30 GENERATION OF CLONED TRANSGENIC GOATS WITH CARDIAC SPECIFIC OVEREXPRESSION OF TRANSFORMING GROWTH FACTOR β1

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Abstract

Transforming growth factor β1 (TGF-β1) has a potent profibrotic function and is central to signaling cascades involved in interstitial fibrosis, which plays a critical role in the pathobiology of cardiomyopathy and contributes to diastolic and systolic dysfunction. In addition, fibrotic remodeling is responsible for generation of re-entry circuits that promote arrhythmias (Bujak and Frangogiannis 2007 Cardiovasc. Res. **74**, 184–195). Due to the small size of the heart, functional electrophysiology of transgenic mice is problematic. Large transgenic animal models have the potential to offer insights into conduction heterogeneity associated with fibrosis and the role of fibrosis in cardiovascular diseases. The goal of this study was to generate transgenic goats overexpressing an active form of TGFβ-1 under control of the cardiac-specific α-myosin heavy chain promoter (α-MHC). A pcDNA3.1DV5-MHC-TGF-β1cys^{33}ser vector was constructed by subcloning the MHC-TGF-β1 fragment from the plasmid pUC-BM20-MHC-TGF-β1 (Nakajima *et al.* 2000 Circ. Res. **86**, 571–579) into the pcDNA3.1D V5 vector. The Neon transfection system was used to electroporate primary goat fetal fibroblasts. After G418 selection and PCR screening, transgenic cells were used for SCNT. Oocytes were collected by slicing ovaries from an abattoir and matured *in vitro* in an incubator with 5% CO_2 in air. Cumulus cells were removed at 21 to 23 h post-maturation. Oocytes were enucleated by aspirating the first polar body and nearby cytoplasm by micromanipulation in Hepes-buffered SOF medium with 10 µg of cytochalasin B mL^{-1}. Transgenic somatic cells were individually inserted into the perivitelline space and fused with enucleated oocytes using double electrical pulses of 1.8 kV cm^{-1} (40 µs each). Reconstructed embryos were activated by ionomycin (5 min) and DMAP and cycloheximide (CHX) treatments. Cloned embryos were cultured in G1 medium for 12 to 60 h *in vitro* and then transferred into synchronized recipient females. Pregnancy was examined by ultrasonography on day 30 post-transfer. A total of 246 cloned embryos were transferred into 14 recipients that resulted in production of 7 kids. The pregnancy rate was higher in the group cultured for 12 h compared with those cultured 36 to 60 h [44.4% (n = 9) *v.* 20% (n = 5)]. The kidding rates per embryo transferred of these 2 groups were 3.8% (n = 156) and 1.1% (n = 90), respectively. The PCR results confirmed that all the clones were transgenic. Phenotype characterization [e.g. gene expression, electrocardiogram (ECG), and magnetic resonance imaging (MRI)] is underway. We demonstrated successful production of transgenic goat via SCNT. To our knowledge, this is the first transgenic goat model produced for cardiovascular research.

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