临床细胞生物学

维生素A对体外培养人脐带间充质干细胞生长的影响

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摘要 该实验以人脐带间充质干细胞(human umbilical cord mesenchymal stem cells, hUCMSCs)为研究对象, 探讨维生素A对其体外培养的影响。结果显示, 添加维生素A培养后, hUCMSCs仍维持其本身生物学特性, 表达其标记基因*CD29*、*CD44*和干细胞标记基因*Oct4*、*Sox2*、*Nanog*。维生素A促进hUCMSCs的体外增殖, 上调增殖基因*PCNA*、*C-myc*和干细胞标记基因*Nanog*的表达, 下调凋亡基因*Bcl-x*的表达。该研究证明了维生素A具有促进hUCMSCs增殖和维持其干细胞特性的作用, 对继续探索hUCMSCs的体外快速增殖和维生素A对hUCMSCs增殖调控的机理具有重要意义。

关键词 人脐带间充质干细胞(hUCMSCs); 维生素A; 增殖

Effects of Retinol (Vitamin A) on Biological Characterization of Mesenchymal Stem Cells from Human Umbilical Cord *In Vitro*

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Abstract This work was designed to investigate the effects of vitamin A on the proliferation of human umbilical cord mesenchymal stem cells (hUCMSCs). The results showed that after *in vitro* treatment of hUCMSCs with vitamin A, the biological characteristics of hUCMSCs could be performed. The expression patterns of hUCMSCs markers and stem cell markers, including *CD29*, *CD44*, *Oct4*, *Sox2* and *Nanog*, were also detected. In addition, we confirmed that vitamin A could stimulate the proliferation of hUCMSCs. The transcriptions of biomolecules, such as *PCNA*, *C-myc* and *Nanog*, were significantly elevated, while the transcript of *Bcl-x* declined. It was identified that the vitamin A could trigger the characteristics maintenance and proliferation of hUCMSCs, which might be crucial to reveal the regulation mechanism of vitamin A on the proliferation of hUCMSCs.

Key words human umbilical cord mesenchymal stem cells (hUCMSCs); vitamin A; proliferation

人脐带间充质干细胞(human umbilical cord mesenchymal stem cells, hUCMSCs)是一类具有自我更新、增殖和多向分化潜能的干细胞。研究表明, hUCMSCs的增殖能力、分化潜能、生物学特性及

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免疫表型都与骨髓间充质干细胞(bone marrow derived mesenchymal stem cells, BMSCs)高度相似[1-4], 可分化为成熟脂肪细胞、骨细胞、神经细胞、心肌样细胞、肝细胞和胰岛样细胞等[5-11], 在神经系统疾病、糖尿病、风湿性关节炎、心血管疾病及肿瘤疾病等的治疗中具有很大的应用潜力[12-16]。与BMSCs相比, hUCMSCs可避免取材困难、给供体造成二次损伤等问题[17]; 与脐血来源的MSCs相比较, hUCMSCs能排除含量稀少、异质细胞多和需要保持酸性培养环境等问题[18-19], 而且hUCMSCs含量丰富、对供体无不良影响, 易于采集、保存和运输, 无免疫排斥反应和伦理争议。

维生素A对视力变化、骨骼发育、精子生成和胚胎发育都有重要影响, 维持着机体的多种生理功能[20-21]。此前研究表明, 维生素A可维持精细胞的形态并保持其活力[22], 还能促进多种细胞的增殖[23-25], 且浓度为1 μmol/L的维生素A相对于其他浓度更有利于干细胞的体外增殖, 对干细胞的发育和减数分裂等具有促进作用, 也有利于干细胞全能性的维持[26]。但是, 关于维生素A对体外培养的hUCMSCs的增殖、分化以及发育的影响, 到目前为止还没有相关报道。本研究以脐带组织来源的MSCs为研究对象, 通过4组不同培养基对体外培养的hUCMSCs生长和相关基因表达的影响进行平行对照研究, 初步探讨了维生素A对体外培养hUCMSCs生长的影响。

1　材料与方法

1.1　材料

在产妇及家属同意的情况下, 脐带标本来源于健康足月剖宫产新生儿, 由四川省雅安市妇幼保健院提供。

1.2　主要试剂和仪器

胎牛血清(fetal bovine serum, FBS) 、血清替代品(knockout serum replacement, KSR)购自Gibco公司; 维生素A、胰蛋白酶、IV型胶原酶、DNA酶、DMEM/F12购自 Sigma公司; 总RNA快速提取试剂盒购自北京百泰克公司; MTT细胞增殖检测试剂盒、PrimeScript™ RT reagent Kit with gDNA Eraser(Perfect Real Time)试剂盒、RNA PCR Kit(AMV) Ver.3.0试剂盒购自 TaKaRa公司; 一抗CD29(1:100稀释)、CD44(1:50稀释)、CD105(1:200稀释)、CD31(1:200稀释)、CD34(1:200稀释)购自 Ycbio公司; 一抗Oct4(1:200稀释)、Nanog(1:500稀释)、Sox2(1:200稀 释)购 自CST公司; SP免疫组化染色试剂盒购自北京中杉金桥公司; DAB显色试剂盒购自博士德生物公司; 二氧化碳培养箱、–80 °C超低温冰箱和DG5033A酶标仪购自Thermo公司; 倒置相差显微镜购自尼康公司; PCR仪、平板电泳仪、凝胶成像系统购自Bio-Rad公司。

1.3　hUCMSCs的分离培养

无菌取足月妊娠剖宫产健康胎儿的脐带, 抽除脐带血, 置于无菌PBS中带回实验室, 用PBS充分冲洗, 剔除羊膜、脐静脉和脐动脉, 然后将组织剪成约1 mm^3大小的组织块, 加入3倍体积的IV型胶原酶4 °C消化过夜, 后加入0.25%胰蛋白酶+0.02% EDTA和0.1% DNA酶消化30 min, 终止消化, 用120目筛网过滤收集细胞, 接种于24孔板培养, 加入含12% FBS的DMEM/F12培养基, 稳定传代后, 基础培养基不变, 设置4个组同时培养: 12% FBS组; 12% FBS+1 μmol/L维生素A组; 14% KSR组和14% KSR+1 μmol/L维生素A组, 在37 °C、5% CO_2及饱和湿度培养箱中培养, 待细胞生长至80%~90%汇合时进行传代。

1.4　hUCMSCs生长曲线的绘制

采用MTT法测定细胞生长曲线, 取第三代hUCMSCs, 以2 500/孔的密度接种于96孔板进行培养, 分别在培养1, 2, 3, 4, 5, 6, 7 d时按试剂盒说明对各组细胞进行MTT检测。加入MTT溶液10 μL/孔, 37 °C孵育4 h, 加formanzan液100 μL/孔, 37 °C孵育至formanzan完全溶解, 在酶联免疫检测仪上波长570 nm处检测各孔的光密度(*D*)值, 根据测得的吸光度值绘制细胞生长曲线, 每组设6孔对照。

细胞以1×10^6/mL密度接种于24孔板培养24 h后, 采用胰酶消化法计算细胞群体倍增时间(population doubling time, PDT), 用以下公式法求得: PDT=t×[lg2/(lgNt–lgNo)], 公式中t代表培养时间, No代表首次计数(细胞培养24 h时)获得的细胞数, Nt代表培养t时间后的细胞数。

1.5　hUCMSCs表型免疫组化

采用免疫组化法在蛋白水平上对FBS+维生素A组稳定培养20代后的hUCMSCs进行标记基因检测, 包括基因*CD29*、*CD44*、*CD105*、*CD31*、*CD34*、*Oct4*、*Sox2*和*Nanog*。用4%多聚甲醛固定细胞10 min, 干燥5 min; PBS冲洗后用0.5% TritonX-100处理15 min(细胞表面标记CD29、CD44、CD105、CD31、CD34不经此步骤); PBS冲洗后加入3% H_2O_2

孵育7 min; PBS冲洗后滴加封闭用山羊血清37 ℃孵育10 min, 滴加一抗4 ℃过夜; 次日PBS冲洗后滴加生物素化二抗37 ℃孵育20 min; PBS冲洗, 滴加辣根酶标记链霉卵白素37 ℃孵育15 min; PBS冲洗, DAB显色阳性表达时细胞着色为蓝色, 阴性表达时细胞不着色, 所有结果均在倒置相差显微镜下拍照记录。

1.6 反转录聚合酶链式反应(RT-PCR)检测相关基因在hUCMSCs中的表达

调整细胞浓度为1×10^6/mL, 接种于24孔板培养, 取四组中分别培养3, 6, 9, 12 d时的细胞样品, 用RT-PCR检测细胞增殖基因*PCNA*、*C-myc*和细胞凋亡基因*Bcl-x*的表达; 取FBS+维生素A组连续培养20代后的细胞样品, 用RT-PCR检测MSCs标记基因*CD29*、*CD44*, 内皮细胞标记基因*CD31*, 造血干细胞标记基因*CD34*及干细胞标记基因*Oct4*、*Sox2*、*Nanog*的表达。

总RNA的提取、反转录、PCR反应均按试剂盒说明操作, PCR反应条件: 95 ℃预变性3 min; 94 ℃变性30 s, 51~59.5 ℃退火30 s, 72 ℃延伸1 min, 30个循环; 最后72 ℃延伸10 min(引物序列见表1)。然后进行凝胶电泳, 每组独立重复3次, 之后将凝胶放置在BIO-RAD凝胶成像仪内, 记录并保存电泳图像, 用Quantity One软件对图像进行灰度值分析, 结果以目的基因吸光度值/内参基因吸光度值的平均数±标准差表示。

1.7 数据统计

统计软件为SPSS 16.0, 方差分析采用*F*检验, 组间均值比较采用*t*检验, $P<0.05$为差异有统计学意义。

2 结果

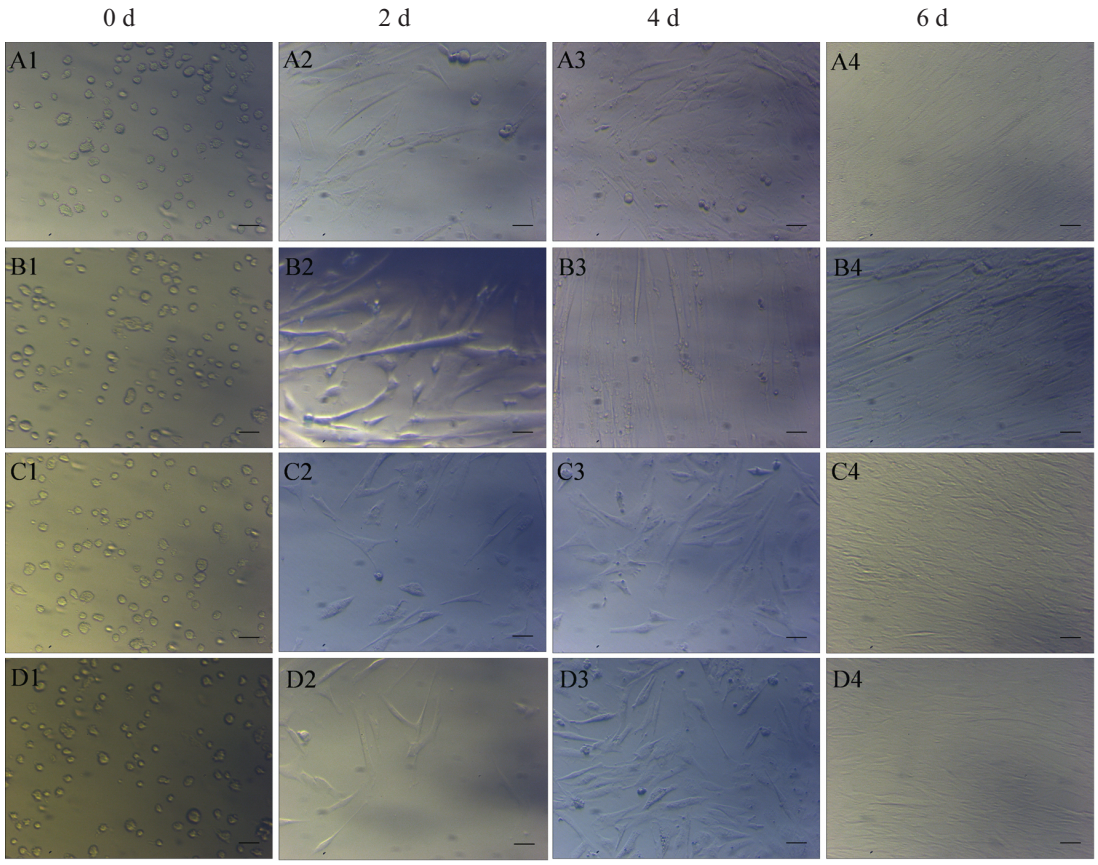
2.1 维生素A对hUCMSCs体外生长的影响

在四组培养基中hUCMSCs均可稳定生长, 呈梭形的成纤维细胞样贴壁生长, 4 d时细胞生长汇合达80%左右后变细长, 6 d时细胞100%汇合, 呈平行排列或漩涡状(图1)。MTT法绘制的生长曲线结果显

表1 RT-PCR引物序列及产物长度

Table 1 Sequence of primers and product length

基因 Gene	引物序列 Sequence of primers	产物长度 Product length
GAPDH	Forward: 5′-AAG GTC GGA GTC AAC GGA T-3′ Reverse: 5′-AAT GAG CCC CAG CCT TCT-3′	290 bp
CD29	Forward: 5′-AAC CAA CCG TAG CAA AGG-3′ Reverse: 5′-CCA CCA AGT TTC CCA TCT-3′	591 bp
CD44	Forward: 5′-CTG AGC ATC GGA TTT GAG-3′ Reverse: 5′-CCA TTT CCT GAG ACT TGC-3′	778 bp
CD105	Forward: 5′-GCT TCT GGT CCT CAG TGT AAA-3′ Reverse: 5′-CTT GAA GCC ACG AAT GTT T-3′	589 bp
CD31	Forward: 5′-AAA ATG GGA AGA ACC TGA-3′ Reverse: 5′-CAT CGG AAG GAT AAA ACG-3′	475 bp
CD34	Forward: 5′-TTG CTG CCT TCT GGG TTC-3′ Reverse: 5′- TTC TGC CTT GAT GTC ACT TA-3′	450 bp
Oct4	Forward: 5′-GGT ATT CAG CCA AAC GAC-3′ Reverse: 5′-CCT GAG AAA GGA GAC CCA-3′	390 bp
Nanog	Forward: 5′-TTT GGA AGC TGC TGG GGA AG-3′ Reverse: 5′-GAT GGG AGG AGG GGA GAG GA-3′	245 bp
Sox2	Forward: 5′-GAG TGG AAA CTT TTG TCG-3′ Reverse: 5′-AGT GGG AGG AAG AGG TAA-3′	550 bp
PCNA	Forward: 5′-AAG AAG GTG TTG GAG GCA-3′ Reverse: 5′-GTG TCC CAT ATC CGC AAT-3′	702 bp
C-myc	Forward: 5′-TCC CAC TGA CGA GTT CTA-3′ Reverse: 5′-CTG TTT CAT GGC GTG CTT C-3′	196 bp
Fas	Forward: 5′-TGC CAA GAA GGG AAG GAG-3′ Reverse: 5′-TGG TGT TGC TGG TGA GTG-3′	238 bp
Bcl-x	Forward: 5′-TGT GCG TGG AAA GCG TAG-3′ Reverse: 5′-AGT GAG CCC AGC AGA ACC-3′	238 bp



从上到下依次为FBS组(A1~A4)、FBS+Vitamin A组(B1~B4)、KSR组(C1~C4)、KSR+Vitamin A组(D1~D4)细胞的生长状况。标尺=50 μm。
Growth situation of cells treated with FBS (A1~A4), FBS+Vitamin A (B1~B4), KSR (C1~C4) and KSR+Vitamin A (D1~D4). Scale bar=50 μm.

图1　不同培养条件下人脐带间充质干细胞的形态特征

Fig.1　*In vitro* morphological characterization of hUCMSCs in different conditions

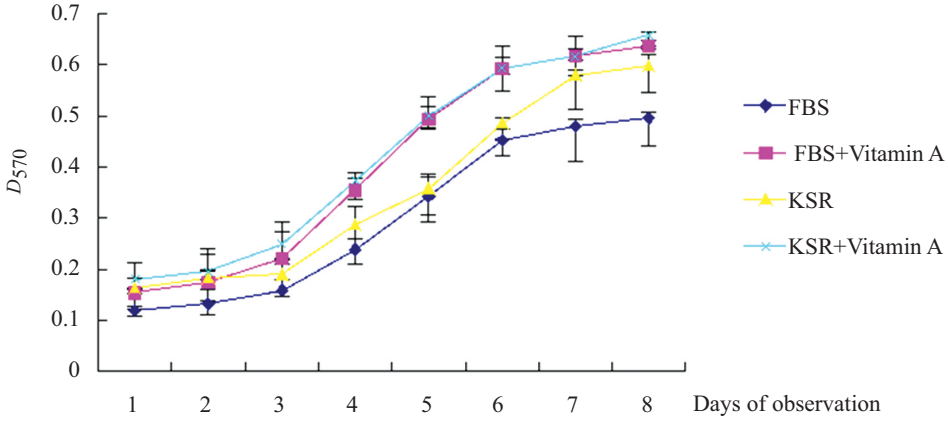


图2　不同培养条件下人脐带间充质干细胞的生长曲线

Fig.2　Growth curve of hUCMSCs in different conditions

示, 4组细胞均经过1~2 d的潜伏期后开始快速增殖进入对数生长期(3~6 d), 在第6 d后逐渐进入平台期(图2)。FBS+维生素A组和KSR+维生素A组在1~5 d内细胞增殖速率高于其他两组, 具有显著性差异。FBS+维生素A组和KSR+维生素A组细胞倍增时间也明显缩短, KSR组细胞增殖稍慢于FBS组, 但在添加维生素A后, 无论在KSR组还是FBS组的细胞均呈现快速增殖趋势(表2)。

通过RT-PCR检测细胞增殖基因*PCNA*、*C-myc*和细胞凋亡基因*Bcl-x*在四组细胞培养中转录水平的变化。*PCNA*、*C-myc*在FBS+维生素A组和KSR+维生素A组显著上调; *PCNA*在细胞培养3 d、6 d时在添加维生素A的组中均显著上调, 到第9 d后有所下降, 但添加维生素A的组仍显著高于其他组; *C-myc*

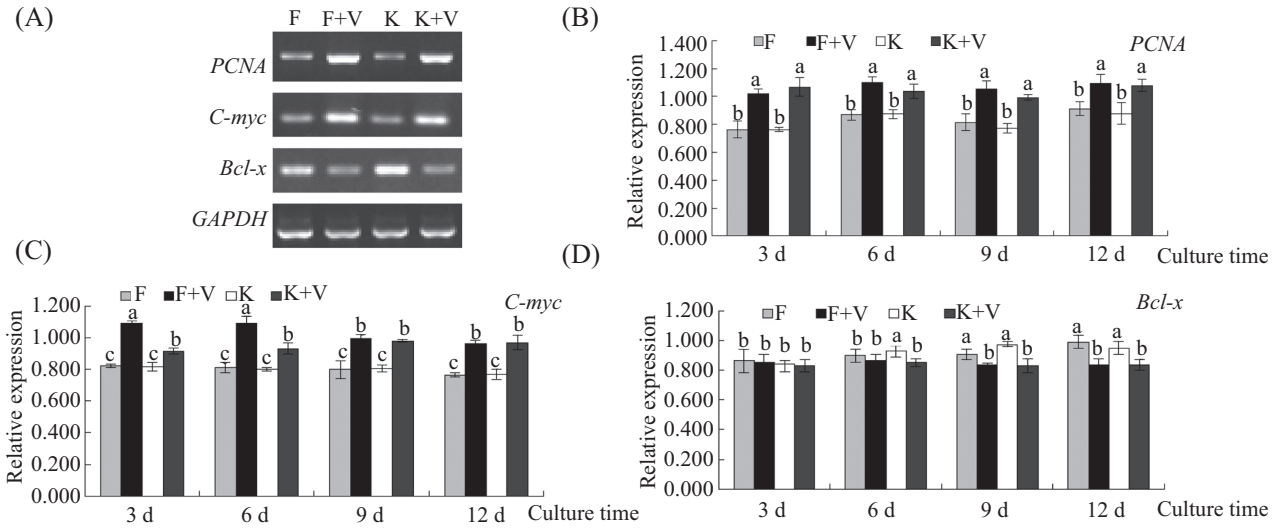
表2 处于对数生长期时各组细胞倍增时间($\bar{x}\pm s$, n=6)

Table 2 The doubling time of each group's cells in logarithmic phase ($\bar{x}\pm s$, n=6)

	FBS 组 FBS group	Vitamin A+FBS组 Vitamin A+FBS group	KSR组 KSR group	Vitamin A+KSR组 Vitamin A+KSR group
细胞倍增时间 Cell doubling time (h)	75.63±0.12[a]	52.15±0.67[b]	80.26±0.21[a]	54.67±0.19[b]

不同字母表示组别之间倍增时间呈显著差异(P<0.05), 相同字母表示组别之间倍增时间无显著差异(P>0.05)。

The different letter indicated significant difference (P<0.05) in cell doubling time, and the same letter indicated no significant difference (P>0.05) in cell doubling time.



A: 目的基因在人脐带间充质干细胞中表达的部分电泳图; B: 基因*PCNA*相对灰度值分析结果; C: 基因*C-myc*相对灰度值分析结果; D: 基因*Bcl-x*相对灰度值分析结果。F代表12% FBS组; F+V代表12% FBS+1 μmol/L维生素A组; K代表14% KSR组; F+V代表14% KSR+1 μmol/L维生素A组。不同字母表示组别之间相对表达量呈显著差异(P<0.05), 相同字母表示组别之间相对表达量无显著差异(P>0.05)。

A: parts of the electrophoresis images of purpose gene in hUCMSCs; B: the analysis of *PCNA* relative transcriptions using Quantity One; C: the analysis of *C-myc* relative transcriptions using Quantity One; D: the analysis of *Bcl-x* relative transcriptions using Quantity One. F represented the group of 12% FBS; F+V represented the group of 12% FBS+1 μmol/L Vitamin A; K represented the group of 14% KSR; F+V represented the group of 14% KSR+1 μmol/L Vitamin A. The different letters indicated significant difference (P<0.05) in relative expression, and the same letters indicated no significant difference (P>0.05) in relative expression.

图3 *PCNA*、*C-myc*及*Bcl-x*在hUCMSCs中的RT-PCR检测

Fig.3 mRNA transcription levels of *PCNA*, *C-myc* and *Bcl-x* in the hUCMSCs detected by RT-PCR

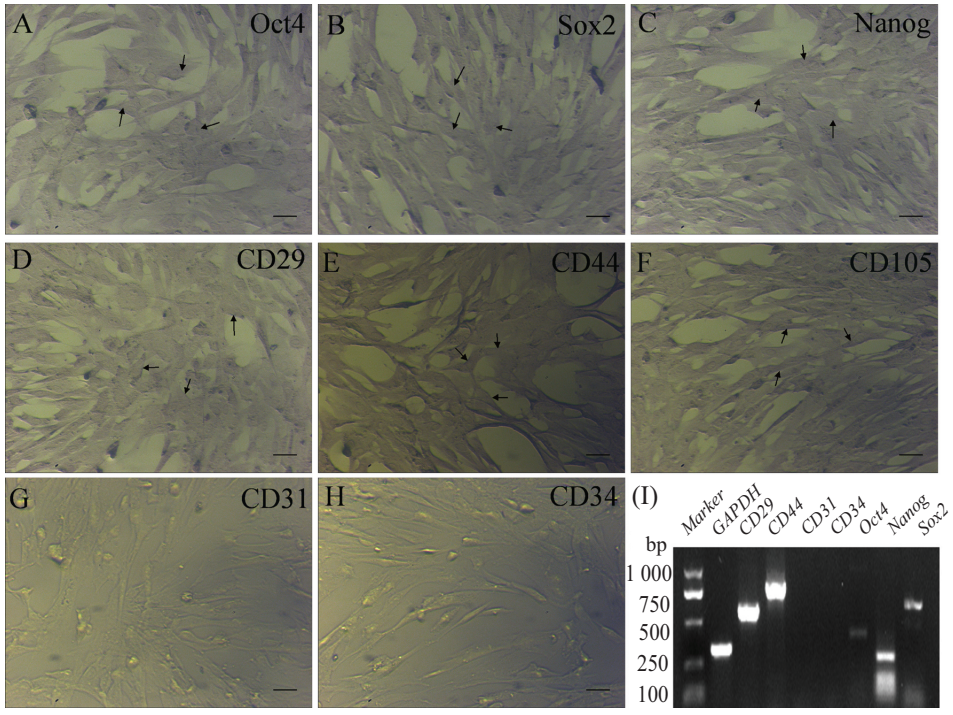
在3 d和6 d组也显著上调, 在9 d和12 d组均有所下降, 且在3 d和6 d组FBS培养的组比KSR培养的组*C-myc*上调更明显, 可能是血清中含有更多的促进细胞增长的因素; 整个培养过程中*Bcl-x*的转录水平变化不明显, 在细胞培养6 d后添加维生素A的组别中表达量有所下降(图3)。以上结果表明, 维生素A可能通过这些基因的激活进一步促进了hUCMSCs的增殖。

2.2 FBS+维生素A组连续培养20代后hUCMSCs标记基因的检测

分别用RT-PCR和免疫组化方法对添加维生素A稳定培养细胞20代后其标记基因的表达进行检测。在基因转录水平上, RT-PCR检测结果显示, 第20代hUCMSCs高表达其标记基因*CD29*、*CD44*, 不表达内皮细胞标志基因*CD31*和造血干细胞标志基因*CD34*, 可稳定表达干细胞标记基因*Oct4*、*Sox2*、*Nanog*基因。在蛋白水平上, 免疫组化检测结果显示, CD29、CD44、CD105、Oct4、Sox2和Nanog阳性表达, 显蓝色; CD31和CD34阴性表达, 不着色, 即20代hUCMSCs表 达CD29、CD44、CD105、Oct4、Sox2和Nanog, 而不表达CD31和CD34(图4)。

2.3 干细胞相关基因和hUCMSCs标记基因在培养中表达的变化

RT-PCR检测干细胞相关基因和MSCs标记基因在细胞中的表达结果显示, MSCs的标记基因*CD29*、*CD44*、*CD105*和干细胞标记基因*Oct4*、*Sox2*在不同培养时间、不同组中表达量均无显著性差异; 干细

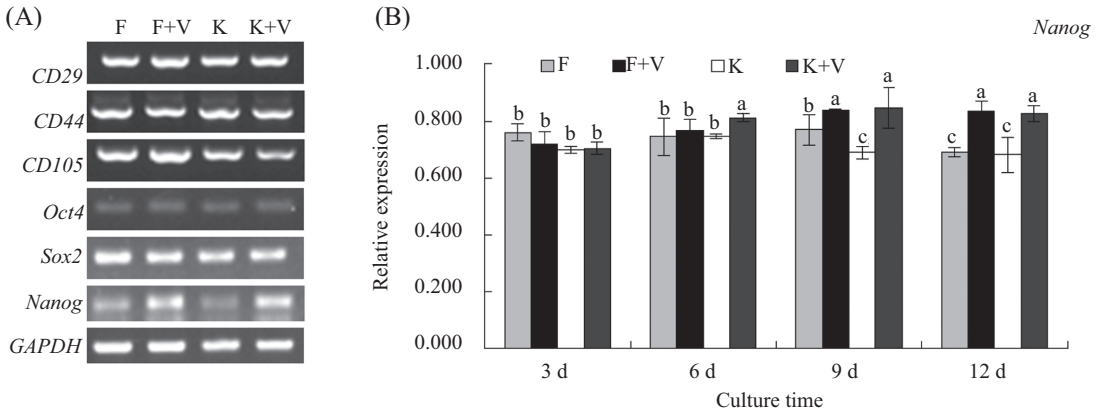


A~H: 免疫组化检测结果, 细胞表达MSCs标志基因CD29、CD44、CD105和干细胞标志基因Oct4、Nanog、Sox2, 箭头所指为部分阳性表达的细胞, 不表达上皮细胞标志基因CD31和造血干细胞标志基因CD34; I: RT-PCR检测结果, 细胞表达基因*CD29*、*CD44*、*CD105*、*Oct4*、*Nanog*、*Sox2*, 不表达*CD31*、*CD34*。标尺=50 μm。

A~H: the results of Elisa test. The expression of the cell markers of MSCs (CD29, CD44, CD105) and the markers of stem cells (Oct4, Nanog, Sox2) were detected, while the marker of epithelial cells (CD31) and the marker of hematopoietic stem cells (CD34) were not invested, and the arrow represented some cells of positive expression. I: the results of RT-PCR test. The expression of the cell markers of MSCs (*CD29*, *CD44*, *CD105*) and the markers of stem cells (*Oct4*, *Nanog*, *Sox2*) were detected, while the marker of epithelial cells (*CD31*) and the marker of hematopoietic stem cells (*CD34*) were not invested. Bar=50 μm.

图4　维生素A连续培养hUCMSCs, 对f20细胞标记基因的免疫组化和RT-PCR检测

Fig.4　Immunohistochemical staining and RT-PCR detection of f20 hUCMSCs incubated with vitamin A



A: 目的基因在人脐带间充质干细胞中表达的部分电泳图片, *GAPDH*为内参基因; B: *Nanog*基因相对灰度值分析结果。*Nanog*基因在培养9 d和12 d的样品中维生素A组的表达量显著上调, 其他基因则无显著变化。不同字母表示组别之间相对表达量呈显著差异(P<0.05), 相同字母表示组别之间相对表达量无显著差异(P>0.05)。

A: parts of the electrophoresis images of purpose gene in hUCMSCs; B: the analysis of *Nanog* relative transcriptions using Quantity One. The transcription of *Nanog* was significantly up-regulated at the 9 d and 12 d after incubated with vitamin A. However, the transcription of other genes did not been affected. The different letter indicated significant difference (P<0.05) in relative expression, and the same letter indicated no significant difference (P>0.05) in relative expression.

图5　hUCMSCs中标记基因及干细胞标记基因的RT-PCR检测

Fig.5　Transcription patterns of the marker genes of hUCMSCs and stem cells in the hUCMSCs by RT-PCR

胞标记基因*Nanog*在各组细胞培养初期无显著变化, 在培养9 d后添加维生素A培养的组中表达量显著上升(图5)。

3 讨论

已有研究表明, hUCMSCs具有与BMSCs相似的生物学特性, 如细胞贴壁生长, 具有特定的表面标志, 表达标志基因*CD29*、*CD44*、*CD73*和*CD105*, 不表达内皮细胞标志基因*CD31*、造血干细胞标记基因*CD34*、*HLA-DR*和白细胞标记基因*CD45*[27], 且具有胚胎干细胞的特性, 表达早期干细胞标记基因*Oct4*、*Sox2*和*Nanog*[28-30]。维生素A又名视黄醇, 主要的衍生物是视磺酸, Bhatia等[31]研究表明, 低浓度的维生素A可促进胚胎干细胞悬浮培养时细胞集落的富集和维持胚胎干细胞在体外长期培养时的未分化状态, 本实验中持续添加维生素A培养hUCMSCs 20代后, RT-PCR和免疫组化检测结果均显示细胞表达CD29、CD44、CD105、Oct4、Sox2和Nanog, 不表达CD31和CD34。已有实验结果证明, $Sox2^+$是多潜能性细胞的标志[32], *Nanog*和*Oct4*基因高表达时细胞具有多潜能性[33], 由此可知本实验中添加维生素A培养20代后的hUCMSCs仍具有一定的多潜能, 但本次实验只是对培养得到的hUCMSCs进行了初步鉴定, 未涉及可衍生细胞标记鉴定和hUCMSCs诱导分化的实验, 因此添加维生素A培养后hUCMSCs是否仍维持其多潜能性以及其分化程度是否改变还需进一步实验证明。

现今多采用异种来源血清FBS体外培养hUCMSCs, 可获得快速增殖, 但也为后期在人类疾病治疗中的应用带来潜在危害。本实验中采用人工合成的血清替代品KSR进行培养, 发现只添加KSR培养时, hUCMSCs增殖相对FBS培养较慢, 加入维生素A后细胞增殖速度显著高于FBS培养, 说明完全可采用KSR代替FBS培养hUCMSCs, 从而排除异源血清可能带来的潜在威胁[34-35]。

Sharow等[36]的研究表明, 视磺酸可维持无血清培养时小鼠胚胎干细胞在体外的稳定生长; Ghiaur等[37]的实验证明, 维生素A参与人造血干细胞自我更新的信号传导, 维持细胞自我更新和分化之间的平衡; Blum等[38]的实验显示, 维生素A促进斑马鱼芽祖细胞增殖从而促使鱼鳍的再生。由此可见, 维生素A可能对细胞增殖有一定的促进作用。本实验中通过MTT检测发现, 在培养液中添加1 μmol/L的维生素A培养细胞, 细胞数目明显增多、细胞增殖速率也显著加快。进一步RT-PCR检测发现, 在添加维生素A组培养的细胞中, 细胞增殖基因*PCNA*、*C-myc*在3~12 d培养的细胞中均有不同程度的上调, 而细胞凋亡基因*Bcl-x*在细胞培养6 d后开始下调表达。由MTT结果可知, 培养6 d后细胞几乎达到100%汇合, 出现生长抑制进入稳定期, 而*Bcl-x*基因在6 d后开始下调, 表明维生素A的添加可能对阻碍细胞生长抑制现象的发生有一定的作用。由此可见, 维生素A可能对细胞生长相关基因有相应的调控作用, 通过激活或抑制其表达而促进细胞增殖。实验中, 在添加维生素A的培养组, *Nanog*基因表达量也有所上调, Park等[39]的研究结果表明, 视黄醇上调*Nanog*基因的表达, 从而促进致瘤生长因子GDF3的表达而诱导胚胎癌细胞增殖。Chen等[40]实验结果也显示, 在培养液中添加维生素A可使*Nanog*基因表达上调, 调控LIF/Stat3信号传导通路从而维持胚胎干细胞的多潜能性和自我更新, 由此可见, *Nanog*基因的上调表达可能与干细胞的增殖相关。

综上, 本实验表明, 维生素A可促进hUCMSCs体外快速增殖, 维持长期培养中未分化状态和多潜能性, 为维生素A促进hUCMSCs体外增殖的作用机理和进一步在临床应用中的研究提供了一定的实验依据。

参考文献 (References)

1 Kita K, Gauglitz GG, Phan TT, Herndon DN, Jeschke MG. Isolation and characterization of mesenchymal stem cells from the sub-amniotic human umbilical cord lining membrane. Stem Cells Dev 2010; 19(4): 491-502.

2 Secco M, Zucconi E, Vieira NM. Mesenchymal stem cells from umbilical cord: Do not discard the cord! Neuromuscul Disord 2008; 18(1): 17-8.

3 Karahuseyinoglu S, Cinar O, Kilic E, Kara F, Akay GG, Demiralp DO, *et al*. Biology of stem cells in human umbilical cord stroma: *In situ* and *in vitro* surveys. Stem Cells 2007; 25(2): 319-31.

4 Dalous J, Larghero J, Baud O. Transplantation of umbilical cord-derived mesenchymal stem cells as a novel strategy to protect the central nervous system: Technical aspects, preclinical studies, and clinical perspectives. Pediatr Res 2012; 71(42): 482-90.

5 Karahuseyinoglu S, Kocaefe C, Balci D, Erdemli E, Can A. Functional structure of adipocytes differentiated from human umbilical cord stroma derived stem cells. Stem Cells 2008; 26(3): 682-91.

6 De la Fuente A, Mateos J, Lesende-Rodríguez I, Calamia V, Fuentes-Boquete I, de Toro FJ, *et al*. Proteome analysis during chondrocyte differentiation in a new chondrogenesis model using human umbilical cord stroma mesenchymal stem cells. Mol Cell Proteomics 2012; 11(2): M111.010496.

7 Fu YS, Shih YT, Cheng YC, Min MY. Transformation of human umbilical mesenchymal cells into neurons *in vitro*. J Biomed Sci 2004; 11(5): 652-60.

8 Pereira WC, Khushnooma I, Madkaikar M, Ghosh K. Reproducible methodology for the isolation of mesenchymal stem cells from human umbilical cord and its potential for cardio myocyte generation. J Tissue Eng RegenMed 2008; 2(7): 394-9.

9 Campard D, Lysy PA, Najimi M, Sokal EM. Native umbilical cord matrix stem cells express hepatic markers and differentiate into hepatocyte-like cells. Gastroenterology 2008; 134(3): 833-48.

10 Chao KC, Chao KF, Fu YS, Liu SH. Islet-like clusters derived from mesenchymal stem cells in Wharton's jelly of the human umbilical cord for transplantation to control type 1 diabetes. PLoS One 2008; 3(1): e1451.

11 Buensuceso C, Seyda A, Colter DC, Dhanaraj S, Kramer BC. Induced pluripotent stem cells from human umbilical cord tissue-derived cells. US Patent 20, 130, 157, 365, 2013.

12 Xiong N, Zhang Z, Huang J, Chen C, Zhang Z, Jia M, *et al*. VEGF-expressing human umbilical cord mesenchymal stem cells, an improved therapy strategy for Parkinson's disease. Gene Therapy 2010; 18(4): 394-402.

13 Tsai PJ, Wang HS, Shyr YM, Weng ZC, Tai LC, Shyu JF, *et al*. Transplantation of insulin-producing cells from umbilical cord mesenchymal stem cells for the treatment of streptozotocin-induced diabetic rats. J Biomed Sci 2012; 19(1): 47.

14 Liu YY, Mu R, Wang SY, Long L, Liu X, Li R, *et al*. Therapeutic potential of human umbilical cord mesenchymal stem cells in the treatment of rheumatoid arthritis. Arthritis Res Ther 2010; 12(6): R210.

15 Salem HK, Thiemermann C. Mesenchymal stromal cells: Current understanding and clinical status. Stem Cells 2010; 28(3): 585-96.

16 Chao KC, Yang HT, Chen MW. Human umbilical cord mesenchymal stem cells suppress breast cancer tumourigenesis through direct cell–cell contact and internalization. J Cell Mol Med 2012; 16(8): 1803-15.

17 Ding F, Wu J, Yang Y, Wen H, Qi Z, Xin T, *et al*. Use of tissue-engineered nerve grafts consisting of a chitosan/poly (1aetic-co-glycolic acid)-based scaffold included with bone marrow mesenchymal cells for bridging 50-mm dog sciatic nerve gaps. Tissue Eng Part A 2010; 16(12): 3779-90.

18 AriewZakay H, Lazarovici P, Nagler A. Tissue regeneration potential in human umbilical cord blood. Best Pract Res Clin Haematol 2010; 23(2): 291-303.

19 Wexler SA, Donaldson C, Denning-Kendall P, Rice C, Bradly B, Hows JM. Adult bone marrow is a rich source of human mesenchymal pstemp cells but umbilical cord and mobilized adult blood are not. Br J Haematol 2003; 121(2): 368-74.

20 Jenkins KJ, Correa A, Feinstein JA, Botto L, Daniels SR, Elixson M, *et al*. Noninherited risk factors and congenital cardiovascular defects: Current knowledge a scientific statement from the American heart association council on ardiovascular disease in the young. Circulation 2007; 115(23): 2995-3014.

21 Zile MH. Function of vitamin a in vertebrate embryonic development. J Nutr 2001; 131(3): 705-8.

22 Riha RL, Yang IA, Rabnott GC, Tunnicliffe AM, Fong KM, Zimmerman PV. Cytokine gene polymorphisms in idiopathic pulmonary fibrosis. Int Med J 2004; 34: 126-9.

23 Bernard H, Davis M D, Angelina V. The effect of retinol on ito cell proliferation *in vitro*. Hepatology 1988; 8(4): 788-93.

24 Margis R, Pinheiro-Margis M, Da Silva LC, Borojevic R. Effects of retinol on proliferation, cell adherence and extracellular matrix synthesis in aliver myofibroblast or lipocyte cell line (GRX). Int J Exp Pathol 1992; 73(2): 125-35.

25 Chen L, Khillan JS. A novel signaling by vitamin a/retinol promotes self renewal of mouse embtyonic stem cells by activating PI3K/Akt signaling pathway via insulin-like growth factor-1 receptor. Stem Cell 2010; 28(1): 57-63.

26 Zhang S, Sun J, Pan S, Zhu H, Wang L, Hu Y, *et al*. Retinol (vitamin A) maintains self-renewal of pluripotent male germline stem cells (mGSCs) from adult mouse testis. J Cell Biochem 2011; 112(4): 1009-21.

27 Hematti P. Role of mesenchymal stromal cells in solid organ transplantation. Transplant Rev 2008; 22(4): 262-73.

28 Tondreau T, Meuleman N, Delforge A, Dejeneffe M, Leroy R, Massy M, *et al*. Mesenchymal stem cells derived from CD133-positive cells in mobilized peripheral blood and cord blood: Proliferation, Oct4 expression, and plasticity. Stem Cells 2005; 23(8): 1105-12.

29 Go MJ, Takenaka C, Ohgushi H. Forced expression of Sox2 or Nanog in human bone marrow derived mesenchymal stem cells maintains their expansion and differentiation capabilities. Exp Cell Res 2008; 314(5): 1147-54.

30 Riggi N, Suvà M, De Vito C, Provero P, Stehle JC, Baumer K, *et al*. EWS-FLI-1 modulates miRNA145 and SOX2 expression to initiate mesenchymal stem cell reprogramming toward ewing sarcoma cancer stem cells. Gene Dev 2010; 24(9): 916-32.

31 Bhatia H, Sharma R, Dawes J, Khillan JS. Maintenance of feeder free anchorage independent cultures of ES and iPS cells by retinol/vitamin A. J Cell Biochem 2012; 113(9): 3002-10.

32 Arnold K, Sarkar A, Yram M A, Polo JM, Bronson R, Sengupta S, *et al*. $Sox2^{+}$ adult stem and progenitor cells are important for tissue regeneration and survival of mice. Cell Stem Cell 2011; 9(4): 317-29.

33 Greco SJ, Liu K, Rameshwar P. Functional similarities among genes regulated by OCT4 in human mesenchymal and embryonic stem cells. Stem Cells 2007; 25(12): 3143-54.

34 Tecirlioglu RT, Nguyen L, Koh K, Trounson AO, Michalska AE. Derivation and maintenance of human embryonic stem cell line on human adult skin fibroblast feeder cells in serum replacement medium. *In vitro* Cell Dev Biol Anim 2010; 46(3/4): 231-5.

35 Guzzo RM, Gibson J, Xu RH, Lee FY, Drissi H. Efficient differentiation of human iPSC-derived mesenchymal stem cells to chondroprogenitor cells. J Cell Biochem. 2013; 114(2): 480-90.

36 Sharow KA, Temkin B, Ann Asson-Batres M. Retinoic acid stability in stem cell cultures. Int J Dev Biol 2012; 56(4): 273-8.

37 Ghiaur G, Yegnasubramanian S, Perkins B, Gucwa JL, Gerber JM, Jones RJ. Regulation of human hematopoietic stem cell self-renewal by the microenvironment's control of retinoic acid signaling. Pro Natl Acad Sci USA 2013; 110(40): 16121-6.

38 Blum N, Begemann G. Retinoic acid signaling controls the formation, proliferation and survival of the blastema during adult zebrafish fin regeneration. Development 2012; 139(1): 107-16.

39 Park SW, Lim HY, Do HJ, Sung B, Huh SH, Song H, *et al*. Regulation of human growth and differentiation factor 3 gene expression by NANOG in human embryonic carcinoma NCCIT cells. FEBS Lett 2012; 586(19): 3529-35.

40 Chen L, Yang M, Dawes J, Khillan JS. Suppression of ES cell differentiation by retinol (vitamin A) via the overexpression of Nanog. Differentiation 2007; 75(8): 682-93.