Function and Evolution of the piRNA Pathway in the Amniote Gonad and Human Ovarian Cancer

by

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Contents

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List of Publications

Three publications will arise from this thesis. One has been submitted and two are in preparation. Another two research papers and a book chapter were published and a research paper has been submitted by the candidate during her PhD, but the research will not be discussed in this thesis.

Published:

1. Tsend-Ayush E., Lim S.L., Pask A.J., Hamdan D.D., Renfree M.B., Grutzner F. 2009. Characterisation of ATRX, DMRT1, DMRT7 and WT1 in the platypus (Ornithorhynchus anatinus). *Reproduction, Fertility, and Development* 21: 985-991.

2. Rowell D.M., Lim S.L., Grutzner F. 2011. Chromosome analysis in invertebrates and vertebrates. *Methods in Molecular Biology* 772: 13-35.

3. Tsend-Ayush, E., Kortschak, R.D., Bernard, P., Lim, S.L., Ryan, J., Rosenkranz, R., Borodina, T., Dohm, J.C., Himmelbauer, H., Harley, V.R., Grutzner, F. 2012. Identification of mediator complex 26 (Crsp7) gametologs on platypus X1 and Y5 sex chromosomes: a candidate testis-determining gene in monotremes? *Chromosome Research* 20: 127-138.

4. Hrdličková, R., Nehyba, J., Lim, S.L., Grützner, F., Bose, H.R.Jr. Platypus TERT preserves features of TERT genes of ancestral amniotes. *BMC Evolutionary Biology*.

Submitted manuscripts:

1. Lim, S.L., Tsend-Ayush, E., Kortschak, R.D., Ricciardelli, C., Oehler, M., Grutzner, F. Conservation and expression of piRNA pathway genes in male and female adult gonads of amniotes suggest ancient role in germ cell development. *Plos one*.

Manuscript in preparation:

1. Lim, S.L., Ricciardelli, C., Oehler, M., Tan, M.D.D.A.I., Russell, D., Grutzner, F. Overexpression of piRNA pathway genes: a role in the progression of epithelial ovarian cancer. *Int J of cancer*.

2. Lim, S.L. and Grützner, F. Friend or foe? A role for the piRNA pathway in the mammalian ovary and ovarian cancers. *Developmental Biology*.

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List of Abbreviations

Aa	Amino acid
Actb	Beta actin
Ago	Argonaute
Ago3	Argonaute 3
Aldh-1	Aldehyde dehydrogenase-1
anti-5-MeC	Anti-5-methylcytosine antibody
Aub	Aubergine
BAC	Bacterial artificial chromosome
BLAST	Basic Local Alignment Search Tool
BLASTn	Nucleotide BLAST
cDNA	Complementary DNA
Chr	Chromosome
Ck7	Cytokeratin 7
CL	Corpus luteum
Crem	cAMP-responsive element upmodulator
DEPC	Diethylpyrocarbonate
Dig	Digoxigenin-11-UTP
Dnmt3a	DNA-methyltransferase 3A
Dnmt3l	DNA-methyltransferase 3L
Dpp	Days post coitum
Dpp	Days post-partum
EMT	Epithelial to mesenchymal transition
EOC	Epithelial ovarian cancer
Ev	Empty vector
FBS	Fetal bovine serum
FISH	Fluorescence *in situ* hybridisation
Flam	Flamenco
Gapdh	Glyceraldehyde-3-phosphate dehydrogenase
gDNA	Genomic DNA
Gon4l	Gon-4-like (C. elegans)
GSC	Germline stem cell
Gtsf1	Gametocyte-specific factor 1
H&E	Hematoxylin and Eosin
HCl	Hydrochloric acid
HMG	High mobility group
Hr	Hour
hTERT	Human telomerase reverse transcriptase
IAP	Intracisternal-A-particle
IPTG	Isopropyl β-D-1-thiogalactopyranoside
ISH	*In situ* hybridisation
Kb	Kilobase
L1	Long interspersed element-1/Line-1
LTR	Long terminal repeat
Mael	Maelstrom
Min	Minute
miRNA	MicroRNA
MMP	Matrix metalloproteinase

Muc1	Mucin 1
MUSCLE	Multiple Sequence Comparison by Log-Expectation
MYA	Million years ago
NBT/BCIP	Nitrobluetetrazolium chloride/ X-phosphate-5-bromo-4-chloro-3-indolylphosphate
NCBI	National Center for Biotechnology Information
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
nrapiRNA	Non repeat associate piRNA
Nt	Nucleotide
OSE	Ovarian surface epithelium
PBS	Phosphate buffered saline
PDAC	Pancreas ductal adenocarcinomas
PFA	Paraformaldehyde
piNG body	piRNA nuage giant body
piRISC	piRNA-induced silencing complex
piRNA	Piwi-interacting RNA
Piwi	P-element induced wimpy testis
Piwil1	Piwi-like 1
Piwil2	Piwi-like 2
Piwil3	Piwi-like 3
Piwil4	Piwi-like 4
PL2L	PIWIL2-like
rapiRNA	repeat associate piRNA
RISC	RNA-induced silencing complex
RNAi	RNA interference
RT	Room temperature
RT-PCR	Reverse transcription-polymerase chain reaction
SC	Serous carcinoma
SC1	Serous carcinoma 1
SD	Segmental duplication
S	Sec
shRNA	Short hairpin RNA
siRNA	Small interfering RNA
snoRNA	Small nucleolar RNA
SNP	Single nucleotide polymorphism
SSC	Saline-sodium citrate
Stat3	Signal transducer and activator of transcription 3
Ste	*Stellate*
Su(Ste)	*Suppressor of stellate*
Tdrd1	Tudor domain containing protein 1
TE	Transposable element
Vim	Vimentin
X-gal	Abbreviated BCIG for 5-bromo-4-chloro-indolyl-β-D-galactopyranoside
Yy1ap1	YY1-associated protein 1

Abstract

The Piwi-interacting RNA (piRNA) pathway is a RNA silencing pathway which represses the expression of gene and transposable elements (TE) in the gonads via the binding of piRNAs (26-30nt) to their complimentary RNA targets and by influencing the epigenetic makeup of chromatin via interacting with other proteins (e.g. HP1). piRNAs interact with PIWI (P-element induced wimpy testis) proteins and other components such as Maelstrom (Mael) for TE silencing. In addition, *Piwi-like (Piwil)* genes and *Mael* are important for germline stem cell (GSC) production from fly to mouse. The expression of these genes was reported exclusively in mammalian testis despite the presence of piRNAs in mouse testis and ovary. Although the pathway is essential for oogenesis in fly, fish and *Xenopus,* an important role in the mammalian ovary has been in doubt, as female knockout mice (*Piwil1, 2 and Mael*) genes are fertile. In addition *Piwil* genes in particular have undergone lineage specific changes leading to up to 4 *Piwil* genes in most eutherian mammals.

Work presented in this thesis investigates the expression of piRNA pathway genes in the amniote gonad. This confirmed the robust expression of *Piwil* genes and *Mael* in the mammalian testis. Importantly, specific expression of these genes in oocyte and growing follicles was detected in mammals. The extraordinary conservation of piRNA pathway gene expression in germ cells and ovarian somatic cells from fly to human suggests an important role in mammalian gonadal development. A comprehensive bioinformatics analysis of *Piwil* genes provided new aspects towards understanding the evolutionary trajectory of *Piwi like* genes in vertebrates. For example the correction of Xenopus *piwil3* as *piwil1* ortholog clearly showing that *Piwil3* evolved exclusively in eutherian mammals. Finally, based on the expression of piRNA pathway genes in the

ovary, we hypothesised that *PIWIL* genes and *MAEL* may play a part in the origin and progression of epithelial ovarian cancer (EOC). To test this hypothesis, expression of these genes was investigated in postovulatory tissues i.e. the corpus luteum (CL) and inclusion cysts. Preliminary data show that piRNA pathway genes are not expressed in the CL but transcripts are detected in the epithelial cells of inclusion cysts. This raises the possibility that piRNA pathway genes may be involved in the cancerous transformation of epithelial cells.

To test if the piRNA pathway plays a part in EOC progression, and is related to the activity of TEs, the expression of *PIWIL genes*, *MAEL* and *L1* (one of the most abundant TEs in human) was investigated in different types of EOC. Significant upregulation of these genes was found in malignant EOC when compared to benign ovarian tumours. This upregulation might be a result of increased *L1* activity in EOC, or may due to the stem cell like characteristic of malignant EOC. Analyses of the *PIWIL1* transcripts from a malignant EOC show that most of the *PIWIL1* transcripts contain premature stop codons. Therefore, although *PIWIL1* is overexpressed in malignant EOC, the function of PIWIL1 is likely to be compromised in these tumours.

To understand the effects of *PIWIL1* and *MAEL* overexpression on cancer cell invasiveness, *PIWIL1* and *MAEL* were transiently overexpressed in ovarian cancer SKOV3 cells. Overexpression of these genes decreases cell invasiveness, suggesting a repressive role in EOC progression. Hypomethylation of *L1* and chromosome instability was found in ovarian cancers. To understand the genome stability in EOC, primary ovarian cancer cells were established from patient ascites of different stages of

ovarian cancers. FISH analyses showed that 20%-40% of the cells are aneuploid. Thus, this is a good model for understanding aneuploidy in EOC development. Our research provides a better understanding of this ancient yet conserved piRNA pathway in mammalian gonads and ovarian cancers.