Towards *in vitro* produced germline stem cells in the bovine

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Abstract

Bovine spermatogonial stem cells (SSCs) have potential to be used in advanced reproductive technologies such as testis cell transplantation, where identification and purification of large numbers of SSCs is required. There are at least two possible sources of SSCs: isolation from the testis, or *in vitro* differentiation from pluripotent stem cells. The long-term goal of this thesis was to work towards the generation of SSCs from bovine somatic cells using induced pluripotent stem (iPS) cell technology. In order to do so, it was first important to characterise molecular markers expressed by bovine SSCs to allow for their identification in culture, and secondly to explore the feasibility of producing bovine iPS cells.

In order to achieve the first goal, a screening platform was developed based on comparative analysis of gene expression levels in SSC enriched and depleted cell populations. Expression of established testis cell markers was used to confirm the validity of the screening platform. This method was then used to examine expression of candidate spermatogonial markers in the bovine testis. STRA8, KIT, GFRA1, CLDN8, DDX6 and NAP1L4 were shown to be putative markers for bovine spermatogonia. Further analysis of CLDN8 showed expression by both a subset of spermatogonia and a subset of Sertoli cells, leading to the hypothesis that CLDN8 plays a role in the maintenance of SSCs in the SSC niche.

Reprogramming of bovine somatic cells was undertaken by introducing canonical reprogramming factors through a lentiviral vector. Initial experiments found that the reprogramming protocol was sufficient to produce cells exhibiting stem cell-like characteristics. Analysis of these cells indicated partial reprogramming had been achieved. A number of small molecules were then tested for their ability to enhance the success of cell reprogramming. A combination of three small molecules was found to accelerate the kinetics

of the reprogramming process and also promoted further reprogramming where cells could differentiate to the three different germ layers. Further research is now required to define the optimal culture conditions for the maintenance and expansion of bovine pluripotent cells in long term culture, and to test whether they can be differentiated towards the germ line.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

BDF: Bovine dermal fibroblast

BEF: Bovine embryonic fibroblast

bFGF: basic fibroblast growth factor

BMP: Bone morphogenetic protein

BTB: Blood-testis barrier

DBA: *Dolichos biflorus* agglutinin

DMEM: Dulbecco's Modified Eagle Medium

Dox: Doxycycline

DPBS: Dulbecco's phosphate buffered saline

EB: Embryoid body

ESC: Embryonic stem cell

FACS: Fluorescent activated cell sorting

FBS: Foetal bovine serum

GFP: Green fluorescent protein

HDAC: Histone deacetylase

iPSC: Induced pluripotent stem cell

LIF: Leukemia inhibitory factor

MACS: Magnetic activated cell sorting

MDF: Modified Davidsons Fixative

MEF: Mouse embryonic fibroblast

MET: Mesenchymal-to-epithelial transition

NEAA: Non-essential amino acids

OSKM: POU5F1 (OCT4); SOX2; KLF4; c-MYC

PCR: Polymerase chain reaction

PGC: Primordial germ cell

RA: Retinoic acid

RT-PCR: Reverse transcribed polymerase chain reaction

SEM: Standard error margin

SCF: Stem cell factor

SSC: Spermatogonial stem cell

TBS: Tris buffered saline

Tet: Tetracycline

Gene Synonyms

Gene Symbol	Synonym	Gene Name
AFP		*Alpha-fetoprotein*
ALPL		*Alkaline phosphatase*
ASB9		*Ankyrin repeat and SOCS box containing 9*
ATIC		*5-aminoimidazole-4-carboxamide ribonucleotide methyltransferase/ IMP cyclohydrolase*
BCL6B		*B-cell CLL/lymphoma 6 member B*
BMP4		*Bone morphogenetic protein 4*
CLDN8		*Claudin-8*
c-MYC		*v-myc avian myelocytomatosis viral oncogene homolog*
CSF1R		*Colony stimulating factor 1 receptor*
DDX4	*VASA*	*DEAD (Asp-Glu-Ala-Asp) box polypeptide 4*
DDX6		*DEAD (Asp-Glu-Ala-Asp) box polypeptide 6*
DES		*Desmin*
FOXA2		*Forkhead box A2*
GATA4		*GATA binding protein 4*
GFRA1		*GDNF family receptor alpha 1*
KIT	*c-KIT*	*v-KIT Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog*
KLF4		*Kruppel-like factor 4*
MTHFD1		*Methylenetetrahydofolate dehydrogenase 1*
NANOG		*Nanog homeobox*
NAP1L4		*Nucleosome assembly protein 1-like-4*
NES		*Nestin*
PARK7	*DJ1*	*Parkinson protein 7*
PFN1		*Profilin 1*
PHGDH		*Phosphoglycerate dehydrogenase*
POU5F1	*OCT4*	*POU class 5 homeobox 1*
PRDX1	*PRX1*	*Peroxiredoxin 1*
REX1		*RNA exonuclease 1 homolog*
SEPT7	*CDC10*	*Septin 7*
SOX2		*SRY (Sex determining region Y) box 2*
STRA8		*Stimulated by retinoic acid gene 8*
THY1		*Thy-1 cell surface antigen*
TKTL1		*Transketolase-like 1*
TUBB3		*Tubulin, beta 3 class III*
UCHL1	*PGP9.5*	*Ubiquitin carboxyl-terminal esterase L1*
ZBTB16	*PLZF*	*Zinc finger and BTB domain containing 16*