

DOUBLE-MUSCLED PIGS CLONED USING MSTN-DISRUPTED FIBROBLASTS

Xi-jun Yin, Wen-Xue Li, Fu-Liang Sun, Jin-Dan Kang

Jilin Provincial Key Laboratory of Transgenic Animal and Embryo Engineering, China

Introduction

Myostatin (MSTN) plays a negative role in regulating the formation and differentiation of pig skeletal muscle, which might prevents the growth of muscle cells and muscle fibers. The deletion or reduction of this gene could cause excessive muscle development of animal body. Also, it has important relation with the growth, development and the fatty deposits of pigs, and it could be used to improve and adjust the development of pigs and the proportion of lean meat and fat.

Materials and Methods

A transcription activator-like effector nuclease (TALEN) pair targeting exon 1 of the swine MSTN gene was constructed and used to transfect porcine fetal fibroblasts (PFFs), along with a surrogate reporter plasmid. MSTN-mutated PFFs were enriched by magnetic separation and used to produce homozygous MSTN-KO boars in a two-step cloning process via somatic cell nuclear transfer (SCNT).

Results and Discussion

In the first step, 428 of SCNT embryos transferred to two recipient sows, five and nine fetuses were obtained on gestation days 27 and 36, respectively. All fetuses were used to establish PFF cell lines, which were analyzed for MSTN gene mutations by T7E1 and Sanger sequencing. Eight of the fourteen fetuses (57%) showed mutations at the MSTN loci, with three having biallelic and five having monoallelic mutations. In the second step, a PFF cell line with biallelic MSTN mutations (MSTN^{-/-}), consisting of a 2-bp deletion in one allele and a 4-bp deletion in the other allele, was used as a donor to generate cloned pigs via SCNT. Of 646 cloned embryos transferred into three surrogates, 14 live and two stillborn piglets were delivered. At birth, these piglets were hardly distinguishable phenotypically from wild-type piglets. However, starting at 2 month of age, they showed visually-clear hypermuscular characteristics. Comparisons of the magnitude of increase in dressing percent and loin eye size and the magnitude of decrease in backfat thickness in 6-month-old cloned and age-matched wild-type pigs support the MSTN^{-/-} genotype of the former. MSTN^{-/-} boars developed and grew normally to sexual maturity, after artificial insemination gave birth to normal offspring.