肿瘤微环境对骨髓间充质干细胞形态、生长及增殖的影响

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摘要 因骨髓间充质干细胞(human mesenchymal stem cells-bone marrow, HMSC-bm)易于体外分离、培养、扩增及外源基因的导入, 肿瘤趋向性和低免疫源性等特点, 近年来已成为肿瘤生物治疗理想的载体。该实验采用Transwell小室建立HMSC-bm与肺腺癌细胞A549非接触共培养体系, 研究肿瘤微环境诱导3 d、7 d后分别传至第3代、第5代HMSC-bm相关生物学特性的改变。结果显示, 随着共培养时间的延长及传代代次的增加, 实验组HMSC-bm细胞形态逐渐发生显著变化; 细胞生长曲线与周期检测结果发现细胞增殖速度逐渐增快, 提示肺腺癌微环境诱导HMSC-bm分化, 导致其形态、生长及增殖等生物学特性改变, 具有向肿瘤转化的可能, 但尚需进一步研究, 为HMSC-bm的临床广泛应用提供科学依据。

关键词 人骨髓间充质干细胞; 肿瘤微环境; 形态学; 细胞周期

The Effect of Tumor Microenvironment to Morphology, Growth and Proliferation of Human Mesenchymal Stem Cells-bone Marrow

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Abstract Because of the advantages of human mesenchymal stem cells-bone marrow (HMSC-bm) such as easy to be isolated, cultured, expanded *in vitro* and imported exogenous gene, the tropism to tumor, the low-down immunogenicity etc., it becomes an ideal target therapeutic vector for tumor's biological treatment in recent years. In this study, a co-culture system of HMSC-bm and lung adenocarcinoma cell line A549 was established by using Transwell chamber. To study the related biological characteristics' changes of HMSC-bm, induced for 3 and 7 days, and then, passaged to the third generation and the fifth generation respectively in tumor microenvironment. The results showed that along with the extension of co-culture time and the increase of the passage, morphology of the experimental groups cells changed gradually. MTT assay and Cell cycle analysis indicated that the experimental

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groups cells growth vigor was reinforced gradually. The results suggested HMSC-bm was induced to differentiate in lung adenocarcinoma microenvironment, its biological characteristics such as morphology, growth and proliferation were changed. The HMSC-bm may be has tumorigenicity. But the mechanism of differentiation should be studied deeply to provide the scientific evidence for widely clinical application of HMSC-bm.

Key words human mesenchymal stem cells-bone marrow; tumor microenvironment; morphology; cell cycle

由于肿瘤复杂的致病因素以及居高的发病率、死亡率, 使得肿瘤临床治疗仍然是医学界面临的首要难题之一。现代肿瘤生物治疗是继传统手术、化学药物和放射治疗之后的第四种肿瘤治疗模式, 为肿瘤临床治疗提供了一种新的策略。因骨髓间充质干细胞(human mesenchymal stem cells-bone marrow, HMSC-bm)可塑性高、取材方便、易于体外分离、培养、扩增及外源基因的导入、极好的迁移能力、肿瘤趋向性和低免疫源性、不涉及伦理问题等特点[1-2], 近年来已成为肿瘤生物治疗理想的载体, 靶向治疗肿瘤的重要手段[3-4]。但近期研究表明, 干细胞生存的微环境组分及信号分子的改变可能导致正常的干细胞(包括HMSC-bm)向恶性肿瘤细胞转化, 并可促进多种肿瘤的发生、发展[5-7]; 还有研究提示HMSC-bm可能是肿瘤发生或复发的起源细胞[8], 因而HMSC-bm在临床应用中的安全性问题日渐突出。本实验通过HMSC-bm与肺腺癌细胞A549非接触共培养, 研究肿瘤微环境不同诱导时间对HMSC-bm形态、生长及增殖等生物学特性的影响, 进一步为评估临床安全应用HMSC-bm治疗提供基础实验数据。

1 材料与方法

1.1 材料

1.1.1 仪器及试剂 流式细胞仪(美国Becton Dickinson公司), 倒置相差显微镜(日本OLYMPUS公司), 酶联免疫检测仪(美国BIO-RAD公司), 细胞培养箱(日本三洋电机公司); Transwell小室(美国Corning公司), RPMI-1640培养基(美国Hyclone公司), DMEM/F-12(美国Hyclone公司), 胎牛血清(美国Hyclone公司), 胰蛋白酶(美国Gibco公司), MTT(美国Sigma公司), DMSO(美国Sigma公司), PI(美国Sigma公司)。

1.1.2 HMSC-bm的培养 实验HMSC-bm购自Cyagen Biosciences Inc.(Catalog number: HUXMA-01001; Registration number: 08795844465781)。HMSC-bm培养体系为含10%的胎牛血清、100 U/mL青霉素和100 U/mL链霉素的DMEM/F-12培养液, 置于5% CO_2、37 °C、饱和湿度的细胞培养箱中培养, 每3 d换液1次。待贴壁细胞达到80%~90%融合后, 用0.25%胰蛋白酶37 °C消化, 然后以1:3的比例传代。第5代细胞用于实验。

1.1.3 A549细胞的培养 实验A549细胞株购自中国科学院上海细胞研究所(Catalog number: TCHu150)。A549培养体系为含10%的胎牛血清、100 U/mL青霉素和100 U/mL链霉素的RPMI-1640培养液, 置于5% CO_2、37 °C、饱和湿度的细胞培养箱中培养, 每3 d换液1次。待贴壁细胞达到80%~90%融合后, 用0.25%胰蛋白酶37 °C消化, 然后以1:3的比例传代。

1.2 方法

1.2.1 共培养体系的建立及分组 采用具有PET膜的Transwell悬挂式培养小室结合6孔板进行非接触共培养, 取对数生长期的细胞用0.25%胰蛋白酶消化制成单细胞悬液, 计数、调整细胞密度后接种, 实验组将A549接种于Transwell共培养系统的下室, 再将Transwell的上室置于孔中, 在Transwell小室内接种HMSC-bm, 让上下室的培养液相互通融, 从而建立起HMSC-bm与A549的共培养体系; 对照组HMSC-bm以HMSC-bm单独接种于Transwell小室内, 下层为基础培养液; 对照组A549以A549单独接种于下层, Transwell小室内为基础培养液。分别培养了3 d、7 d后终止培养, 收集细胞移到培养瓶中继续培养, 再分别传代至第3代、第5代的细胞用于实验。实验分组及各组细胞种植密度见表1。

1.2.2 细胞形态学观察 倒置相差显微镜下观察各组细胞的形态变化, 并照相记录。

1.2.3 MTT比色试验绘制细胞生长曲线 收集对数生长期的各组细胞, 用0.25%胰蛋白酶消化制成单细胞悬液, 并调整浓度为1×10^4/mL, 接种于96孔培养板(每组细胞每个培养板接种6孔, 每孔200 μL, 共7块培养板), 在5% CO_2、37 °C、饱和湿度培养箱中

表1 实验分组及各组细胞种植密度

Table 1 Cell densities in experimental and control groups

分组 Group			Transwell小室(个/孔) Transwell chamber (cell number/chamber)	六孔板(个/孔) 6-well plate (cell number/chamber)
co-cultured for 3 days	the 3rd passage	control group HMSC-bm(HMSC-bm 3d-P3)	HMSC-bm 5×10^4	RPMI-1640
		experimental group HMSC-bm co-cultured with A549(CO-HMSC-bm 3d-P3)	HMSC-bm 5×10^4	A549 1×10^5
		control group A549(A549 3d-P3)	DMEM/F-12	A549 1×10^5
	the 5th passage	control group HMSC-bm(HMSC-bm 3d-P5)	HMSC-bm 5×10^4	RPMI-1640
		experimental group HMSC-bm co-cultured with A549(CO-HMSC-bm 3d-P5)	HMSC-bm 5×10^4	A549 1×10^5
		control group A549(A549 3d-P5)	DMEM/F-12	A549 1×10^5
co-cultured for 7 days	the 3rd passage	control group HMSC-bm(HMSC-bm 7d-P3)	HMSC-bm 2×10^4	RPMI-1640
		experimental group HMSC-bm co-cultured with A549(CO-HMSC-bm 7d-P3)	HMSC-bm 2×10^4	A549 4×10^4
		control group A549(A549 7d-P3)	DMEM/F-12	A549 4×10^4
	the 5th passage	control group HMSC-bm(HMSC-bm 7d-P5)	HMSC-bm 2×10^4	RPMI-1640
		experimental group HMSC-bm co-cultured with A549(CO-HMSC-bm 7d-P5)	HMSC-bm 2×10^4	A549 4×10^4
		control group A549(A549 7d-P5)	DMEM/F-12	A549 4×10^4

培养, 每隔48 h换液一次。分别于接种后第l, 2, 3, 4, 5, 6, 7 d进行MTT法检测。每天固定时间每孔加20 μL MTT(5 mg/mL), 温箱继续孵育4 h后弃去液体, 沉淀内加入150 μL DMSO, 摇床上震荡10 min, 用酶联免疫检测仪于490 nm波长处测定吸光度(*D*)值, 以时间为横坐标, 吸光度值为纵坐标, 绘制细胞生长曲线。

1.2.4 流式细胞术(FCM)检测细胞周期 收集对数生长期的各组细胞, 消化成单细胞悬液, 1 000 r/min、5 min离心后弃上清, PBS洗涤2次, 缓慢加入–20 ℃预冷的70%的乙醇4 ℃固定过夜, 然后1 000 r/min、5 min离心, 收集固定的细胞, PBS洗涤2次后离心弃上清, 加入500 μL的PBS重悬细胞, 再加入2.5 μL(10 μg/μL) RNase A混匀, 37 ℃反应30 min, 过300目细胞筛, 加入含1% Triton X-100的PI 50 μL(0.1 mg/mL)混匀, 于室温避光反应30 min, 上流式细胞仪检测, 分析细胞DNA含量, 计算G_1期及S期细胞比例。每组实验重复3次。

1.3 统计学分析

应用SPSS 17.0统计软件分析结果, 计量资料结果采用$\bar{x}\pm s$表示, 多组间比较采用单因素方差分析, 组间比较用配对样本的*t*检验, $P<0.05$为差异有统计学意义。

2 结果

2.1 形态学观察

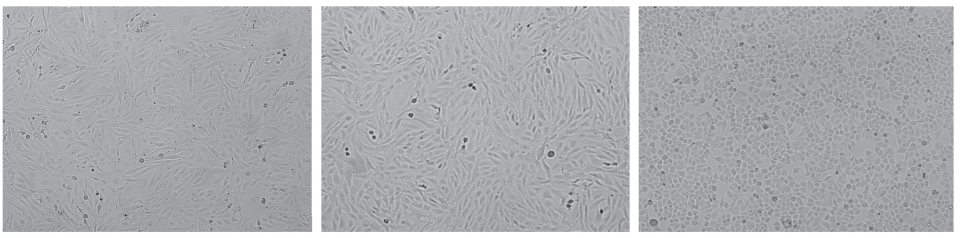
倒置相差显微镜下观察, 对照组HMSC-bm细胞边界清晰, 形态完全均一, 为成纤维细胞样, 扁平、呈长梭形, 分布均匀, 排列有序, 呈集落样贴壁生长, 折光性较强; CO-HMSC-bm 3d-P3未见明显改变; CO-HMSC-bm 3d-P5偶见细胞缩短变小成梭形或多角形; CO-HMSC-bm 7d-P3部分细胞缩短变小成短梭形或多角形; CO-HMSC-bm 7d-P5形态发生显著变化, 细胞呈不规则多角形, 排列紊乱, 成团生长,类似于A549细胞(图1)。

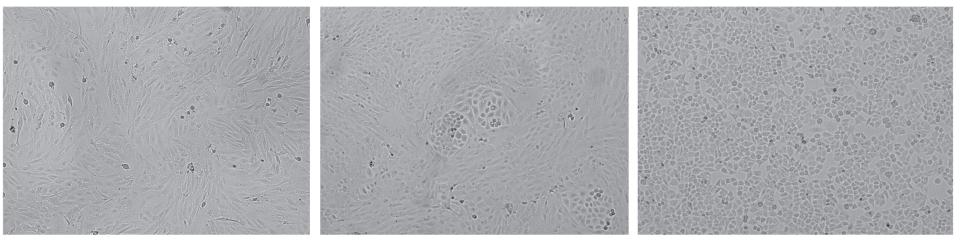
2.2 细胞生长曲线

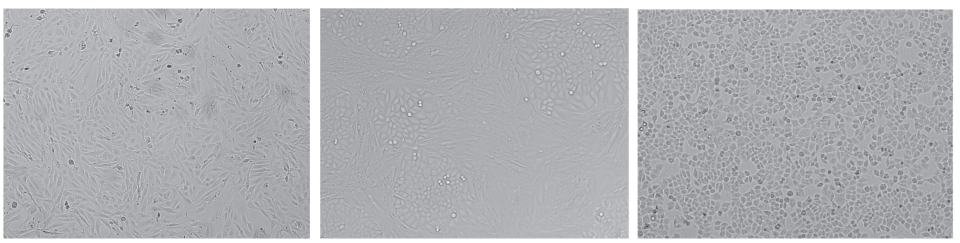
与单独培养的HMSC-bm比较, CO-HMSC-bm 3 d未见明显改变($P>0.05$), CO-HMSC-bm 7 d细胞增殖速度增快($P<0.05$, 表2、图2)。

2.3 FCM检测细胞周期的变化

通过流式细胞分析术检测各组细胞 G_1期、S期、G_2/M期细胞比例的变化, 从而反应细胞增殖力的改变。结果显示除CO-HMSC-bm 3d-P3外, CO-HMSC-bm 3d-P5、CO-HMSC-bm 7d-P3、CO-HMSC-bm 7d-P5处于G_1期细胞的比例逐渐减少, S期细胞比例逐渐增加($P<0.05$), 可见各实验组细胞随着共培养时


HMSC-bm 3d-P3 CO-HMSC-bm 3d-P3 A549 3d-P3


HMSC-bm 3d-P5 CO-HMSC-bm 3d-P5 A549 3d-P5


HMSC-bm 7d-P3 CO-HMSC-bm 7d-P3 A549 7d-P3

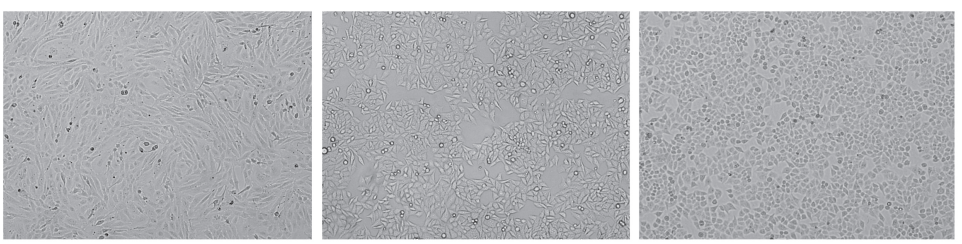

HMSC-bm 7d-P5 CO-HMSC-bm 7d-P5 A549 7d-P5

图1 各组细胞形态观察(100×)

Fig.1 Observation cell morphography of groups(100×)

表2 各组细胞吸光度(*D*)值的结果

Table 2 Absorbance(*D*) value of the groups cells

组别 Group	第3代(the 3rd passage)			第5代(the 5th passage)		
	第3天 the 3rd day	第5天 the 5th day	第7天 the 7th day	第3天 the 3rd day	第5天 the 5th day	第7天 the 7th day
HMSC-bm 3d	0.210±0.004	0.356±0.001	0.419±0.039	0.216±0.001	0.382±0.004	0.464±0.013
CO-HMSC-bm 3d	0.210±0.007	0.378±0.003	0.446±0.003	0.220±0.006	0.388±0.002	0.489±0.036
A549 3d	0.988±0.039	1.979±0.050	1.979±0.050	1.009±0.036	2.011±0.040	2.011±0.040
HMSC-bm 7d	0.203±0.007	0.344±0.006	0.460±0.026	0.207±0.007	0.346±0.009	0.464±0.023
CO-HMSC-bm 7d	0.262±0.007*▲	0.480±0.002*▲	0.640±0.007*▲	0.279±0.002●△	0.532±0.003●○△	0.699±0.013●○△
A549 7d	1.103±0.037	2.337±0.014	2.436±0.045	1.115±0.036	2.344±0.015	2.458±0.046

*P<0.05, CO-HMSC-bm 7d-P3与HMSC-bm 7d-P3比较; ●P<0.05, CO-HMSC-bm 7d-P5与HMSC-bm 7d-P5比较; ○P<0.05, CO-HMSC-bm 7d-P5与CO-HMSC-bm 7d-P3比较; ▲P<0.05, CO-HMSC-bm 7d-P3与CO-HMSC-bm 3d-P3比较; △P<0.05, CO-HMSC-bm 7d-P5与CO-HMSC-bm 3d-P5比较。n=6。

*P<0.05, CO-HMSC-bm 7d-P3 compare with HMSC-bm 7d-P3; ●P<0.05, CO-HMSC-bm 7d-P5 compare with HMSC-bm 7d-P5; ○P<0.05, CO-HMSC-bm 7d-P5 compare with CO-HMSC-bm 7d-P3; ▲P<0.05, CO-HMSC-bm 7d-P3 compare with CO-HMSC-bm 3d-P3; △P<0.05, CO-HMSC-bm 7d-P5 compare with CO-HMSC-bm 3d-P5. n=6.

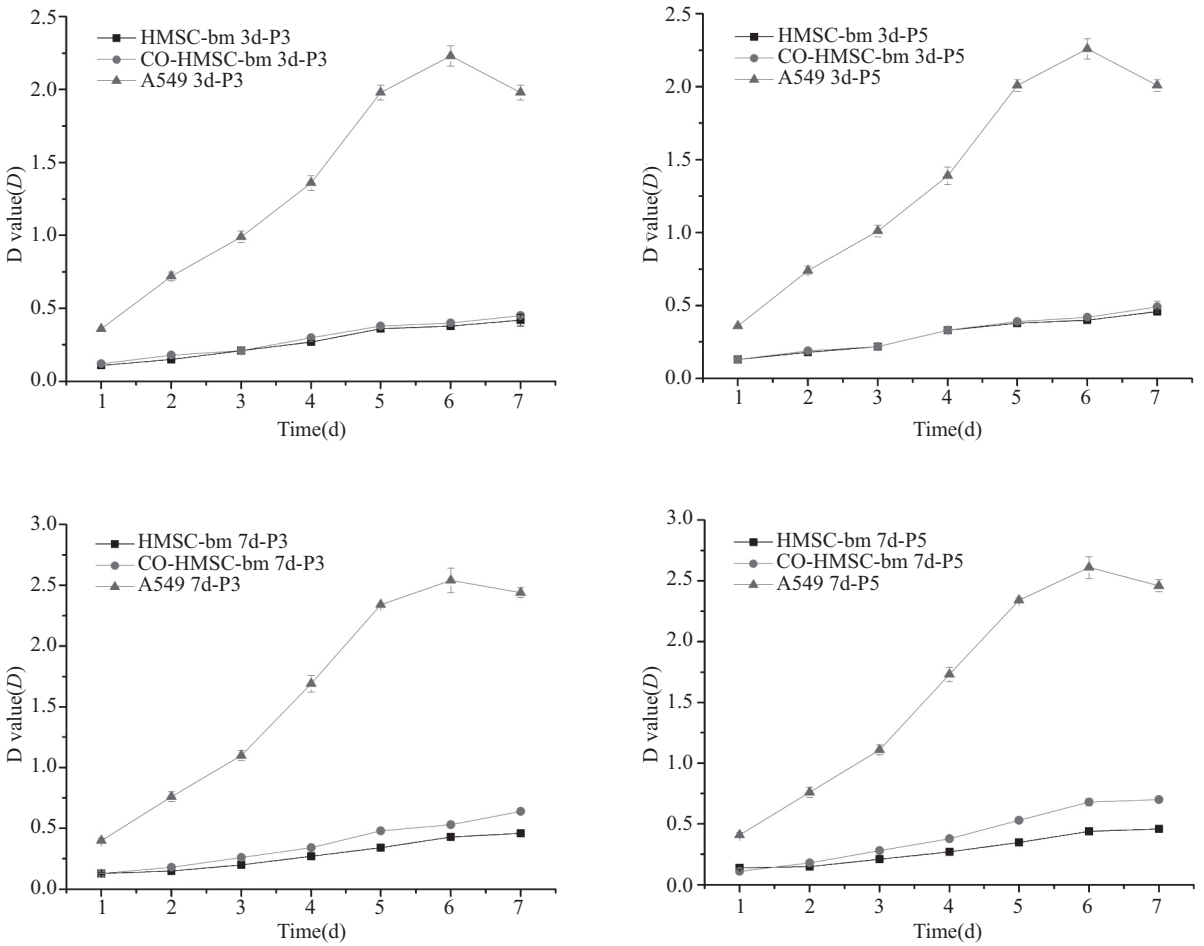


图2 各组细胞生长曲线的变化

Fig.2 Growth curves of the groups cells

表3 FCM分析细胞周期的结果

Table 3 Analysis of results in cell cycle by flow cytometry

组别 Group	第3代 the 3rd passage(P3)			第5代 the 5th passage(P5)		
	G_1	S	G_2/M	G_1	S	G_2/M
HMSC-bm 3d	(71.663±5.829)%	(12.230±3.774)%	(16.107±2.567)%	(71.750±1.138)%	(10.203±2.267)%	(18.047±3.278)%
CO-HMSC-bm 3d	(65.403±1.785)%	(17.000±7.524)%	(17.597±6.323)%	(62.243±0.924)%*●	(21.810±6.335)%*●	(15.913±5.606)%
A549 3d	(45.367±0.809)%	(35.500±1.852%	(19.133±2.570)%	(41.000±5.575)%	(21.800±7.238)%	(37.200±2.339)%
HMSC-bm 7d	(70.860±3.709)%	(10.790±1.991%	(18.350±3.591)%	(72.723±6.492)%	(11.840±2.789)%	(15.433±7.322)%
CO-HMSC-bm 7d	(59.333±0.351)%○■	(24.233±2.811)%○■	(16.400±2.406)%	(55.433±0.404)%▲△□	(27.833±1.955)%▲△	(16.767±2.363)%
A549 7d	(43.447±1.289)%	(32.990±9.020)%	(20.797±7.059)%	(42.043±3.938)%	(35.280±7.381)%	(19.670±0.154)%

*P<0.05, CO-HMSC-bm 3d-P5与HMSC-bm 3d-P5比较; ●P<0.05, CO-HMSC-bm 3d-P5与CO-HMSC-bm 3d-P3比较; ○P<0.05, CO-HMSC-bm 7d-P3与HMSC-bm 7d-P3比较; ▲P<0.05, CO-HMSC-bm 7d-P5与HMSC-bm 7d-P5比较; △P<0.05, CO-HMSC-bm 7d-P5与CO-HMSC-bm 7d-P3比较; ■P<0.05, CO-HMSC-bm 7d-P3与CO-HMSC-bm 3d-P3比较; □P>0.05, CO-HMSC-bm 7d-P5与CO-HMSC-bm 3d-P5比较。

*P<0.05, CO-HMSC-bm 3d-P5 compare with HMSC-bm 3d-P5; ●P<0.05, CO-HMSC-bm 3d-P5 compare with CO-HMSC-bm 3d-P3; ○P<0.05, CO-HMSC-bm 7d-P3 compare with HMSC-bm 7d-P3; ▲P<0.05, CO-HMSC-bm 7d-P5compare with HMSC-bm 7d-P5; △P<0.05, CO-HMSC-bm 7d-P5 compare with CO-HMSC-bm 7d-P3; ■P<0.05, CO-HMSC-bm 7d-P3 compare with CO-HMSC-bm 3d-P3; □P>0.05, CO-HMSC-bm 7d-P5compare with CO-HMSC-bm 3d-P5.

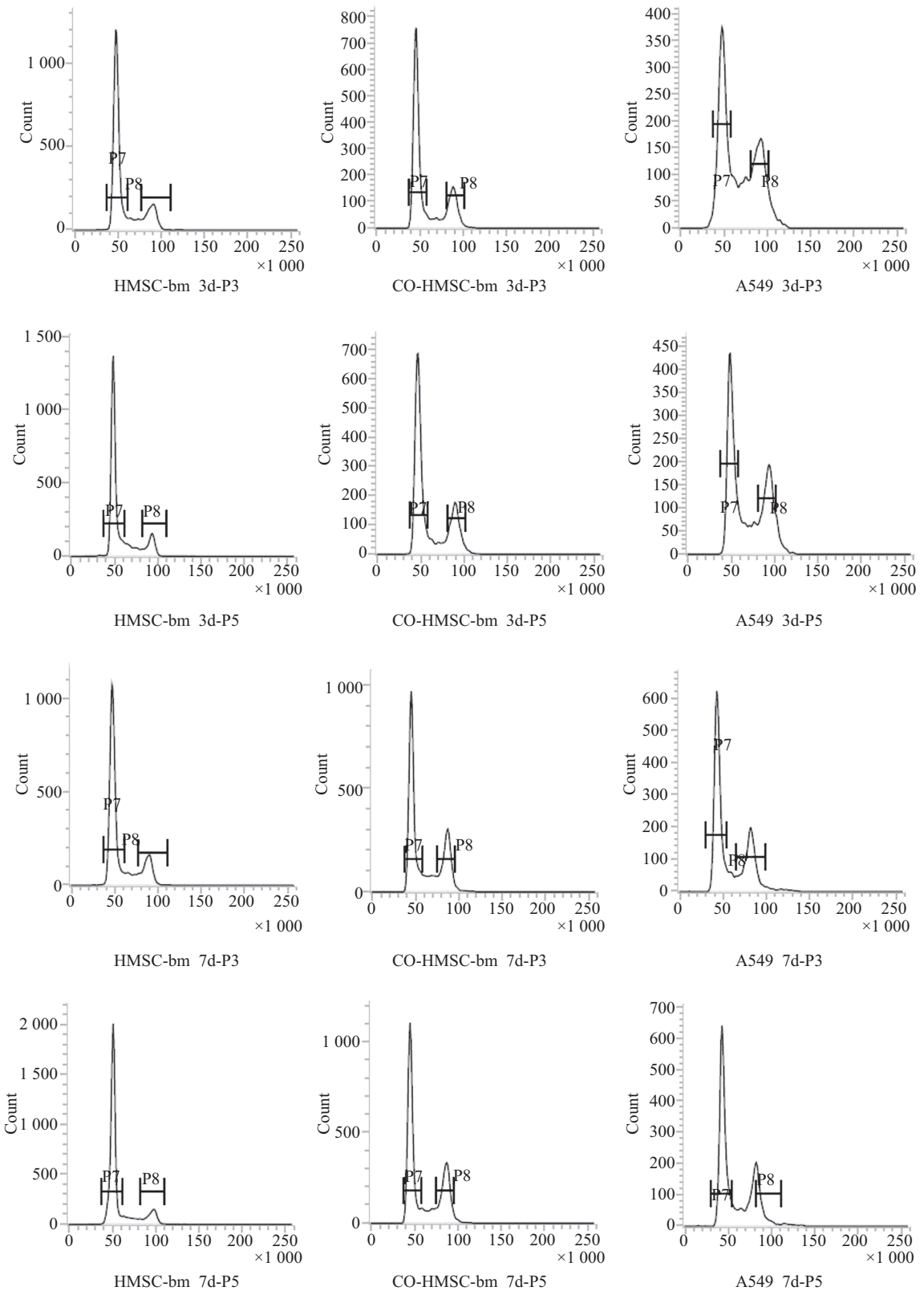


图3 流式细胞仪检测细胞周期的结果

Fig.3 Analysis of results in cell cycle by flow cytometry

间的延长及传代代次的增加, 其增殖能力逐渐增强(表3、图3)。

3 讨论

HMSC-bm是中胚层发育的早期细胞, 具有较强的自我增殖和多向分化潜能。目前研究表明[9-12], 在特定的诱导条件下, HMSC-bm不仅可以定向诱导分化为中胚层细胞, 而且还可跨胚层分化为内胚层及外胚层来源的所有细胞, 所以被认为是组织工程领域的理想种子细胞来源, 已成为骨科、肿瘤治疗、心血管疾病、血液系统、神经系统、再生医学等领域临床细胞学治疗的最佳干细胞之一[13-14]。在肿瘤治疗方面, 研究发现, HMSC-bm不仅可直接抑制某些恶性肿瘤的生长, 激活肿瘤抗原特异性免疫应答, 而且还可导入外源基因, 作为抗癌药物载体, 使治疗性细胞因子及相关药物在肿瘤组织浓度提高从而达到抑瘤的作用[3,15]。

但最近研究表明, 一方面HMSC-bm具有肿瘤趋向性, 并可分化为肿瘤间质成分, 参与并促进肿瘤的生长、增殖、迁移与侵袭, 同时, 抑制机体免疫功能帮助肿瘤免疫逃逸等[16-18]; 另一方面HMSC-bm本身也可发生恶性转化, 主要是干细胞巢, 即干细胞生存的微环境, 巢成分及相关信号分子可能促进正常干细胞向肿瘤转化。已有研究证实HMSC-bm在肿瘤等环境中出现形态、染色体、端粒酶、蛋白、基因等生物学特征的改变, 甚至自发恶性转化[5-8], 这提示HMSC-bm安全性问题已成为广泛应用于临床前必需首先解决的问题。

本实验利用Transwell小室建立非接触共培养体系, 小室底层为通透性的PET膜(孔径为0.4 μm), 这种膜允许生物大分子自由通过膜的微孔, A549分泌的细胞因子和信号分子可以通过这层膜作用于HMSC-bm细胞, 而细胞不可以通过膜的微孔。以此系统来模拟肺腺癌微环境, 探讨肺腺癌微环境对HMSC-bm生物学特性的影响。实验中通过HMSC-bm与A549细胞共培养, 发现随着诱导时间的延长及传代代次的增加, 实验组细胞形态逐渐发生显著变化; 细胞生长曲线与周期检测结果发现细胞增殖速度逐渐增快。上述结果提示肿瘤微环境诱导HMSC-bm分化, 导致其形态、生长及增殖等生物学特性改变。这与Chen等[19]报道的结果相类似。

干细胞的数量、分裂、自我复制和分化受细胞内在因素和周围微环境的外在信号的共同调节, 这些周围微环境被称为干细胞小生境。发育生物学中有观点认为不同信号微环境可以引导干细胞选择不同的命运。干细胞与周围细胞密切接触, 后者作为控制干细胞行为重要信号的来源, 调控着干细胞自我复制与分化。微环境组分及信号通路的改变, 则可能细胞二次突变, 导致肿瘤发生。同时, 干细胞与肿瘤细胞通过分泌多种细胞因子、信号分子等相互作用, 发生级联反应, 最终影响干细胞形态、结构、生长、分化、代谢等生物学特性的改变, 进而诱导其向肿瘤转化。但本实验对于HMSC-bm是否被诱导分化为类肿瘤细胞或是其他终末细胞及其具体分化机制, 还需进一步从基因、蛋白水平及体内成瘤实验等方面进行验证, 为HMSC-bm的临床广泛应用提供科学依据。

参考文献 (References)

1 Xu F, Shi J, Yu B, Ni W, Wu X, Gu Z. Chemokines mediate mesenchymal stem cell migration toward gliomas *in vitro*. Oncol Rep 2010; 23(6): 1561-7.

2 Yang SH, Park MJ, Yoon IH, Kim SY, Hong SH, Shin JY, *et al*. Soluble mediators from mesenchymal stem cells suppress T cell proliferation by inducing IL-10. Exp Mol Med 2009; 41(5): 315-24.

3 Duan X, Guan H, Cao Y, Kleinerman ES. Murine bone marrow-derived mesenchymal stem cells as vehicles for interleukin-12 gene delivery into Ewing sarcoma tumors. Cancer 2009; 115(1): 13-22.

4 Komarova S, Roth J, Alvarez R, Curiel DT, Pereboeva L. Targeting of mesenchymal stem cells to ovarian tumors via an artificial receptor. J Ovarian Res 2010; 3: 12.

5 Kolf CM, Cho E, Tuan RS. Mesenchymal stromal cells. Biology of adult mesenchymal stem cells: regulation of niche, self-renewal and differentiation. Arthritis Res Ther 2007; 9(1): 204.

6 Li Q, Hisha H, Takaki T, Adachi Y, Li M, Song C, *et al*. Transformation potential of bone marrow stromal cells into undifferentiated high-grade pleomorphic sarcoma. J Cancer Res Clin Oncol 2010; 136(6): 829-38.

7 刘永琦, 达　瑞, 窦娟娟, 苏菊萍, 颜春鲁. 氯化镉对小鼠骨髓间充质干细胞微核率和染色体畸变率的影响. 中国组织工程研究(Liu Yongqi, Da Rui, Dou Juanjuan, Su Juping, Yan Chunlu. Effects of cadmium chloride on micronucleus rate and chromosome aberration rate in mouse bone marrow mesenchymal stem cells. Chinese Journal of Tissue Engineering Research) 2012; 16(41): 7612-6.

8 Rosland GV, Svendsen A, Torsvik A, Sobala E, McCormack E, Immervoll H, *et al*. Long-term cultures of bone marrow-derived human mesenchymal stem cells frequently undergo spontaneous malignant transformation. Cancer Res 2009; 69(13): 5331-9.

9 Arminán A, Gandía C, García-Verdugo JM, Lledó E, Mullor JL, Montero JA, *et al*. Cardiac transcription factors driven lineage-

specification of adult stem cells. J Cardiovasc Transl Res 2010; 3(1): 61-5.
10 Dong XJ, Zhang H, Pan RL, Xiang LX, Shao JZ. Identification of cytokines involved in hepatic differentiation of mBM-MSCs under liver-injury conditions. World J Gastroenterol 2010; 16(26): 3267-78.
11 Tian H, Bharadwaj S, Liu Y, Ma H, Ma PX, Atala A, *et al*. Myogenic differentiation of human bone marrow mesenchymal stem cells on a 3D nano fibrous scaffold for bladder tissue engineering. Biomaterials 2010; 31(5): 870-7.
12 Jiang TS, Cai L, Ji WY, Hui YN, Wang YS, Hu D, *et al*. Reconstruction of the corneal epithelium with induced marrow mesenchymal stem cells in rats. Mol Vis 2010; 16: 1304-16.
13 Yoshioka T, Mishima H, Kaul Z, Ohyabu Y, Sakai S, Ochiai N, *et al*. Fate of bone marrow mesenchymal stem cells following the allogeneic transplantation of cartilaginous aggregates into osteochondral defects of rabbits. J Tissue Eng Regen Med 2011; 5(6): 437-43.
14 Lee MJ, Jung J, Na KH, Moon JS, Lee HJ, Kim JH, *et al*. Antifibrotic effect of chorionic plate-derived mesenchymal stem cells isolated from human placenta in a rat model of CCl(4)-injured liver: Potential application to the treatment of hepatic diseases. J Cell Biochem 2010; 111(6): 1453-63.
15 Ren C, Kumar S, Chanda D, Kallman L, Chen J, Mountz JD, *et al*. Cancer gene therapy using megenchymal stem cells expressing interferon-beta in a mouse prostate cancer lung metastasis model. Gene Ther 2008; 15(21): 1446-53.
16 Heo SC, Lee KO, Shin SH, Kwon YW, Kim YM, Lee CH, *et al*. Periostin mediates human adipose tissue-derived mesenchymal stem cell-stimulated tumor growth in a xenograft lung adenocarcinoma model. Biochim Biophys Acta 2011; 1813(12): 2061-70.
17 Do EK, Kim YM, Heo SC, Kwon YW, Shin SH, Suh DS, *et al*. Lysophosphatidic acid-induced ADAM12 expression mediates human adipose tissue-derived mesenchymal stem cell-stimulated tumor growth. Int J Biochem Cell Biol 2012; 44(11): 2069-76.
18 Nishimura K, Semba S, Aoyaqi K, Sasaki H, Yokozaki H. Mesenchymal stem cells provide an advantageous tumor microenvironment for the restoration of cancer stem cells. Pathobiology 2012; 79(6): 290-306.
19 Chen Y, Cong L, Yin X, Dong B, Han Y, Tu G. The culture of temporary tumor-like bone marrow mesenchymal stem cells (TT-BMSC) and the detection of cell biology property. Ann Transplant 2011; 16(3): 49-58.

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胞宫颈癌Hela细胞和肺癌A549细胞时发现其组织特异性不是很显著, 这方面有待进一步的研究和探讨。

参考文献 (References)

1 Schmidhauser C, Casperson GF, Myers CA, Sanzo KT, Bolten S, Bissell MJ. A novel transcriptional enhancer is involved in the prolactin and extracellular matrix-dependent regulation of β-casein gene expression. Mol Biol Cell 1992; 3(6): 699-709.
2 王付龙, 徐 祥, 刘 昕. 转录因子圈套策略研究进展. 生物化学与生物物理进展(Wang Fulong, Xu Xiang, Liu Xin. Research development of transcription factor decoy strategy. Progress in Biochemistry and Biophysics) 2001; 28(6): 802-4.
3 Jiminez-Flores R, Richardson T. Genetic engineering of the caseins to modify the behaviour of milk during processing: A review. J Dairy Sci 1988; 71: 2640-54.
4 Morris DR, Geballe AP. Upstream open reading frames as regulators of mRNA translation. Mol Cell Biol 2000; 20(23): 8635-42.
5 Ou YC, Gardner TA, Kao C, Zhau HE, Chung LW. A potential of tissue restrictive gene therapy in renal cell carcinoma using MN/CA IX promoter. Anticnacer Res 2005; 25(2A): 881-6.
6 卢圣栋. 现代分子生物学实验技术, 第2版. 北京: 中国协和医科大学出版社(Lu Shengdong. Current Protocols for Molecular Biology, 2nd ed. Beijing: Peking Union Medical College Press), 1999, 539-40.
7 Barash I, Nathan M, Kari R, Ilan N, Shani M, Hurwitz DR. Elements within the β-lactoglobulin gene inhibit expression of human serum albumin cDNA and minigenes in transfected cells but rescue their expression in the mammary gland of transgenic mice. Nucleic Acids Res 1996; 24(4): 602-10.