## Switch off of $\alpha$ –Gal epitope expression in hepatocyte like cells derived from ear fibroblast of genetically modified pig Ran Lee\*, Ullah Imran\*, Youngim Kim, Hayeon Wi, Keon Bong Oh, Seunghoon Lee, Jae-Seok Woo, Sun A Ock†

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# <sup>국민의 내일을 위한 정부혁신</sup> 보다나은 농촌진흥청

Introduction

- Hyperacute rejection (HAR) is one of the major barrier in successful transplantation (Schroeder et al., 2005).
- Control of the pig GalT gene prevents acute anti-Gal antibody-mediated rejection (Ko et al., 2012).

### **Materials and Methods**

- **Experimental animals**
- White Yucatan as a control for non genetically modified miniature pig were provided by Optifarm Inc.
- GalT KO pig was provided by National Institute of Animal Science



Figure 1. Schematic view of isolation of porcine Ear fibroblast (EF)

#### **Culture medium**

The cells were maintained in Advanced Dublbecco's modified Eagle's medium (A-DMEM; Gibco), supplemented with 10 % fetal bovine serum (Invitrogen), 1X antibiotic/ antimitotic (Invitrogen), 1X glutamax (Invitrogen), + 1% streptomycin penicilin (invitrogen), 1% gentamicin (Invitrogen)

#### Generation of piHeps

FOXA3



Figure 2. Schematic design for the generation of piHeps

#### Immunocytochemistry (ICC)

- First antibody : α-Gal monoclonal antibody (MBS 603355), Albumin antibody (Abcam ab112980),
- Second antibody: Alexa A21042, Santacruz SC3916

#### • **RT-PCR**

 Table 1. Primer used for gene analysis of GalT KO pig

Target	Sequence $(5' \rightarrow 3')$		
GalT	Forward	ACC AGT CAG GTA AGC CAC TCC ACC TC	
	Reverse	GTG CTG AAC ATC AAG TCA GTG CAA TGG CTC	



A		400
	ve fluorescence level	380 360
	Relativ	20
		0
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gure 6. Immunofluorescence intensity level of  $\alpha$ -Gal and albumin in piHep Relative fluorescence density of (A)  $\alpha$ -Gal and (B) Albumin in two type of piHeps, respectively.



piHep like cells using a confocal microscopy A, piHep derived from GalTKO pig showed positive expression of

Albumin while lack  $\alpha$ -Gal expression. B, piHep derived from control pigs showed expression of both Albumin as well as  $\alpha$ -Gal.

#### Conclusion

• The ear fibroblasts derived from both pigs (wi/wo  $\alpha$ -Gal gene) were successfully induced into hepatocytes like cells by overexpressing three transcription factors (HNF1A, HNF4A, FOXA3) using lentiviral vector and they expressed albumin.

• Positive expression of  $\alpha$ -Gal was found in cell membrane of piHep from wild mini pig while this expression was found negligible in piHep derived

These results confirmed the absence of  $\alpha$ -Gal epitope in piHep derived from GalT KO pig which can be implemented in future regenerative medicine for possibly curing the human chronic liver diseases without the risk of hyper-acute rejection.

#### Reference

Schroeder et al., 2005 Hyperacyte Rejection Is Attenuated in GalT Knockout Swine Lungs Perfused Ex Vivo With Human Blood Transplantation

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