Production of Multiple Transgenic Pig for Xenotransplantation of Non-human Primate

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BACKGROUND

Previously, a transgenic pig of alpha-1,3-galactosyltransferase knockout (GT-/-) with a membrane cofactor protein (MCP) overexpressed pig (GT-MCP/-MCP) was generated to control hyperacute and acute immune rejection. However, cardiac xenotransplantation results of GT-MCP/-MCP pigs xenografted into cynomolgus monkeys showed that the survivability after grafting still remained insufficient and required to be improved.

PURPOSE

A thrombomodulin (TBM) is a part of the protein C pathway which is well known to have some roles on anti-coagulation anti-inflammatory. Thus, it is needed that a multiple transgenic pig with overexpression of MCP and TBM using the GT-/- cells.

MATERIALS AND METHOD

Briefly, we constructed two types of cassettes for MCP and TBM concurrent expression using wild-type MCP and TBM cDNA. Subsequently, we transfected and selected the cells with highly expressed MCP and TBM, and thereby generated a single clone genotyped as GT-/-/MCP/TBM. Using GT-/-/MCP/TBM cells, cloned-embryos were produced and transferred into surrogates by surgical method.

Table 1. Primer sets for PCR analysis

Gene		Sequences
Gal	Forward	*5'-ACCAGTCAGGTAAGCCACTCCACC TC-3'*
	Reverse	*5'-GTGCTGAACATCAAGTCAGTGCAATGGCTC-3'*
Modified MCP	Forward	*5'-CGGCAAGAAGTTCTACTACAAGGC-3'*
TBM	Reverse	*5'-TGCAGGACCACCTCTTTGCTAG-3'*

RESULTS AND CONCLUSIONS

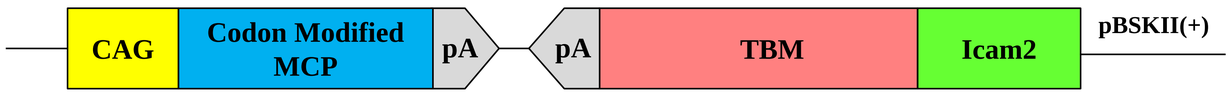

Figure 1. Constructionof expression vector of *MCP/TBM*, CAG, *CMV enhancer/chicken β-actin promoter;* pA, *bGH poly A signal;* Icam2, *porcine Icam2 promoter*

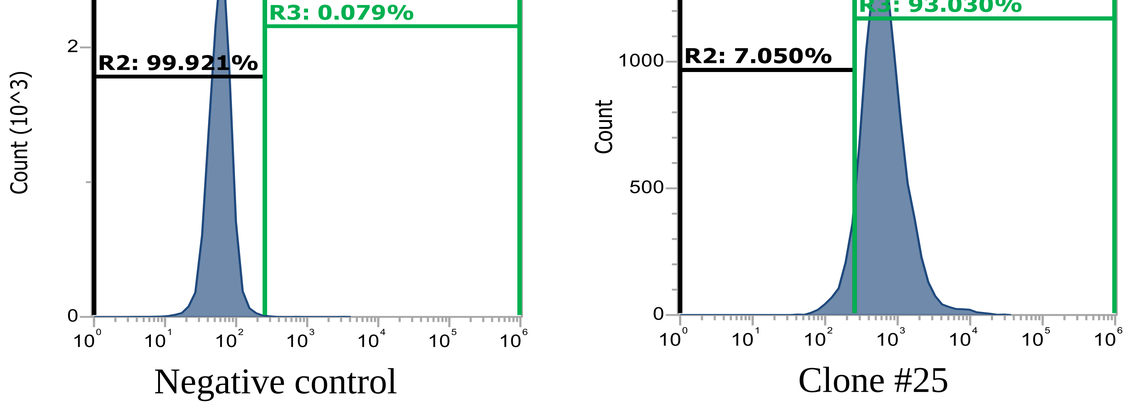

Figure 2. Confirmation of MCP expression

Table 2. Selection of cell lines by MCP expression level

Cell	No. of isolated clones	No. of analyzed clones	No. of MCP-positive <50% clones	No. of MCP-positive ≥50% and <90% clones	No. of MCP-positive ≥90% clones
Gal$^{-/-}$ #242(M)	114	33	10	9	14

Table 3. Production of transgenic piglets expressing *MCP/TBM*

IVM(%)	Fusion rate	Embryo transfer	Pregnancy (%)	Delivery status		
				Live	Dead	Total
620 ± 23 (78 ± 2)	58 ± 2	310 ± 10	1/4(25%)	6	3	9

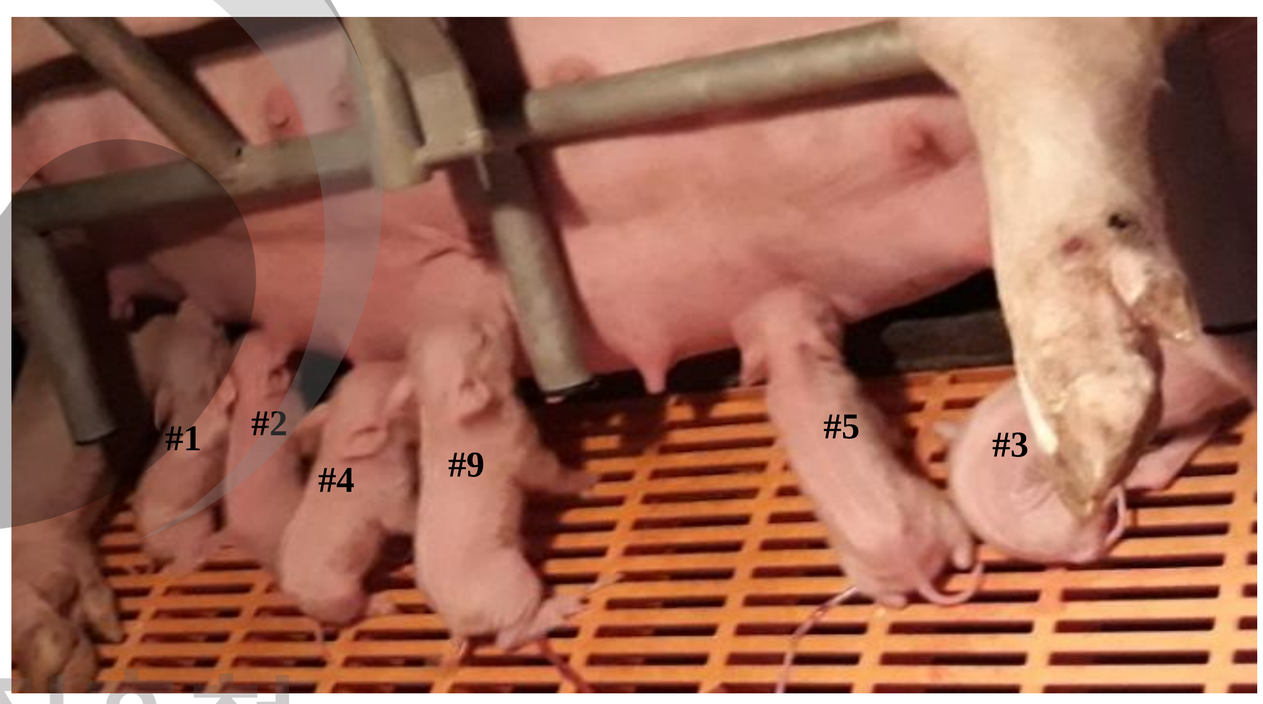

Figure 3. Transgenic piglets produced by SCNT

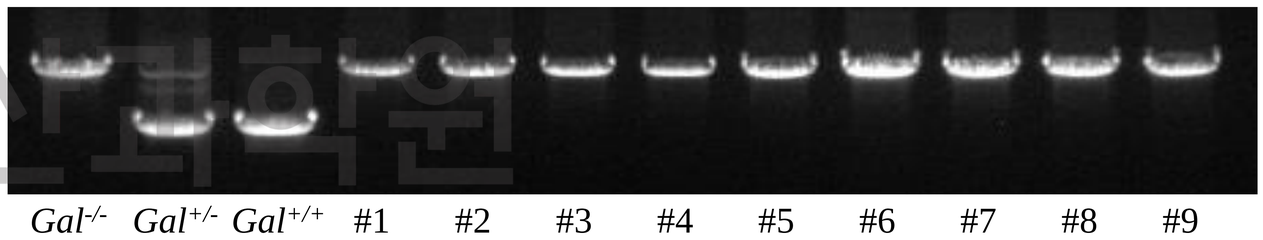

Figure 4. *Alpha-galactosyltransferase* expression analysis

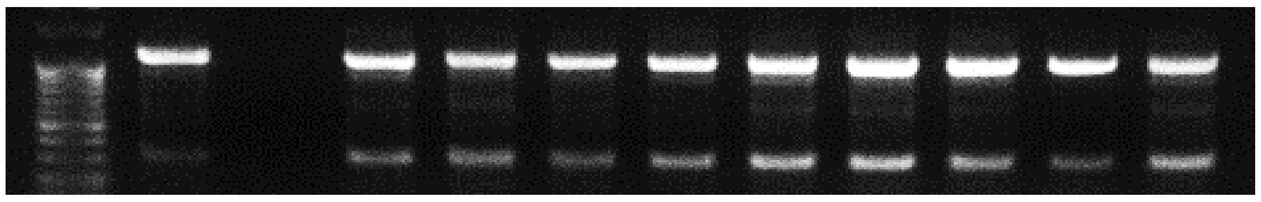

Figure 5. MCP/T*BM* expression analysis

Specifically, cumulus-oocytes complexes were matured in vitro (78%), enucleated, injected, and fused (58%) with GT-/-/MCP/TBM cells. After reconstruction, cloned-embryos were transferred into four surrogates (310 cloned-embryos each). One of four surrogate was pregnant (25%) and delivered (25%) 9 piglets (6 live, 3 dead) successfully. All 9 piglets were confirmed as GT-/-/MCP/TBM transgenic pigs by PCR analysis using genomic DNA.