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论著

诱导人卵黄囊间质干细胞向成骨细胞及神经细胞定向分化

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摘要 目的：分离纯化及体外定向诱导人卵黄囊间质干细胞 (hYS-MSC)分化为成骨细胞及神经细胞。方法：卵黄囊细胞经贴壁培养、传代纯化得到hYS-MSC，检测其表面抗原表达、对其进行核型分析、细胞周期检测并测定AKP活性；采用地塞米松、β-甘油磷酸钠、维生素C作成骨诱导剂、β-巯基乙醇或复方丹参注射液作为神经诱导剂诱导hYS-MSC向成骨细胞及神经细胞定向分化。组织化学方法作成骨检测；免疫组化方法检测NSE、NF及GFAP在经神经诱导hYS-MSC中的表达。结果： hYS-MSC易于纯化，在培养过程中保持正常核型，具有较大增殖能力。hYS-MSC CD29、CD44、CD166及CD105表达阳性, CD34、CD45和CD86为阴性；AKP弱阳性。hYS-MSC经成骨诱导AKP强阳性，诱导两周后形成钙盐沉积形成的矿化区。hYS-MSC经神经诱导可见NSE、NF或GFAP阳性细胞，符合神经元及胶质细胞的生物学特征。结论： hYS-MSC在体外培养过程中具有较大增殖能力并保持正常核型，与成体MSC表型一致，在体外可以诱导分化为成骨细胞、神经元及胶质细胞。

关键词 干细胞； 胎儿发育； 分化； 成骨细胞； 神经元

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Osteogenic and neurogenic differentiation of human yolk sac mesenchymal stem cells

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Abstract

<FONT face=Verdana>AIM: To purify human yolk sac mesenchymal stem cells (hYS-MSC) and investigate its osteogenic and neurogenic differentiation potentials. METHODS: hYS-MSC were separated from yolk sac and purified via passage culture. The karyotype of hYS-MSCs was analyzed via G-banded characteristics. Flow cytometric analysis was used to determine the cell cycle and phenotype of hYS-MSC. The AKP expression of hYS-MSC was also tested. Osteogenic differentiation of hYS-MSCs was induced by 10-8mol/L dexamethasone, 10 mmol/L β-glycerophosphate and 50 mg/L vitamin C. Alizarin red S stain was used for identification of mineralization. β-mecaptoethanol or salviae miltiorrhizae were used to induce neurogenic differentiation of hYS-MSCs. The expressions of NSE, NF and GFAP were identified by immunohistochemical method. RESULTS: hYS-MSCs could be purified at passages 2 or 3. The cell cycle analysis suggested that hYS-MSCs showed strong proliferational potentials by which the cells kept normal diploid karyotype during the in vitro culture. Flow cytometry showed the phenotype of purified hYS-MSCs was uniformly positive for CD29, CD44, CD105, and CD166, and negative for reactivity to antigens CD34, CD45, or CD86. hYS-MSCs were weakly but clearly positive in AKP. Osteogenic differentiation was appeared after induction of osteogenic differentiation. hYS-MSCs, which were of spindle shape, uniform in size, were induced to pleomorphism osteoblast-like cells which expressed high level of AKP. Aggregates or nodules were formed at day 7 and calcium accumulation was detected by alizarin red S staining on day 10 or day 14. Neurogenic differentiation

of hYS-MSCs was induced by β-mecaptoethanol or salviae miltiorrhizae. NSE, NF or GFAP positive cells were detected by immunohistochemical staining. CONCLUSIONS: hYS-MSCs have strong proliferation potential and the normal diploid karyotype is kept during the in vitro culture. The phenotype of hYS-MSCs is coincident with adult hMSCs. hYS-MSCs could be induced to differentiate into osteogenic or neurogenic cells.</FONT>

Key words Stem cells Fetal development Differentiation； Osteoblasts Neurons

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