WOMEN IN CANCER PROFILE

My pathway to understanding the role of the tumour microenvironment in cancer progression

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I grew up in an Italian family in Adelaide, South Australia, with an older sister and younger brother. Both my parents left their families and migrated to Australia after the Second World War for a better life. Growing up in southern Italy in the 1930s, it was uncommon for children to study beyond primary school as older children needed to help their parents on the farm and help raise the younger children. My father started working as a labourer at General Motors Holden a week after he arrived in Adelaide in 1958 and also worked as a market gardener throughout his life. My mother worked in a fruit preserving factory, stopping formal work after she had her first child, but always helped my father in the market garden. We regularly helped our parents in the market garden too during school holidays and got a small glimpse of what it was like for them growing up in Italy. My dad always regretted that his parents did not give him the opportunity to complete a secondary education. Growing up we were always encouraged to study hard as my father believed it would be an advantage for us in the long run. Our Mum played an active role in our early education even though she herself could barely read and write. She only completed 2 years of primary school, but always encouraged us and patiently listened to us read. I remember dad testing me on my times tables and being scorned when I got them wrong. I commenced school a year younger than other students in my class and was asked to repeat grade 2 when I was found to be behind. I was devastated, but this failure at a young age contributed to my strong determination to succeed and not give up when I found something difficult.

I went to an all girls' Catholic High School (St Mary's College) in Adelaide, South Australia, where my final year maths and chemistry classes were small, just 12 and 7 girls, respectively. I was very shy but thrived in this non-threatening environment. I loved chemistry the most at school and particularly thank my chemistry teacher for her patience and encouragement that I could succeed. My older sister led the way and all three of us completed tertiary qualifications, which was unusual for Italo-Australians in the 1990s. She completed a Ph.D. in psychology in 1990 and my younger brother graduated with a medical degree in 1996. Although our parents never openly told us, I know they are proud of what we have achieved. I owe them for providing me with an education and for instilling in me a strong determination to succeed.

I was the dux of my class in the Bachelors of Applied Science in Medical Laboratory Science, 1985, at the South Australian Institute of Technology (now University of South Australia). I intended to commence a career in a diagnostic medical laboratory but accidently got involved in research after several unsuccessful job applications. My first job was as a research assistant in the Department of Surgery, Flinders University of South Australia with Professor Wayne Tilley. There, I worked on a project that examined the role of oestrogen in the normal development of the prostate gland and found that oestrogen receptor was expressed by the stromal cells (Tilley *et al.* 1989). I will always be indebted to Wayne Tilley for giving me the opportunity to be involved in my first research project. It was this project that gave me an appreciation of stromal–epithelial interactions and encouraged me to continue my

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own research in this field. I was fascinated by the work of Cunha and coworkers who showed that it was the stromal cells that could instruct the development of glandular tissues (Cunha *et al*. 1980, Cunha 1984). I realised too that extracellular matrix (ECM) was not just amorphous connective tissue 'goo' but provided instructive clues to epithelial cells and wanted to understand this more. My sister greatly inspired me to embark on a higher degree after I finished my undergraduate degree in medical laboratory science. I went on to do a Masters Qualifying and initially a part-time Masters, which was converted to a full-time Ph.D. candidature after I was successful at attaining a Flinders University Scholarship. As a Ph.D. student, I did get the opportunity to work part-time in a diagnostic laboratory, but it did not take long for me to realise that research was much more fulfilling for me.

The focus of my Ph.D. studies was on the role of the stroma in regulating growth and function of the prostate gland. Although earlier studies by other investigators had implicated the stroma in the development of benign prostatic hyperplasia (BPH) and associated clinical problems (urinary retention), at this time very little was known about the contribution of the stroma to normal and abnormal growth of the prostate gland. I thought a better understanding of the mechanisms controlling normal prostatic growth and function of the prostate would provide insight into the development of abnormal growth of the prostate gland. I established an *in vitro* system for studying prostatic smooth muscle cell (SMC) growth and function (Ricciardelli *et al*. 1989) and showed that SMC proliferation and steroid hormone receptor expression could be controlled by opposing effects of androgen and oestrogen (Ricciardelli *et al*. 1994). The complex interplay of steroid and growth factor regulation of SMC proliferation demonstrated in these studies suggested that an imbalance in the production or modulation of growth factors in the prostate gland with age could lead to SMC hyperplasia and consequent development of BPH pathology. I will always be grateful to my supervisor Associate Professor David Horsfall for his encouragement to complete my Ph.D. studies.

I did not take up any postdoctoral position outside of Australia due to family commitments in Adelaide. My husband was the only child in his family and we both needed to remain in Adelaide to help care for his elderly parents. After commencing my own family in 1997 and working in part-time appointments for several years (1997–2004), it became too difficult to move to another country to further my research career. I often wonder about the opportunities I missed but greatly value my postdoctoral experiences in Adelaide, as I still had the opportunity to learn many new techniques and be involved in world-class research. Balancing family life and work was always a juggle but also very rewarding. Staying in Adelaide I had the continual support from my family and the inspiration to keep going. I received several highly competitive fellowships along the way (Faculty of Health Sciences, University of Adelaide and Cancer Council SA), which enabled me to build a Reproductive Cancer Research Group in the Discipline of Obstetrics and Gynaecology at Robinson Research Institute, University of Adelaide. I was recently awarded the inaugural Lin Huddleston Ovarian Cancer Research Fellowship from Cancer Council SA (2016–2020).

My first postdoctoral position focussed on the expression of ECM proteoglycans in prostate cancer and their role in prostate cancer progression with Associate Professor David Horsfall funded by National Health and Medical Research Council (NHMRC), Australia. Although studies in the 1980s had demonstrated that prostate cancer tissues were enriched for the glycosaminoglycan, chondroitin sulphate (CS) compared with non-malignant prostate tissues (De Klerk 1983), my seminal studies demonstrated that the peritumoural stroma was the source of the increased CS (Ricciardelli *et al*. 1997). Furthermore, I demonstrated that the level of CS in the peritumoural stroma was strongly associated with development of metastatic disease and an independent predictor of progression-free survival in a cohort of clinically localised prostate cancer (Ricciardelli *et al*. 1997, 1999). These results demonstrated for the first time that a component of the reactive stroma could be useful in predicting the metastatic potential of early stage prostate cancer.

Our subsequent work led to the identification of versican as the CS proteoglycan being associated with metastatic disease in prostate cancer (Ricciardelli *et al*. 1998). Versican levels correlated with CS levels in the prostate and showed a similar association between elevated expression in the peritumoural stroma and disease progression to that observed for CS (Ricciardelli *et al*. 1998). Patients with high stromal versican had a significantly higher incidence of progression than patients with low versican. I also showed that versican expression was also strongly associated with cancer relapse in a cohort of early stage breast cancer (Ricciardelli *et al*. 2002). These studies suggested that regulating stromal cell secretion of versican into the peritumoural stromal matrix could be an important factor in cancer progression.

Studies with Ph.D. students Andrew Sakko and Sue Suwiwat funded by Cancer Council SA demonstrated

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that cancer cells could induce the secretion of versican by cancer-associated fibroblasts (Sakko *et al*. 2001, Ricciardelli *et al*. 2002, Suwiwat *et al*. 2004). This was mediated predominantly by the cancer cell-derived mediator, transforming growth factor beta 1 (TGFβ1). Functional studies went on to demonstrate that versican purified from prostatic fibroblast culture medium could inhibit prostate cancer cell attachment to ECM components (Sakko *et al*. 2003). Studies with a Ph.D. student Miranda Ween showed that cancer cells could assemble versican and hyaluronan (HA) into a pericellular sheath to aid motility and invasion of prostate and ovarian cancer cells (Ricciardelli *et al*. 2007, Ween *et al*. 2011*a*). These landmark studies showed that cancer cells could recruit stromal components to remodel their pericellular environment to promote their motility and invasion.

I moved to the newly established Dame Roma Mitchell Cancer Research Laboratories (Hanson Institute, Department of Medicine, University of Adelaide) in January 2002 with Professor Wayne Tilley and worked on NHMRC-funded projects during 2002–2004 including 'The role of androgen receptor in breast cancer' and 'Prognostic importance of androgen receptors in epithelium and stroma in early stage prostate cancer'. During this time, I set up the immunohistochemistry laboratory and developed immunofluorescence and confocal microscopy techniques to investigate the trafficking of androgen receptor and androgen receptor-interacting proteins in breast and prostate cancer cells (Jia *et al*. 2004, Butler *et al*. 2006, Buchanan *et al*. 2007). I showed that androgen receptor in the prostatic stroma cells is an important determinant of disease relapse in early stage prostate cancer (Ricciardelli *et al*. 2005). Elevated expression of HER-2/neu and androgen receptor could identify a subgroup of patients with non-organ-confined disease (Ricciardelli *et al*. 2008). I also contributed to key research showing that androgen receptor expression is associated with breast cancer outcome in ERα-positive disease (Peters *et al*. 2009).

As a Cancer Council SA Fellow, I moved to the Department of Obstetrics and Gynaecology, University of Adelaide, in 2005, in order to enhance my ability to study ECM proteins and their role in stromal–epithelial interactions. The move to this department allowed me to closely collaborate with Professor Ray Rodgers who has an international strength in ECM proteins, including a focus on the role of versican in ovarian function (McArthur *et al*. 2000, Rodgers *et al*. 2000). I also established a collaboration with Associate Professor Darryl Russell from the same department, who has a strong interest in a protease enzyme (ADAMTS1), which can specifically cleave versican protein and plays an important role in ovulation (Russell *et al*. 2003*a*,*b*). Using the transgenic mammary cancer model (MMTV-*PymT*), we found high levels of cleaved versican in the peritumoural stroma of primary tumours that metastasise to the lung whilst ablation of the protease gene for Adamts1 resulted in reduced versican and reduced pulmonary metastasis (Ricciardelli *et al*. 2011). This study provided important *in vivo* evidence that Adamts1 could specifically promote mammary tumour growth and progression to metastasis.

I have continued my work on the tumour microenvironment and recently explored the ovarian cancer–peritoneal cell interaction together with Professor Martin Oehler to increase our understanding of the molecular mechanisms involved in ovarian cancer metastasis. These studies have led to the identification of several proteins that are mechanistically involved in the peritoneal spread of ovarian cancer and may serve as novel ovarian cancer biomarkers and/or therapeutic targets. Studies with Ph.D. students Noor Lokman and Miranda Ween identified a key link between the annexin A2 signalling pathway and activation of the plasminogen–plasmin system (Ween *et al*. 2011*b*, Lokman *et al*. 2013, Ricciardelli *et al*. 2015). Our findings, together with published literature, support the notion that ECM processing via the plasminogen–plasmin pathway promotes the colonisation and attachment of ovarian cancer cells to the peritoneum and actively contributes to the early stages of ovarian cancer metastasis. Targeting the annexin A2 signalling pathway with annexin A2 neutralising antibodies (Lokman *et al*. 2013) or the plasminogen–plasmin system with plasmin inhibitors are both promising strategies to inhibit ovarian cancer metastasis.

Recent studies focussing on the tumour microenvironment have identified that the ECM molecule HA, plays an important role in both ovarian cancer invasion and the development of chemotherapy resistance (Ween *et al*. 2011*a*, Ricciardelli *et al*. 2013). Our studies have confirmed that HA plays an important role in promoting attachment of cancer cells to peritoneal cells via interactions with the HA receptor, CD44 (Ween *et al*. 2011*a*). We have also shown that HA can induce resistance to the chemotherapeutic drug carboplatin and increase the expression of ABC transporters that function as drug efflux pumps in ovarian cancer cell lines (Ricciardelli *et al*. 2013). These *in vitro* observations concur with a recent study highlighting that acquired chemotherapy resistance in ovarian cancer patients is associated with

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the increased expression of the ABC transporter, *ABCB1* (Patch *et al*. 2015). Importantly, we have demonstrated that serum HA levels are increased in ovarian cancer patients following chemotherapy and at recurrence, and can predict prognosis (Ricciardelli *et al*. 2013). Reducing HA production is, therefore, a promising strategy to improve ovarian cancer survival in patients with chemoresistant disease and a focus of ongoing research.

All of this work would not have been possible without my husband Leo's unconditional support (who has been with me in every step of the way) and my children Laura and Michael for their understanding when I was home late or did not attend all their school activities. My ultimate aim is to make a difference and improve survival outcomes for people diagnosed with cancer. Recently, I was touched by cancer in a personal way when my sister was diagnosed with advanced breast cancer. My personal experience has made me even more determined to work as hard as possible to make a difference.

Declaration of interest
The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of this profile.

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