Overexpression of Mammalian *Nanog* mRNA Hyperdorsalises Zebrafish Embryos

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Abbreviations

PGCs	primordial germ cells
ES cells	embryonic stem cells
LIF	leukemia inhibitory factor
hsnanog	human *NANOG* cDNA
mnanog	mouse *Nanog* cDNA
hsnanog-pCS2+	recombinant construct of human *NANOG* cDNA subcloned into pCS2+
mnanog-pCS2+	recombinant construct of mouse *Nanog* cDNA subcloned into pCS2+
reverse transcription polymerase chain reaction	RT-PCR
microlitre	µl
nanogram	ng
milligram	mg
Tris-acetate-EDTA Buffer	TAE Buffer
deoxyribonuclease I	DNase I
dithiothreitol	DTT
New England Biolabs	NEB

Abstract

The work presented in this thesis is an investigation of the effects of misexpression of mammalian *NANOG/Nanog* mRNA in zebrafish embryos. I measured whether there was any effect of overexpression of mammalian *NANOG/Nanog* mRNA on the specification of germ line and on dorsal-ventral patterning. I subcloned human *NANOG* and mouse *Nanog* cDNAs into the pCS2+ vector for in vitro transcription, synthesized RNA and injected embryos. I then observed changes in the morphology of zebrafish embryos and counted the number of primordial germ cells. I used quantitative RT-PCR to quantify expression of five genes involved in dorsal-ventral patterning, and used a t-test to determine the significance of gene expression changes. The results show that the overexpression of either human *NANOG* mRNA or mouse *Nanog* mRNA by injection leads to significantly dorsalized changes in the morphology of the zebrafish embryos (25% and 56% of embryos show significant changes in their morphology after injection with 50 pg and 100 pg human *NANOG*; 81% and 82% of embryos show significant changes after injection with 50 pg and 100 pg mouse *Nanog*). I could detect no difference in the number of primordial germ cells between control and *NANOG*-injected embryos. I found, however, that expression of *goosecoid* was significantly upregulated and expression of *wnt8a* was significantly downregulated in *NANOG/Nanog*-injected embryos, which is consistent with the dorsalized phenotypes of *NANOG/Nanog*-injected embryos.

Preface

This thesis is the result of my own work and not the product of any collaboration. Other scientific results are referenced throughout the body of this thesis and in a bibliography. This thesis is not substantially the same as any that I have submitted for a degree or diploma or other qualification at any other University.

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