[1]徐梦遥,王延洲,徐惠成.大鼠骨髓间充质干细胞向膀胱平滑肌细胞分化的标志基因表达受组蛋白乙酰化调控[J].第三军医大学学报,2013,35(21):2295-2300.

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Title: Histone acetylation regulates expression of marker genes in differentiation of rat BMSCs to BSMCs

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关键词: 骨髓间充质干细胞; 膀胱平滑肌细胞; 组蛋白乙酰化; 启动子

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摘要: 目的 通过骨髓间充质干细胞(bone marrow mesenchymal stem cells, BMSCs)与膀胱平滑肌细胞(bladder smooth muscle cells, BSMCs)接触共培养探讨组蛋白乙酰化对BMSCs分化能力的影响。 方法 分别采用密度梯度离心法和胶原酶消化法培养大鼠BMSCs及BSMCs至第3代，并对BMSCs进行了鉴定；以单独培养的BMSCs作为对照组，接触共培养的BMSCs为实验组；运用免疫荧光化学染色法分别对2组BMSCs中的平滑肌标志性蛋白平滑肌α肌动蛋白(α-smooth muscle actin, α-SMA)、平滑肌肌球蛋白重链(smooth muscle-myosin heavy chain, SM-MHC)、Calponin进行检测，Western blot检测α-SMA蛋白、Calponin蛋白在BMSCs中的表达,并用微球菌核酸酶(micrococcal nuclease, MNase)对平滑肌3个标志性基因的启动子区进行了染色质开放性的检测，同时运用了ChIP技术分析了α-SMA、SM-MHC启动子区组蛋白乙酰化水平对BMSCs向平滑肌分化的影响。 结果 大鼠原代BMSCs流式检测显示CD29表达阳性,阳性率为99.8%,CD31、CD45表达阴性，阳性率为6.30%、1.04%；免疫荧光显示平滑肌3种标志性蛋白的表达均随时间的延长而增加；Western blot结果显示随着共培养天数的增加α-SMA蛋白、Calponin蛋白在实验组中的表达相比对照组有明显的增加；MNase显示实验组的BMSCs启动子区染色质对核酸酶的敏感性明显高于对照组，ChIP显示实验组中

BMSCs平滑肌标志性基因α-SMA、SM-MHC、Calponin启动子区H4乙酰化水平较未分化前明显增加(*P*<0.05)。　　结论　　组蛋白乙酰化程度的增加促进了BMSCs向BSMCs分化。

Abstract: Objective To investigate the effect of histone acetylation on the differentiation capacity of bone marrow mesenchymal stem cells (BMSCs) directly co-cultured with bladder smooth muscle cells (BSMCs). Methods Density gradient centrifugation and collagenase digestion were used to train the rat BMSCs and BSMCs to the 3rd passage. After the cells were identified, BMSCs co-cultured with BSMCs were regarded as experimental cells, while the cells cultured alone as the control cells. Immunofluorescence assay was used to detect the expression of BMSCs landmark proteins, α-smooth muscle actin (α-SMA), smooth muscle myosin heavy chain (SM-MHC), and calponin in the 2 kinds of BMSCs. Western blotting was adopted to detect the protein levels of α-SMA and calponin. Micrococcal nuclease (MNase) was used to detect the promoter regions of the 3 marker genes for open chromatin, while ChIP technical analysis was used to detect the histone acetylation levels in the promoter regions of α-SMA and SM-MHC, and the effects of the levels on BMSCs to smooth muscle differentiation were determined. Results Our obtained primary rat BMSCs were positive to CD29 (99.8%), negative to CD31 and CD45 (positive rate of 6.30% and 1.04%). Immunofluorescence assay showed the expression of the 3 BMSCs landmark proteins was increased with time elapsing. Western blotting showed that the protein levels of α-SMA and calponin were significantly higher in experimental cells than in control cells with the elapse of co-culture time. MNase assay displayed that experimental BMSCs had significantly higher chromatin nuclease sensitivity in promoter regions than the control cells. ChIP showed the experimental BMSCs had significantly higher H4 acetylation level in the promoter regiong of α-SMA, SM-MHC and calponin when compared with the undifferentiated cells (*P*<0.05). Conclusion The enhanced histone acetylation promotes BMSCs differentiation to BSMCs.

参考文献/REFERENCES:

徐梦遥, 王延洲, 徐惠成. 大鼠骨髓间充质干细胞向膀胱平滑肌细胞分化的标志基因表达受组蛋白乙酰化调控[J].第三军医大学学报,2013,35(21):2295-2300.

相似文献/REFERENCES:

[1]张蕾,陈沅,田杰,等.心肌细胞介导骨髓间充质干细胞的心肌样分化[J].第三军医大学学报,2005,27(16):1681.

[2]王颖楠,范雪梅,赵敏,等.脱细胞膀胱基质复合大鼠骨髓间充质干细胞体外构建组织工程化吊带治疗压力性尿失禁的初步研究[J].第三军医大学学报,2012,34(22):2269.

Wang Yingnan,Fan Xuemei,Zhao Min,et al.Preliminary study on the construction of a tissue-engineered sling with BMSCs and UBM in vitro for treating stress urinary incontinence[J].J Third Mil Med Univ,2012,34(21):2269.

[3]周长立,任先军,蒋涛,等.Wnt7a基因对大鼠骨髓间充质干细胞增殖及向神经元样细胞分化的影响[J].第三军医大学学报,2013,35(08):702.

Zhou Changli,Ren Xianjun,Jiang Tao,et al.Wnt7a gene stimulates mesenchymal stem cell proliferation and differentiation into neuron-like cells[J].J Third Mil Med Univ,2013,35(21):702.

[4]郝磊,邹仲敏,王军平,等.hPDGF-A/hBD2双基因共表达腺病毒载体的构建及表达[J].第三军医大学学报,2007,29(10):859.

HAO Lei,ZOU Zhong-min,WANG Jun-ping,et al.Construction and identification of recombinant adenovirus expressing hPDGF-A and hBD2[J].J Third Mil Med Univ,2007,29(21):859.

[5]郭书权,吴雪晖,许建中,等.两种方法分离小型猪骨髓间充质干细胞的比较[J].第三军医大学学报,2007,29(10):988.

[6]黄文秋,黄宏,徐祥,等.mTOR及其下游信号通路在骨髓间充质干细胞氧化应激损伤中的变化及作用[J].第三军医大学学报,2013,35(02):114.

Huang Wenqiu,Huang Hong,Xu Xiang,et al.Changes and roles of mTOR and its downstream signaling passway in mouse

bone marrow stem cells with oxidative stress injury[J].J Third Mil Med Univ,2013,35(21):114.

[7]冯一梅,徐辉,邹仲敏,等.hPDGF-A/hBD2双基因转染对大鼠骨髓间充质干细胞生物学特性的影响[J].第三军医大学学报,2008,30(06):472.

FENG Yi-mei,XU Hui,ZOU Zhong-min,et al.Effects of hPDGF-A/hBD2 genes transfection on rat bone marrow mesenchymal stem cells[J].J Third Mil Med Univ,2008,30(21):472.

[8]姚青,宋治远,马显光.脉冲微交流电刺激促进体外诱导大鼠骨髓间充质干细胞向心肌分化[J].第三军医大学学报,2008,30(05):410.

YAO Qing,SONG Zhi-Yuan,MA Xian-guang.Electrical stimulation promotes the differentiation of rat bone marrow mesenchymal stem cells to cardiomyocyte induced by 5-azacytidine in vitro[J].J Third Mil Med Univ,2008,30(21):410.

[9]朱淑霞,李永华,宋治远,等.电磁场促进骨髓间充质干细胞体外诱导分化时细胞增殖[J].第三军医大学学报,2008,30(05):421.

ZHU Shu-xia,LI Yong-hua,SONG Zhi-yuan,et al.Pulsed electromagnetic fields improve proliferation of rat marrow mesenchymal stem cells induced by 5-azacytidine[J].J Third Mil Med Univ,2008,30(21):421.

[10]金旭红,杨柳,段小军,等.体外纳米磁标记骨髓间充质干细胞生物学特性及其MR成像[J].第三军医大学学报,2008,30(04):275.

JIN Xu-hong,YANG Liu,DUAN Xiao-jun,et al.Biological characteristics and in vitro MRI of superparamagnetic ironoxide labeled bonederived mesenchymal stem cells from rabbits[J].J Third Mil Med Univ,2008,30(21):275.

[11]杨婧,徐惠成,王延洲,等.丁酸钠对大鼠骨髓间充质干细胞向平滑肌细胞分化的影响[J].第三军医大学学报,2011,33(10):1016.

Yang Jing,Xu Huicheng,Wang Yangzhou,et al.Effects of sodium butyrate on differentiation of rat bone marrow mesenchymal stem cells into smooth muscle cells in vitro[J].J Third Mil Med Univ,2011,33(21):1016.