Genetics and Molecular Research

Targeting exogenous *GDNF* gene to the bovine somatic cell beta-casein locus for the production of transgenic bovine animals

X.M. Zhang[1], F.H. Luo[2], H.M. Ding[1], B. Li[1], J.J. Zhang[1] and Y.J. Wu[2]

[1]Baotou Medical College, Inner Mongolia University of Science and Technology, Baotou, China
[2]Key Laboratory of National Education Ministry for Research of Mammal Reproductive Biology and Biotechnology, Inner Mongolia University, Hohhot, China

Corresponding author: Y.J. Wu
E-mail: wuyj1211@163.com

Genet. Mol. Res. 14 (4): 14989-14998 (2015)
Received April 9, 2015
Accepted June 26, 2015
Published November 24, 2015
DOI http://dx.doi.org/10.4238/2015.November.24.6

ABSTRACT. Considerable attention is currently being directed toward methods for producing recombinant human proteins in the mammary glands of genetically modified transgenic livestock. However, the expression of inserted genes in transgenic animals is variable and often very low because of the randomness of the site of transgene integration. One possible strategy to avoid the expression problem associated with random integration is to use site-specific integration by targeting integration to a high expression locus and, thereby, to improve expression of the transferred gene. In the present study, we focused on glial cell line-derived neurotrophic factor (GDNF), a novel type of neurotrophic factor first cloned in 1993. Research has shown that GDNF may have potential applications in the treatment of Parkinson's disease and other diseases of the central nervous system since it acts as a protective factor for central dopaminergic neurons. Here, we constructed a gene targeting vector to knock-in the human *GDNF* gene at the bovine beta-casein gene locus as a

first step to producing transgenic animals with a high level of expression of human GDNF protein in their mammary glands. Bovine fetal fibroblast cells were transfected with linearized *pNRTCNbG* by electroporation. Three cell clones were identified with successful targeting to the beta-casein locus; and were confirmed using both polymerase chain reaction analysis and sequencing. Gene-targeted cells were used as nuclear donors; a total of 161 embryos were reconstructed, 23 of which developed to the blastocyst stage. These blastocysts were transferred to 8 recipient cows, but no offspring were obtained.

Key words: Gene targeting; Somatic cell nuclear transfer; GDNF; Beta-casein locus

