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单细胞克隆肝癌干细胞向间充质样细胞的分化潜能 点此下载全文

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摘要：

探讨单细胞克隆肝癌干细胞（liver cancer stem cell，LCSCs）向间充质样细胞分化的潜能。方法：通过有限稀释法获得单个细胞来源的LCSC克隆，采用RT-PCR法鉴定干细胞标志物;将该单细胞克隆分别用成骨、软骨和脂肪诱导分化培养基培养3周后，采用Real-time PCR及特殊染色技术比较诱导前后LCSC表达成骨、软骨及脂肪细胞特异标志物的差异。结果：单细胞克隆的LCSC表达多种干细胞标志物、干细胞因子（stem cell factor，SCF）、干细胞因子受体、C-kit、巢蛋白（nestin）、CD34、三磷酸腺苷结合转运蛋白G超家族成员2（ATP-binding cassette sub-family G member 2，ABCG2）、CD133。分化诱导培养3周后，成骨方向诱导的细胞茜素红染色呈现橘红色钙结节形成，软骨方向诱导的细胞阿尔新蓝染色显示蓝色蛋白多糖沉积，脂肪方向诱导的细胞油红O染色显示大量脂滴形成。Real-time PCR结果显示，诱导后成骨细胞特异标志物骨钙素和Ⅰ型胶原、软骨细胞特异标志物蛋白聚糖和Ⅱ型胶原、脂肪细胞特异标志物脂联素和过氧化物酶体增殖物激活受体γ的mRNA表达均较对照组显著上调（Ⅰ型及Ⅱ型胶原间为P<0.05，其他指标间均为P<0.01）。结论：LCSC具有可塑性，在特定的微环境下具有向间充质样细胞分化的潜能。

关键词：肝癌干细胞 单细胞克隆 间充质样细胞 多向分化潜能 可塑性

Mesenchymal differentiation potential of single-cell cloned liver cancer stem cells Download Fulltext

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Abstract:

To investigate the multilineage differentiation potential of single-cell-cloned liver cancer stem cells (LCSCs). Methods: Single cell cloned LCSCs were generated by limiting dilution cloning and confirmed by RT-PCR analysis of stem cell markers. Phenotype-confirmed LCSCs were induced to differentiate into osteoblasts, chondrocytes and adipocytes, respectively, by culturing them in corresponding differentiation-inducing media for 3 weeks. Both before and after the differentiation induction culture, the expression of markers specific for osteoblasts, chondrocytes and adipocytes was comparatively analyzed by real-time PCR and cell type-specific staining techniques.Results: Stem cell markers including CD133, CD34, ATP-binding cassette sub-family G member 2 (ABCG2), nestin, stem cell factor (SCF) and stem cell growth factor receptor (C-kit) were detected in undifferentiated single cell cloned LCSCs. After three weeks of differentiation induction, cells cultured in the osteoblast-specific medium formed orange calcium nodules as demonstrated by Alizarin red staining; cells cultured in the chondrocyte-specific medium showed blue proteoglycan depositions as revealed by Alcian blue staining; and cells cultured in the adipocyte-specific medium showed Oil Red O stained lipid droplets. In the three corresponding differentiation induction cultures, LCSCs acquired the expression of markers for osteoblast (osteocalcin and collagen), chondrocyte (aggrecan and collagen type Ⅱ), and adipocyte (adiponectin and peroxisome proliferator-activated receptor) respectively. All the differences were significant (P<0.01) except for collagen type Ⅰ and collagen type Ⅱ (P<0.05). Conclusion: These preliminary observations suggest that single cell cloned LCSCs possess a plasticity and may potentially differentiate into mesenchymal-like cells under specific microenvironments.

Keywords:liver cancer stem cell (LCSC) single-cell clone mesenchymal-like cell multilineage differentiation potential plasticity

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