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·基础研究·

外泌体在ⅢA型慢性前列腺炎发病机制中的作用及意义

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[摘 要] **目的** 探讨前列腺液来源的外泌体在ⅢA型慢性前列腺炎发病机制中的作用。**方法** 选取我院2018年4月至2018年8月收治的30例ⅢA型慢性前列腺炎患者作为ⅢA组,14例健康志愿者作为对照组。利用超速离心法从前列腺按摩后尿液中提取外泌体。通过透射电镜和Western blot鉴定提取的外泌体,使用激光粒度仪测量外泌体粒径,应用外泌体测量试剂盒(ExoELISA-ULTRA CD63-1)及BCA法测定外泌体浓度及外泌体蛋白浓度。**结果** 电镜及Western blot显示,从前列腺按摩后尿液中成功提取出外泌体。对照组外泌体平均粒径为(157.5±2.27) nm,ⅢA组外泌体平均粒径为(155.4±1.73) nm,2组比较差异无统计学意义($t=0.7$,$P>0.05$)。对照组外泌体浓度为$(6.13\pm1.19)\times10^{13}$/L,ⅢA组为$(9.77\pm1.05)\times10^{13}$/L,2组比较差异有统计学意义($t=2.09$,$P<0.05$)。对照组外泌体蛋白浓度为(1.24±0.16) g/L,ⅢA组为(1.84 ±0.17) g/L,2组比较差异有统计学意义($t=2.18$,$P<0.05$)。**结论** ⅢA组前列腺按摩后尿液中外泌体数量多于对照组,外泌体可能在ⅢA型慢性前列腺炎发病机制中具有重要作用。

[关键词] ⅢA型慢性前列腺炎;外泌体;前列腺按摩后尿液;CD9;CD63

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Effect of exosomes on pathogenesis of ⅢA chronic prostatitis and its significance

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Abstract: Objective To investigate the effect of exosomes in the pathogenesis of ⅢA chronic prostatitis (CP). **Methods** A total of 30 patients with ⅢA CP in our hospital from April to August 2018 were selected as ⅢA group, and 14 healthy people were selected as control group. Differential ultracentrifugation was performed to isolate exosomes from the expressed prostatic secretion in urine (EPS-urine). Exosomes were characterized by transmission electron microscopy and Western blot. The size of exosomes were analyzed by particulate size description analyser. Exosomes were quantified using Exosome Quantification Assay Kit (ExoELISA-ULTRA CD63-1). Proteins were quantified by BCA assay. **Results** Exosomes were successfully isolated from EPS-urine and identified by transmission electron microscopy and Western blot. The average size of exosomes in control group was (157.5±2.27) nm, that in ⅢA group was (155.4±1.73) nm, the difference was not significant ($t=0.7$, $P>0.05$). The exosomes concentration in control group was $(6.13\pm1.19)\times10^{13}$/L, that in ⅢA group was $(9.77\pm1.05)\times10^{13}$/L, the difference was significant ($t=2.09$, $P<0.05$). The protein concentration in control group was (1.24±0.16) g/L, that in ⅢA group was (1.84±0.17) g/L, the difference was significant ($t=2.18$, $P<0.05$). **Conclusion** The level of exosomes in ⅢA group was significantly higher than that in control group. Exosomes may have an important role in the etiology of ⅢA CP.

Keywords: ⅢA chronic prostatitis; exosome; expressed prostatic secretion in urine; CD9; CD63

前列腺炎是泌尿外科的常见疾病,约占门诊就诊患者的25%[1]。根据NIH分类,前列腺炎分为4类:Ⅰ型急性细菌性前列腺炎、Ⅱ型慢性细菌性前列腺炎、Ⅲ型慢性前列腺炎/骨盆疼痛综合征(chronic prostatitis/chronic pelvic pain syndromes, CP/CPPS)和Ⅳ型无症状性炎症性前列腺炎。其中CP/CPPS约占所有前列腺炎的90%[2]。国外CP/CPPS发病率为5%~9%,我国CP/CPPS发病率为6%~32.9%[3-6]。CP/CPPS临床表现为长期、反复的骨盆区域疼痛或不适,持续超过3个月,伴有不同程度的排尿症状和性功能障碍。根据前列腺按摩液中白细胞计数,将前列腺炎分为ⅢA型(炎症性CP/CPPS)和ⅢB型(非炎症性CP/CPPS)。在临床上ⅢA型慢性前列腺炎更为常见,其病因目前还不明确,泌尿系感染、前列腺导管内反流、免疫改变、神经内分泌失调、盆底肌功能障碍、心理因素等都可能导致疾病的发生[1-2,4]。

外泌体是细胞分泌的小囊泡,广泛存在于血液、尿液、唾液、脑脊液等体液中,由Trams等[7]于1981年发现。外泌体直径30~150 nm,来源于细胞中的晚期胞内体。晚期胞内体膜向内出芽形成腔内囊泡,转变为多泡体,多泡体与细胞膜融合,腔内囊泡释放到细胞

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外,即为外泌体。外泌体中含有多种生物活性物质,如蛋白质、脂质、mRNA、microRNA、DNA 等,是细胞间信息传递的重要工具,可以与周围或远处靶细胞相互作用,发挥相应的生物学效应[8-12]。有研究表明外泌体在系统性红斑狼疮、慢性阻塞性肺疾病等炎症性疾病的发生、发展中起到重要作用[13-14]。

Ⅲ A 型慢性前列腺炎与外泌体之间的相互联系目前还不清楚,外泌体是否在Ⅲ A 型慢性前列腺炎的发生中发挥作用还不得而知。本研究利用超速离心法从前列腺按摩后尿液中提取外泌体,研究其在Ⅲ A 型慢性前列腺炎发病机制中的作用,以期为慢性前列腺炎的治疗提供参考。

1 资料与方法

1.1 尿液样本收集

选取 2018 年 4 月至 2018 年 8 月陆军军医大学第一附属医院收治的 30 例Ⅲ A 型慢性前列腺炎患者作为Ⅲ A 组,14 例健康志愿者作为对照组。Ⅲ A 型慢性前列腺炎患者平均年龄 31 岁;健康志愿者平均年龄 36 岁,2 组患者年龄差异无统计学意义($P>0.05$)。Ⅲ A 组患者前列腺按摩前、后尿液细菌培养阴性,前列腺液常规检查示白细胞数目大于等于 10 个/高倍视野。对照组患者无慢性前列腺炎相关临床表现,前列腺液常规检查正常。留取前列腺按摩后尿液 30 mL。本研究经过陆军军医大学第一附属医院伦理委员会的批准同意,患者及健康志愿者均签署知情同意书。

1.2 外泌体分离

参照 Théry[15] 及 Wang 等[16] 的分离方法,收集前列腺按摩后尿液 30 mL,5 000 g 离心(4 ℃)15 min,去除沉淀,离心后的上清 15 000 g 离心(4 ℃)30 min,去除沉淀,上清液 150 000 g 离心(4 ℃)70 min,弃上清,PBS 溶液重悬沉淀,0.22 μm 滤器过滤,悬液150 000 g 离心(4 ℃)70 min,弃上清,PBS 溶液重悬沉淀,悬液 150 000 g 离心(4 ℃)70 min,弃上清,100 μL PBS 溶液重悬沉淀,即为外泌体,-80 ℃保存。

1.3 透射电镜观察

取外泌体样本 30 μL 置于载样铜网上,室温静置 5 min,滤纸从侧面吸干液体,铜网上滴加 4% 磷钨酸溶液约 20 μL,室温负染 5 min,滤纸吸干负染液,白炽灯下烤干约 10 min,样品置于 TECNAI 10 electron microscope 下观察。

1.4 BCA 法测量蛋白浓度

取 10 μL 外泌体样本,加入 100 μL RAPA 裂解液(上海碧云天)、1 μL PMSF(上海碧云天),充分混匀,冰上孵育 30 min,充分裂解,12 000 g 离心 5 min,取上清,按照 BCA 试剂盒(上海碧云天)标准方法测定蛋白浓度。

1.5 Western blot 鉴定

取Ⅲ A 组及对照组外泌体各约 10 μg,加入上样缓冲液,95 ℃、5 min 蛋白变性。15% SDS-PAGE 凝胶电泳(80 V,40 min;120 V,60 min)分离蛋白,湿法(300 mA,60 min)蛋白转移至 PVDF 膜上。5% 脱脂奶粉室温封闭 2 h,加入一抗鼠抗人 CD9、CD63 抗体 1∶1 000,4 ℃过夜,TBST 液漂洗 5 次,每次 5 min,二抗兔抗鼠 1∶3 000 室温孵育 2 h。TBST 液漂洗 5 次,每次 5 min,ECL 发显色后,进行结果分析。

1.6 激光粒度仪测外泌体粒径

取外泌体样本 10 μL 溶于 1 mL PBS 溶液中,涡旋 1 min,随后使用激光粒度仪(英国马尔文公司)测量外泌体粒径。

1.7 外泌体浓度定量测定

取外泌体 20 μL,按照外泌体测量试剂盒(ExoELISA-ULTRA CD63-1,SBI)的操作步骤进行定量测定。该试剂盒依赖于外泌体表面标记蛋白 CD63,通过 ELISA 方法进行定量测定。

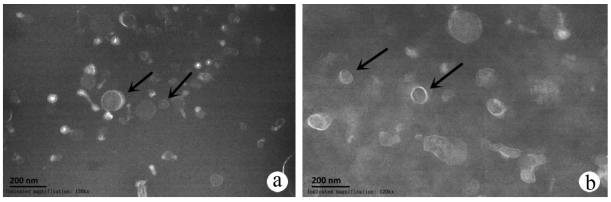
1.8 统计学处理

数据以均数 ± 标准差($\bar{x} \pm s$)表示,采用 GraphPad Prism 5 软件进行数据处理。2 组均数比较采用 t 检验,$P<0.05$ 为差异具有统计学意义。

2 结果

2.1 透射电镜结果

透射电镜下观察 2 组外泌体样本,均有完整双层膜包裹,呈圆形、椭圆形囊泡,直径约 100 nm,外泌体形态无明显区别(图 1)。



a:对照组;b:Ⅲ A 组 箭头:外泌体

图 1 外泌体透射电镜形态

2.2 Western blot 检测外泌体标志物蛋白

采用 Western blot 检测外泌体表面标志物蛋白,结果显示对照组及Ⅲ A 组患者的外泌体均呈阳性表达 CD9、CD63,成功提取外泌体(图 2)。

2.3 外泌体粒径分布

激光粒度仪检测显示,对照组外泌体平均粒径为(157.5 ± 2.27)nm,Ⅲ A 组外泌体平均粒径为(155.4 ± 1.73)nm,2 组比较差异无统计学意义($P>0.05$),见图 3。

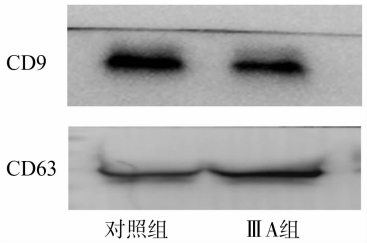
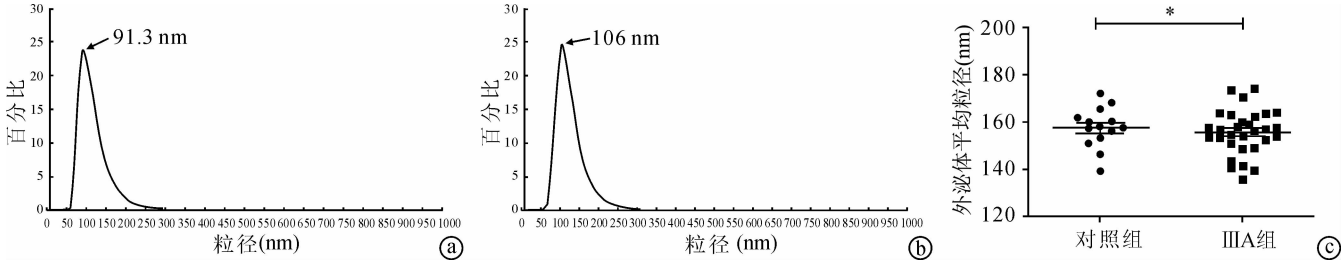


图 2 Western blot 检测外泌体标志物蛋白 CD9、CD63

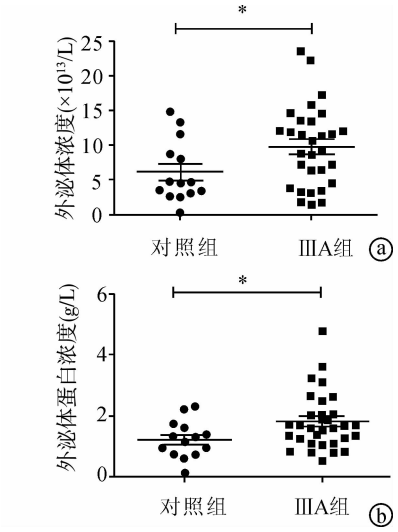
2.4 外泌体浓度及蛋白浓度测定

经外泌体测量试剂盒测定,对照组外泌体浓度为 $(6.13 \pm 1.19) \times 10^{13}$/L,ⅢA 组为 $(9.77 \pm 1.05) \times 10^{13}$/L,2 组比较差异有统计学意义($P < 0.05$)。BCA 法测定外泌体蛋白浓度,对照组外泌体蛋白浓度为 (1.24 ± 0.16)g/L,ⅢA 组为 (1.84 ± 0.17)g/L,2 组比较差异有统计学意义($P < 0.05$),见图 4。



a:对照组外泌体粒径分布;b:ⅢA 组外泌体粒径分布;c:对照组与ⅢA 组外泌体平均粒径比较 *:$P > 0.05$

图 3 外泌体粒径分布图



a:外泌体浓度;b:外泌体蛋白浓度 *:$P < 0.05$

图 4 外泌体浓度及蛋白浓度测定

3 讨论

前列腺按摩后尿液主要用于前列腺炎的诊断,尿常规及尿培养有助于疾病的诊断。前列腺按摩可以促进前列腺液排出,按摩后尿液中前列腺来源的外泌体也会相应增加[17-18]。本研究采集前列腺按摩后尿液,利用超速离心法提取其中的外泌体,经电镜及 Western blot 鉴定,证实成功提取出外泌体,进一步检测发现ⅢA型慢性前列腺炎患者前列腺按摩后尿液中外泌体数量及蛋白含量均明显增加。

外泌体是细胞外囊泡的一种,来源于细胞中的晚期胞内体,CD9、CD63、CD81、TSG101、Alix 等为其标志蛋白[8,11]。细胞分泌外泌体,释放至细胞外,作用于靶细胞,产生相应的生物学效应,在免疫应答、炎症反应中发挥重要的作用;巨噬细胞分泌的外泌体,可以将其表面携带的抗原传递给树突细胞,进而激活 CD4 + T 细胞,产生免疫应答[19]。成熟的树突状细胞分泌的外泌体,可以刺激未成熟树突细胞分泌 IL-1、IL-6、TNF,以及 CD4 + T 细胞分泌 IL-2、MIP-1α、MIP-1β、MCP-1,诱导炎症反应的发生,这一过程受到外泌体表面趋化因子受体(CC chemokine receptor 7,CCR7)的调控[20]。Lee 等[13]研究发现系统性红斑狼疮患者血液中外泌体数量增多,并且可作用于外周血单核细胞,诱导IFN-α、TNF-α、IL-1β、IL-6 等细胞因子的生成。慢性阻塞性肺疾病患者血液中的外泌体数量增多,与 CRP、IL-6 炎症因子水平呈正相关[8]。Ye 等[21]发现慢性心力衰竭患者血液中外泌体数量增多,外泌体中包含的线粒体 DNA,通过激活 TLR9-NF-κB 信号通路,刺激人脐静脉内皮细胞分泌 IL-1β、IL-8。Wang 等[22]发现急性心梗动物模型中,巨噬细胞分泌富含 mir-155 的外泌体,mir-155 通过外泌体,刺激心脏成纤维细胞分泌 IL-1β、IL-6、TNF-α,诱导炎症反应发生。几乎在所有的炎症条件下,外泌体的分泌都会增加。外泌体通过影响细胞间的相互作用、刺激黏附因子的表达、细胞因子的释放,形成一个正反馈环路。

ⅢA 型 CP/CPPS 病程长、易反复,影响患者日常生活质量。目前研究表明多种因素在疾病发生中发挥作用。Pontari 等[23]认为 CP/CPPS 是精神因素、免疫功能紊乱、神经和内分泌功能紊乱等相互作用的结果,其中免疫功能紊乱发挥着重要作用。Doble 等[24]发现 CP 患者前列腺组织中 IgM、IgA 沉积,患者前列腺液、精液和尿液中多种炎症因子发生改变。IL-1β、IL-2、IL-6、IL-8、IL-10、TNF-α、IFN-γ 等都被认为与疾病有

关,IL-8 和 IL-10 与临床表现相关,但具体机制目前还不清楚[4,25-29]。

外泌体在多种炎症性疾病中发挥重要作用,病理情况下,细胞分泌外泌体增加,通过外泌体影响靶细胞,促进相关炎症因子分泌,进而影响疾病进展。本试验证实Ⅲ A 型慢性前列腺炎患者外泌体分泌增加,外泌体在Ⅲ A 型 CP/CPPS 中发挥着一定作用。我们推测在Ⅲ A 型慢性前列腺炎中,外泌体会作用于周围靶细胞,诱导炎症因子表达,进而引起正常细胞、组织损伤,炎症播散,疾病进一步发展。外泌体在Ⅲ A 型 CP/CPPS 中的可能作用及相关机制,还有待于进一步的研究探索。

[参考文献]

[1] Khan FU, Ihsan AU, Khan HU, et al. Comprehensive overview of prostatitis[J]. Biomed Pharmacother, 2017, 94: 1064 - 1076. doi: 710.1016/j.biopha.2017.08.016.

[2] Seong KM, Jang G, Kim DW, et al. Hwanglyunhaedok pharmacopuncture versus saline pharmacopuncture on chronic nonbacterial prostatitis/chronic pelvic pain syndrome[J]. J Acupunct Meridian Stud, 2017, 10(4): 245 - 251. doi: 10.1016/j.jams.2017.06.001.

[3] Schaeffer AJ. Epidemiology and demographics of protatitis[J]. Andrologia, 2003, 35(5): 252 - 257. doi: 10.1111/j.1439 - 0272.2003.tb00852.

[4] Breser ML, Salazar FC, Rivero VE, et al. Immunological mechanisms underlying chronic pelvic pain and prostate inflammation in chronic pelvic pain syndrome[J]. Front Immunol, 2017, 8: 1 - 11. doi: 10.3389/fimmu.2017.00898.

[5] Huang TR, Li W, Peng B. Correlation of inflammatory mediators in prostatic secretion with chronic prostatitis and chronic pelvic pain syndrome [J]. Andrologia, 2018, 50(2): e128600. doi: 10.1111/and.12860.

[6] 米 华,陈 凯,莫曾南. 中国慢性前列腺炎的流行病学特征[J]. 中华男科学杂志, 2012, 18(7): 579 - 582. doi: 10.13263/j.cnki.nja.2012.07.005.

[7] Trams EG, Lauter CJ, Salem N Jr, et al. Exfoliation of membrane ectoenzymes in the form of micro-vesicles[J]. Biochim Biophys Acta, 1981, 645(1): 63 - 70. doi: 10.1016/0005 - 2736(81)90512 - 5.

[8] Colombo M, Raposo G, Théry C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles[J]. Annu Rev Cell Dev Biol, 2004, 30: 255 - 289. doi: 10.1146/annurev-cellbio-101512 - 122326.

[9] Junker K, Heinzelmann J, Beckham C, et al. Extracellular vesicles and their role in urologic malignancies[J]. Eur Urol, 2016, 70(2): 323 - 331. doi: 10.1016/j.eururo.2016.02.046.

[10] Karpman D, Stahl AL, Arvidsson I. Extracellular vesicles in renal disease[J]. Nat Rev Nephrol, 2017, 13(9): 545 - 562. doi: 10.1038/nrneph.2017.98.

[11] Simons M, Raposo G. Exosomes-vesicular carriers for intercellular communication[J]. Curr Opin Cell Biol, 2009, 21(4): 575 - 581. doi: 10.1016/j.ceb.2009.03.007.

[12] Yamamoto S, Azuma E, Muramatsu M, et al. Significance of extracellular vesicles: pathobiological roles in disease[J]. Cell Struct Funct, 2016, 41(2): 137 - 143. doi: 10.1247/csf.16014.

[13] Lee JY, Park JK, Lee EY, et al. Circulating exosomes from patients with systemic lupus erythematosus induce an proinflammatory immune response[J]. Arthritis Res Ther Arthritis Res Ther, 2016, 18(1): 264. doi: 10.1186/s13075 - 016 - 1159 - y.

[14] Tan DBA, Armitage J, Teo TH, et al. Elevated levels of circulating exosome in COPD patients are associated with systemic inflammation[J]. Respir Med, 2017, 132: 261 - 264. doi: 10.1016/j.rmed.2017.04.014.

[15] Théry C, Amigorena S, Raposo G, et al. Isolation and characterization of exosomes from cell culture supernatants and biological fluids[J]. Curr Protoc Cell Biol, 2006, 30(1): 3.22.1 - 3.22.29. doi: 10.1002/0471143030.cb0322s30.

[16] Wang L, Skotland T, Berge V, et al. Exosomal proteins as prostate cancer biomarkers in urine: From mass spectrometry discovery to immunoassay-based validation[J]. Eur J Pharm Sci, 2017, 98: 80 - 85. doi: 10.1016/j.ejps.2016.09.023.

[17] Duijvesz D, Versluis CY, van der Fels CA, et al. Immuno-based detection of extracellular vesicles in urine as diagnostic marker for prostate cancer[J]. Int J Cancer, 2015, 137(12): 2869 - 2878. doi: 10.1002/ijc.29664.

[18] Drake RR, White KY, Fuller TW, et al. Clinical collection and protein properties of expressed prostatic secretions as a source for biomarkers of prostatic disease[J]. J Proteomics, 2009, 72(6): 907 - 917. doi: 10.1016/j.jprot.2009.01.007.

[19] Xu YP, Liu Y, Yang CQ, et al. Macrophages transfer antigens to dendritic cells by releasing exosomes containing dead-cell-associated antigens partially through a ceramide-dependent pathway to enhance CD4 + T-cell responses[J]. Immunology, 2016, 149(2): 157 - 171. doi: 10.1111/imm.12630.

[20] Gao W, Yuan J, Liu HB, et al. Dendritic cells derived exosomes migration to spleen and induction of inflammation are regulated by CCR7[J]. Sci Rep, 2017, 7: 42996. doi: 10.1038/srep42996.

[21] Ye W, Tang XJ, Yang ZQ, et al. Plasma-derived exosomes contribute to inflammation via the TLR9-NF-κB pathway in chronic heart failure patients[J]. Mol Immunol, 2017, 87: 114 - 121. doi: 10.1016/j.molimm.2017.03.011.

[22] Wang CX, Zhang CC, Liu LX, et al. Macrophage-derived mir-155-containing exosomes suppress fibroblast proliferation and promote fibroblast inflammation during cardiac injury[J]. Mol Ther, 2017, 25(1): 192 - 204. doi: 10.1016/j.ymthe.2016.09.001.

[23] Pontari MA, Ruggieri MR. Mechanisms in prostatitis/chronic pelvic pain syndrome[J]. J Urol, 2008, 179(5 Suppl): S61 - S67. doi: 10.1016/j.juro.2008.03.139.

[24] Doble A, Walker MM, Harris JR, et al. Intraprostatic antibody deposition in chronic abacterial prostatitis[J]. Br J Urol, 1990, 65(6): 598 - 605. doi: 10.1111/j.1464 - 410X.1990.tb14827.

[25] Nadler RB, Koch AE, Calhoun EA, et al. IL-1β AND TNF-α in prostatic secretions are indicators in the evaluation of men with chronic prostatitis [J]. J Urol, 2000, 164(1): 214 - 218. doi. org/10.1016/S0022 - 5347(05)67497 - 6.

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·基础研究·

不同代昆明小鼠胚胎成纤维细胞的生长特性研究

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[摘 要] **目的** 分离培养昆明小鼠成纤维细胞,研究其体外生长特性并用于胚胎干细胞培养。**方法** 消化法分离培养获得昆明小鼠胚胎成纤维细胞,利用MTT法绘制各代细胞生长曲线,免疫荧光对各代细胞进行细胞骨架分析;用不同浓度丝裂霉素C(10 μg/mL、20 μg/mL和30 μg/mL)分别处理MEFs 1、2、3 h,MTT法筛选饲养层制备的最佳条件,制备饲养层用于小鼠胚胎干细胞的培养。**结果** 分离的小鼠胚胎成纤维细胞3~5代细胞增殖能力较好;1~3代细胞微管微丝排列整齐,5代以后的细胞微管微丝排列紊乱;10 μg/mL丝裂霉素C处理2 h有利于小鼠胚胎干细胞的培养。**结论** 体外分离培养的3~5代小鼠胚胎成纤维细胞可用于小鼠胚胎干细胞的培养。

[关键词] 小鼠胚胎成纤维细胞;细胞骨架;饲养层
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Growth characteristics of murine embryonic fibroblasts of Kunming species in vitro

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Abstract: Objective Isolation and culture of Kunming mouse embryonic fibroblasts (MEFs) for growth feature in vitro are used to culture the embryonic stem cell. **Methods** Kunming MEFs were isolated and cultured by digestion. MTT assay was used to plot the growth curve of each generation of cells. The cytoskeleton of each generation of cells was analyzed by immunofluorescence. The MEFs were dealt with different concentration of Mitomycin C(10 μg/mL, 20 μg/mL and 30 μg/mL) for 1 hour, 2 hours and 3 hours. The optimum condition of feeder layer preparation was screened by MTT assay. The feeder layer was prepared for the culture of mouse embryonic stem cells. **Results** The proliferation ability of generation 3 to 5 of MEFs was good. The cytoskeleton immunofluorescence staining showed that the generation 1 to 3 of cell microtubules and microfilaments arranged in neat rows, the generation 5 to 7 of cells microtubules and microfilaments were disorganized. The feeder cells dealt with 10 μg/mL Mitomycin C for 2 hours was better for the culture of mouse embryonic stem cell. **Conclusion** The generation 3 to 5 of MEFs can be used for the culture of mouse embryonic stem cells.

Keywords: mouse embryonic fibroblasts; cytoskeleton; feeder cells

近年来,胚胎干细胞在早期胚胎发育研究、基因打靶技术、定向诱导分化及细胞移植治疗疾病方面得到广泛的应用而成为生物医学领域的研究热点[1-4]。目前体外进行胚胎干细胞的培养需依赖大量的饲养层细胞,而市售的饲养层细胞成本较高且运输不便,还存在复苏成活率低等缺点。目前对饲养层细胞的分离培养研究较多,但对于制备小鼠胚胎成纤维细胞(mouse embryonic fibroblasts, MEFs)的小鼠最适胎龄、最佳消化浓度和时间、分离时间等结论不一,用于制备饲养层的处理浓度和时间及饲养层细胞的冻存方法有较大差异[5-8]。本研究参照现有的小鼠胚胎成纤维细胞分离培养方法,利用胰酶消化法体外获得小鼠胚胎成纤维

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[26] Khadra A, Fletcher P, Luzzi G, et al. Interleukin-8 levels in seminal plasma in chronic prostatitis/chronic pelvic pain syndrome and non-specific urethritis[J]. BJU Int, 2006, 97(5): 1043-1046. doi: 10.1111/j.1464-410X.2006.06133.

[27] Miller LJ, Fischer KA, Goralnick SJ, et al. Interleukin-10 levels in seminal plasma: implications for chronic prostatitis-chronic pelvic pain syndrome[J]. J Urol, 2002, 167(2 Pt 1): 753-756. doi: 10.1016/S0022-5347(01)69139-0.

[28] 贺大林,周 锋,何 辉,等.前列腺按摩液中IL-8和TNF-α测定在慢性前列腺炎诊断、分型中的临床意义[J].中华男科学杂志,2002,10(10):740-742. doi:10.13263/j.cnki.nja.2004.10.005.

[29] 李宏军,商学军,黄宇烽.IL-10、IL-8在慢性前列腺炎中的改变及意义[J].中华男科学杂志,2004,10(7):486-487. doi:10.13263/j.cnki.nja.2004.07.002.

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