Genesis of ovarian cancer: understanding the mechanisms of oviductal epithelial cell homoeostasis

Thesis Submitted

In Fulfilment of the Requirements for the Degree of

Doctor of Philosophy

By

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TO

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STATEMENT OF ORIGINALITY

I hereby certify that the work embodied in the thesis is my own work, conducted under normal supervision. The thesis contains no material which has been accepted, or is being examined, 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 reference has been made. 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 and any approved embargo.

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DECLARATIONS (PART A)

I hereby certify that the work embodied in the thesis is my own work, conducted under normal supervision.

The thesis contains scholarly work of which I am a co-author. For each such work a written statement, endorsed by my supervisor, attesting to my contribution to the joint work has been included.

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DECLARATIONS (PART B)

I hereby certify that to the best of my knowledge the work for this thesis entitled "**Genesis of ovarian cancer: understanding the mechanisms of oviductal epithelial cell homoeostasis**" has been carried out under my supervision, in the School of Biomedical Sciences and Pharmacy at The University of Newcastle, Australia, and that all of the scholarly work described in chapters 2, 3 and 4 has been carried out by the Research Higher Degree candidate Arnab Ghosh. Outlined below are the items that the candidate has contributed towards the fulfilment of the work described in this thesis:

- Contributed to the conception and design of the studies
- Conducted and designed most of the experiments
- Critically analysed and interpreted the results
- Prepared and organised the figures
- Contributed in drafting and conceptualising the thesis chapters
- Contributed in formatting initial and revised versions of the thesis chapters.

Supervisor Signature: …… Date: 24/ 04/ 2019

Pradeep S. Tanwar dd/mm/yyyy

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List of abbreviations

3D	3-dimensional
HGSC	High-grade serous carcinoma
CK8	Cytokeratin 8
FT	Fallopian tube
FRT	Female reproductive tract
WRN	Wnt3a-Rspodin3-Noggin
CM	Conditioned media
DAPI	4', 6-diamidino-2-phenylindole
E2	Estradiol
ECM	Extracellular matrix
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
BRCA	Breast cancer antigen
OSE	Ovarian surface epithelium
ER	Estrogen receptor
PAX8	Paired box 8
Ac-TUB	Acetylated Tubulin
YFP	Yellow fluorescent protein
FACS	Fluorescence-activated cell sorting
FOXJ1	Forkhead Box J1
FFPE	Formalin-fixed paraffin-embedded
FBS	Fetal bovine serum
DMEM	Dulbecco Modified Eagle Medium
DPBS	Dulbecco's phosphate-buffered saline
WNT	wingless-type MMTV integration site family
GFP	Green fluorescence protein
TBST	Tris-buffered saline-Tween 20
H2B-GFP	H2bj protein and green fluorescent protein fusion protein complex
LGR5	Leucine-rich repeat-containing G-protein coupled receptor 5
H & E	Hematoxylin and Eosin
HBSS	Hanks balanced salt solution
HGFP	H2bj protein and green fluorescent protein fusion protein complex
IGF	Insulin-like growth factor
LEF	Lymphoid enhancer binding factor

LRCs	Label retaining cells
PFA	Paraformaldehyde
PND	Post-natal day
PTEN	Phosphatase and tensin homolog
PR	Progesterone receptor
TAM	Tamoxifen
TCF	T-cell factor
eGFP	Enhanced green fluorescence protein
TGF β	Transforming growth factor β
TP53	Tumor suppressor p53
DBZ	Dibenzazepine
SCE	Secretory cell expansions /
SCOUT	Secretory cell outgrowths
STIC	Serous tubal intraepithelial carcinoma
STIN	Serous tubal intraepithelial neoplasia
BMP	Bone morphogenetic protein

Abstract

Fallopian tube (FT) (also known as oviduct in mouse) is a tubular structure that connects the ovary with the uterus. It is an important part of the female reproductive tract (FRT), as it provides the site for fertilisation inside its lumen. There are two separate events around the process of fertilisation, which are known as pre- and post-fertilisation event. The pre-fertilisation event requires the successful transfer of the healthy and viable gametes into the site of fertilisation to form an embryo. Whereas, the post-fertilisation event entails with embryo survival and successful embryo transport into the uterus for implantation. All these processes require an efficient function of every epithelial cell of FT lumen because they are in direct contact with the gametes and the embryo. Studies showed the faulty function of this epithelium leads to multiple reproductive disorders such as infertility, ectopic pregnancy and ovarian cancer. Clinical examination revealed that the FT epithelium of BRCA1/2 germline mutated patients who are more susceptible to ovarian cancer consist of the secretory cell only precancerous lesions. Thus in requirement of understanding this disease biology, there is a need to understand the healthy FT epithelial homeostasis first. Therefore, in my thesis, first I identified secretory cells as the stem/progenitor cells of mouse FT epithelium by using lineage tracing approach. This secretory cells can self-renew and differentiate into the ciliated cell to maintain the FT epithelial homeostasis. Upon identification of a stem/progenitor cell population, there is a need to identify the signalling pathways that are involved in the process of stem/progenitor cell self-renewal and differentiation. In the same chapter (Chapter 2), we also identified the Wnt/β-Catenin signalling pathway that guides the secretory cell self-renewal and stemness. We have provided evidence for the Wnt/β-Catenin signalling involvement in ciliated cell differentiation process (Chapter 3). We showed the role of Wnt/β-Catenin signalling pathway is indispensable for ciliated cell differentiation in mouse oviduct epithelium.

Cells in the FRT are highly sensitive to ovarian steroid hormones such as estrogen and progesterone. Fluctuations in these hormones level during the oestrous/menstrual cycle also alter the morphology of the FT epithelial cells. In chapter 4, we showed the effect of ovarian hormone estrogen for a single epithelial cell fate determination. Our result showed estrogen could induce ciliated cell differentiation in mouse oviduct epithelium. However, the underlying mechanism of this estrogen-mediated ciliated cell differentiation has been remained unclear. We showed in the absence of the Wnt/β-catenin signalling pathway, estrogen do not induce any ciliated cell differentiation in the mouse oviduct epithelium. Overall, my thesis first time identified a stem/progenitor cell population in the mouse oviduct epithelium. Later we also defined the requirement of Wnt/β-catenin signalling pathway for the process of these stem/progenitor cell self-renewal and differentiation in mouse oviduct epithelium. In addition,

we also proved in mouse oviduct signals from the Wnt pathway are the link between estrogen and ciliated cell differentiation process.

Thesis overview

1. Introduction:

Reproduction is an essential biological process that is required for the survival of all mammalian species. This process entails the interaction between female and male gametes to form the first form of life, the zygote, which eventually develops into an embryo. This whole process of gamete interaction, known as fertilisation, takes place in the fallopian tube, a part of the female reproductive tract (FRT) in mammalian species. The fallopian tube (FT) (in mice, referred to as oviduct) is a narrow tubular structure that serves its purpose by providing an optimum environment for fertilisation and early embryo development. These two functions are mainly divided into two separate events during the process of fertilisation. The first part is considered as a pre-fertilization event when the female gamete travels from the ovarian side, and the male gamete travels from the uterine side to meet at the site of fertilisation named the ampulla. Whereas, the second event is considered as the post-fertilisation period where the newly formed embryo spends its initial 3-4 days in the FT lumen and then is transported into the uterus for implantation (1). In both events, the FT epithelium plays a crucial role as it is in direct contact with the gametes and the embryo. The FT epithelium is pseudostratified and mainly consists of two cell types: ciliated and secretory cells. Both of these cell types have their unique functions for a successful pregnancy, where ciliated cells transport the gametes and the embryo. On the other hand, secretory fluids secreted from the secretory cells create an optimum environment for gamete and embryo survival. The existence of two other cell types named peg/intercalated cells and basal/reserve cells were also described in the literature (2-4). However, many studies have now identified that the peg cells are exhausted secretory cells that have lost a considerable amount of apical cytoplasm as secretion into the FT lumen and the basal cells are not epithelial cells but T lymphocytes (5-7).

Other than the FT epithelium, the contraction of smooth muscle cells is also essential for successful gamete transport. Interestingly, FT epithelium here also plays an indirect role by secreting Prostaglandin E (PGE) and Prostaglandin F (PGF) that eventually regulate smooth muscle contraction (8). Overall, the importance and significance of FT epithelium in reproductive functions highlight the need for in-depth investigations into the FT epithelial biology and the underlying mechanisms of its maintenance during homoeostasis and disease.

2. Aims:

Studies presented in this thesis aim to,

2.1. Identify the stem/progenitor cell population in the fallopian tube epithelium.

2.2. Role of Wnt/β-catenin signalling and ovarian hormones to maintain fallopian tube epithelial homeostasis.

2.3. To Understand the role of Wnt pathway genes and the interplay of ovarian hormones to maintain fallopian tube epithelial homeostasis and disease biology.