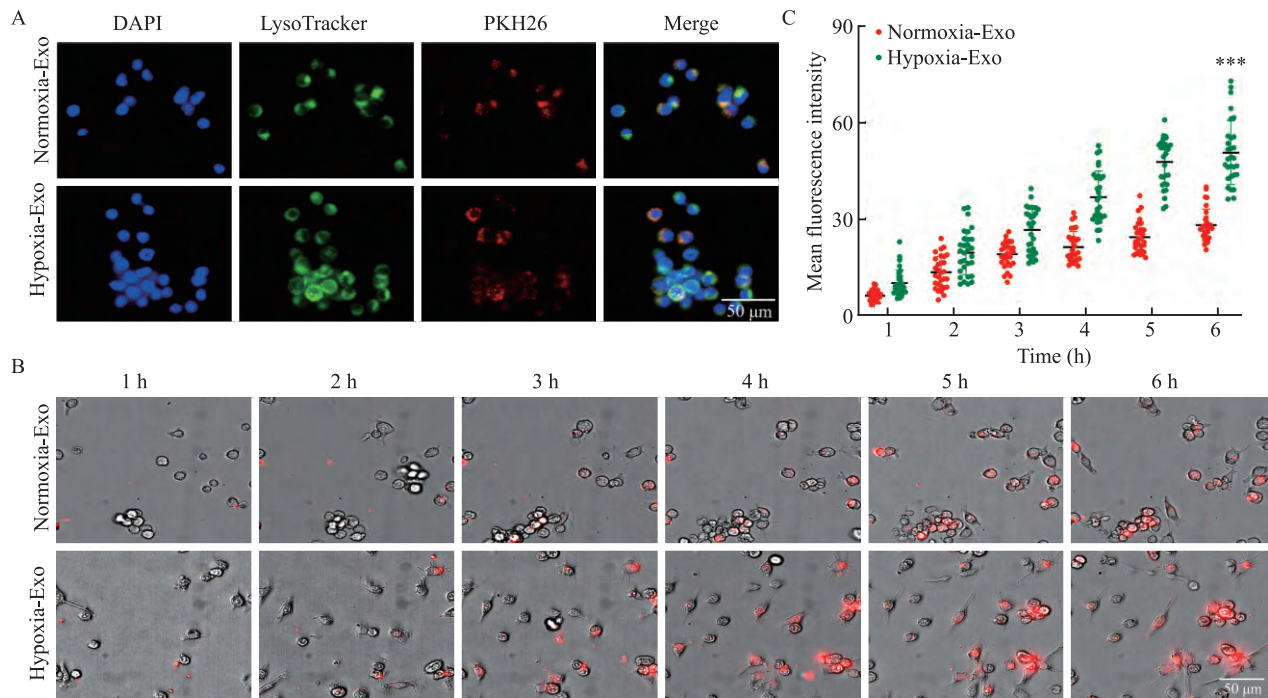


图3 小胶质细胞摄取外泌体

Fig. 3 The uptake of exosomes by microglial cells

A: Confocal imaging showing the uptake of Normoxia-Exo and Hypoxia-Exo by BV2 cells $\times 200$; B: The uptake of Normoxia-Exo and Hypoxia-Exo by BV2 cells in 1-6 hours detected by high content imaging system $\times 200$; C: Statistics of the uptake of Normoxia-Exo and Hypoxia-Exo by BV2 cells in 1-6 hours; *** $P < 0.001$ vs Normoxia-Exo.

2.5 荷载姜黄素的常氧外泌体和荷载姜黄素的缺氧外泌体的制备与表征 测定外泌体对姜黄素的

包封率,其中常氧外泌体: 36.25%, 缺氧外泌体: 36.38%。为了评估单独姜黄素和外泌体姜黄素的

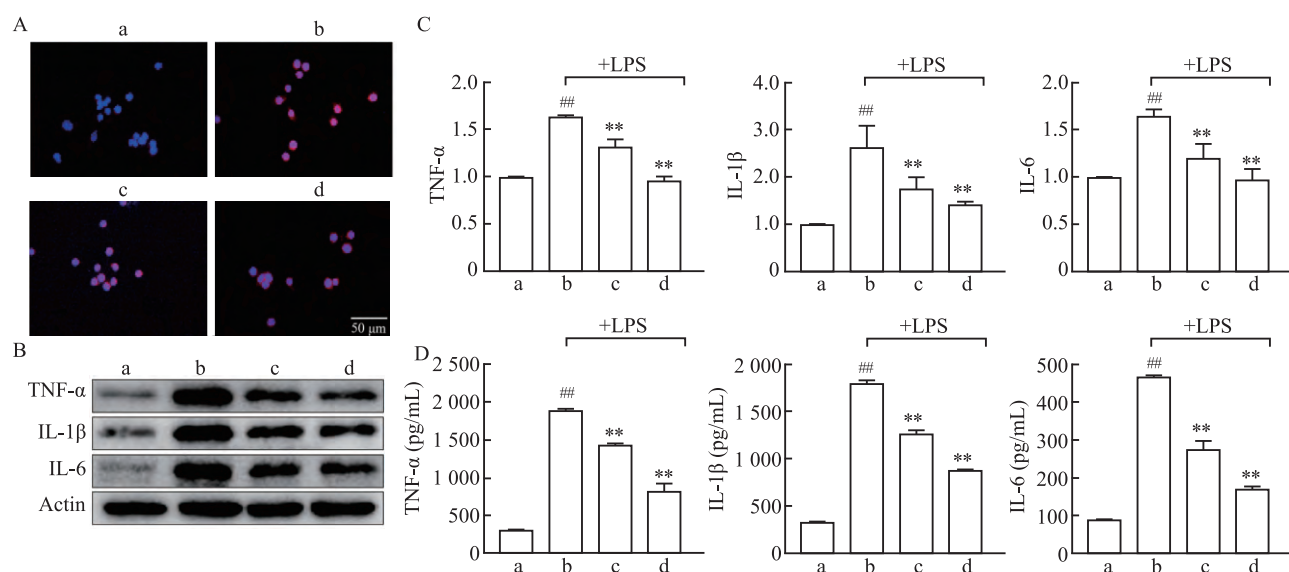


图4 外泌体抑制小胶质细胞M1极化与炎症因子释放

Fig. 4 Exosomes inhibited the polarization of microglia to M1 and released inflammatory factors

A: The expression of iNOS detected by immunofluorescence $\times 200$; B: The expression of TNF- α , IL-1 β and IL-6 protein detected by Western blot; C: Statistics of the expression of TNF- α , IL-1 β and IL-6 protein; D: The secretion of inflammatory factors TNF- α , IL-1 β and IL-6 detected by Elisa; a: Control group; b: LPS group; c: Normoxia-Exo group; d: Hypoxia-Exo group; $##P < 0.01$ vs Control group; $**P < 0.01$ vs LPS group.

稳定性,将单独姜黄素和外泌体姜黄素加入到PBS中,测定不同时间点的姜黄素浓度,结果表明游离在PBS中的姜黄素迅速降解,与0 min样品(设置为1.0)相比,孵育150 min后只剩下35%,而外泌体中的姜黄素被保护免于降解,剩余70%以上(图5A);为了检测荷载姜黄素后外泌体的形态变化,通过透射电镜对其进行观察,结果显示荷载姜黄素的常氧外泌体和荷载姜黄素的缺氧外泌体与天然外泌体一样,均呈现出双层膜结构,形态呈“杯托状”,但直径均稍有增大(图5B)。

2.6 缺氧处理的外泌体和姜黄素联合用药减轻BV2细胞的炎症反应 通过IF检测BV2细胞iNOS的表达,结果显示荷载姜黄素的常氧外泌体和荷载姜黄素的缺氧外泌体组BV2细胞中iNOS的表达较姜黄素组均降低,但荷载姜黄素的缺氧外泌体组降低更显著(图6A);通过Western blot检测BV2细胞中促炎因子的表达情况,结果显示荷载姜黄素的常氧外泌体和荷载姜黄素的缺氧外泌体组细胞中TNF- α 、IL-1 β 和IL-6的表达较姜黄素组降低,且荷载姜黄素的缺氧外泌体组降低更显著(图6B、6C);通过ELISA检测BV2细胞上清液中促炎因子的分泌情况,结果证实荷载姜黄素的常氧外泌体和荷载姜黄素的缺氧外泌体组细胞上清液中炎症因子TNF- α 、IL-1 β 和IL-6的分泌量较姜黄素组显著减少,且荷载姜黄素的缺氧外泌体组减少更显著

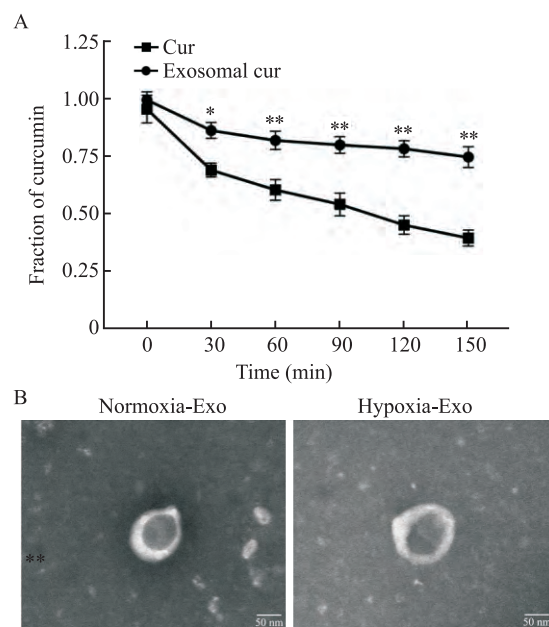


图5 外泌体荷载姜黄素与表征

Fig. 5 Characterization of exosomes loaded with curcumin

A: The stability of curcumin and exosome-loaded curcumin within 150 min; B: Morphological characterization of Normoxia-Exo-Cur and Hypoxia-Exo-Cur by transmission electron microscopy $\times 80\ 000$; $*P < 0.05$, $**P < 0.01$ vs Cur group.

(图6D)。

3 讨论

MSCs是具有自我更新和分化特性的多能干细

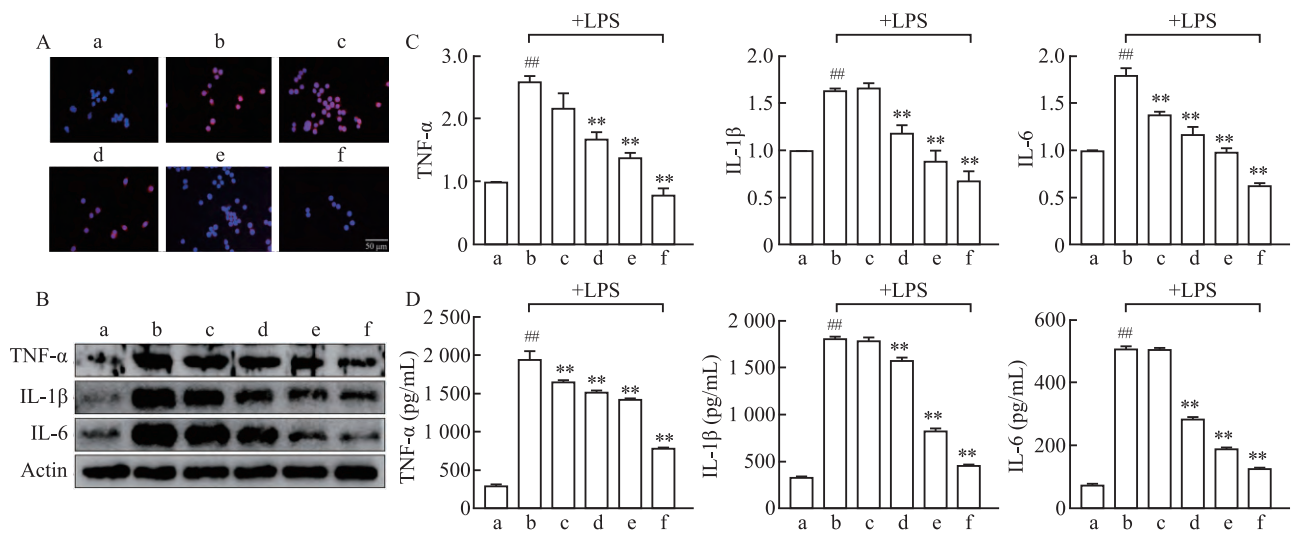


图6 姜黄素外泌体抑制小胶质细胞M1极化与炎症因子释放

Fig. 6 Curcumin-loaded exosomes inhibited the polarization of microglia to M1 and released inflammatory factor

A: The expression of iNOS detected by immunofluorescence $\times 200$; B: The expression of TNF- α , IL-1 β and IL-6 protein detected by Western blot; C: Statistics of the expression of TNF- α , IL-1 β and IL-6 protein; D: The secretion of inflammatory factors TNF- α , IL-1 β and IL-6 detected by Elisa; a: Control; b: LPS group; c: DMSO group; d: Curcumin group; e: Normoxia-Exo-Cur group; f: Hypoxia-Exo-Cur group; ^{##} $P < 0.01$ vs Control group; ^{**} $P < 0.01$ vs LPS group.

胞,分布在人体的许多部位,包括骨髓、脂肪和脐带等^[5]。MSCs的疗效已在许多临床前研究中充分证明,但低植入率和不良反应等缺陷仍需改进^[6]。研究^[7]显示MSCs分泌的外泌体不仅可以继承其疗效,且具有低毒性、高稳定性、低免疫原性等特点,是确定优化MSCs疗法的一种新途径。有研究^[8]表明, MSCs的特性受环境变化的影响,其在体内处于缺氧生态位,多存在于氧含量为2%~8%的环境中。因此,对比体外21% O₂环境,低氧培养更符合MSCs的体内环境,有助于保持其生理特性。本研究结果说明, hUC-MSCs在缺氧条件下培养时表现出更好的细胞活性,能分泌更多内容物丰富的外泌体,有利于其被细胞摄取并发挥作用。

除此之外,与常氧间充质干细胞外泌体相比,缺氧处理的间充质干细胞外泌体已被证明在调节炎症方面具有更积极的治疗效果。如:Wang et al^[9]发现脐带来源的缺氧处理的间充质干细胞外泌体可以增加神经元活力,并抑制细胞死亡和炎症。除此之外,也有研究证明缺氧处理的间充质干细胞外泌体在小胶质细胞的极化转变中发挥有效作用。如:Qian et al^[10]发现脂肪来源的缺氧处理的间充质干细胞外泌体可以减轻结肠炎中的过度炎症,促进巨噬细胞M2型极化。本研究实验结果也表明,与常氧间充质干细胞外泌体相比,缺氧处理的间充质

干细胞外泌体能够更精准地靶向小胶质细胞,减少其炎症因子的表达与分泌。这些研究进一步表明,缺氧培养是一种有效增强hUC-MSCs-Exo治疗潜力的策略,为神经炎症相关疾病的治疗提供了新的见解。

姜黄素是一种天然疏水性多酚,其强大的抗炎作用可以改善小胶质细胞异常功能导致的神经炎症^[11]。例如,Gao et al^[12]发现姜黄素可以通过调控小胶质细胞功能来减轻LPS诱导的神经炎症; Zhang et al^[13]发现姜黄素可以通过调节小胶质细胞(M1/M2)极化,从而显著缓解LPS诱导的炎症。本研究结果也显示姜黄素具有一定的抗炎效果,但同时也发现其溶解性弱、稳定性差,这可能导致其在临床应用中的生物利用度较低。因此,课题组设想通过外泌体荷载的方式来改善姜黄素的缺陷。

在多种药物递送载体中,外泌体因其良好的生物相容性和极低的免疫原性,是一种更为安全、可靠的药物递送载体^[4]。Liu et al^[14]发现荷载miR-29b的BM-MSCs-Exo可以通过激活自噬改善角膜炎症。Yan et al^[15]发现使用ADSCs-Exo荷载淫羊藿苷可以调节巨噬细胞从M1到M2的极化来抑制关节炎。上述例子都是使用常氧间充质干细胞外泌体作为递送载体,本研究进一步探究了缺氧处理的间充质干细胞外泌体作为递送载体的治疗价值。实验结

果证明,通过缺氧外泌体荷载姜黄素对BV2细胞向M1型极化及炎症因子的表达与分泌具有更显著的抑制作用。说明缺氧处理的间充质干细胞外泌体是更好的递送载体,在改善姜黄素缺陷的同时,也通过与姜黄素联合用药,双管齐下,发挥了更强的抗炎作用。综上所述,未来缺氧处理的间充质干细胞外泌体可能会成为一种与其他药物联合用药治疗神经炎症相关疾病的更优选择。

然而,缺氧环境如何调节hUC-MSCs-Exo的分泌并影响其功能,以及缺氧处理的间充质干细胞外泌体和姜黄素联合作用的机制仍需继续进行实验探究。所以,本研究后续将深入研究缺氧处理的间充质干细胞外泌体的活性蛋白,以发掘其新的治疗功能。同时,进一步完善缺氧处理的间充质干细胞外泌体在相关动物模型中的治疗效果验证,为缺氧处理的间充质干细胞外泌体工程化及其未来的临床转化和应用提供理论基础。

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Curcumin-loaded exosomes from hypoxia-treated mesenchymal stem cells alleviate microglial inflammatory response in a combined therapy approach

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Abstract Objective To investigate the effects of hypoxia-treated mesenchymal stem cell (MSCs) exosomes (Exo) and their loading with curcumin on microglial inflammatory responses, and to explore the enhancing effect of hypoxia treatment on the function of MSCs Exo. **Methods** The supernatants of human umbilical cord (hUC)-MSCs cultured under normal and hypoxic conditions were collected, and Exo were isolated using ultracentrifugation. After identification by transmission electron microscopy and Western blot, curcumin was loaded using the co-incubation method. The lipopolysaccharide (LPS)-induced microglial inflammation model was treated with dimethyl sulfoxide (DMSO), curcumin, normoxia Exo, hypoxia Exo, normoxic Exo loaded with curcumin, and hypoxic Exo loaded with curcumin, respectively. The expression of the M1-type marker inducible nitric oxide synthase (iNOS) in BV2 cells was detected by immunofluorescence (IF). Western blot and enzyme-linked immunosorbent assay (ELISA) were used to measure the expression and secretion levels of tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), and IL-6 in the cells and their culture supernatants. **Results** Normoxia Exo, hypoxia Exo, normoxic Exo loaded with curcumin, and hypoxic Exo loaded with curcumin exhibited a "saucer-like" shape with a diameter ranging from 30~150 nm, and the expression of exosomal markers CD9, CD81, and TSG101 were positive. After treating the BV2 cell inflammation model, IF results showed that, compared with the normoxia Exo group, treatment with hypoxic Exo significantly reduced the expression of iNOS. Moreover, when compared with the curcumin group and the normoxic Exo loaded with curcumin group, the expression level of iNOS significantly decreased after treatment with hypoxic Exo loaded with curcumin. The results of Western blot and ELISA indicated that, in comparison with the normoxia Exo group, treatment with hypoxic Exo significantly reduced the expression and secretion of the inflammatory cytokines TNF- α , IL-1 β , and IL-6. Additionally, when compared with the curcumin group and the normoxic Exo loaded with curcumin group, both the expression and secretion of TNF- α , IL-1 β , and IL-6 significantly decreased after treatment with hypoxic Exo loaded with curcumin. **Conclusion** Hypoxia preconditioning can enhance the ability of hUC-MSCs-Exo in the inhibition of microglial polarization and inflammatory factors' secretion. Additionally, using Hypoxia-MSCs-Exo as a drug-delivery carrier of curcumin can improve its solubility and stability, facilitating its absorption by cells and exerting the therapeutic effect of combination therapy.

Key words hypoxia; mesenchymal stem cells; exosomes; curcumin; microglia; inflammatory

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