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白藜芦醇诱导大鼠骨髓基质细胞的神经元样细胞分化伴Shh信号激活

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Title: Resveratrol induces differentiation of rat bone marrow stromal cells into neuron-like cells *via* Shh signaling activation *in vitro*

作者： 黄家贵；沈长波；刘舒；徐兰；杨琴
重庆医科大学附属第一医院神经内科

Author(s): Huang Jiagui; Shen Changbo; Liu Shu; Xu Lan; Yang Qin
Department of Neurology, First Affiliated Hospital, Chongqing Medical University, Chongqing, 400016, China

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摘要：
目的　研究Sonic Hedgehog(Shh)信号在白藜芦醇诱导大鼠骨髓基质细胞(marrow stromal cells，MSCs)分化为神经元样细胞中的作用。 方法　采用全骨髓贴壁法分离培养MSCs。MSCs分为3组，白藜芦醇诱导组：用含白藜芦醇的无血清DMEM/F12诱导MSCs分化；对照组：用无血清DMEM/F12诱导MSCs分化；正常组：不做诱导处理。倒置显微镜下观察细胞形态，免疫荧光法、免疫印迹法检测NSE、MAP-2、GFAP、Smo和Gli1蛋白的表达和移位。 结果　白藜芦醇诱导后细胞胞体收缩，伸出长突起，类似神经元。免疫荧光显示正常组及对照组MSCs轻度表达神经元NSE蛋白，白藜芦醇诱导组MSCs NSE、MAP-2阳性，但诱导前后细胞始终未表达胶质细胞GFAP蛋白。免疫荧光显示，MSCs具有初级纤毛，并表达Smo和Gli1。白藜芦醇诱导组Smo从细胞质进入初级纤毛，Gli1从细胞质进入细胞核，同时，Smo、Gli1蛋白的表达增加（*P*<0.01）。对照组则无明显变化。 结论　白藜芦醇能诱导MSCs分化为神经元样细胞；此过程中Shh信号通路被激活，推测Shh信号在MSCs分化过程中可能发挥着重要作用。

Abstract:
Objective　To determine the role of sonic hedgehog (Shh) signaling in the differentiation of rat bone marrow stromal cells (MSCs) into neuron-like cells induced by resveratrol *in vitro*. Methods　The rat MSCs were isolated and cultured by whole bone marrow adherent culture, and then induced to differentiate into neuron-like cells with serum-free DMEM/F12 medium containing resveratrol. Cell morphology was observed with invert microscopy. Indirect immunofluorescence assay and Western blotting were applied to detect the protein expression of NSE, MAP-2, Smo and Gli1, and the translocation of Smo and Gli1. Results　Almost all MSCs and the control cells were poor positive for NSE. The differentiated cells showed neuron-like morphology with the cell body shrinkage and extend long processes after by resveratrol induction, and were positive to NSE and MAP-2 but negative to GFAP. Immunofluorescence assay confirmed that MSCs had primary cilia, and expressed Smo and Gli1 in the cytoplasm. After resveratrol induction, Smo entered into the primary cilia from the cytoplasm while Gli1 translocated to the nucleus from the cytoplasm. Meanwhile, Western blot analysis indicated that the expression of Smo and Gli1 protein was gradually enhanced with the prolongation of induction (*P*<0.01). However, the control cells had no such marked changes. Conclusion　Resveratrol induces MSCs to differentiate into neuron-like cells. Moreover, Shh signaling pathway is activated during induced process, suggesting that Shh signaling may play an important role in the differentiation.

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