Early serous ovarian carcinogenesis:

Understanding the genetic and lifestyle factors

Thesis Submitted

In Fulfilment of the Requirements for the Degree of

Doctor of Philosophy

By

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B.Eng (Biotechnology); M.Sc. (Molecular Genetics)

To

Faculty of Health,

School of Biomedical Sciences and Pharmacy

The University of Newcastle, Australia

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DECLARATIONS PART A

TESTIMONY OF ORIGINALITY

*I hereby certify that this thesis is my own work and contains no material which has been accepted for the award of any other degree or diploma 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 references and acknowledgement has been made in the text. I give consent to the final version of my thesis being made available worldwide when deposited in the University's Digital Repository**, subject to the provisions of the Copyright Act 1968.*

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I hereby certify that the work embodied in this thesis contains a published paper/s/scholarly work of which I am a joint author. I have included as part of the thesis, written statements for each published work, endorsed by my supervisor, attesting to my contribution to the joint publication/s/scholarly work.

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I hereby certify that this thesis is in the form of series of published papers of which I am a joint author. I have included as part of the thesis written statement from each co-author endorsed by the Faculty Assistant Dean (Research Training), attesting to my contribution to the joint publications.

COLLABORATION

I hereby certify that that the work embodied in this thesis has been done in collaboration with other researchers. I have included as part of the thesis a statement clearly outlining the extent of collaboration and under what auspices.

Signature: Date: 05/09/2019

Prathima B Nagendra dd/mm/yyyy

DECLARATIONS PART B

Acknowledgement of authorship

I hereby certify that the work embodied in this thesis contains submitted/published papers of which I am a joint author. I have included a written declaration below endorsed in writing by my supervisor, attesting to my contribution to the joint publication/s/scholarly work.
Publications included as a part of this thesis are listed below:

I the undersigned corresponding author of following publications:

1. Nagendra PB and Tanwar PS. Role of Fallopian tube in pelvic serous ovarian carcinogenesis. Gynaecology Oncology (Submitted)
2. Nagendra PB, Goad J, Nielsen S, Rassam L, Lombard JM, Nahar P, et al. Ovarian hormones through Wnt signalling regulate the growth of human and mouse ovarian cancer initiating lesions. Oncotarget. 2016;7(40):64836-53.
3. Nagendra PB and Tanwar PS. Evidence of early Fallopian tube precursor escape in omental serous ovarian carcinoma. Cancer research (Submitted)
4. Nagendra PB and Tanwar PS. Molecular characterisation of papillary tubal hyperplasia: the putative precursors of low grade serous carcinoma. Gynaecology Oncology (submitted)

Authorize the inclusion of these works and declare that Research Higher Degree candidate, Prathima B Nagendra contributed to the paper/publication. Outlined below are the items that the candidate has contributed towards the fulfilment of these papers:

- Conducted and designed most of the experiments
- Critical analysis and interpretation of results
- Prepared and organized the figures
- Drafting and conceptualizing the manuscripts
- Contributed in formatting initial and revised versions of manuscripts

Signature:
Pradeep S. Tanwar

Date: 05/09/2019
dd/mm/yyyy

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LIST OF PUBLICATIONS INCLUDED AS A PART OF THIS THESIS

Contained in:

CHAPTER 1

Nagendra PB and Tanwar PS. Role of Fallopian tube in pelvic serous ovarian carcinogenesis. Gynaecology Oncology (Submitted)

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List of abbreviations

A	
ANZGOG	Australia New Zealand Gynaecology Oncology Group
ALDH1	Aldehyde dehydrogenase 1 family, member A1
APE	Atypical proliferative endosalpingiosis
B	
BRCA1	Breast cancer type 1 susceptibility protein 1
BRCA2	Breast cancer type 1 susceptibility protein 2
BSA	Bovine serum albumin
BRaf	serine/threonine-protein kinase B-Raf kinase
C	
CK8	Cytokeratin 8
CNV	Copy number variations
D	
DAB	Diaminobenzidine
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulfoxide
DEG	Differentially expressed genes
2D	Two dimensional
3D	Three dimensional
E	
EGFR	Epidermal growth factor receptor
EDTA	Ethylenediamine tetraacetic acid
EOC	Epithelial Ovarian Cancer
4E-BP1	Eukaryotic initiation factor 4E-binding protein 1
EZH2	Enhancer of zeste homolog 2
ERBB2	Erythroblastic oncogene B, a gene isolated from avian genome
ER	Estrogen Receptor
F	
FBS	Fetal bovine serum
FSH	Follicle stimulating hormone
FTE	Fallopian tube epithelium
FIGO	International Federation of Gynecology and Obstetrics
FTSEC	Fallopian Tube Secretory Epithelial Cells
FTCEC	Fallopian tube Ciliated Epithelial Cells
G	
GRH	Gonadotropin releasing hormone
H	
HGSOC	High grade serous ovarian carcinoma
HGSC	High grade serous carcinoma
I	
IgG	Immunoglobulin G
IHC	Immunohistochemistry
IRS1	Insulin receptor substrate 1

IMP3	U3 small nucleolar ribonucleoprotein

K	
KDa	Kilodalton
KRas	Kirsten RAt Sarcoma virus protein
L	
LH	Luteinizing hormone
LKB1	Liver Kinase B 1
LGSC	Low Grade Serous Carcinoma
LGSOC	Low Grade Serous Ovarian Carcinoma
LEF1	Lymphoid enhancer-binding factor 1
LOH	Loss of heterozygosity
M	
Mg	Milligram
Ml	Millilitre
mM	Millimolar
µg	Microgram
µl	Microlitre
µM	Micromolar
nM	Nanomolar
N	
NIH	National Institute of Health
O	
OvCa	Ovarian cancer
OSE	Ovarian surface epithelium
P	
Pax2	Paired box gene 2
Pax8	Paired box gene 8
PBS	Phosphate buffered saline
PI3K	Phosphatidylinositol-4,5-biphosphate-3-kinase
PIP2	Phosphatidylinositol 4, 5-biphosphate
PSC	Pelvic Serous Carcinoma
PTEN	Phosphatase and tensin homolog
PCGA	Pre-Cancerous Genome Atlas
PTH	Papillary tubal hyperplasia
p16	p16INK4a, cyclin-dependent kinase inhibitor 2A, multiple tumor suppressor 1
PR	Progesterone Receptor
R	
RPMI media	Rosewell Park Memorial Institute media
RTK	Receptor tyrosine kinase
RT	Room Temperature
RCN1	Reticulocalbin 1
RRSO	Risk Reducing Salpingo Oophorectomy
RNASeq	RNA Sequencing

S	
SDS- PAGE	Sodium dodecyl sulfate Polyacrylamide gel electrophoresis
SE	Standard error
SCE	Secretory Cell Expansions
SCOUTS	Secretory Cell Outgrowths
STIC	Serous Tubal Intraepithelial Carcinoma
Stmn1	Stathmin-1/ Metablastin /Oncoprotein 18
SBT	Serous Borderline Tumours

T	
TBS	Tris buffered saline
TCGA	The Cancer Genome Atlas
TP53	Tumour Protein 53
TAH-BSO	Total Abdominal Hysterectomy-Bilateral Salpingo Oophorectomy
W	
WES	Whole exome sequencing

Abstract

For the last century, carcinoma has been a consistent Emperor of all maladies. Carcinoma is not a single disease, but a family of diseases which have one common characteristic. Cancer is the loss of cellular regulation, norm and function in a particular cell, leading to uncontrolled growth and disruption of the normal physiology and biology of the organ where it occurs. In the last century, we have succeeded in recognising each of the carcinomas that humans encounter, and have characterised their anatomical, physiological, histological and in most cases molecular features. This has greatly enhanced our ability to treat carcinomas.

40% of all cancer incidences are today treatable with more than 60% of the patients surviving 5 years post incidence. We have not had this kind of success in cases of heterogenous tumours. These are carcinomas which depend on multiple signalling cascades for their growth advantage, thus circumventing traditional chemotherapy and targeted therapies. The other failure is lack of accurate and scalable detection mechanisms, especially in deep seated organs. The combination of these two factors leads to high mortality rates in such tumours.

One such example is the serous ovarian carcinoma. It has two histological subtypes, High grade serous ovarian carcinoma (HGSOC) and Low grade serous ovarian carcinoma (LGSOC). HGSOC is a high heterogeneity carcinoma due to which relapse rates are 60% within 5 years. LGSOC although mostly detected in early stages is inherently chemoresistant leading to poor prognosis. The best way to circumvent such carcinomas is early detection. This needs evolutionary understanding of the disease. Our work focuses on understanding the earliest stages of SOC, drivers of these changes and deciphering the mechanisms of prevention. To achieve this, we probe dysplasia and preneoplasia in the Fallopian tubes, which are one of the sites of origin of SOC. The Fallopian tube secretory epithelial cells (FTSEC) are the closest molecular phenotype to SOC.

Secretory cell outgrowths (SCOUTS), p53 signatures and papillary tubal hyperplasia are the dysplastic lesions acting as precursors of SOC in the Fallopian tube epithelium (FTE). FTE is primarily constituted of secretory and ciliated cells and the ratio between these cells is key to maintaining homeostasis. The disruption of this balance is the first step to SOC. The secretory cell type dominates and outgrows ciliated cells leading to dysplasia. All three aforementioned preneoplasia are made up of secretory cells.

We undertook molecular characterisation of these three lesions. The morphological and immunohistochemical aspects of the lesions are well charted. Through histopathological analysis, use

of microdissection, next generation animal models and next generation sequencing we sought to characterise molecular nature of these lesions.

We found that Wnt signalling pathway is the driver of SCOUTS. We have established an accurate mouse model by constitutive activation of β-Catenin specifically in the FTSECs. We have further used this model to probe the role of ovarian hormonal milieu in progression of HGSOC. We found progesterone mitigates progression of SCOUT lesions and oestrogen enhances this progression. This is the first mouse model to accurately mimic early serous ovarian carcinogenesis.

By molecular probing of morphologically normal Fallopian tubes in a case of p53 null-omental high grade serous carcinoma three years post risk reducing salpingo oophorectomy (RRSO), we found tumour associated aberrations, specifically identical *TP53* mutations in FTE before RRSO and the omental tumour. This establishes the clonal identity and proves the precursor escape model in pelvic serous ovarian carcinomas.

Papillary tubal hyperplasia are known to be putative precursors for LGSOC, atypical endosalpingiosis (AES) in the peritoneum and also intermediate Serous Borderline tumours (SBT). They are the only known precursors in the FT. LGSC is also the closest to the FTSECs. As no molecular attributes were known, through Whole exome sequencing (WES) and RNA sequencing (RNASeq) we have characterised molecular aberrations and also have established them as precursors for the accompanying AES. Study of these three lesions suggests, salpingectomy can be a good preventive measure in at least patients with high risk of ovarian cancer incidence. Progesterone can also be used as an effective preventive measure.