论著

大鼠心肌微血管内皮细胞的培养及基因芯片分析的研究

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摘要 目的:用血管内皮细胞生物学功能基因芯片研究培养的大鼠心肌微血管内皮细胞特征。 方法: 用植块法培养大鼠心肌微血管内皮细胞,利用倒置显微镜、扫描电子显微镜和透射电子显微镜观察培养细胞的形态学特征;利用免疫细胞化学方法显示血管内皮细胞特异性表面标志;用细胞计数方法绘制细胞生长曲线;用血管内皮细胞生物学功能基因芯片研究细胞基因表达谱,并比较原代细胞和传代细胞基因表达的变化。 结果: 利用植块法培养的大鼠心肌微血管内皮细胞具备多种典型微血管内皮细胞(MVEC)特征:细胞呈多边形或梭形并呈铺路石样生长;存在管腔样结构(TLS)和毛细血管网络,细胞表面有丰富的微绒毛; CD34、CD31、CD105、VW因子和Tie-2阳性;多种与正常血管功能密切相关的基因不同程度表达,前2代细胞特征稳定。 结论: 用植块法培养的大鼠心肌微血管内皮细胞,具备微血管内皮细胞的特征,原代和第2代培养细胞特征稳定,可用于心血管疾病的基础与临床研究。

关键词 细胞培养; 毛细血管; 内皮细胞; 寡核苷酸序列分析

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Study on the culture of rat myocardium microvascular endothelial cells and microarray analysis

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Abstract

AIM: To study the cytological characteristics of rat myocardium microvascular endothelial cells (RMMVEC) by microarray. METHODS: The RMMVEC were cultured by the method of planting myocardium tissue. The morphology of RMMVEC was studied by light and electronic microscopy. Its molecular markers were observed by immunocytochemistry. Cell proliferation kinetic was analyzed by counting the number of cells. The gene expression of the RMMVEC was studied by endothelial cell biology gene microarray and compared the change of gene expression among the cultured cells of primary, 2nd and 5th passage. RESULTS: The RMMVEC showed morphological characteristics of microvascular endothelial cells (MVEC): growing in a cobblestone pattern, forming tube-like structure or capillary network and having microvilli on cell surface. At the same time, the RMMVEC showed positive staining for vWF, CD34, CD31, CD105 and Tie-2. Gene microarray analysis indicated expression of VEGFR, ICAM-1, VCAM-1, angiopoietin1, PECAM1 (CD31) and other genes closely related to microvascular endothelial functions at relatively high level. But in cultured cells of 5th passage the characteristic gene expression of microvascular endothelial cells disappeared. CONCLUSION: The RMMVEC cultured by this method possess typical characteristics of MVEC. The cytological characteristics are steady in the cultured cells of primary and 2nd passage. It can be utilized to study the mechanisms of some cardiovascular diseases.

Key words Cell culture Capillaries Endothelial cells Oligonucleotide array sequence analysis

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