BIOGRAPHICAL SKETCH

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NAME: Linda A. Schuler

eRA COMMONS USER NAME (credential, e.g., agency login): lschuler

POSITION TITLE: Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Wisconsin, Madison	B.S.	12/1974	Zoology
University of Pennsylvania, Philadelphia, PA	Ph.D.	12/1980	Physiology
University of Pennsylvania, School of Veterinary Medicine, Philadelphia, PA	V.M.D.	05/1981	Veterinary Medicine
University of Wisconsin, Madison	Post-doc	04/1983	Biochemistry

A. Personal Statement

Hormones dictate the cycles of proliferation, differentiation and apoptosis that drive mammary function in reproduction. These same hormones have been implicated in breast tumorigenesis and resistance to existing cancer therapies. The ERα+ "luminal" subtype is the most plentiful type of breast cancer. Although many patients respond to anti-estrogen therapies, about 25% of these patients exhibit *de novo* or acquired therapeutic resistance, accounting for the majority of breast cancer deaths. The Schuler laboratory has developed unique in vivo and in vitro models to investigate the basis of the epidemiologic data implicating prolactin in the pathology of metastatic ERα+ breast cancer.

Our NRL-PRL murine model, which mimics the high local prolactin in breasts of women, leads to aggressive ERα+ carcinomas, modeling therapy resistant luminal breast cancers. This model has permitted examination of the dynamic *in vivo* processes that drive the development and progression of this subset of clinical cancers, including metastasis and insensitivity to therapies. Transplantation of these carcinomas to syngeneic, immunocompetent hosts enables examination of treatment sensitivity. In vitro systems permit dissection of the molecular mechanisms that enable crosstalk among hormones, growth factors and the extracellular microenvironment. Together, these studies have pointed to the different outcomes of the different signaling cascades initiated by prolactin, and the importance of factors that modulate this repertoire in breast tumorigenesis.

Mentoring is a core value in my work. My laboratory is organized so that trainees are the driving forces behind these studies, providing them with opportunities to develop the confidence, and intellectual and experimental skills to grow into independent scientists (trainee names bolded in citations below). I am intensely involved in graduate training. Four of my former graduate students hold tenured or tenure track positions at prominent universities, and I have served on over 120 graduate mentoring committees. Furthermore, mentoring junior faculty with related research interests is one of my most rewarding professional activities. I was humbled and gratified to receive the Doris Slesinger Award for Excellence in Mentoring for these activities. Thus my research interests and professional experiences make me well suited to mentor trainees, guiding them to the next step of their careers.

B. Positions and Honors

Employment:

1983-89 Assistant Professor, Department of Comparative Biosciences, School of Veterinary Medicine, University of Wisconsin-Madison
1989-97 Associate Professor, Department of Comparative Biosciences, School of Veterinary Medicine, University of Wisconsin-Madison
1997-present Professor, Comparative Biosciences, School of Veterinary Medicine, University of Wisconsin-Madison
1995-present Member, University of Wisconsin Comprehensive Cancer Center
1999-present Adjunct Professor, Department of Medicine, School of Medicine, University of Wisconsin
2011-present Member, Stem Cell and Regenerative Medicine Center
2014 Interim Chair, Department of Comparative Biosciences, University of Wisconsin-Madison,
2015-present Associate Chair for Faculty Development, Department of Comparative Biosciences, University of Wisconsin-Madison

Honors:

Fellow, Veterinary Medical Scientist Training Program, 1976-1981
Phi Zeta Honor Society, 1979; Phi Zeta Research Award, 1981
V.M.D. magna cum laude, 1981
Shaw Scholarship, 1986
Smith Kline Beecham Award for Research Excellence, 1993
UW Renk Distinguished Professor, 1999
UW Doris Slesinger Award for Excellence in Mentoring, 2014

Other Professional Experience:

USDA Growth and Development review panel, 1989, 1990, Molecular Genetics review panel, 1992
DOD Congressionally Directed Medical Research Breast Cancer review panels, 1994, 1995, 1996, 1997
Committee of Visitors, Physiological Processes, NSF, 1991
NIH Human Embryonic Development (HED-1); regular member 1997-2001
ad hoc reviewer for NSF, BARD, March of Dimes, USDA Competitive Grants Program, Small Business Innovative Research,
ad hoc reviewer for NIH Biochemical Endocrinology (BCE), NIH Endocrinology (END), NIH Molecular and Cellular Endocrinology (MCE), Cancer Molecular Pathobiology (CAMP), Integrative and Clinical Endocrinology and Reproduction (ICER), EMNR Fellowships, NCI program projects, NCI Grand Opportunities, NCI Provocative Questions
co-editor, Prolactin and Growth Hormone in Mammary Gland Development and Breast Cancer, J. Mammary Gland Biology and Neoplasia, 2008.
Chair-elect, FASEB Conference, The Growth Hormone/ Prolactin Family in Biology and Disease, 2017

C. Contribution to Science

Trainees as authors in publications in bold:

1. Preclinical models to study prolactin actions in the development and progression of breast cancer

Despite clear evidence of the importance of prolactin in lobuloalveolar development during pregnancy and lactation, relatively little is known about its actions in breast cancer. Lack of robust preclinical models has been a major limitation. Many human breast cancer cell lines that express the prolactin receptor also express prolactin ligand, making it difficult to elicit a robust response. We developed a MCF7-derived cell line, which together with phenotypically distinct T47D cells, have permitted dissection of the molecular mechanisms that enable crosstalk of prolactin with ovarian steroids, growth factors and the extracellular microenvironment. In order to develop an in vivo model to probe the dynamic contributions of prolactin to pathogenesis without altering ovarian steroids, we generated the NRL-PRL transgenic mouse. This model gives rise to diverse, aggressive primarily ERα+ adenocarcinomas, which express the transcript signature of luminal clinical tumors. This model permits examination of the dynamic interactions of prolactin and other oncogenic factors in the development and progression of this breast cancer subtype. Moreover,

transplantation of these carcinomas or derived cell lines to syngeneic, immunocompetent hosts enables study of cancer stem cells and treatment sensitivity.

a. **Schroeder, M., J. Symowicz**, and L.A. Schuler. Prolactin modulates cell cycle regulators in mammary epithelial tumor cells, Mol. Endocrinol. 16:45-57, 2002.
b. **Rose-Hellekant, T.A., L.M. Arendt, M.D. Schroeder**, K. Gilchrist, E.P. Sandgren and L.A. Schuler. Prolactin induces ERα positive and ERα negative mammary cancer in transgenic mice, Oncogene 22:4664-4674, 2003. PMCID: 1630768
c. **Arendt, L.M.**, D.E. Rugowski, **T.A. Grafwallner-Huseth**, **M.J. Garcia-Barchino**, H. Rui and L.A. Schuler. Prolactin-induced mouse mammary carcinomas model estrogen resistant luminal breast cancer, Breast Cancer Research 13:R11, 2011. *Recognized by the weekly Mammary Cell News.* PMCID: 3109579
d. **O'Leary, K.A., M.P. Shea** and L.A. Schuler. Modeling prolactin actions in breast cancer in vivo: insights from the NRL-PRL mouse. Adv. Expt. Med. Biol. 846:201-220, 2015. PMC 4770878

2. Crosstalk of prolactin with estrogen and growth factors in breast cancer

Epidemiologically, prolactin increases the risk for particularly ER+ breast cancer, and is linked to resistance to anti-estrogen treatments. Therefore, understanding interactions with estrogen and ER is critical for understanding the role of prolactin in this disease, and exploiting those findings to develop preventive and therapeutic strategies. We have demonstrated that prolactin cooperates with estrogen in vitro and in vivo by multiple mechanisms. Prolactin can activate ERα in the absence of ligand, and cooperate with 17β-estradiol to increase proliferation of luminal epithelial cells and tumorigenesis *in vivo*. With addition of the EGFR ligand, TGFα, prolactin accelerates tumorigenesis, but the combination reduces sensitivity to estrogen despite high ERα expression.

a. **Arendt, L.M.**, L.C. Evans, D.E. Rugowski, **M.J. Garcia-Barchino**, H. Rui, and L.A. Schuler. Ovarian hormones are not required for PRL-induced mammary tumorigenesis, but estrogen enhances neoplastic processes, J. Endocrinol. 203: 99-110, 2009. PMCID: 2841967
b. **Arendt, L.M.**, **T.L. Grafwallner-Huseth** and L.A. Schuler. Prolactin and growth factor crosstalk reduces mammary estrogen responsiveness despite elevated ERα expression. Am. J. Pathol. 174:1065-1074, 2009. PMCID: 2665765
c. **O'Leary, K.A., F. Jallow**, D.E. Rugowski, R. Sullivan, **K.W. Sinkevicius**, G.L. Greene and L.A. Schuler. Prolactin activates ERα in the absence of ligand in female mammary development and carcinogenesis in vivo. Endocrinology 154:4883-4892, 2013. PMCID: 3836081

3. Matrix density/stiffness alters hormone signals in ERα+ breast cancer epithelium

In normal mammary function, prolactin signals are mediated primarily by the JAK2-STAT5 pathway, although prolactin is able to activate multiple other signaling cascades, as reported in vitro. In clinical breast cancer, high activation of STAT5A is associated with better outcomes, which is at apparent odds with clinical studies linking high prolactin activity with treatment resistance and metastasis. In order to examine the effect of properties of the extracellular matrix on hormone actions, we employed 3-dimensional collagen cultures, tunable polyacrylamide substrates, and a mouse model of increased collagen 1a1 to investigate hormone driven signals. We found that stiff extracellular matrices increase localization of prolactin receptors in focal adhesions, strengthening prolactin signals to via the src-FAK-ERK1/2 pathway, promoting invasion and re-orienting collagen fibers. Moreover, the outcomes of prolactin crosstalk with estrogen are strongly modulated by stiffness of the extracellular matrix: stiff matrices increase invasion and cell growth and reduce responsiveness to the anti-estrogen, tamoxifen. In vivo, increased collagen 1a1 density/ stiffness promotes intravasation and metastasis. These studies begin to resolve the paradox of prolactin action in normal mammary function compared to breast cancer.

a. **Barcus, C.E.**, P.J. Keely, K.W. Eliceiri and L.A. Schuler. Stiff collagen matrices increase tumorigenic prolactin signaling in breast cancer cells. J. Biol. Chem. 288:12722-12732, 2013. *Featured commentary in Matrix Biology 32:iii-iv, 2013. Recognized by the weekly Extracellular Matrix News*. PMCID: 3642318.
b. **Barcus, C.E., E.C. Holt**, P.J. Keely, K.W. Eliceiri, and L.A. Schuler. Dense collagen-I matrices enhance pro-tumorigenic estrogen-prolactin crosstalk in MCF-7 and T47D breast cancer cells. PLoS One. 2015 Jan 21;10(1):e0116891. PMCID: 4301649

c. **Barcus C.E.**, P.J. Keely, K.W. Eliceiri, L.A. Schuler. Prolactin signals through focal adhesion complexes are increased by a stiff extracellular matrix environment in breast cancer cells, Oncotarget 7: 48093-48106, 2016. *Top Story, Recognized by Extracellular Matrix News* 7.25 June 30, 2016. PMCID: 5217003

d. **Barcus C.E.**, K.A. O'Leary, **J.L. Brockman**, D.E. Rugowski, Y. Liu, N. Garcia, M. Yu, P.J. Keely, K.W. Eliceiri, L.A. Schuler. Elevated collagen-I augments tumor progressive signals, intravasation and metastasis of prolactin-induced estrogen receptor alpha positive mammary tumor cells. Breast Cancer Res 19:1-13, 2017. PMCID: 5244528.

4. Prolactin receptor signaling, trafficking, and interplay with trafficking of growth factor receptors

Understanding how prolactin signals, and how it initiates trafficking of its receptor are crucial to understanding its actions in breast cancer and crosstalk with other oncogenic factors. We demonstrated that ligand-induced downregulation of the prolactin receptor in MCF7 breast cancer cells is mediated in part by proteasomes. Moreover, ligand-activated src family kinases are essential for this process, and prolactin receptor internalization is necessary for optimal activation of ERK1/2 and Akt, but not Stat5. Src-family kinases also mediated prolactin-enhanced IGF-1R phosphorylation, and prolactin-reduced IGF-1R internalization via SHP-2. Together with our studies under (3) above, these data point to an understudied role for c-src as an important proximal kinase which mediates prolactin actions in breast cancer.

b. **Lu, J-C., T.M. Piazza**, and L.A. Schuler. Proteasomes mediate prolactin-induced receptor downregulation and fragment generation in breast cancer cells, J. Biol. Chem. 280: 33909-33916, 2005. PMCID: 1976473

c. **Piazza, T.M., J-C. Lu, K.C. Carver** and L.A. Schuler. Src family kinases accelerate prolactin receptor internalization, modulating trafficking and signaling in breast cancer cells. Mol. Endocrinol. 23: 202-212, 2009. PMCID: 19056863

d. **Carver, K.C., T.M. Piazza** and L.A. Schuler. Prolactin enhances IGF-IR phosphorylation by decreasing its association with the tyrosine phosphatase SHP-2 in MCF-7 breast cancer cells. J. Biol. Chem. 285:8003-8012, 2010. *Recognized by the weekly Mammary Cell News.* PMCID: 2832951

5. Interactions of prolactin and downstream signals to AP-1 with loss of p53 in breast cancer

TP53 is one of the most commonly mutated genes in breast cancers. In order to test if prolactin can interact with this pathway in carcinogenesis, we exploited our *in vivo* model to examine the interplay of p53 loss and elevated prolactin exposure. We demonstrated that prolactin strongly cooperated with p53 loss, decreasing tumor latency and strong shifting tumor histotype toward triple negative claudin-low carcinomas. These carcinomas, which resemble clinical metaplastic carcinomas, were more aggressive than those that developed from $p53^{-/-}$ epithelium without prolactin. They also exhibited higher levels of the AP-1 components, c-Jun and FosL1, which are associated with worse clinical outcomes. These studies confirm the ability of prolactin to contribute to multiple subtypes of breast cancer, and also point to the importance of prolactin driven non-STAT5 signals in breast pathogenesis.

a. **Gutzman, J.H.**, D.E. Rugowski, **M.D. Schroeder**, J.J. Watters and L.A. Schuler. Multiple kinase cascades mediate prolactin signals to Activating Protein-1 in breast cancer cells, Mol. Endocrinol. 18: 3064-3075, 2004. PMCID: 1634796

b. **Gutzman, J.H.**, D.E. Rugowski, **S.E. Nikolai**, L.A. Schuler. Stat5 activation inhibits prolactin-induced AP-1 activity: distinct prolactin initiated signals in tumorigenesis dependent on cell context, Oncogene 26:6341-6348, 2007. PMCID: 3190200

c. **O'Leary, K.A.**, D.E. Rugowski, R. Sullivan and L.A. Schuler. Prolactin cooperates with loss of p53 to promote claudin low mammary carcinomas. Oncogene 33:3075-3082, 2014. PMCID: 4007359

Partial list of peer-reviewed published work in MyBIbliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/linda.schuler.1/bibliography/47748480/public/?sort=date&direction=ascending

D. Additional Information: Research Support and/or Scholastic Performance

Research Support

ONGOING

NIH/NCI R01 CA179556-01A1 (co-PIs, Schuler, Keely) 04/01/14-02/28/19
Matrix density promotes pro-tumorigenic hormone actions in breast cancer
The major goal of this project is to examine the effect of increased stromal tension on hormonal responses in breast cancer, utilizing defined in vitro models, transgenic animals and xenografts. Aim 1: To determine how three hallmark features of ECM structure-stiffness, density, and alignment can shift the spectrum of PRL- and estrogen-induced signals in breast cancer cells in vitro. Aim 2: To examine PRL and estrogen regulation of altered matrix deposition, composition, and structure in vitro. Aim 3: To elucidate the interactions of PRL, estrogen and extracellular matrix structure in vivo in the development and progression of breast cancer using mouse models and clinical samples.

EPA G2013-STAR-L1 (PI, Murphy) 12/1/14-11/30/19
Organotypic Culture Models for Predictive Toxicology Center
Project 3: Cancer MAPS (co-I, Schuler)
To develop a novel in vitro approach which will enable identification of chemicals that may contribute to the development or progression of breast cancer, we will expand our established high throughput 3D microfluidic in vitro platform to mimic important structures and functional relationships of human mammary ducts. Aim 1: To develop the 3D microfluidic multi-culture platform to be used for chemical library screening and the adaptation for use in other projects that are part of this center. Aim 2: To conduct a primary screen using the ToxCast library to identify chemicals that affect normal or cancerous epithelial cells in our synchronous cancer model. The identified “hits” will be validated by conducting high-resolution imaging and evaluating dose-response relationships. Aim 3: To investigate the disruption of endocrine signaling responsible for cancer initiation and progression by certain chemicals by integrating a novel sample isolation platform into the 3D microfluidic multi-culture platform enabling genomic endpoints.

NIH / NCI 1 R01 CA157675 (PI, Schuler) 08/01/11-5/31/16
Prolactin: mammary progenitors and tumor initiating cells in luminal carcinomas
The major goals of this project are to establish the effect of PRL and crosstalk with ovarian steroids on mammary epithelial subpopulations in nonparous females prior to lesions and identify key PRL-induced mediators, to determine characteristics of tumor initiating cells in PRL-induced ERα+ carcinomas, and to elucidate interplay between PRL and a well- characterized oncogenic signal, β-catenin, in regulation of normal mammary subpopulations and tumor subtype. (no cost extension)

EPA G2013-STAR-L1 (PI, William Murphy; PI, Project 3, David Beebe, Schuler co-I) 12/1/14-11/30/19
Organotypic Culture Models for Predictive Toxicology Center
Project 3: Cancer MAPS

To develop a novel in vitro approach which will enable identification of chemicals that may contribute to the development or progression of breast cancer, we will expand our established high throughput 3D microfluidic in vitro platform to mimic important structures and functional relationships of human mammary ducts. Aim 1: To develop the 3D microfluidic multi-culture platform to be used for chemical library screening and the adaptation for use in other projects that are part of this center. Aim 2: To conduct a primary screen using the ToxCast library to identify chemicals that affect normal or cancerous epithelial cells in our synchronous cancer model. The identified “hits” will be validated by conducting high-resolution imaging and evaluating dose-response relationships. Aim 3: To investigate the disruption of endocrine signaling responsible for cancer initiation and progression by certain chemicals by integrating a novel sample isolation platform into the 3D microfluidic multi-culture platform enabling genomic endpoints.