

绵羊 *FGF5* 基因的克隆、表达及RNA干扰

刘伍限¹, 贾斌¹, 石国庆^{1,3}, 任建功², 刘卡², 马润林²

1. 石河子大学动物科技学院, 石河子 832000 2. 中国科学院遗传与发育生物学研究所, 北京100101 3. 新疆农垦科学院畜牧兽医研究所, 石河子 832000

LIU Wu-Xian¹, JIA Bin¹, SHI Guo-Qing^{1,3}, REN Jian-Gong², LIU Ka², MA Run-Lin²

1. College of Animal Science, Shihezi University, Shihezi 832000, China 2. Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China 3. Institute of Animal Husbandry and Veterinary Medicine, Xinjiang Academy of Agricultural and Reclamation Science, Shihezi 832000, China

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摘要 根据牛的成纤维细胞内生长因子5(Fibroblast growth factor 5, *FGF5*)基因cDNA序列设计引物, PCR扩增得到绵羊*FGF5*基因cDNA的开放阅读框序列, 并比较和其他6种高等哺乳动物的序列同源性; 同时研究该基因在绵羊多种组织的表达情况, 以及研究以细胞模型RNA干扰下的表达情况。结果表明, 绵羊*FGF5*基因ORF全长为813 bp, 编码270个氨基酸, 分子量约为29.58 kDa, 理论等电点10.59。绵羊*FGF5*基因cDNA序列与牛、人、小鼠、大鼠、犬和猫的对应该序列同源性高度保守, 预测氨基酸序列同源性同样具有高度保守性。RT-PCR分析表明*FGF5*在绵羊皮肤、小肠、肾脏、心脏、肝脏、脾脏、胰脏和肺中均有表达, 皮肤中表达量最高。构建该基因的原核表达载体和RNAi载体, IPTG诱导在大肠杆菌中融合表达获得55 kDa的蛋白条带, 设计的RNA干扰片段能显著抑制*FGF5*基因的表达。文章为进一步阐明绵羊*FGF5*的功能尤其是在羊毛生长发育中的作用提供了理论和实验基础。

关键词: 绵羊 *FGF5* 基因克隆 表达 RNAi

Abstract: The cDNA of fibroblast growth factor 5 (*FGF5*) gene in sheep was cloned, and the nucleotides sequence homology of *FGF5* was compared with other six mammal. In addition, the expression of *FGF5* in different tissues was analysed. Gene *FGF5* was then recombined into prokaryotic expression vector (pGEX-4T-2) and RNA interference vector (pSilencer 5.1 H1) to study its expression in fibroblast cell lines. Results showed that the open reading frame (ORF) of cDNA in sheep consisted of 813 nucleotide acids encoding 270 amino acids, with the molecular mass of 29.58 kDa and theoretical pI of 10.59. The amino acids sequence of *FGF5* gene in sheep shared high identity with those in cow, human, mouse, rat, dog, cat and rabbit. In addition, analysis on tissue expression showed that *FGF5* expressed in skin, heart, kidney, liver, pancreas, spleen, lung, and small intestine, especially presenting high levels in skin. The expression of *FGF5* in *E. coli* was induced with IPTG, which produced a protein band with the expected size of 56 kDa on SDS-PAGE, while the expression of *FGF5* in sheep fibroblast cell line was knocked down remarkably with the help of integrated RNAi vector.

Keywords: sheep, *FGF5*, gene cloning, expression, RNAi

收稿日期: 2010-12-03; 出版日期: 2011-09-25

基金资助:

优质转基因细毛羊新品种培育(编号: 2009ZX08008-001B)

通讯作者 贾斌 Email: jabin65@sina.com

引用本文:

刘伍限, 贾斌, 石国庆, 任建功, 刘卡, 马润林. 绵羊*FGF5*基因的克隆、表达及RNA干扰. 遗传, 2011, 33(9): 982-988.


LIU Wu-Wen, GU Bin, DAN Guo-Qiang, LIN Jian-Gong, LIU Ka, MA Run-Lin. Cloning, expression analysis and RNA interference of *FGF5* gene in sheep. HEREDITAS, 2011, V33(9): 982-988.

链接本文:

http://www.chinagene.cn/Jwk_yc/CN/10.3724/SP.J.1005.2011.00982 或 http://www.chinagene.cn/Jwk_yc/CN/Y2011/V33/I9/982

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