BIOGRAPHICAL SKETCH

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NAME: Kanako Hayashi

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

POSITION TITLE: Associate 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
Obihiro University of Agriculture and Veterinary Medicine, Japan	B.S.	03/1997	Animal Science
Obihiro University of Agriculture and Veterinary Medicine, Japan	M.S.	03/1999	Animal Science
Iwate University, The United Graduate School of Agriculture Sciences, Japan	Ph.D.	03/2002	Reproductive Physiology
Texas A&M University, College Station, TX	Postdoctoral	05/2003	Reproductive Physiology

A. Personal Statement

I have extensive experience in gynecological diseases including endometriosis and ovarian/endometrial cancer, uterine biology and reproductive toxicology. Furthermore, I have a good track record for leadership of research teams, which are necessary to successfully carry out the proposed study. I also have a broad background in cellular, molecular, genetic and epigenetic techniques to understand the etiology and mechanisms of the diseases using animal models, primary human tissues and cells, and data sets from public sources. I have expertise in the targetable signaling mechanisms for developing therapeutic strategies. For example, we have published our findings in *FASEB J*, *Oncogene*, *Biol Reprod* and *Tox Sci*. In those studies, we have found not only the signaling mechanisms of the disease but also therapeutic target(s) by small molecule(s) in the signaling pathway. Currently, my team is further developing niclosamide for clinical use for the treatment of endometriosis. Thus, I have been successfully expanding potential therapeutic drugs in a pre-clinical setting to clinical translational research. In addition, I laid the groundwork for the research by developing necessary animal and cell culture models. In the past, we developed a new mouse model to examine and understand the etiology of endometrial cancer. I have successfully collaborated with many top-notch researchers and produced several peer-reviewed publications from each project.

Ongoing and recently completed projects that I would like to highlight:

1 R01 HD104619-01 NIH/NICHD 04/01/21-03/31/26
“Complex inflammatory mechanisms and therapeutic targeting in endometriosis”
Aim: To examine how loss of LPM impacts pathophysiology of endometriosis, how ELL induction alters the functionally heterogenic population of ELL and peritoneal exudate cells, and how their inflammatory dysfunction is inhibited by niclosamide, and how inhibitory interactions from niclosamide correlate with pain-related symptomology.
Role: PI

1 R21 ES031607-01 NIH/NIEHS 09/01/20-08/31/22
“Transgenerational epigenetic alterations on male germ cells caused by bisphenol S”
The objective is to determine how BPS affects DNA methylomes and transcriptomes in male germ cells to identify genes whose expression and DNA methylation status are altered in the subsequent generation.

Role: PI

1 R21 HD092739-01 NIH/NICHD 09/01/17-08/31/20
"Development of new therapeutic strategies for endometriosis"
The objective is to study potential therapeutic drugs and their inhibitory mechanisms focusing on inflammatory activity and macrophage-dependent neuroangiogenesis.
Aim1: To determine whether niclosamide inhibits inflammatory activity via STAT3 signaling.
Aim2: To determine whether niclosamide inhibits macrophage-dependent angiogenesis
Role: PI

1 R13 HD0792007-01 NIH/NICHD 04/01/18-03/30/19
"Illinois Symposium on Reproductive Sciences (ISRS) Annual Meeting 2018"
Role: MPI with Dr. James MacLean

1 R15 CA179214-01 NIH/NCI 09/01/13-06/30/18
"Mechanisms of WNT7A-FGF1 signaling and their therapeutic potential in ovarian cancer"
The objective is to study whether FGF1 is an oncogenic downstream target of WNT7A/β-catenin signaling, and whether WNT7A-FGF1 signaling has activity to promote tumor progression, dissemination and/or metastasis. Further, we will determine whether niclosamide can be a useful drug for ovarian cancer treatment through inhibition of WNT7A/β-catenin-FGF1 signaling.
Role: PI

1. Shi M, Sekulovski N, Whorton AE, MacLean JA II, Greaves E, **Hayashi K**. Efficacy of niclosamide on the intra-abdominal inflammatory environment in endometriosis. *FASEB J* 2021 PMID: 33860549
2. Sekulovski N, Whorton AE, Tanaka T, Hirota Y, Shi M, MacLean JA II, Loret de Mola JR, Groesch K, Diaz-Sylvester P, Wilson T, **Hayashi K**. Niclosamide suppresses macrophage induced inflammation in endometriosis. *Biol Reprod* 2020. PMID: 31950153 PMCID: PMC7186788
3. Shi M, Whorton AE, Sekulovski N, MacLean JA II, **Hayashi K**. Prenatal exposure to bisphenol A, E and S induces transgenerational effects on male reproductive functions in mice. *Toxicol Sci* 2019. PMID: 31532523.
4. King ML, Lindberg ME, Stodden GR, Okuda H, Ebers SD, Johnson A, Montag A, Lengyel E, MacLean JA II, **Hayashi K.** WNT7A/β-catenin signaling induces FGF1 and influences sensitivity to niclosamide in ovarian cancer. *Oncogene* 2015; 34:3452-3462. PMID: 25174399. PMCID: PMC4345161.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2002-2003: Postdoctoral Fellow. Center for Animal Biotechnology and Genomics, Department of Animal Science, Texas A&M University, College Station.
2004-2005: Assistant Research Scientist. Center for Animal Biotechnology and Genomics, Department of Animal Science, Texas A&M University, College Station.
2005-2007: Research Assistant Professor. Center for Animal Biotechnology and Genomics, Department of Animal Science, Texas A&M University, College Station.
2008-2010: Research Assistant Professor. Department of Physiology, Southern Illinois University School of Medicine, Carbondale.
2010-2016: Assistant Professor. Department of Physiology, Southern Illinois University School of Medicine, Carbondale.
2016-2020: Associate Professor. Department of Physiology, Southern Illinois University School of Medicine, Carbondale.
2017-2020: Associate Professor. Department of Obstetrics and Gynecology, Southern Illinois University School of Medicine.
2020-2021: Associate Professor. Center for Reproductive Biology, Department of Animal Science, Washington State University

2020-2021: Associate Faculty. School of Molecular Biosciences, College of Veterinary Medicine, Washington State University
2021-present: Associate Director. Center for Reproductive Biology, Washington State University
2021-present: Associate Professor. School of Molecular Biosciences, College of Veterinary Medicine, Washington State University

2018: Reviewer, NIH Peer Review Committee: Integrative and Clinical Endocrinology and Reproductive Study Section (ICER)
2018 Reviewer, DoD Congressionally Directed Medical Research Programs (CDMRP): Peer Review Medical Research Program (PRMRP), the Discovery Endometriosis (DIS-EM)
2019: Reviewer, NIH Peer Review Committee: Integrative and Clinical Endocrinology and Reproductive Study Section (ICER)
2020 Reviewer, DoD Congressionally Directed Medical Research Programs (CDMRP): Peer Review Medical Research Program (PRMRP), PRE-EM (Endometriosis) panel
2020 Reviewer, DoD Congressionally Directed Medical Research Programs (CDMRP): Peer Review Medical Research Program (PRMRP), EM (Endometriosis) panel
2020-2024 Regular Member, NIH Peer Review Committee: CHHD R Study Section, Reproduction, Andrology and Gynecology (RAG) Subcommittee
2021 Reviewer, NIH Special Emphasis Panel, HD-21-002 “Centers to Advance Research in Endometriosis (CARE) (P01 Clinical Trial Not Allowed)”
2021: Reviewer, DoD Congressionally Directed Medical Research Programs (CDMRP): Peer Review Medical Research Program (PRMRP), PRE-EM (Endometriosis) panel
2021: Reviewer, DoD Congressionally Directed Medical Research Programs (CDMRP): Peer Review Medical Research Program (PRMRP), Discovery Endometriosis (DIS-EM) panel

1998-current: Member, Society for the Study of Reproduction
2009-current: Member, American Association for Cancer Research
2016-current: Member, Society of Toxicology
2019-current: Member, World Endometriosis Society

Honors

1993-2000: Scholar, The Japan Scholarship Foundation
1998: Research Fellow, Japan-Germany Joint Research Project of the Japan Society for the Promotion of Science
1998: Research Fellow, H. Wilhelm Schaumann-Stiftung, Germany
2000-2002: Research Fellow, Japan Society for the Promotion of Science Research for Young Scientist
2001: Award, Trainee Research Competition Finalist, USDA NRI Travel Fellow, Society for the Study of Reproduction (SSR)
2003: Award, Lalor Foundation Post-doctoral Fellow
2014: Award, **a New Investigator Award**, Society for the Study of Reproduction (SSR)
2014: Award, **Best of American Association for Cancer Research** (AACR: one of the most highly-cited *Molecular Cancer Research* articles published in 2012; Yoshioka S, King ML, Ran S, Okuda H, MacLean JA II, McAsey ME, Sugino N, Watabe K, **Hayashi K**. WNT7A regulates tumor growth and progression in ovarian cancer through the WNT/β-catenin pathway. *Mol Cancer Res* 2012; 10:469-482. PMID:22232518. PMCID: PMC3307825

C. Contributions to science

Endometriosis:

We focus on inflammatory mechanisms as a druggable target to inhibit endometriosis and improve endometriosis-associated hyperalgesia. We have demonstrated that large peritoneal MΦ (LPM) are specifically increased in the peritoneal fluid (PF) of endometriotic lesion-induced mice and invaded into the lesions. Moreover, elevated LPM populations in the PF are reduced by niclosamide. Niclosamide also inhibits aberrant inflammation established in the PF, lesions, pelvic organs (uterus and vagina) and dorsal root ganglion (DRG), as well as MΦ infiltration, vascularization and innervation in the lesions. I have created Endometriosis Team with Drs. Julie Christianson and Warren Nothnick at KUMC, and Dr. Nash Moward at the University of Florida

to further understand the immune system and endometriosis-associated pain and develop new therapeutic strategies in endometriosis.

a. Shi M, Sekulovski N, Whorton AE, MacLean JA II, Greaves E, **Hayashi K**. Efficacy of niclosamide on the intra-abdominal inflammatory environment in endometriosis. *FASEB J* 2021 PMID: 33860549
b. Sekulovski N, Whorton AE, Tanaka T, Hirota Y, Shi M, MacLean JA II, Loret de Mola JR, Groesch K, Diaz-Sylvester P, Wilson T, **Hayashi K**. Niclosamide suppresses macrophage induced inflammation in endometriosis. *Biol Reprod* 2020. PMID: 31950153 PMCID: PMC7186788
c. Sekulovski N, Whorton AE, Shi M, MacLean JA II, **Hayashi K**. Endometriotic inflammatory microenvironment induced by macrophages can be targeted by niclosamide. *Biol Reprod* 2019. PMID: 30329025. PMCID: PMC6378864
d. Prather GR, MacLean JA II, Shi M, Boadu DK, Paquet M, **Hayashi K**. Niclosamide as a potential nonsteroidal therapy for endometriosis that preserves reproductive function in an experimental mouse model. *Biol Reprod* 2016; 95:76. PMID: 27535961. PMCID: PMC5333938

Reproductive Toxicology:

One of my research focuses on studying environmental toxicants in reproduction. We have found that prenatal exposure or postnatal exposure to bisphenol S (BPS) and BPE, prevalent BPA substitutes, disrupt reproductive functions in male and female mice such as reduced sperm counts and motility, altered germ cell development in males, impaired estrous cyclicality, follicle numbers, steroidogenesis and fertility in females. Specifically, a low-dose of BPS and BPE treatment, which mimics environmentally relevant daily-base concentration in human, causes more significant abnormalities compared with those in higher doses, indicating that a daily-base low dose exposure to BPA substitutes (BPS and BPE) *in utero* induces changes in early germ cell development and adult reproductive functions that may exhibit lasting toxic effects through transmission to progeny. Now, we are further studying transgenerational effects of BPS and BPE on germ cell development and reproductive functions.

a. Shi M, Whorton AE, Sekulovski N, MacLean JA II, **Hayashi K**. Prenatal exposure to bisphenol A, E and S induces transgenerational effects on male reproductive functions in mice. *Toxicol Sci* 2019. PMID: 31532523.
b. Shi M, Whorton AE, Sekulovski N, MacLean JA II, **Hayashi K**. Prenatal exposure to bisphenol A, E, and S induces transgenerational effects on female reproductive functions in mice. *Toxicol Sci* 2019. PMID: 31132128.
c. Shi M, Sekulovski N, MacLean JA II, Whorton A, **Hayashi K**. Prenatal exposure to bisphenol A analogues on female reproductive functions in mice. *Toxicol Sci* 2019. PMID: 30629253.
d. Shi M, Sekulovski N, MacLean JA II, **Hayashi K**. Prenatal exposure to bisphenol A analogues on male reproductive functions in mice. *Toxicol Sci* 2018. PMID: 29741722.

Ovarian Cancer:

One of my projects focuses on WNT signaling, in which I have extensive experience (see below). First finding is that WNT7A-activated β-catenin signaling plays a major role in the primary tumorigenesis and the metastatic progression of serous ovarian carcinomas. These results were published in *Mol Cancer Res* 2012 and received "**Best of American Association for Cancer Research 2012"** (one of the most highly-cited *Mol Cancer Res* articles published in 2012). Then, we have identified that FGF1 is a direct downstream target of WNT7A/β-catenin signaling and this pathway has potential as a therapeutic target in ovarian cancer. Moreover, niclosamide is a promising inhibitor of this pathway and may have clinical relevance. These results were published in *Oncogene* 2015. In addition, we have further reported that WNT7A is post-transcriptionally regulated by *miR-15b*, which could be down-regulated by promoter hypermethylation, potentially via DNMT1, in ovarian cancer. Now, we are expanding the studies on niclosamide's direct binding targets, RNA binding proteins (RBPs: FXR1 and IGF2BP2) and their oncogenic activities, as well as their regulatory mechanisms and target mRNAs in ovarian cancer.

a. Sekulovski N, MacLean JA II, Bheemireddy SR, Yu Z, Okuda H, Pru C, Plunkett KN, Matzuk M, **Hayashi K**. Potential niclosamide's direct targets in ovarian cancer. *Biol Reprod* 2021 PMID: 33855343
b. King ML, Lindberg ME, Stodden GR, Okuda H, Ebers SD, Johnson A, Montag A, Lengyel E,

MacLean JA II, **Hayashi K.** WNT7A/β-catenin signaling induces FGF1 and influences sensitivity to niclosamide in ovarian cancer. *Oncogene* 2015; 34:3452-3462. PMID: 25174399. PMCID: PMC4345161.

c. MacLean JA II, King ML, Okuda H, **Hayashi K**. WNT7A regulation by miR-15b in ovarian cancer. *PloS One* 2016:e0156109. PMID: 27195958. PMCID: PMC4873135.
d. Yoshioka S, King ML, Ran S, Okuda H, MacLean JA II, McAsey ME, Sugino N, Brard L, Watabe K, **Hayashi K**. WNT7A regulates tumor growth and progression in ovarian cancer through the WNT/β-catenin pathway. *Mol Cancer Res*. 2012; 10:469-482. PMID: 22232518. PMCID: PMC3307825. **Best of AACR 2012**, one of the most highly-cited *Mol Cancer Res* articles published in 2012

Endometrial Cancer:

I have created several endometrial cancer models. My lab generated mice with conditional ablation of *Trp53* and *Cdh1* in the mouse uterus based on mutation and inactivation status of type II endometrial cancer using the innovative *Pgr-Cre* mice to understand the mechanisms of tumorigenesis necessary for early stage diagnosis as well as rational design of therapies to increase long-term survival. Our results indicate that absence of CDH1 and TP53 in endometrial cells initiates chronic inflammation, promotes tumor microenvironment development following the recruitment of macrophages, and promotes aggressive endometrial carcinomas. The results were published in *Oncogene* 2015. We also generated and characterized conditional ablation of *Cdh1* and *Pten* in the mouse uterus. The uteri of $Cdh1^{d/d}$ $Pten^{d/d}$ mice were abnormally structured with curly horns, disorganized epithelial structure, and accelerated cellular invasiveness and angiogenesis, these mice died at postnatal day 15-19 with massive blood loss. These results were published in *Biol Reprod* 2013.

a. Stodden GR, Lindberg ME, King ML, Paquet M, MacLean JA II, Mann JL, DeMayo FJ, Lydon JP, **Hayashi K.** Loss of *Cdh1* and *Trp53* in the uterus induces chronic inflammation with modification of tumor microenvironment. *Oncogene* 2015; 34:2471-2482. PMID: 24998851. PMCID: PMC4551401.
b. Lindberg ME, Stodden GR, King ML, MacLean JA II, Mann JL, DeMayo FJ, Lydon JP, **Hayashi K**. Loss of *Cdh1* and *Pten* accelerates cellular invasiveness and angiogenesis in the mouse uterus. *Biol Reprod* 2013; 89:8. PMID: 23740945. PMCID: PMC4076352.
c. Reardon SN, King ML, MacLean JA II, Mann JL, Demayo FJ, Lydon JP, **Hayashi K**. *Cdh1* is essential for endometrial differentiation, gland development and adult function in mouse uterus. *Biol Reprod* 2012; 86:141. PMID: 22378759. PMCID: PMC3364924.

A full list of my publications:

http://www.ncbi.nlm.nih.gov/sites/myncbi/1fypobkdbAKkF/bibliography/40353383/public/?sort=date&direction=descending