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论著

用CD133免疫磁珠分离脐血内皮祖细胞的实验研究

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摘要 目的：从脐血中分离、培养血管内皮祖细胞,研究内皮祖细胞的生长特性和诱导分化条件。 方法： 应用MACS磁球抗体标记法纯化脐血中的CD133+细胞，通过流式细胞仪、免疫细胞化学、免疫荧光等技术及形态学（光镜、电镜）观察研究内皮祖细胞；将细胞接种于添加（或未添加）VEGF、bFGF、干细胞因子（SCF）的含20%胎牛血清（FBS）的IMDM培养基中,观察内皮祖细胞的生长特性。 结果： 分离新鲜脐血所得CD133阳性细胞占单个核细胞的(1.41±1.14)%,经流式细胞仪鉴定CD133+细胞纯度为75%-85%；将分离细胞接种于纤维连接蛋白包被的24孔板内，培养1-2 h即有细胞贴壁，7-10 d可见贴壁细胞呈铺路石样排列；14 d后细胞出现小圆形、梭形等多样性变化，可见毛细血管管腔样结构，电镜观察可见胞浆内典型的Weibel-Palade小体；在VEGF、bFGF、SCF存在条件下,检测贴壁细胞培养14 d后细胞表面抗原表达情况：与培养开始时相比，祖细胞标志CD133和CD34阳性率呈明显下降趋势，分别由(77.0±3.3)%和(93.1±4.7)%降至(1.6±2.2)%和(37.4±4.9)%，P<0.05，内皮细胞特异性标志Flk-1表达明显增加，由(22.3±3.3)%增至(94.3±4.1)%，P<0.05，同时vWF抗原呈强阳性表达，阳性率为(77.9±3.3)%。 结论： 根据细胞表面特异性分子标志（CD133+/CD34+/Flk-1+）可以从脐血中分离出EPCs，EPCs可在体外一定的诱导因子作用下，培养7-10 d分化为成熟内皮细胞。

关键词 胎血； 内皮祖细胞； 细胞分离； 纯化； 分化

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Isolation of endothelial progenitor cells from cord blood with CD133 immunomagnetic sorting

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Abstract

<FONT face=Verdana>AIM: To isolate, purify and differentiate endothelial progenitor cells from cord blood in vitro and to study their biological characteristics. METHODS: CD133+ cells were selected from fresh cord blood mononuclear cells (MNC) by magnetic activated cell-sorting system (MACS). EPC was studied by flow cytometry, immunocytochemistry and immunofluorescence staining. Isolated cells were cultured in IMDM medium supplemented with or without VEGF, bFGF, SCF. RESULTS: The percentage of CD133+ cells of cord blood MNC was (1.41±1.14)%, and purity was 75%-85% (FACS method). CD133+ cells were grown on fibronectin-coated chamber slides in the presence of VEGF, bFGF, SCF. Within 1-2 hours of culture cells became adherent. On day 7-10, the adherent cells displayed a typical “cobblestone” morphology. After 14 days of culture, the adherent cells revealed a heterogeneous cell population, comprising small-sized round cells, spindle-like cells and formed tube-like structure. Weibel-Palade bodies were shown on the transmission electron microscopy photomicrographs. Compared with the original, cell markers CD133 and CD34 decreased significantly (77.0%±3.3% to 1.6%±2.2% and 93.1%±4.7% to 37.4%±4.9%, P<0.05), while Flk-1 increased significantly (from 22.3%±3.3% to 94.3%±4.1%, P<0.05) after 14 days of culture with VEGF, bFGF, SCF. The vWF was strongly expressed (77.9%±3.3%) on the 14th day later. CONCLUSION: Vascular endothelial progenitor cells were isolated from cord blood with specific expression of CD133/CD34/Flk-1. With the stimulation of the growth factors, seven-ten days after culture EPCs could be turned to endothelial cells.</FONT>

Key words Fetol blood Endothelial progenitor cells Cell separation Purification Differentiation

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