

[1]张可洲,熊亚,杨希川,等.MAD2B与TCF4相互作用及其对毛乳头细胞功能的影响[J].第三军医大学学报,2013,35(11):1071-1075.

Zhang Kezhou,Xiong Ya,Yang Xichuan,et al.Interaction of MAD2B and T cell factor 4 and its effects on function of dermal papilla cells[J].J Third Mil Med Univ,2013,35(11):1071-1075.

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《第三军医大学学报》[ISSN:1000-5404/CN:51-1095/R] 卷: 35 期数: 2013年第11期 页码: 1071-1075 栏目: 论著 出版日期: 2013-06-15

Title: Interaction of MAD2B and T cell factor 4 and its effects on function of dermal papilla cells

作者: 张可洲; 熊亚; 杨希川; 宋志强; 钟白玉; 郝飞  
第三军医大学西南医院皮肤科, 重庆市皮肤性病研究所

Author(s): Zhang Kezhou; Xiong Ya; Yang Xichuan; Song Zhiqiang;  
Zhong Baiyu; Hao Fei

Department of Dermatology, Chongqing Institute of  
Dermatovenerology, Southwest Hospital, Third Military Medical  
University, Chongqing, 400038, China

关键词: MAD2B; TCF4; 毛乳头细胞; 旁分泌因子; Wnt

Keywords: MAD2B; T cell factor 4; dermal papilla cells; paracrine factor;  
Wnt

分类号: R322.991; R329.26; R394.2

文献标志码: A

摘要: 目的 探讨MAD2B基因过表达对毛乳头细胞 (dermal papilla cells, DPCs) 生物学功能的影响。 方法 构建真核表达载体pIRES2-MAD2B和pIRES2-TCF4质粒, 在Lipofectamine? 2000脂质体的介导下转染毛乳头细胞, 荧光显微镜下观察转染效率, 使用Western blot检测目的蛋白的表达, 并验证两种蛋白的相互作用, 比较细胞增殖状况, Wnt通路信号强度, 毛乳头分泌胰岛素样生长因子-1 (insulin-like growth factor-1, IGF-1) 、血管内皮生长因子 (vascular endothelial growth factor, VEGF) 、肝细胞生长因子 (hepatocyte growth-promoting factors, HGF) 的变化情况。 结果 成功构建pIRES2-MAD2B和pIRES2-TCF4重组质粒, 且转染后发现MAD2B和TCF4蛋白过表达于毛乳头细胞。免疫共沉淀实验结果提示在凝集生长的毛乳头细胞中存在相互

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作用的MAD2B和TCF4蛋白质复合物。在MAD2B过表达于毛乳头细胞之后，发现Wnt途径信号通路强度下调。CCK-8实验结果提示MAD2B过表达后细胞增殖活性下降，与对照组比较有统计学差异( $P<0.05$ )；同时实时荧光定量PCR检测结果显示，与对照组相比，IGF-1、VEGF、HGF mRNA的表达有所下降( $P<0.05$ )。 结论 免疫共沉淀提示MAD2B与TCF4在毛乳头细胞中存在相互作用，MAD2B过表达后可能通过抑制Wnt信号通路而对毛乳头细胞增殖和其旁分泌因子IGF-1、VEGF、HGF产生抑制作用。

**Abstract:** Objective To determine the efficiency of over-expression of MAD2B gene on biological functions of dermal papilla cells (DPCs). Methods Eukaryotic expression plasmid vectors pIRES2-MAD2B and pIRES2-T cell factor 4 (TCF4) were constructed and transfected in the DPCs with Lipofectamine 2000. The transfection efficiency was evaluated by green fluorescence microscopy, and the objective proteins were detected with Western blotting. Further, the interaction of 2 proteins was verified with co-immunoprecipitation analysis, and the change of cell proliferation and signal strength of Wnt signal pathway, DPFs secreting insulin-like growth factor-1 (IGF-1), vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) were detected with real-time PCR. Results MAD2B and TCF4 genes were expressed in DPFs successfully. Co-