研究论文

成年核移植山羊生长相关基因的表达分析

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摘要 体细胞核移植过程有可能影响克隆动物生长相关基因尤其是印迹基因的表达水平。本研究运用同源 引物PCR扩增、RACE技术并结合同源克隆策略,克隆了7个山羊生长相关基因包括3个印迹基因(H19、1GF2 和 IGF2R) 和4个非印迹基因(IGF1、IGF1R、GHR 和 GHSR)的完全CDS或者部分cDNA序列,经生物信息学技术确认后,用荧光实时定量PCR对8只成年克隆山羊中这些基因的表达水平进行分析,结果表明3个印迹基因中IGF2R基因表达水平极显著高于对照组的自然繁殖山羊(P<0.01),而H19和IGF2的表达则没有很大区别;4个非印迹基因中只有IGF1R的表达水平极显著高于对照组(P<0.01),IGF1、GHR和GHSR的表达与对照组相似。表明即使在表型正常的成年克隆动物也存在一定的表观遗传异常。通过对获得完全CDS和3′UTR的IGF2基因经过生物信息学分析表明,山羊IGF2基因包含一个540 bp的开放阅读框(ORF)编码179个氨基酸。IGF2基因cDNA序列和氨基酸序列以及其它基因部分序列比较分析表明,山羊所有这些基因与绵羊的同源性要高于同牛的同源性。

关键词 山羊;核移植;基因表达;荧光实时定量 PCR;印迹基因 分类号

Overexpression of *IGF2R* and *IGF1R* mRNA in SCNT-produced Goats Survived to Adulthood

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<P>The procedure of somatic cell nuclear transfer (SCNT) is likely to affect the expression level of growth-related genes especially imprinting genes. In this study, expressions of growth-related genes including three imprinting genes (H19, IGF2, and IGF2R) and four non-imprinting genes (IGF1, IGF1R, GHR, and GHSR) in adult nuclear transferred (NT) goats were investigated by real-time PCR. The expressions of these genes in adult clones were found largely normal, but IGF2R and IGF1R were more highly expressed in cloned goats than in non-NT goats (P < 0.01). Analysis on mono-allelic expression pattern of imprinting genes indicated that mono-allelic expression patterns of H19 and IGF2 in cloned goats were similar to that in non-NT goats. In addition, the sequence of goat IGF2 gene and the putative amino acid sequence were obtained. The 986 nucleotide cDNA of goat IGF2 gene contained an openreading frame of 540 nucleotides coding for 179 amino acids. Both cDNA sequence and amino acid sequence of IGF2 in goat showed their higher homology with that in sheep than in cattle; the partial cDNA fragments of H19, IGF2R, GHSR, IGF1R, and GHR in goat were also cloned and sequenced, which shared higher sequence identities with those in sheep than in cattle. </P>

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Key words goat nuclear transfer gene expression real-time PCR imprinting gene

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