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Investigating the dynamics of interchromosomal interactions and CTCF site methylation at the IGF2 locus in mammalian evolution and human disease

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Abbreviations

^{o}C	degree Celcius
µg	microgram
µl	microlitre
µm	micrometre
3C	Chromosome conformation capture
5aza	5-aza-2-deoxycytidine
ACTB	Actin, beta
ACRF	Australian Cancer Research Foundation
APBβ	Amyloid precursor protein
BAC	Bacterial artificial chromosome
BWS	Beckwith-Wiedemann syndrome
cDNA	Complementary DNA
CHORI	Children's Hospital Oakland Research Institute
CpG	5'-C-phosphate-G-3'
CSC	Cancer stem-like cell
CTCF	CCCTC-binding factor protein
ChIP	Chromatin immunoprecipitation
DAPI	4',6-diamidino-2-phenylindole
DMR	Differentially methylated region
DMSO	Dimethyl sulphoxide
ESCs	Embryonic stem cells
FBS	Foetal bovine serum
FISH	Fluorescence *in situ* hybridisation
gDNA	Genomic DNA
IAS1	ICR associated site
IC	Imprinting centre
ICD	Interchromatin domain model
ICN	Interchromosomal network model
ICR	Imprinting control region
IFNγR1	Interferon gamma receptor
IFN-γ	Interferon gamma
IG	Immunoglobin
IGF2	Insulin-like growth factor II
IGF2R	Insulin-like growth factor II receptor
INS	Insulin
iPSC	Induced pluripotent stem cell
LAD	Laminar-associated domain
LCR	Locus control region
LOI	Loss of imprinting
MEF	Mouse embryonic fibroblasts
NCBI	National Centre for Biotechnology Information
ncRNA	Non-coding RNA
NF1	Neurofibromatosis 1
ORc	Olfactory receptor
PBL	Peripheral blood lymphocyte
PCR	Polymerase chain reaction
qPCR	Quantitative polymerase chain reaction
RAH	Royal Adelaide Hospital
ROS	Reactive oxygen species
SINE	Short interspersed nuclear element
SNPs	Single nucleotide polymorphisms

SNRPN	Small nuclear ribonucleoprotein polypeptide N
TCR	T cell receptor
tRNA	Transfer RNA
UBE3A	Ubiquitin-protein ligase 3A
UCSC	University of California, Santa Cruz
WSB1	WD repeat and SOCS box-containing 1

Nomenclature

Throughout this thesis, various forms of conventional notation are used in relation to species-specific nomenclature, particularly for mouse, human, bovine and platypus.

Abstract

Long-range physical interactions between distant sections of DNA have been shown to form complex networks of loops controlling gene regulation and other nuclear functions, which are essential throughout development and disease. These chromatin interactions are remarkably frequent, with interaction patterns varying between cell types, developmental stage and in disease. The chromatin insulator CTCF mediates many of these interactions, and is also thought play a role in the definition of topological domains and preventing the spread of heterochromatin. Binding of the CTCF protein can be methylation sensitive, and few studies have investigated the impact of specific methylation changes at CTCF binding sites on long-range interactions at a particular locus. This form of regulation is particularly important to many imprinted genes, which are important for foetal growth and development, such as the growth factor *IGF2*. Altering the regulation at this locus can affect foetal development and has also been shown to be linked to poor prognosis in several cancers.

The aim of this project was to investigate the important *IGF2/H19* locus in relation to long-range interaction and CTCF binding site methylation, in both developmental and disease contexts. We investigated expression of *IGF2* and *H19* as well as the frequency of long range chromatin interactions at the locus in cattle embryos, comparing purebred and hybrid crosses with known differences in birthweight. This work identified different levels of *H19* expression between the different crosses, although no significant difference was observed in the frequency of the *IGF2/H19-WSB1* long-range chromatin interaction. We have suggested that a different mechanism of regulation at the *IGF2/H19* locus may occurring at this early developmental stage. We also investigated the methylation status of seven CTCF binding sites in the *Igf2/H19* imprinting control region in several ovarian cancer tumours and cell lines, as well as looking at expression of key genes and interaction frequency using DNA Fluorescence *in situ* hybridisation. We identified highly variable DNA methylation patterns at CTCF binding sites in serous ovarian cancer tumours at different disease stages and noted that methylation at each site responded with variable sensitivity to treatment with a common demethylating drug in ovarian cancer cell lines.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree. I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968. The author acknowledges that copyright of published works contained within this thesis resides with the copyright holder(s) of those works. I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

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