IGF2-H19 LOCUS METHYLATION STATUS IN CLONED GOAT FIBROBLAST CELLS

Zifei Liu, Mingtian Deng, Caifang Ren, Yongjie Wan, Feng Wang *

Nanjing Agricultural University, China

Introduction

Previous study showed a decrease in efficiency or even a failure over repeated iterations in somatic cell nuclear transfer (SCNT). DNA methylation in cloned offspring should be responsible for this inefficiency or failure. Hypothetically, SCNT itself may influence the methylation of *IGF2-H19* locus in cloned goat fibroblast cells that could be donor cells in SCNT, which resulted in inefficient serial recloning.

Materials and Methods

Goat ear fibroblast cells (GFC, control group) were obtained from the ear of a 2-month-old healthy female Saanen dairy goat (Yangling, China).Cloned goat ear fibroblast cells (CFC, experimental group) were obtained from the ear of a 2-month-old healthy female cloned goat that was produced by SCNT (using GFC as donor cells). When two groups were cultured to passage 5*,* using cytometry for cell growth curves, flow cytometer for cell apoptosis, Real-Time PCR for gene relative expression level and bisulfite sequencing PCR for the methylation status of differentially methylated region (DMR).

Results and Discussion

In this study, we found that there is no obvious morphological difference between CFC and GFC, while cell proliferation and apoptosis in CFC had significantly changes. Furthermore, we also observed dysregulation on *Dnmts* and *Tets* genes expression, and aberrant DNA methylation on *IGF2-H19* locus. Compared with amphigory, SCNT embryo had a low survival rate and cloned offspring had a high morbidity and mortality rates. Ear fibroblast cells played an important role in SCNT, and its DNA methylation had a great impact on recloning efficiency. Therefore, we concluded that SCNT resulted in aberrant methylation of *IGF2-H19* locus by influencing *Dnmts* and *Tets* genes expression, which could affect genomic imprinting, and then influenced the efficiency on serial recloning.