WOMEN IN REPRODUCTIVE SCIENCE

Discovering science and the ovary: a career of joy

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Abstract

My career has been about discovering science and learning the joys of the discovery process itself. It has been a challenging but rewarding process filled with many exciting moments and wonderful colleagues and students. Although I went to college to become a French major, I ultimately stumbled into research while pursuing a Masters Degree in teaching. Thus, my research career began in graduate school where I was studying NAD kinase in the ovary as a possible regulator of steroidogenesis, a big issue in the late 1960s. After a short excursion of teaching in North Dakota, I became a postdoctoral fellow at the University of Michigan, where radio-immuno assays and radio receptor assays had just come on the scene and were transforming endocrinology from laborious bioassays to quantitative science and of course these assays related to the ovary. From there I went to Baylor College of Medicine, a mecca of molecular biology, cloning genes and generating mouse models. It has been a fascinating and joyous journey.

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Early days, growing up

My career in science developed by default and a whole lot of luck. I was born in Exeter and grew up in Durham, New Hampshire. My father was the only person in his family to go to college, all others worked in the shoe mills. My mother was a graduate of physiology at Mount Holyoke College; somehow they met at a gas station in NH where my Dad was pumping gas. They married and eventually, after starting as an English major, my Dad became a professor of geology at the University of New Hampshire in Durham. My father's expertise in geology meant that he surveyed prospective sites for mica mines in Vermont during WWII; I was a WWII baby.

I went to Oyster River High School in Durham; I loved Latin and French. Chemistry and Biology were just OK. My passions were horses and figure skating. I took riding lessons and competed; blue ribbons were the goal. I took figure skating lessons in Durham from talented UNH graduate students on an outdoor rink in the winter and in Boston at the Boston Figure Skating Club from September to May. My dear mother drove me to Boston on Friday afternoons and I would study along the way leaving her to make the 3-h round trip essentially by herself. I passed some of the required compulsory figure skating tests but never had the time or the temperament (too shy) to excel in free style. I dearly loved the sport but was clearly not bound for the Olympics!

Most memorable of all were the many summers my family spent on my grandparents' farm in NH where we had horses and farmed the old-fashioned way ([Fig. 1](#)). We made hay with a horse-drawn wooden hay wagon, using old-fashioned hay forks and pitched loose hay into the mow of an 18th century barn constructed with huge wooden beams and wooden pegs. There were no nails! My mother's stern discipline and demand for excellence ensured that the hay was made to perfection. Her barn would not burn down with hay that was not dry! When we were not haying or weeding the vegetable garden and if the day started with clear blue skies and sunshine (rare in NH), we would head for the mountains to enjoy a vigorous hike or to the seashore (NH has all of 11 miles!) for a swim (dip!) in the cold Atlantic. I had a wonderful life growing up! And I still treasure all the memories. Luckily for me, I inherited the old farm and still have Mother's helpers to run it! I just don't get there very often. A brief personal profile along with a list of some of my published research is presented in [Box 1](#).

College days, more growing up

From this wonderful, idyllic life surrounded by the lakes, mountains and the seashore of New Hampshire, I went off to Oberlin College (Ohio) in the middle of a corn field to become a French major. I had been inspired by

Box 1: Prof. JoAnne S Richards, PhD

I am currently a professor in the Department of Molecular and Cellular Biology (MCB) and the Dan L Duncan Cancer Center at Baylor College of Medicine in Houston, Texas. I have been in MCB Department since 1981 and have been honored to have many wonderful faculty colleagues, postdoctoral fellows and graduate students as well as visiting faculty such as Dr Larry Espey from Trinity University in San Antonio, Texas and Dr. Masayuki Shimada, Hiroshima University, Japan. Collectively, we have worked to understand the molecular and cellular mechanisms that regulate ovarian follicular development, ovulation and ovarian cancer. There have been many exciting moments in my career but perhaps the most memorable was discovering a second antigenic form of cyclooxygenase, now known as cyclooxygenase 2 (COX2) or prostaglandin synthase 2 (PTGS2). It was memorable because it was so unexpected and because it took us so long to figure it out. But once we had the right reagents the story unfolded rapidly. Many people influenced my career but I did not have any one female role model that inspired my decision to work in science. I sort of stumbled into research. However, my mother was a mighty force, a strong and independent woman. Although getting grants and maintaining funding affects everyone in science, I have always enjoyed the pursuit of science and the people in science. One has to have a love of discovery and adventure and be willing to take failure. Science is a mindset and I think one either has it or not. Science is not about making money; rather, it is the people and the hope and joy of discovery. It is the ideas and the puzzles of nature that provide the rewards and inspiration in science, with the ultimate hope of improving health for everyone.

Some important articles

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an engaging teacher who taught our French class in my senior year of high school and who had just graduated from Oberlin! The summer after my freshman year, I went on a French summer program in France and that was my first time abroad. The adventure was a scary but eye-opening and wonderful experience for a naïve NH girl! But my goal to become a French major changed in my sophomore year when I had difficulty using headphones to distinguish recorded French conversation (little did I

know that I had a hearing problem). I also grew tired of analyzing French literature. Luckily, I was taking all the premed biology courses, not because I was intending to be a premed and go to medical school but because I was keenly interested in biology and eventually, like my mother, biology became my major. I had some traumatic times at Oberlin adjusting to a confining environment away from the mountains, seashore and farm. There was no way to escape by air by sea or by land. Luckily,

New Hampshire Farm in the Spring and Fall

Figure 1 New Hampshire, growing up. New Hampshire is a beautiful state, especially in the spring with lilacs and apple blossoms and in the fall when the landscape is resplendent in flaming colors. Our family farm dating to 1820 provided me with a rich love of nature, hard work in the hay fields and independence. The farm also provided me with a sense of purpose that by a circuitous route eventually led me to science.

there was an ice skating rink, where I could escape but only in the winter!! I did discover tennis but played only sporadically. In the end, I graduated from Oberlin and learned that despite all these years of study and acquisition of 'knowledge', I was absolutely useless and unemployable in the big world. My dear parents had deceived me!!

So, after spending a wonderful summer working in beautiful and exotic Yellowstone Park, I trudged off to graduate school to become a high school biology teacher through the MAT (Master's in Arts and Teaching) program at Brown University. I learned the first day of practicing teaching that this was not my calling because I had absolutely no disciplinary powers whatsoever! Luckily, the program required a research experience and that evolved into my applying for the PhD program in Physiological Chemistry or Biochemistry at Brown. This was my first exposure to research and research in the ovary, with a focus on steroidogenesis, NAD kinase and electron microscopy. Isolating steroid hormones was a big thing in the late 1960s and it was an exciting time! The steroidogenic pathway was being mapped out and the biological functions of these hormones was being discovered. But steroid hormone receptors had not quite yet come on the scene.

Postdoctoral days and the call of research

After receiving my PhD degree from Brown University, I spent a year teaching in the Biology Department at the University of North Dakota. This was my second exposure to the American West and the wide-open spaces. It was captivating. I spent two summers teaching on a Native American reservation in Killdeer, ND, hoping

to inspire some of Native Americans to teach in their own school systems. But the University Faculty urged me not to stay in ND but to go on for a postdoctoral fellowship. The question was where ... I did not really know anyone and the schools in the West did not have money. So I went through the journal 'Endocrinology', figuring that anyone who was publishing had money and consequently, I joined the Reproductive Endocrinology Program (fondly known as REP) at the University of Michigan (1972). REP had Rockefeller and Ford Foundation grants. This was a time when there was a keen interest to measure peptide and steroid hormones and develop contraceptives! This is where my career in reproductive endocrinology started. Radioactivity had entered the biological world. Radio-immunoassays were being developed and for the very first time allowed endocrinologists to accurately quantitate hormones in serum! Rosalyn Yalow was a co-winner of the 1977 Nobel Prize for the first insulin radioimmunoassay. Prior to this development, hormones could only be measured by laborious bioassays! Now using radioactively labeled reagents and computer technology, many samples could be analyzed rapidly! (The computer at the University of Michigan at that time encompassed an entire building!! Now a laptop can do the same thing.) What a revolution! Radio-immunoassays soon led to radio-receptor assays and the realization that not only hormones in serum but also hormone receptors in tissues were hormonally regulated in a cell-specific manner and changed during follicular development. REP was one of the MECCAs for these new assays.

In the early 1970s, the concept of receptors was just beginning to emerge based on new technologies for radiolabeling protein and steroid hormones. The

'retention' of these radiolabeled hormones in target tissues led to the concept of specific binding factors or receptors. My research in REP provided some of the first evidence for nuclear estradiol receptors in granulosa cells using nuclear radio-receptor assays (Richards 1975). Subsequently, I with others showed that estradiol- and FSH-induced receptors for luteinizing hormone (LHCGR) in granulosa cells of rat preovulatory follicles. The acquisition of LH receptors in granulosa cells of preovulatory follicles determined which follicles ovulate in response to the LH surge. The induction of the LHCGR in preovulatory follicles is now the hallmark of healthy preovulatory follicles in all mammals (Richards 1978, 1979, 1980) (Fig. 2). We also showed that responses of granulosa cells to FSH increased as follicles developed and in response to estradiol. This was not associated with marked changes in FSH receptor levels, but to other downstream events that actually still remain to be clearly defined but involve enhanced production of cAMP by adenylyl cyclase (Jonassen *et al.* 1982). We also showed that in corpora lutea of rats, the induction of the LH receptor was dependent on prolactin, not FSH and estradiol! It was a truly awesome and exciting time in ovarian biology including the very first Ovarian Workshop and contacts with ovarian experts of the time (Cornelia Channing, Roger Short, Joanne Fortune, David Armstrong, Jennifer Dorrington, Roy Greep, Griff Ross, Jock Findlay, Mary Hunzicker-Dunn, Gilbert Greenwald; to name just a few). Even though the REP labs were housed in a dark, gloomy, subterranean basement of the medical center, this did not suppress the magnificent glow of research. The world of cell signaling had burst on the scene.

Faculty: A Rees Midgely, Gordon Niswender, P Landis Keyes, Anita Payne, Jerome Menon (Jan Uilenbroek; visiting from the Netherlands).

Postdoctoral Fellows: James Ireland, Riaz Farooqui, John Holt, Ron Carson, Katryna Bogovich (Geula Gibori, postdoc with Landis Keyes).

Graduate students: Meena Rao, Julie Jonassen (Graduate Students with A Rees Midgely: Anthony J Zeleznik, Anne Hirshfield, P Bagavandoss, Michel Sanders).

Establishing a research career in Texas

Unfortunately and regrettably, things in REP changed and dissolved when a new Chair of Pathology came on board at the University of Michigan. He wanted the space that housed REP! The lesson learned is that institutions don't care about you. Do what you do because you are dedicated to a cause. It was time to move on. I wrote many letters and was surprised, and honored, to hear from Dr Bert O'Malley. I never dreamed I would live in Texas, but I do remember leaving Ann Arbor in a blizzard and arriving on a different flight for Houston where Lutz Birnbaumer was hosting my interview. Although I was

Radio-receptor assays and the induction of the LH receptor

Figure 2 Early-day radio-receptor assays: The 1970s heralded in the use of radioactivity in radio-immuno assays and radio-receptor assays allowing endocrinologists to quantitate hormones and hormone receptors such as the LH receptor for the very first time. The induction of the LH receptor in granulosa cells of preovulatory follicles was documented not only by radio receptor assays following an i.v. injection of radio-iodinated hCG (panel A; black liver grain) but also following the cloning of the LH receptor gene and Northern Blot analyses of RNA prepared from hypophysectomized (H) rats treated with estradiol (HE), FSH (HF) or estradiol and FSH (HEF) (panel B). A major question plaguing endocrinologists for years was how does LH acting on mural granulosa cells impact the function of cumulus cells surrounding the oocyte (panel C). (Adapted from Segaloff *et al.* 1990, Richards *et al.* 2015).

not on the scheduled flight, we eventually connected ... this was before cell phones! Lutz is also memorable at this time in my career because he was the only person who ever recognized that I was hard of hearing. He came to my defense one time to say, 'She is not stupid, she just can't hear'. I was shocked, and needless to say, quickly got hearing-aids. My ultimate move to the Cell Biology Department at Baylor College of Medicine allowed me to enter yet another new world, that of modern molecular biology and the cloning of genes. It was clear as soon as I arrived that if I didn't have a cloned gene in my pocket I wasn't going anywhere in biology or endocrinology. Surrounded by eminent faculty who supported my early career, my path in molecular endocrinology of the ovary had begun. I am forever indebted. I was also fortunate to have talented postdoctoral fellows from Norway and Sweden who generated the first lambda phage ovarian cDNA library and cloned the type II beta regulatory subunit of cAMP-dependent protein kinase (PKA RII beta) using a highly specific antibody from colleagues in Germany, Suzanne Lohmann and Ulrich Walter (Jahnsen *et al.* 1986). This

led to further studies to sequence and describe the RII β promoter to determine the hormonal regulation RII β expression and to analyze the phosphorylation of the protein during follicular development (Ratoosh and Richards 1985, Ratoosh *et al.* 1987, Kurten *et al.* 1992).

Following the cloning of RII β , we then cloned and analyzed promoter activation and the hormonal regulation of genes encoding cholesterol side chain cleavage (CYP11A1) and aromatase (CYP19A1) (Golding *et al.* 1987, Hickey *et al.* 1988, 1990, Oonk *et al.* 1989, 1990). We collaborated with Deborah Segaloff to analyze the induction of LH receptor expression in mouse granulosa cells (Segaloff *et al.* 1990). We also analyzed the hormonal regulation of α 2-macroglobulin (Dajee *et al.* 1996, 1998) and WNT signaling pathway members (Gaddy-Kurten *et al.* 1989, Hsieh *et al.* 2002, 2003, 2005), the latter of which led to analyzing the role of beta-catenin (CTNNB1) in ovarian cancer (Boerboom *et al.* 2005, 2006). We were excited about the hormonal regulation of gene expression patterns during follicular development (Richards 1994).

Many studies have shown that the FSH and LH receptors are G-protein-coupled receptors that activate adenylyl cyclase leading to the production of cAMP, activation of cyclic AMP-dependent protein kinase (PKA) and phosphorylation of the transcription factor CREB in ovarian cells. However, CREB is not the only transcription factor regulated by activation of PKA; induction of serum and glucocorticoid-induced kinase (SGK) in granulosa cells depends on GC-rich binding factors such as Sp1 (Alliston *et al.* 1997, 2000). Moreover, canonical cAMP/PKA signaling is not the only pathway activated by FSH in granulosa cells. We showed for the first time that FSH rapidly stimulates the phosphorylation of AKT/PKB in granulosa cells and that this phosphorylation is not mediated directly by PKA (Gonzalez-Robayna *et al.* 1999, 2000, Richards 2001). These studies coupled with our analyses of the functions of the AKT target factors, FOXO1 and FOXO3, in granulosa cells have revealed that these transcription factors play key roles in ovarian follicular development by acting with either the activin or BMP signaling pathways to regulate granulosa cell fate decisions, proliferation or apoptosis, respectively (Liu *et al.* 2009, 2013, 2015b).

Enter the world of transgenic mice! More recently, we have shown that targeted disruption of *Foxo1*, *Foxo3* and *Pten* in granulosa cells leads to the formation of granulosa cell tumors that exhibit many characteristics of adult granulosa cell tumors (AGCTs) in women; they express FOXL2, WNT4 and GATA4 and show high levels of phosphoSMAD2/3 (Liu *et al.* 2013, 2015b) (Fig. 3).

The ovulation years

Prior to 1991, the scientific community thought that a single gene encoded the rate-limiting enzyme in prostaglandin biosynthesis, namely, cyclooxygenase.

Prostaglandin biosynthesis was of keen interest: (1) because ovulation had been coined an 'inflammatory response' by Dr Espey (Espey 1980), (2) because it was associated with increased prostaglandin biosynthesis and (3) because inhibiting prostaglandin biosynthesis with indomethacin blocked ovulation. Although a cyclooxygenase gene had been cloned, its expression pattern did not match the increase in prostaglandin levels in ovulating follicles. My laboratory had been generating antibodies to purified sheep seminal vesicle cyclooxygenase. Based on differential antibody recognition and molecular weight on Western blots, we unveiled the presence of two immunologically distinct forms of cyclooxygenase, one of which was markedly induced by LH in granulosa cells of ovulating follicles, the other was constitutively expressed in theca cells (Wong & Richards 1991, Sirois & Richards 1992, Sirois *et al.* 1992, 1993, Wong & Richards 1992, Sirois *et al.* 1993). Our subsequent purification of the induced protein coincided with the discovery by others of a cyclooxygenase-like gene in chicken embryo fibroblasts that was induced by the Rous sarcoma virus. The deduced amino acid sequence of the chicken gene matched the N-terminal amino sequence of the mouse protein and paved the way for our documenting that ovulating follicles expressed a second, distinct form of cyclooxygenase, now known as COX-2 or PTGS2 (prostaglandin synthase-2) (Sirois & Richards 1992, 1993, Sirois *et al.* 1992, 1993). The induction of this inducible PTGS2 protein is essential for cumulus cell-oocyte complex (COC) expansion. Disruption of the *Ptgs2* gene by others confirmed that it was essential for ovulation and that COC expansion is obligatory for ovulation (Fig. 4).

These discoveries in the early 1990s paved the way for our later studies on the molecular events that control LH-mediated induction of ovulation and the essential roles of specific genes, in addition to *Ptgs2*, that control COC expansion. Genes induced by the LH surge and determined to be critical for COC expansion include the EGF-like factors (*Areg*, *Ereg*, *Btc*), hyaluronic acid synthase 2 (*Has2*) and tumor necrosis factor alpha-induced protein 6 (*Tnfaip6*) that with pentraxin 3 (*Ptx3*) and the serum protein inter alpha inhibitor (α) form a scaffold of proteins that stabilize the expanded COC matrix; this stabilization is critical for the release of COCs during ovulation (Richards *et al.* 2002, 2008, 2015, Richards 2005, 2007, Hernandez-Gonzalez *et al.* 2006, Richards & Pangas 2010, Richards & Ascoli 2018). We identified these genes by microarray analyses of COCs isolated from periovulatory follicles at specific times following administration of hCG/LH to eCG-primed immature mice and by culturing isolated COCs *in vitro* in the presence of AREG or EREG (Shimada *et al.* 2005, 2006, Hernandez-Gonzalez *et al.* 2006). Other genes that are induced by the LH surge and critical for follicle rupture and ovulation include the

Granulosa Cell Tumor Formation/Altered Cell Fate

Figure 3 Granulosa cell tumor formation/altered cell fate: The transcription factor FOXO1 is highly expressed in granulosa cells where it acts to control follicle growth and apoptosis by interacting with the activin or BMP2 pathways, selectively and respectively, to regulate specific genes. Depletion of FOXO1/3 in granulosa cells leads to granulosa cell tumor growth that is even more pronounced if the *Pten* gene is also depleted. Some of the mutant granulosa cells acquire distinct cell fates as indicated by the expression of SOX9, a marker of Sertoli cells and epithelial cells. (Adapted from Liu *et al.* 2015b).

progesterone receptor (PGR) (Park & Mayo 1991), a PGR target gene *Adamts1* (Espey *et al.* 2000, Russell *et al.* 2003a) and versican (Russell *et al.* 2003b). Although the PGR-null mice fail to ovulate, the granulosa cells luteinize, indicating that the targets of PGR controlling rupture, such as endothelin 2 (Ko *et al.* 2006, 2012) are highly specific and restricted: new targets are still being analyzed by others. Disruption of cyclin D2 also leads to ovulation failure, likely because there are so few granulosa cells and cumulus cells present in the developing follicles (Robker & Richards 1998). The Toll-receptor pathway is also expressed in cumulus cells and may impact ovulation as well as fertilization (Liu *et al.* 2008, Richards *et al.* 2008) (Fig. 4).

Because LHCGR receptors are localized to mural granulosa cells but not cumulus cells, the molecular mechanisms by which LH-mediated COC expansion eluded endocrinologists for years until the discovery that LH induced the expression of the EGF-like factors that then (1) bound EGF receptors in granulosa cells and cumulus cells, (2) activated the RAS-MAP-kinase and ERK1/2 pathways (3) that then activated specific

transcription factors including C/EBP alpha and C/EBP beta (Fan *et al.* 2008, 2009b, 2011). By targeted depletion of *Erk2* in *Erk1*-null mice, we documented unequivocally that ERK1/2 control the master switch that suppresses the FSH-driven molecular program in granulosa cells of growing follicles and induces COC expansion (all genes controlling matrix formation and stabilization), meiotic resumption in oocytes (cGMP), ovulation (genes involved in rupture; PGR) and luteinization (cytokines and vascularization factors) in preovulatory follicles. By targeted depletion of *Cebpa* and *Cebpb*, we have documented further that these transcription factors are obligatory for ovulation and the regulation of specific genes controlling vascularization of the newly formed corpus luteum (Fan *et al.* 2011).

Entry into the world of ovarian cancer

The critical role of RAS activation of ERK1/2 in granulosa cells of ovulating follicles led us to ask what effects premature expression of RAS would exert in small growing follicles. To address this, we targeted the expression of a constitutively active form of RAS, namely *KRAS*^{G12D}, to granulosa cells using mice expressing *Amhr-2-Cre*. We were surprised that the expression of active *KRAS*^{G12D} blocked follicle growth, reduced ovarian steroid production and led to increased expression of the tumor suppressor gene, *PTEN*; granulosa cell proliferation and apoptosis ceased and small abnormal follicles accumulated in the ovaries (Fan *et al.* 2008). When we disrupted *Pten* in the *Kras* mutant mice to reverse the effects of *KRAS*, follicle development was still blocked (Fan *et al.* 2009a). In striking contrast, in the same ovaries, the ovarian surface epithelial cells (that are also targeted by *Amhr2-Cre*) were transformed and provided us with the first model of ovarian surface epithelial cancer that occurred early and was 100% penetrant (Fan *et al.* 2009a). These mice are also unique because endogenous ovarian steroid hormone production is drastically reduced thereby allowing us to determine the effects of exogenous estradiol and/or progesterone on primary ovarian tumor growth and metastasis *in situ*. By depleting the tumor repressor protein 53 (*Trp53*; *p53*) gene in the *Kras/Pten* mutant mice, we discovered that wild-type *p53* is essential for tumor growth, whereas cells lacking *p53* develop only small tumor lesions (Mullany *et al.* 2014). However, the *p53*-null cells, unlike cell expressing WT *p53*, are exquisitely sensitive to steroid hormones and become highly metastatic when exposed to exogenous estradiol (Mullany *et al.* 2014). Expressing the *p53* R172H mutant in this *Kras/Pten* mouse strain leads to rampant metastasis if the mutant alleles are homozygous and also to serous and mucinous-type tumors in cells expressing a wild-type and mutant allele. The heterozygous tumors are also highly sensitive to estradiol (Ren *et al.* 2016) (Fig. 5).

Adapted from J.S. Richards and S.A. Pangas, JCI 2010; Richards et al, 2015

Figure 4 Ovulation-related processes. The LH surge initiates a remarkable cascade of signaling and molecular events (panel A) that control cumulus cell-oocyte complex (COC) expansion, ovulation and luteinization (panel B). Key to these events is the induction of the EGF-like factors, AREG, EREG and BTC, in granulosa cells that bind and activate EGF receptors in granulosa and cumulus cells and lead to activation of ERK1/2 (panel C). Also critical is the induction of the progesterone receptor (PGR). Furthermore, the LH surge leads to the initiation of meiosis in oocytes by regulating cGMP levels in cumulus cells and the oocyte (panel D). (Adapted from Richards & Pangas 2010, Richards et al. 2015).

There are many mutations in p53 in ovarian cancer cells. Some p53 mutant proteins are inactive; other mutant proteins acquire gain-of-function (GOF) oncogenic activities that promote ovarian cancer growth. We have become interested in these GOF mutant proteins and their impact on tumor growth and metastasis, responses to steroid hormones and their abilities to evade immune attack. Thus, our current studies are focused on determining how specific GOF R175H, R273H and R248Q mutant p53 alleles impact the growth of ovarian cancer cells in the same *Kras/Pten* genetic background. We already know that the R175H p53 mutant protein behaves differently than the R273H and R248Q mutant p53 proteins in response to cytotoxic drugs and in tumor morphology (Padmanabhan et al. 2018). Thus, we anticipate finding distinct mechanisms by which each of these regulates responses to steroids and the immune cell microenvironment.

The power and force by which these cancer cells grow and metastasize is impossible to ignore and has had a huge impact on my view of biological regulatory mechanisms. One wants to conquer these cells and learn how to kill them, but how? It is amazing that man has not yet figured this out ... maybe we are coming closer with our understanding of the immune system. Moreover, the power by which different oncogenes interact is sometimes surprising. For example, in transgenic mice expressing either KRAS^{G12D} or lacking PTEN profoundly affects the ability of a constitutively active form of beta-catenin (CTNNB1) to promote granulosa cells tumor formation (Richards et al. 2012). What is most remarkable is that the gene profiles in the granulosa cell tumors expressing mutant CTNNB1

and KRAS^{G12D} are essentially identical to those tumors expressing CTNNB1 in the absence of PTEN. One conclusion is that mutant active CTNNB1 is driving the tumor phenotype and either KRAS^{G12D} or loss of PTEN promotes the CTNNB1-mediated program.

New projects: theca cell functions and dysfunction in women's health

Premature ovarian failure (POF), polycystic ovarian syndrome (PCOS) and ovarian hyperthecosis (OH) are disorders in women where theca cell functions appear to be abnormal. In some cases of POF, theca cell recruitment to primary/secondary follicles may be impaired because oocyte production of GDF9 is reduced. This leads to impaired induction of hedge-hog signaling ligands (IHH and DHH) in granulosa cells that, in turn, prevents the recruitment of stromal theca cells. Hence, theca androgen-producing cells are lacking (Liu et al. 2015a). Conversely, theca cells in PCOS and OH patients overproduce androgens leading to complex local and systemic effects, including insulin resistance, obesity and a proinflammatory state (Dumesic & Richards 2013, Richards et al. 2018). We have recently analyzed and identified inflammatory events associated with peri-ovulatory follicles of PCOS patients undergoing IVF and showed that granulosa cells obtained from follicles of obese PCOS patients produce more cytokines than those of lean PCOS patients (Adams et al. 2016). These studies have led to our current interest in how elevated androgens and cytokines interact to control ovarian follicle growth, PCOS and the follicular microenvironment.

A Mouse Model of Ovarian Cancer

Adapted from Mullany et al, 2014

Figure 5 A mouse model of ovarian cancer. Mutant mice were generated to express granulosa cell-specific expression of a constitutively active form of KRAS, KRAS^{G12D}, by mating mice harboring the LSL-Kras^{G12D} gene to mice with *Amhr2*-cre recombinase (panel A). Expression of the mutant KRAS in granulosa cells blocked follicular development (pane A: transition from white to black circles) leading to ovaries with severe loss of steroidogenesis. These abnormal follicles expressed elevated levels of the tumor suppressor PTEN. In an attempt to revert follicular development back to a normal state, *Pten* was deleted in these mice by mating the *Kras*; *Amhr2*-cre mutant mice to *Pten*^{fl/fl} mice. Although disrupting *Pten* in the granulosa cells had no effect on follicle growth, it caused ovarian surface epithelial (OSE) cells to transform. These mutant OSE cells expressed WTp53 that when deleted led to severe suppression of tumor growth indicating that WT p53 supported tumor growth. Remarkably, depletion of p53 impaired tumor growth and only small lesions were observed (panel B). However, estradiol potentially stimulated tumor growth of the p53 null tumor cells; progesterone blocked this effect (panels A and B). This mouse model has provided a unique *in vivo* system in which to analyze the effects of steroids on tumor formation and metastasis. (Adapted from Mullany et al. 2014).

The role of androgens and the androgen receptor (AR, NR3C4) in follicular development has primarily focused on how they impact granulosa cell functions, whereas the potential impact of androgens and the role of AR on theca and stromal/interstitial cells have been largely overlooked (Richards et al. 2018). The oversight is

especially evident at the molecular level, despite the fact that mouse and human theca cells express both the AR and the modulator of AR action co-repressor COUPTF-II/NR2F2. A recent theca cell-specific androgen receptor (AR)-knockout mouse model documents that the loss of AR selectively in theca cells prevents androgens from promoting a PCOS-like phenotype in an androgen-treated mutant mouse model (Ma et al. 2017). This key observation clearly implicates not only elevated theca cell androgens but also theca cell AR as potential culprits of changes in theca cell functions, including inflammatory events that contribute to PCOS. Using a DHT-treated mouse model, we have observed profound changes in AR-positive and NR2F2-positive cells in the theca/stroma compartment and the induction of specific genes. We hypothesize that elevated androgens alter AR regulation of theca cell functions leading to specific changes in cellular and molecular events that underlie and/or contribute to ovarian dysfunction in PCOS and OH (Candelaria et al. 2018, 2019) (Fig. 6).

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What makes science so much fun and rewarding?

My life in science came as a surprise, a discovery! It was not a childhood dream; it was not planned. Even today, I do not actually think of myself as a 'scientist'. What I enjoy and what has captured me is the process of discovery and the challenges to decipher the secrets of mother nature. Most rewarding have been the unexpected results that have led me down new paths and reminded me that one should always keep an open mind! When experiments do not seem to work but when it is not due to technical issues, then there is likely something significant and important to be found. For my lab it has been: identifying a new cyclooxygenase gene, generating an unexpected mouse model of ovarian cancer, identifying novel pathways regulating ovulation and documenting a novel role for androgens in theca/stromal cells. In these discoveries, I have enjoyed the enthusiasm of young students and colleagues (Fig. 7).

Figure 6 (A) Schematic of a mouse model of androgen excess and PCOS. (B) Key roles of AR, NR2F2 and VCAM1 in theca cells. The androgen receptor (AR) is expressed in theca cells as well as granulosa cells of follicles in control Day 25 and DHT-treated mice (panels A and B) and impacts theca cell functions along with the AR co-modulatory regulator NR2F2. NR2F2 is exclusively expressed in theca cells and together with AR they comprise a regulatory feedback system (panels A and B). Excess androgen DHT leads to the expansion of theca/stromal cells that express not only AR and NR2F2 but also elevated levels of vascular cell adhesion molecule 1 (VCAM1). VCAM1 appears to be essential for follicular integrity. A similar AR, NR2F2 and VCAM1 regulatory system appears to occur in theca cells of PCOS patients. (White arrowheads indicate theca AR (green, upper panels) and VCAM1 (green, lower panels)). (Adapted from Candelaria et al., SSR Meeting 2018)

I have enjoyed being the Director of the Graduate Program in the Molecular and Cellular Biology Department for over 25 years. Luckily, my life and research career has been blessed with many wonderful students, fellows, colleagues and collaborators. I have never really felt that being a woman hindered my science or my career path because both women and men have supported my efforts. I enjoy the interactions, discussions and trust in sharing ideas with others; the trust in sharing precious reagents. As an example, Dan Simmons at the University of Utah had just published his paper on the cyclooxygenase-like gene in chicken embryo fibroblasts that was induced by the Rous Sarcoma Virus. I did not know him, but I called him (this was before email!) to see if he would collaborate and send a cDNA clone for us to test in granulosa cells of ovulating follicles. By then, he had the mouse cDNA and sent it immediately. This cDNA was a treasure in our efforts to determine whether or not a distinct gene encoded the inducible cyclooxygenase-like protein observed in ovulating follicles (Simmons et al. 1992, Sirois et al. 1992, 1993). We were thrilled to see a positive, highly induced signal on Northern Blots (this was before qPCR!). The inducible RNA was there and only induced by LH! It was like winning the Wimbledon or an Olympic medal. Of special note, I have yet to meet Dan Simmons in person! He certainly gave us a great gift for which we have been forever grateful. There are many other examples: Deborah Segaloff and her long-standing efforts to clone the LH receptor and then share the probe with my lab (Segaloff et al. 1990), Dagmar Wilhelm for sending her treasured FOXL2 antibody (and I still have not met her!) (Liu et al. 2015b), and many more.

Science is driven by ideas and technology; scientists are rewarded by discovering new pathways, genes and

approaches to understand basic biological processes such as ovulation and PCOS and to open doors for improving and controlling cancer. I have been richly rewarded throughout my career. None of this would be possible without the support of the Society for the Study of Reproduction, the Endocrine Society and the National Institutes of Health. These organizations and the wonderful, dedicated people who maintain these scientific and educational opportunities are the ones who sustain our research in endocrinology and reproductive biology.

My career in science has been balanced by my dedication to sports. In Houston, I continued my love of ice-skating and managed to pass all of the USFSA compulsory ice dancing tests to the gold level. In addition, I love to jog, swim and hike. My husband Phil and I have backpacked across and camped in the Grand

Figure 7 Follow your instincts and dreams. Life is not linear. So follow your instincts and follow your dreams. But listen to others and if asked, participate! There is always something new to learn and discoveries to be made! When something is not working like it should, pay attention. Something new may emerge. What seems like a failure can lead to success. Persevere! And most of all, just when you think you have hiked the Grand Canyon for the last time, you will just keep trekking.

Canyon for the last 9 years. Because I cannot have horses in my back yard in Houston, I have cats!

Declaration of interest

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

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