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[1]王海,黄博,周跃,等.Fibronectin差别黏附法筛选、纯化软骨终板干细胞[J].第三军医大学学报,2013,35(10):965-968.

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## Fibronectin差别黏附法筛选、纯化软骨终板干细胞 本期目录/Table of Contents

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Title: Fibronectin differential adhesion assay for selection and purification

of cartilage endplate-derived stem cells

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Keywords: fibronectin; cartilage endplate-derived stem cells; differential adhesion assay

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摘要: 目的 通过Fibronectin介导进行差别黏附筛选软骨终板干细胞,观察筛选的效果及

其初步生物学性能。 方法 从脊柱融合术中获取椎间盘软骨终板标本,机械-酶消化法获取原代软骨终板细胞,体外扩增至第2代后接种于 Fibronectin 包被过的培养瓶,孵育20 min后将未贴壁细胞及培养液转移至新培养瓶再孵育40 min,吸弃未贴壁细胞及培养液,所得2瓶细胞进行体外扩增,流式细胞仪检测细胞表面标志物。对筛选后的细胞进行向多系细胞诱导分化。 结果 第2代细胞筛选前,细胞形态个体差异较大,呈三角形或多角形,融合时呈铺路石样外观,筛选后细胞形态比较均匀,形成细胞克隆群。流式细胞仪检测发现:经Fibronectin筛选后所获软骨终板干细胞CD90、CD73表达阳性率>95%, CD105表达阳性率>85%, 经诱导能向骨、软骨及脂肪细胞方向分

CD/3表达阳性率>95%, CD/105表达阳性率>85%; 经诱导能向骨、软骨及脂肪细胞方向分化。 结论 Fibronectin筛选所得的软骨终板细胞基本符合国际细胞治疗协会对间充质干细胞的定义标准。Fibronectin差别黏附筛选法得够有效筛选、纯化软骨终板

干细胞。

Abstract: Objective To select cartilage endplate-derived stem cells by fibronectin

differential adhesion assay, to evaluate the efficiency, and to observe the preliminary biological properties of obtained stem cells. Methods The samples of intervertebral disc cartilage endplate were collected from spine fusion surgery, and primary cartilage endplate cells were obtained by mechanical method combined with collagenase. Primitive cells expanding to passage 2 were

transferred to culture flasks coated with fibronectin. After 20 min the non-

adherent cells and media were transferred to a second flask to incubate for 40 min, and both of the two flasks were added with fresh media and cultured after the media and non-adherent cells in the second flask were removed. The cells after selection were detected by flow cytometry and were induced to differentiate into osteoblasts, chondrocytes and adipocytes. Results The discrepancy of cell morphology among cells of passage 2 was observed, but the cells became comparatively uniform after selection. The results of flow cytometry showed these cells were positive for 3 cell markers including CD73, CD105 and CD90, but negative for CD34 and CD45. The percentages of CD73- and CD90-positive cells were more than 95%, while the percentage of CD105-positive cells was more than 82%. The cells could differentiate into osteoblasts, chondrocytes and adipocytes. Conclusion The results of cartilage endplate-derived stem cells obtained by fibronectin differential adhesion assay fulfill the majority of criteria used to define mesenchymal stem cells stated by the International Society for Cellular Therapy. By the method we can obtain cartilage endplate-derived stem cells efficiently and reliably.

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