Theriogenology 79 (2013) 660–666

Contents lists available at SciVerse ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Production of cloned embryos from caprine mammary epithelial cells expressing recombinant human β-defensin-3

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ARTICLE INFO

Article history:
Received 22 July 2012
Received in revised form 20 November 2012
Accepted 25 November 2012

Keywords:
Transgenic goat
Mammary epithelial cell
Human β-defensin-3
Somatic cell nuclear transfer

ABSTRACT

Transgenic animals that express antimicrobial agents in their milk can inhibit bacterial pathogens that cause mastitis. Our objective was to produce human β-defensin-3 (HBD3) transgenic embryos by nuclear transfer using goat mammary epithelial cells (GMECs) as donor cells. Three GMEC lines (GMEC1, GMEC2, and GMEC3) were transfected with a HBD3 mammary-specific expression vector by electroporation. There was a difference ($P < 0.05$) in the rate of geneticin-resistant colony formation among cell lines GMEC1, GMEC2, and GMEC3 (39 and 47 vs. 19 colonies per 3×10^6 cells, respectively). After inducing expression, the mRNA and protein of HBD3 were detected by reverse transcription polymerase chain reaction and Western blot analysis in transgenic cells. Transgenic clonal cells expressing HBD3 were used as donor cells to investigate development of cloned embryos. There were no significant differences in rates of cleavage or blastocyst formation of cloned embryos from transgenic (GMEC1T2 and GMEC2T3) and nontransgenic (GMEC1 and GMEC2) GMECs (72.3 ± 5.0%, 69.5 ± 2.3%, 61.8 ± 4.8%, and 70.0 ± 2%; and 16.8 ± 0.5%, 17.5 ± 0.7%, 16.7 ± 0.9%, and 17.5 ± 0.6%, respectively). However, the fusion rate, cleavage rate, and blastocyst formation rate of cloned embryos from a transgenic clonal cell line (GMEC2T6, 50.7 ± 2.1%, 55.5 ± 2.0%, and 11.1 ± 0.6%) were lower than those of other groups ($P < 0.05$). We concluded that genetic modification of GMECs might not influence the *in vitro* development of cloned embryos, but that some of the transgenic clonal cells were not suitable for nuclear transfer to produce transgenic goats, because of low developmental rates. However, transgenic GMECs expressing HBD3 might be used as donor cells for producing transgenic goats that express increased concentrations of β-defensins in their milk.

1. Introduction

Human β-defensin-3 (HBD3), a kind of antimicrobial peptide, is widely expressed in many tissues [1,2]. Human β-defensin-3 has broad-spectrum antimicrobial activity against bacteria, fungi, and enveloped viruses, and has an important role in immunity [3]. In previous studies, transgenic livestock expressing some kinds of antimicrobial peptides in milk inhibited bacterial pathogens causing mastitis [4–6]. Therefore, HBD3 might be a candidate gene for enhancing mastitis resistance. In addition, recombinant HBD3 purified from the milk might be useful for medical research and clinical applications.

Somatic cell nuclear transfer (SCNT) is an efficient method for producing recombinant therapeutic proteins in the milk of transgenic animals. Because fetal fibroblast cells grow rapidly and have more proliferative ability, they have been commonly used as sources of donor nuclei to produce transgenic animals [7–11]. However, mammary-specific expression vectors cannot be evaluated in transgenic fibroblast cells. Therefore, if the transgene is integrated in a transcriptionally silent region of chromatin, it will not be

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http://dx.doi.org/10.1016/j.theriogenology.2012.11.021

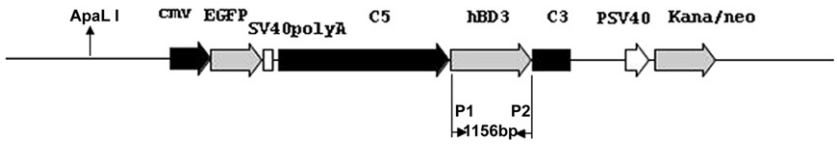

Fig. 1. Schematic representation of the recombinant plasmid pEBB. The pEBB plasmid was constructed by inserting the 2.2 kb promoter region (C5, including the 1.7 kb 5′-flanking sequence, exon 1 and part of intron 1) and 0.6 kb 3′-untranslated region (C3, including part of the last intron and exon) of the bovine beta-casein gene (GenBank: X14711), and 1156 base pair (bp) human β-defensin-3 DNA sequence (GenBank: 55894) into a pEGFP-C1 plasmid.

expressed correctly in transgenic animals, (so-called chromosomal position effect [12–14]). Mammary epithelial cells (MECs) can be used as a model for directly monitoring the expression of mammary-specific vector before SCNT [15]. The use of genetically modified MECs expressing high levels of recombinant protein for SCNT will produce high levels of the recombinant protein in the milk of transgenic cloned offspring. Moreover, a previous study indicated that goat MECs at 25 to 27 passages can support cloned embryo development to term [16]. However, little is known about the developmental competence of cloned embryos derived from genetically modified MECs.

Our objective was to establish an upstream system for producing increased expression of HBD3 in the milk of transgenic goats. Goat MECs were transfected with a HBD3 expression vector by electroporation. The transgenic clonal

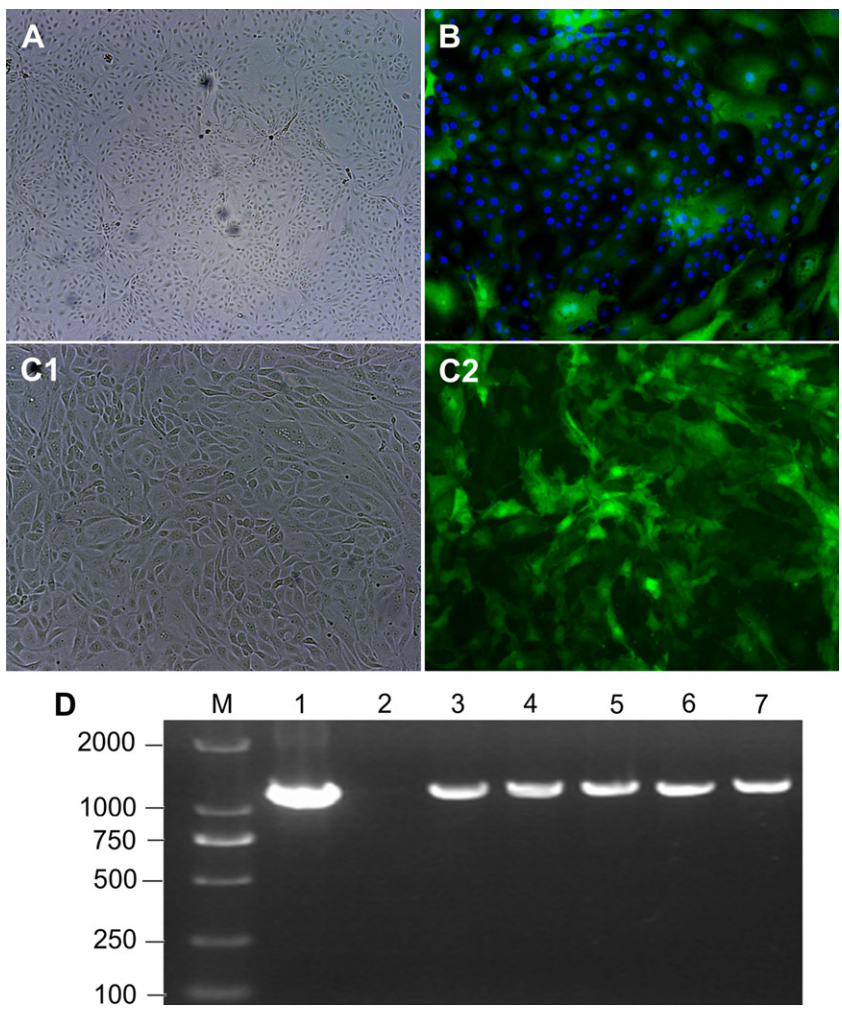

Fig. 2. Isolation and transfection of goat mammary epithelial cells (GMECs). (A) GMECs at passage 1 (magnification ×40). (B) Immunofluorescence staining of cytokeratin 18 (green) and nuclei labeled with 4′,6-diamidino-2-phenylindole (blue) (magnification ×100). (C) Geneticin-resistant colony expressing enhanced green fluorescent protein under a bright field (C1) and fluorescence (C2) (magnification ×100). (D) Amplification of the human β-defensin-3 transgene by polymerase chain reaction. M represents the DNA marker; lane 1, plasmid pEBB (positive control); lane 2, GMECs (negative control); lanes 3 to 7, transgenic clonal cell lines (GMEC1T2, GMEC1T3, GMEC2T3, GMEC2T5, and GMEC2T6).