BIOGRAPHICAL SKETCH

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NAME: Kanako Hayashi

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

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)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Obihiro University of Agriculture and Veterinary Medicine, Japan	B.S.	04/1993	03/1997	Animal Science
Obihiro University of Agriculture and Veterinary Medicine, Japan	M.S.	04/1997	03/1999	Animal Science
Iwate University, The United Graduate School of Agriculture Sciences, Japan	Ph.D.	04/1999	03/2002	Reproductive Physiology
Texas A&M University, College Station, TX	Postdoctoral	04/2002	05/2005	Reproductive Physiology

A. Personal Statement

I have extensive experience in gynecological diseases, including endometriosis and ovarian/endometrial cancer, uterine biology, and reproductive toxicology. I have also expanded my research to focus on cannabis in reproduction. Furthermore, I have a good track record for leadership of my research team and research communities, 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 reproductive functions and diseases using animal models, primary human tissues and cells, and data sets from public sources. I also have expertise in the targetable signaling mechanisms for developing therapeutic strategies. In addition, I laid the groundwork for the research by developing necessary animal and cell culture models. In the past, we have developed a new mouse model to examine and understand the etiology of ovarian and endometrial cancer, as well as endometriosis. Together these studies have been well received, and as a result, in 2014, I was named the recipient of the **New Investigator Award** from The Society for the Study of Reproduction. I have successfully collaborated with many top-notch researchers and produced several peer-reviewed publications from each project.

Research Support (Ongoing and recently completed projects that I would like to highlight):

1 R01 HD104619-01 NIH/NICHD
04/01/21-03/31/26 Role: PI-Hayashi
“Complex inflammatory mechanisms and therapeutic targeting in endometriosis”
Aim: To examine how the loss of LPM impacts the 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.

1 R21 ES031607-01 NIH/NIEHS
09/01/20-08/31/22 Role: PI-Hayashi
“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.

Alcohol and Drug Abuse Research Program (ADARP) The State of Washington Initiative Measure No. 171
01/01/22-07/31/23 Role: PI-Hayashi
"Evaluate transgenerational effects of prenatal and postnatal cannabis vapor exposure on male and female reproductive phenotypes and parameters in male and female germ cells"

Dedicated Marijuana Account (DMAc) The State of Washington Initiative Measure No. 502
08/01/21-06/30/23 Role: Multi-PI (Hayashi, McLaughlin, Delevich)
"Validation of a novel cannabis vapor delivery approach in mice"
To establish pharmacokinetics and dose-response effects of cannabis vapor exposure in mice.

1 R13 HD107728-01 NIH/NICHD and NIEHS
12/1/21-11/30/22 Role: Multi-PI (MacLean, Hayashi)
"Northwest Reproductive Science Symposium (NWRSS) 2022"

1 R21 HD092739-01 NIH/NICHD
09/01/17-08/31/20 Role: PI-Hayashi
"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

B. Positions, Scientific Appointments, and Honors

Positions

2022-current Professor. School of Molecular Biosciences, Washington State University
2021-2022 Associate Professor. School of Molecular Biosciences, Washington State University
2020-2021 Associate Professor. Department of Animal Science, Washington State University
2017-2020: Associate Professor. Department of Obstetrics and Gynecology, Southern Illinois University School of Medicine
2016-2020: Associate Professor. Department of Physiology, Southern Illinois University School of Medicine
2010-2016: Assistant Professor. Department of Physiology, Southern Illinois University School of Medicine
2008-2010: Research Assistant Professor. Department of Physiology, Southern Illinois University School of Medicine
2005-2007: Research Assistant Professor. 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.
2002-2003: Postdoctoral Fellow. Center for Animal Biotechnology and Genomics, Department of Animal Science, Texas A&M University, College Station

Scientific Appointments

2020-current Member, NIH Peer Review Committee: CHHD R Study Section, Reproduction, Andrology and Gynecology (RAG) Subcommittee
2021 Feb Reviewer, NIH Special Emphasis Panel, HD-21-002 "Centers to Advance Research in Endometriosis (CARE)"
2020-current Scientific Reviewer, DoD Congressionally Directed Medical Research Programs (CDMRP): Peer Review Medical Research Program (PRMRP), Endometriosis, PRE-EM, EM and DIS-EM panels
2019 Feb: Ad-hoc reviewer, NIH Peer Review Committee: Integrative and Clinical Endocrinology and Reproductive Study Section (ICER)
2018 Ad-hoc reviewer, DoD Congressionally Directed Medical Research Programs (CDMRP): Peer Review Medical Research Program (PRMRP), the Discovery Endometriosis (DIS-EM)
2018 June: Ad-hoc reviewer, NIH Peer Review Committee: Integrative and Clinical Endocrinology and Reproductive Study Section (ICER)

2021-current: Society for Reproductive Investigation
2019-current: World Endometriosis Society
2016-current: Member, Society of Toxicology
2009-current: Member, American Association for Cancer Research
1998-current: Member, Society for the Study of Reproduction

Honors

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
2014: Award, **a New Investigator Award**, Society for the Study of Reproduction (SSR)
2003: Award, Lalor Foundation Post-doctoral Fellow
2001: Award, Trainee Research Competition Finalist, USDA NRI Travel Fellow, Society for the Study of Reproduction (SSR)
2000-2002: Research Fellow, Japan Society for the Promotion of Science Research for Young Scientist
1998: Research Fellow, H. Wilhelm Schaumann-Stiftung, Germany
1998: Research Fellow, Japan-Germany Joint Research Project of the Japan Society for the Promotion of Science
1993-2000: Scholar, The Japan Scholarship Foundation

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

Cannabis:

We study how cannabis exposure affects reproductive functions, especially how *in utero* and *nursing* exposure to cannabis disrupts reproductive functions in offspring, altering epigenetic reprogramming in germ cells. We have reported that cannabis vapor exposure decreases sperm counts in the F0 males (=direct effect) and the F1 male offspring (=generational effect) and increases DNA damage and DNMT1 in the F1 neonatal germ cells, as well as altered differentially methylated regions (DMR).

a. Shi M, Langholt EM, Butler LC, Harvey ME, Wheeler EC, Zhao L, MacLean II JA, Oh Y, Sabrowsky E, Yu S, Watson S, Davis JF, **Hayashi K**. Vapor cannabis exposure generationally affects male reproductive functions in mice. *Toxicol Sci* 2022. PMID: 34865136

Selected for the cover page *Toxicol Sci* Feb 2022

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 (total 62 peer-reviewed publications):
http://www.ncbi.nlm.nih.gov/sites/myncbi/1fypobkdbAKkF/bibliography/40353383/public/?sort=date&direction=descending