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人脐带干细胞来源的外泌体促进体外培养雪旺细胞迁移的研究 *

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摘要 目的：外泌体是活细胞分泌的来源于多囊泡体的膜性囊泡，其主要作用包括携带与运输。雪旺细胞是周围神经再生中非常优秀的种子细胞，但其迁移能力较差，影响修复效果。本文旨在探讨外泌体和雪旺细胞共培养是否可以促进雪旺细胞迁移。**方法：**本实验通过分离纯化人脐带干细胞外泌体和大鼠坐骨神经雪旺细胞并鉴定，随后将其共培养于 Transwell 小室观察雪旺细胞迁移率。**结果：**通过人脐带干细胞超高速离心法得到的外泌体高表达干细胞标志物 CD44（92.2 ± 3.6 %）、CD73（99.1 ± 0.6 %），并且低表达单核细胞表面抗原 CD14（0.5 ± 0.06 %）以及造血干细胞表面抗原 CD34（0.4 ± 0.07 %），外泌体鉴定高表达 CD81 和 CD9；雪旺细胞培养鉴定纯度达（92.3 ± 2.7）%；均符合实验要求。通过 Transwell 小室实验发现外泌体可以明显促进雪旺细胞的迁移，并且具有一定剂量关系。**结论：**外泌体可以提高雪旺细胞的迁移能力，从而使雪旺细胞在组织工程领域中的应用产生巨大突破。

关键词：外泌体；人脐带间充质干细胞；雪旺细胞；神经再生

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Exosomes Secreted from hUC-MSCs Promoted Schwann Cell Migration*

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ABSTRACT Objective: The exosomes are membranous vesicles?which are derived from polycystic vesicles secreted by living cells. Their main functions include carrying and transporting substances. Schwann cells are one of the excellent seed cells in peripheral nerve regeneration. However, Schwann cells have a relatively poor migration ability which limite the biological effect on nerve regeneration. To investigate whether exosomes can promote Schwann cell migration under cell co-culture. **Methods:** In this experiment, we isolated and purified human umbilical cord stem cells' exosomes and Schwann cells from rat sciatic nerve and then identified them. Subsequently, they were co-cultured in Transwell chamber to observe the migration rate of Schwann cells. **Results:** High expression of stem cell markers CD44(92.2 ± 3.6 %), CD73(99.1 ± 0.6 %) and low expression of mononuclear cell surface antigen CD14(0.5 ± 0.06 %), hematopoietic stem cell surface antigen CD34(0.4 ± 0.07 %) in hUC-MSCs which were obtained by ultrahigh speed centrifugation. The exosomes were identified with high expression of CD81 and CD9. The identification of Schwann cells were factual and the purity of cells were 92.3 ± 2.7 %. All the cultured cells and exosomes were met the experimental requirements. And the exosomes can significantly promote Schwann cells' migration under cell co-culture in Transwell chambers with dose relationship. **Conclusions:** The exosomes can accelerate Schwann cell migration obviously, which made Schwann cells more suitable for seed cells in nerve regeneration tissue engineering .

Key words: Exosomes; Human umbilical cord derived-mesenchymal stem cell; Schwann Cells; Nerve regeneration

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前言

外泌体（exosomes）被定义为活细胞分泌的来源于多囊泡体的膜性囊泡。多项研究表明，外泌体不仅可以通过 miRNA 调节癌症相关基因的改变和分选[1-3]。又可以促进细胞增殖，并进一步影响细胞的表型和功能[4]。外泌体甚至可以介导炎症反应和免疫系统疾病[5-7]。以上研究表明，外泌体参与机体的各项生理病理调节。而越来越多的科学家将目光聚焦在外泌体携带与运输 miRNA 修复和保护机体细胞的作用方面[8,9]。综上所述，外泌体可以成为新型的治疗和修复机体的方法和手段之一。

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周围神经损伤(peripheral nervous injury,PNI)尤其是长节段神经缺损长期以来一直是外科医生棘手的问题之一[10]。虽然随着科学技术的发展,各种新型组织工程材料以及治疗方法层出不穷[11-13],但是,自体神经移植仍然是目前长节段神经损伤修复的"金标准"[14,15]。自体神经修复有助于 Bugner 带形成并生成最佳再生内环境 [16,17]。而 Bugner 带则是雪旺细胞(Schwann Cells,SCs)迁移形成的。雪旺细胞不仅具有神经细胞骨架作用,还能够分泌各种神经营养因子 [18,19]。然而,雪旺细胞本身对很多组织工程材料的组织相容性并不好[20,21],更重要的是,在神经损伤后,雪旺细胞迁移比较缓慢[22],严重影响神经修复速度。因此,能够解决雪旺细胞的迁移速度,将是神经修复领域的一个重大突破。

综上,课题组采用人脐带间充质干细胞(human umbilical cord derived-mesenchymal stem cell, hUC-MSCs)来源的外泌体和雪旺细胞共培养,观察外泌体是否能促进体外培养的雪旺细胞迁移。分离培养高纯度的脐带干细胞并鉴定后,通过电镜、免疫荧光和 Western blot 的方法, 鉴定分离来源于干细胞的外泌体。将纯化培养的雪旺细胞和外泌体共培养于 Transwell 小室检测迁移,结果发现外泌体可以有效促进雪旺细胞迁移。因此,利用外泌体促进雪旺细胞迁移,有望成为一种新型的有效的治疗周围神经损伤的方法。

1 材料与方法

1.1 主要试验材料

新生(1d)SD 大鼠 24 只(空军军医大学实验动物中心);足月剖宫产新生儿脐带(空军军医大学西京医院妇产科,n=6);Transwell 小室培养皿(0.8 mm,NUCN,Denmark)若干;DMEM/F12 培养基(Gibco);胰蛋白酶(Gibco);II 型胶原酶(Gibco);CD14-FITC、CD34-FITC、CD44-FITC、CD73-PE 流式检测抗体(艾美捷);成骨诱导分化培养基试剂盒、成脂诱导分化培养基试剂盒、成软骨诱导分化培养基试剂盒(赛业生物科技);磷钨酸染液;茜素红染液;油红 O 染液;阿立辛蓝染液;HE 染液;Anti-CD9 WB 抗体、Anti-CD81 WB 抗体(Abcam);Western blot 常规试剂(甘氨酸、裂解液、Tris-HCl、SDS、0.01M PBS 等);forskolin(Sigma);牛脑垂体浸提物(sciencell);Triton(北化试剂);Rabbit-S-100 一抗(Sigma);mouse-SOX-10 一抗(Sigma);TRTIC-Rabbit 二抗(Sigma);FITC- mouse 二抗(Sigma);蓝色荧光 DAPI 染液(Sigma);D`hank 液(Gibco);阿糖胞苷(Sigma);4%甲醛溶液等。

1.2 试验方法

1.2.1 脐带干细胞的收集、分离培养及鉴定 经医学伦理委员会批准并且在与受试产妇签署知情同意书后,无菌操作摘取新生儿脐带组织约 5 - 8 cm。于 4℃ 无菌 PBS 冲洗去除残留血块,将脐带放置于培养皿中后置于冰枕上,将脐带剪成约 2 cm 段。沿纵轴剖开脐带后剔除血管及血管外膜,暴露华通氏胶(Wharton's Jelly)。小心剥离华通氏胶后于冰枕上剪碎,加入 0.25 % II 型胶原酶。37℃ 消化 18 h 后用 10% DMEM /F12 培养液终止消化。收集上清后经过 200 目细胞筛,高速离心后弃去上清,下部沉淀即得 hUC-MSCs。利用培养 P3 hUC-MSCs 于 10 cm 培养皿中,隔天更换培养基,并观察 hUC-MSCs 形态,当细胞增殖融合至 80 %,按照 1:5 进行传代。P4 细胞融合至对数生长期时,弃培养基,无菌 PBS 漂洗两次后加入无 FBS 的 DMEM/F12 培养基 6 mL/ 皿,24h 后取培养基于离心管中。计数细胞总数后取生长良好的 P3 或 P4 细胞进行实验。

将上述所得的 hUC-MSCs 调整细胞数为 1. 0× 10 ⁶/mL,加入流式抗体 CD14-FITC、CD34-FITC、CD44-FITC、CD73-PE 后室温孵育 30 min,随后 1200 rpm 离心 5 min,无菌 PBS 漂洗 3 次后,用 PBS 重悬细胞,使用流式细胞仪进行分析。

为进一步检验所得细胞性质是否为脐带干细胞,分别采用成骨诱导分化培养基、成脂诱导分化培养基与成软骨诱导分化培养基对上述所得细胞进行培养,具体步骤为:① 成骨诱导分化培养基:将试剂盒中抗坏血酸、β- 甘油磷酸钠、双抗、谷氨酰胺在室温加入基础培养基溶解摇匀并用培养基洗涤离心管。最后将地塞米松加入培养基后混匀, 即成成骨诱导分化培养基。随后将上文所得细胞进行成骨诱导分化,将细胞按 2× $10^4/cm^2$ 密度接种于基础培养基中。当细胞融合达到 70 %时,吸走基础培养基后加入成骨诱导分化培养基,每隔 2-3 天换液,诱导 2-4 周,光镜观察细胞形态变化及生长情况。② 成脂诱导分化培养基:分为 A 液(成分包括基础培养基 175 mL、专用胎牛血清 20 mL、双抗 2 mL、谷氨酰胺 2 mL、胰岛素 400 μL、3- 异丁基 -1- 甲基黄嘌呤 200 μL、罗格列酮 200 μL、地塞米松 200 μL)和 B 液(成分包括基础培养基 175 mL、专用血清 20 mL、双抗 2 mL、谷氨酰胺 2 mL、胰岛素 400 μL)。进行成脂细胞诱导分化时,按照 2× $10^4/cm^2$ 密度接种于基础培养基中,至细胞 100 %融合后加入 A 液,诱导 3 天后换为 B 液维持培养。24 h 后吸走 B 液换为 A 液继续诱导 3 天。A 液 B 液交替作用 2-3 周后,继续使用 B 液维持培养 4-7 天直至脂滴足够大为止。③ 成软骨诱导分化培养基:将试剂盒中的抗坏血酸、ITS+ 补体、丙酮酸钠、脯氨酸加入基础培养基中, 配制成为不完全诱导分化培养基,2 ℃ -8 ℃ 避光保存。将 TGF-β3 加入不完全诱导分化培养基(比例为 10 μL TGF-β3:1 mL 不完全诱导分化培养基)使其变为完全诱导分化培养基。随后进行成软骨细胞诱导,将上述细胞混悬液 150× g 离心 5 min,取出上清液。使用 1 mL 不完全培养基重悬细胞, 密度为 7.5× 10^5/mL,150× g 离心 5 min 后去除上清液。随后使用完全培养基重悬细胞,每 mL 细胞浓度为 5× 10^5。将 0.5 mL 细胞悬液 150× g 离心 5 min,将离心管轻轻拧松后放入 37℃ ,5 % CO_2 孵箱中培养 24 h。每 2-3 天全量换液,观察细胞团变化。诱导 21 天后细胞团直径增大至约 2 mm,诱导分化基本完成。

将上述成骨诱导分化培养基、成脂诱导分化培养基与成软骨诱导分化培养基中细胞进行特异性染料染色观察结果。分别为:① 成骨诱导:进行茜素红染色,步骤为将细胞 PBS 冲洗 2 次后加入 4%中性甲醛溶液固定 30 min, 随后 PBS 清洗 2 遍,加入茜素红染液 1 mL/ 孔 染色 3 - 5 min,PBS 清洗 2 次后显微镜下观察成骨染色效果。② 成脂诱导:进行油红 O 染色分析。将 6 孔板中培养基吸走,PBS 漂洗 2 次, 加入 4 %中性甲醛溶液固定 30 min。加入 PBS 洗 2 次后每孔加入 1 mL 油红 O 染液染色 30 min。PBS 漂洗 2 次后显微镜下观察。③ 成软骨诱导:将成软骨细胞团加入 4 %中性甲醛溶液固定 30 min,随后用石蜡包埋切片 4-8 μm 后, 脱蜡冲洗, 加入阿立辛蓝染液浸染 30

min，双蒸水冲洗 2 min 后显微镜下观察。

1.2.2 **外泌体的分离和鉴定** 采用 P3 或 P4 的 hUC-MSCs。预冷低温超速离心机至 4 ℃后 300× g 离心细胞 10 min，随后去除细胞碎片。将离心后得到的上清液移入超速离心管中，并用无菌 PBS 补齐配平，在 4℃下继续使用 29500× g 离心细胞悬液 20 min，随后进一步去除细胞碎片。使用 0.22 μm 过滤器过滤离心的上清液，随后将过滤后的上清液收集到另一个超速离心管中，继续用 4 ℃下 120000× g 超速离心 90 min，随后弃去上清，用无菌滤纸小心吸干净管中残液，管底剩余则为相应外泌体。加入无菌 PBS 后存入 -80℃冰箱备用。

外泌体的大体形态采用透射电镜进行观察。将备用外泌体的离心管取出复温 15 min 后，用 50 μL 无菌 PBS 溶液加入离心管溶解外泌体，取 20-30 μL 外泌体悬液于载体铜网上室温下静置 1 min。随后使用滤纸沿铜网外侧吸干液体，在铜网上滴加 20 mL/L 磷钨酸溶液 30 μL，室温染色 1 min。用滤纸吸掉磷钨酸溶液后将铜网置于烤灯下烤干后即可进行电镜观察，并对外泌体直径进行分析统计。

外泌体的膜蛋白富含跨膜蛋白家族 CD81 和 CD9，因此我们利用 Western blot 进行外泌体表面标记物 CD9 和 CD81 对所得到的外泌体进行检测以鉴定。简要步骤为，将分离收集的外泌体蛋白裂解后进行蛋白定量。电泳后将条带分别加入 Anti-CD9 抗体、Anti-CD81 抗体孵育，显色后观察条带相应位置是否显色。

1.2.3 **雪旺细胞的培养与鉴定** 将新生大鼠置于 75%酒精缸内 15 min 消毒处死，随后在体视显微镜下取出大鼠坐骨神经，置于冰冷 D`hank 液中，将坐骨神经组织剪碎至 1 mm^3 小块。用 0.25 %胰蛋白酶和 0.03%Ⅱ型胶原酶；随后于 37℃、5 % CO_2 孵箱中孵育消化 25 min，加入 DMEM/F12+10 %胎牛血清培养基终止消化，1500 rpm 离心 8 min 收集上清液。随后加入 DMEM/F12+10 %胎牛血清培养基吹打成细胞悬液。以 1× $10^5/cm^2$ 接种，差速贴壁纯化后继续培养。24 h 后换液，加入阿糖胞苷（2 μg/mL），第 5 d 更换含 forskolin（2 μM/mL）和牛脑垂体浸提物（20 μg/mL）的培养液进行培养。传代 P3-P4 期细胞进行下步实验。

对上述细胞进行特异性标记物 S-100 免疫组化染色进行细胞鉴定。采用 P3 细胞，以 1× 10^5 的密度接种到接种于载玻片上，置于 5 % CO_2、37℃孵箱内培养 24 h，用 PBS 液漂洗 3 次后用 4 %甲醛固定细胞 30 min。PBS 液漂洗后加入 0.2% Triton 打孔液和牛血清白蛋白封闭液 37 ℃孵育 15-20 min 。取出载玻片加入 Rabbit-S-100 一抗（1:500）和 mouse-SOX-10 一抗（1:500）4 ℃冰箱过夜。复温后 PBS 漂洗 3 次，加入 TRTIC-Rabbit 二抗和 FITC- mouse 二抗室温孵育 4 h。将 DAPI 染液（1:500）滴加在玻片上，孵育 10 min。甘油封片后准备观察。

1.2.4 Transwell **小室实验观察外泌体促雪旺细胞迁移试验** 将上述 P3 雪旺细胞以 1× $10^5/cm^2$ 密度接种于 Transwell 小室上层，继续培养 2 d。分别在外层小室中接种不同浓度的外泌体，其中第一组为对照组，即不接种外泌体（0 ng/mL）。其余各组按照 10 ng/mL、50 ng/mL、100 ng/mL 进行接种，随后放入 37℃，5 % CO_2 孵箱中继续孵育。于 48 h 后，取各组 Transwell 小室上层擦去正面未迁移到小室下方的细胞，酒精固定小室底面后进行 H-E 染色。应用显微镜观察迁移雪旺细胞形态，并计数各组小室迁移细胞数量。

1.3 **统计学处理**

运用 SPSS 20. 0 统计软件统计分析，实验数据以 $\bar{x}\pm s$ 表示，组间比较采用 One-Way ANOVA 进行统计分析。统计结果输入 GraphPad 软件进行制图。

2 结果

2.1 **脐带干细胞纯化鉴定，证实具有三系分化潜能**

采用上述方法原代分离培养人脐带干细胞，可见细胞贴壁生长良好，形态呈双极长梭形或类似于成纤维细胞的扁平形，细胞饱满折光性好（图 1A）。通过流式细胞检测，经过上述培养的 hUC-MSCs 高表达干细胞标志物 CD44（92.2 ± 3.6 %）、CD73（99.1 ± 0.6 %），并且低表达单核细胞表面抗原 CD14（0.5 ± 0.06 %）以及造血干细胞表面抗原 CD34（0.4 ± 0.07 %）（图 2B）。以上结果表明，本实验分离培养的 hUC-MSCs 具有良好的细胞活性，并且具有较高的纯度，符合实验要求。

而通过对上述实验细胞进行成骨、成脂和成软骨诱导分化，分别运用特异性茜素红染色（图 1C）、油红 O 染色（图 1D）以及阿立辛蓝染色（图 1E），证实本实验分离培养的细胞确实为 hUC-MSCs，并且具有良好的分化潜能，细胞状态良好。

2.2 **外泌体的分离鉴定**

通过经典的超速离心分离法得到的人脐带干细胞外泌体，经磷钨酸染色后通过透射电镜观察(图 2A)，可见试验所得颗粒成圆形，并且可以观察到典型的外泌体凹陷半球形结构，表明通过超速离心分离法可以得到符合试验条件的人脐带间充质干细胞外泌体。动态光散射法对镜下纳米颗粒的直径进行统计计算，可以发现所得的纳米颗粒全部符合外泌体 40-140 nm 范围内（图 2B），平均粒径为 58.50 ± 5.88 nm，进一步证明通过上述实验方法所得的颗粒符合外泌体性质。而对实验所得颗粒中外泌体膜蛋白中标志性的 CD81 和 CD9 进行 Western blot 检测，均发现深染条带（图 2C）。综合上述结果，可以明确所得的纳米颗粒确实为人脐带干细胞外泌体，且形态良好，符合下一步实验要求。

2.3 **雪旺细胞的培养与鉴定**

雪旺细胞的培养方法已非常成熟。经过 P3-P4 的雪旺细胞能够保证细胞纯度和状态，符合下步实验条件。通过 S-100 + SOX-10 + DAPI 三标免疫荧光染色，共聚焦显微镜观察镜下红色荧光的即为雪旺细胞标记物 S-100，绿色荧光的为神经鞘细胞标志物 SOX-10，蓝色为 DAPI 胞核衬染（图 3A-D）。雪旺细胞形态呈双极梭形，两极发出部分细胞足。按下述公式 (1) 随机记录并计算 20 个视野的细胞核数量和红色细胞数量，计算出雪旺细胞纯度。

雪旺细胞纯度 =S-100 免疫荧光染色阳性细胞数 /DAPI 染色阳性细胞数× 100 %。 (1)

计算结果约为（92.3 ± 2.7）%，细胞纯度较高，满足下步实验需求。

2.4 **外泌体促进雪旺细胞迁移**

为了研究 hUC-MSCs 的外泌体是否能够促进雪旺细胞迁移，我们采用 Transwell 小室实验观察。通过观察我们发现，对

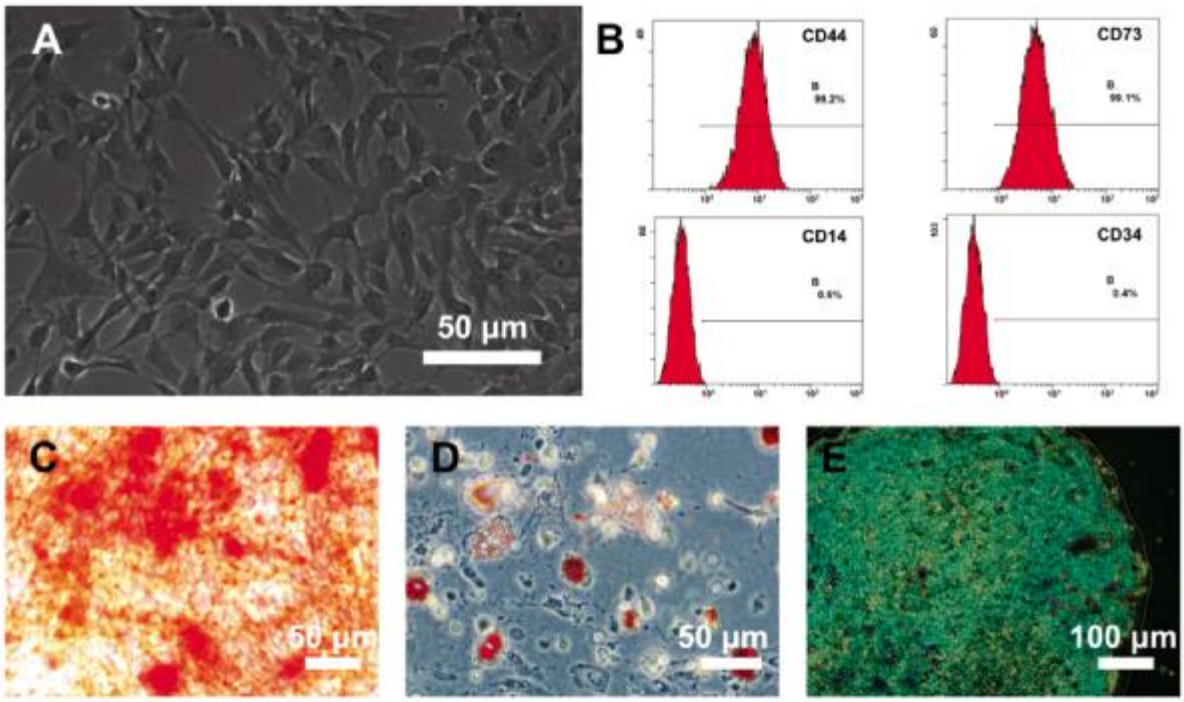

图 1 人脐带干细胞 hUC-MSCs 收集、分离和培养结果

Fig.1 The results of hUC-MSCs collection, separation and cultivation

A: The hUC-MSCs separation and cultivation is successful. The cells are in good condition, with a spindle or flat shape. bar=50 μm. B: Flow cytometry results showed that the hUC-MSCs are high expression on mesenchymal stem cell markers CD44 and CD73, low expression on monocytes surface antigen CD14 and hematopoietic stem cell surface antigen CD34. C: The results of osteoinduced differentiation specificity alizarin red staining. D: The results of fat-induced differentiation specific oil red O staining and E: The results of chondrogenic differentiation of Alcian blue staining. All the staining above indicated that the hUC-MSCs we cultured have the potential of osteoinduced, fat-induced and chondrogenic differentiation.

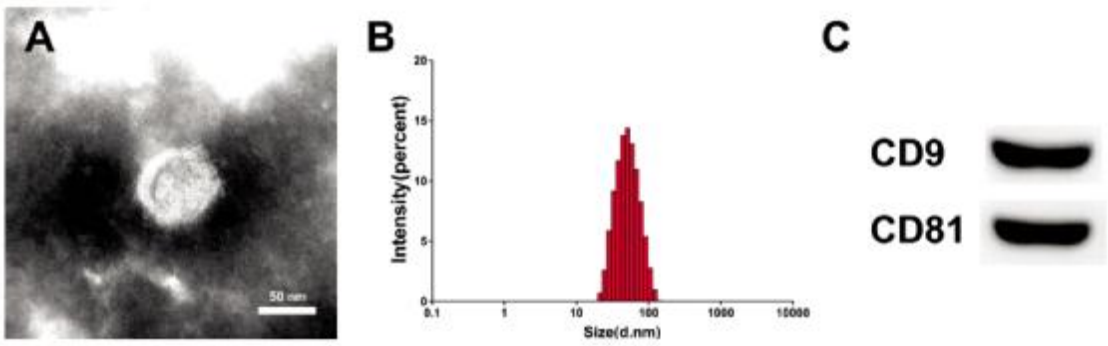

图 2 外泌体分离与鉴定

Fig.2 Separation and identification of exosomes

A: TEM figure observed that the nanoparticles were of typical exosomes structure, bar=50 nm. B: The diameter of the nanoparticles under the microscope was measured in the range of 30-100 nm, and the average particle size were 58.50 ± 5.88 nm, which were in line with the exosomes standard. C: Western blot results showed that the exosomes signature CD9 and CD81 were highly expressed . The results above showed that the nanoparticles were indeed exosomes from the human umbilical cord stem cells.

照组(外室不接种外泌体 0 ng/mL 组)仅有少量雪旺细胞迁移至下室(图 4A)。而按照 10 ng/mL 浓度在外室加入外泌体后,雪旺细胞迁移出现增多趋势(图 4B,和对照组相比 $P>0.05$),随着浓度增加至 50 ng/mL,雪旺细胞迁移明显增多(图 4C,和对照组相比 $P<0.05$),而当浓度提升至 100 ng/mL 时,雪旺细胞迁移数则没有发生明显的增多 (图 4D, 和 50 ng/mL 组相比 $P>0.05$;和对照组相比 $P<0.05$)。由此可见,外泌体可以明显促进雪旺细胞的迁移(图 4E),并且具有一定剂量关系。

3 讨论

周围神经损伤尤其是长节段神经缺损,长期以来一直是困扰临床医生的难题[23]。虽然自体神经修复是治疗长节段神经损伤的金标准,但是由于其来源受限、供体部位神经缺失等诸多问题,目前越来越多的科学家关注采用类似的神经支架材料复合种子细胞来替代治疗[24,25]。雪旺细胞作为神经的骨架细胞,在神经再生中起着非常重要的作用。在瓦勒变性至神经再生的病

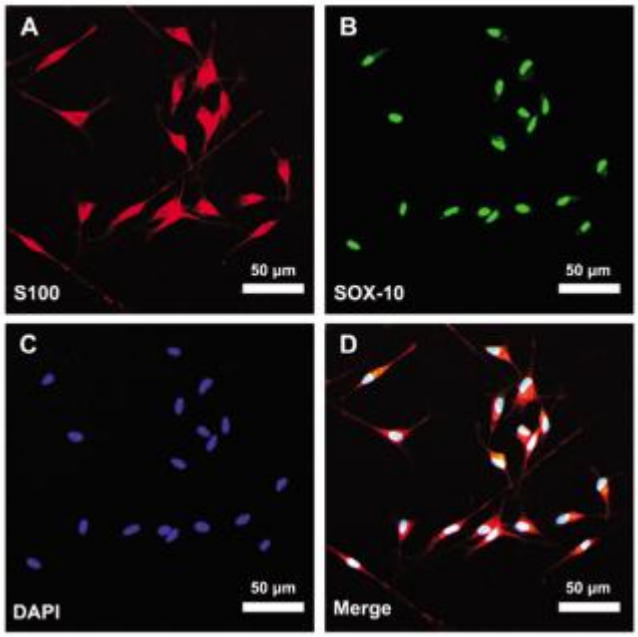

图 3 雪旺细胞鉴定

Fig.3 Schwann cell identification

A: The red fluorescence is the S-100 specific markers staining of schwann cell. B: The green fluorescence was specific marker SOX-10 staining for the nerve sheath cell. C: Blue fluorescence is DAPI, the nuclear staining; D: Merge figure above. The bar = 50 μm.

理生理过程中,都离不开雪旺细胞的迁移,而只有当雪旺细胞形成 Bugner 带, 神经丝才能够长入其中并完成神经修复。再者,雪旺细胞也是多种神经营养因子的分泌细胞,对神经再生和诱导有着极大的辅助作用。然而,许多科学家在运用雪旺细胞作为神经支架种子细胞时,并未发现其修复效果具有明显优势[26],其主要原因很可能就是雪旺细胞作为种子细胞时的迁移受限问题。因此,寻找一种安全有效的促进雪旺细胞迁移的方法,可以成为提高雪旺细胞复合神经支架材料治疗长节段神经缺损疗效的方法之一。

外泌体近些年成为研究热点, 从炎症反应到动脉粥样硬化,从肿瘤基因编码到免疫系统调节[27-31],外泌体几乎参与了身体中绝大多数病理生理过程,而完成这些过程全都归因于外泌体辅助和促进细胞转运和迁移的能力。正是因为外泌体的这种特性,让我们关注它是否能够促进雪旺细胞的迁移。通过传统的人胚胎干细胞的分离培养法,超高速离心分离法获得的外泌体,以及新生大鼠坐骨神经雪旺细胞培养,并对上述细胞和颗粒进行鉴定,实验结果表明上述细胞都具有很好的细胞状态和纯度,实验结果可信。随后将外泌体和雪旺细胞 Transwell 小室共培养发现,外泌体可以明显促进雪旺细胞迁移,并且存在一定剂量依赖关系。然而,相比较加入外泌体浓度为 50 ng/mL 的实验组,加入 100 ng/mL 浓度外泌体并没有使雪旺细胞迁移明显增加,这是因为当加入 50 ng/mL 浓度外泌体时,雪旺细胞已经迁移率已接近饱和,所以再加入外泌体也无法使更多雪旺细胞迁移。但是,外泌体确实能够促进雪旺细胞迁移,而这为雪旺细胞成为优良的神经组织工程种子细胞找到新的突破。

由于外泌体广泛存在于体液中且也能够分离提取 [32],因

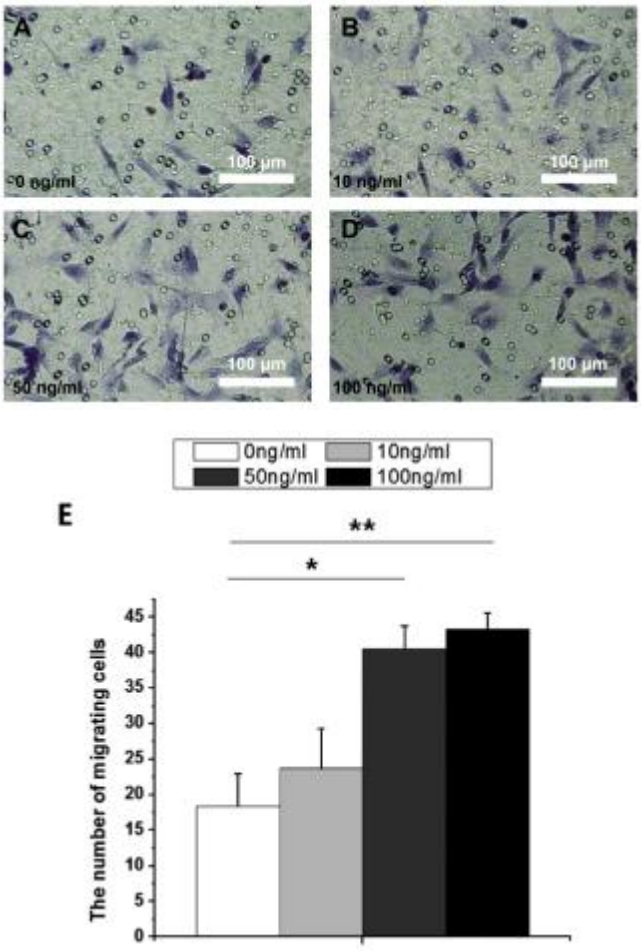

图 4 Transwell 小室实验观察外泌体促进雪旺细胞迁移

Fig. 4 Exosomes promoting the migration of schwann cells in Transwell chambers

A: In the control group(0 ng/mL), there were just a few Schwann cells migration. B: The outer chamber was inoculated with 10 ng/mL exosomes, and the migration of schwann cells began to increase. C: Compared with the control group, when the outer chamber exosomes concentration reached 50 ng/mL, The Schwann cells migration increased significantly. ($P < 0.05$, n=6); D: When the exosomes concentration reached 100 ng/mL, the migration of Schwann cells did not increase significantly.(compared with the control group, $P< 0.01$, n=6; compared with the 50 ng/mL group, $P > 0.05$, n=6); E: Statistics figure about the results.

此对于其它来源的外泌体是否能促进雪旺细胞迁移目前并不得而知。实验中采用分离 hUC-MSCs 分离的外泌体是由于此方法是目前普遍公认的也普遍采用的获得纯度较高外泌体的方法, 而对于促进雪旺细胞迁移这一现象是否和 hUC-MSCs 来源有关,后续我们将进行更多实验进行验证。但是就 Transwell 小室实验本身而言,足以证明外泌体能够很好的促进雪旺细胞迁移。

实验中我们采用较为经典的超高速离心法进行外泌体的分离。在前期预实验中课题组也采用密度梯度离心法和试剂盒提取法尝试进行外泌体分离,然而,密度梯度离心法步骤较为繁琐,并且较超速离心法耗时长,和雪旺细胞培养周期不同步,无法满足实验需要。而采用试剂盒分离(Exo-Quick exosome 提

取试剂盒,System Bioscience),虽然也可以高效率收集外泌体,然而超高速离心法所收集的外泌体已经完全可以满足实验需要,并未出现明显的囊泡损伤等情况发生,更经济高效。因此实验中采用经典的超高速离心法进行外泌体的分离。

关于外泌体促进雪旺细胞迁移的体内实验,可以通过解决外泌体组织相容性后,以胶原微球或凝胶的方式复合进入壳聚糖复合材料中,并在另一端复合雪旺细胞后进行长节段神经缺损的修复,随后观察其疗效。此部分实验目前正在进行中。

总之,采用 hUC-MSCs 分离而来的外泌体可以有效促进雪旺细胞的迁移,并且存在一定的剂量依赖关系。外泌体复合雪旺细胞,有望成为一种新的组织工程种子细胞,促进长节段神经再生,提高治疗效果。试验结果为雪旺细胞在组织工程领域中的应用提供了强有力的理论指导和新的思路。

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