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Double-Muscled Phenotype in Mutant Sheep Directed by the CRISPRCas9 System

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Abstract

Myostatin (MSTN) is a well-known negative regulator of muscle growth. The double-muscled sheep caused by natural loss-of-function mutations of MSTN have very strong skeletal muscle. In this study, our results demonstrate the successful generation of MSTN mutant sheep via specific targeting of an exon 1 site using Cas9 technology.

Keywords

Genome editing; Knockout; Targeted mutagenesis; Sheep; Myostatin.

Introduction

Myostatin (MSTN), a transforming growth factor-β family member, functions as a negative regulator of skeletal muscle development and growth. MSTN is also directly or indirectly involved in regulation of fat and glucose metabolism [1-5], Animals with mutated MSTN genes show an enhanced phenotype. He doublemuscled cattle Caused by natural loss-of-function mutations of MSTN have very strong skeletal muscle and contain much less fat [11]. MSTN-knockout mice have a remarkable increase in muscle mass and significant decrease in fat compared to their corresponding wild-type littermates [12,13]. Herefore, MSTN disruption provides a potential agricultural strategy for promoting animal growth and performance [9]. Gene targeting is the most eٔجective means of introducing mutations in animals and can be used for analyzing gene function, generating animal models for human genetic diseases and optimizing livestock production. CRISPR/Cas9 has been vigorously pursued as an eٹcient method for genetic modification in a wide variety of animals, including livestock species [14]. He components of the prokaryotic clustered, regularly interspaced, short palindromic repeats (CRISPR)/ CRISPR-associated (Cas) system is a recently developed technology for targeted genome modification in mammalian cells, bacteria, zebra fish, mice, monkey and pigs.

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He sizes of the RT-PCR products were estimated by electrophoresis of a 5 ml aliquot on a 2.0% agarose gel. Western blotting of MSTN protein For Western blot analysis, total proteins were isolated from the samples by homogenization in lysis buٔ؏er (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 0.25% sodium deoxycholate, and complete protease inhibitor cocktail (Beyotime, Beijing, China). He concentration of proteins was measured by Bradford reagent (Sigma), separated on 10% SDS-PAGE gels and transferred to Immobilon-P membranes (Millipore). $ier blocking in 5% low-fat milk in PBST (0.1% Tween 20 in PBS) for 1 h, the membranes were incubated with tGFP antibody (1:500, Santa Cruz Biotechnology), Firsts antibody (1:500, Santa Cruz Biotechnology) or mouse GAPDH antibody (1:2000, Santa Cruz Biotechnology) overnight at 4°C. $ier washing in PBST, the membranes were incubated in goat anti-rabbit antibody conjugated with horseradish peroxidase (1:5000) for 1381h, followed by three washes in PBST. He signals were detected by ECL Chemiluminescent kit (Amersham Pharmacia Biotech, Arlington Heights).

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