BIOGRAPHICAL SKETCH

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NAME: Bhandari, Ramji Kumar

eRA COMMONS USER NAME: ------

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
Bangladesh Agricultural University, Bangladesh	B.S.	06/1997	Fisheries (Hons)
Bangladesh Agricultural University, Bangladesh	M.S.	12/1998	Aquaculture
Hokkaido University, Sapporo, Japan	Ph.D.	03/2002	Fish Biology
National Institute for Basic Biology, Japan	Postdoctoral Fellow	11/2003	Developmental Plasticity/ Reproductive Biology
Washington State University, Pullman, WA	Postdoctoral Fellow	11/2006	Developmental Biology/ Environmental Epigenetics

A. Personal Statement

Nearly all diseases result from a complex interaction between an individual's genetic make-up and the environmental agents that he or she is exposed to (NIEHS, 2016). Some exposure effects appear as direct toxic effects, while others appear as adverse health effects in the subsequent generations due to past ancestral exposures. My research mission is to understand molecular underpinnings of environmentally induced developmental origins of adult onset and transgenerational health abnormalities. My laboratory takes comparative, molecular, cellular, and bioinformatics approaches and utilizes *in vitro* human cell culture and *in vivo* animal models. My research group has demonstrated reproductive effects in fish in the third and fourth generations caused by ancestral exposure to a plasticizer (bisphenol A, BPA), birth control pill component (17α-ethinylestradiol, EE2) and herbicide (atrazine). Our studies and others suggest that ancestrally inherited epigenome that is modified by environmental or nutritional exposures may predispose descendants to altered health conditions. Current research is focused on mechanisms underlying gene-environment interactions and inheritance of environmentally induced alterations on epigenome.

Relevant publications are:

1. Bhandari, R.K., vom Saal, F.S., Tillitt, D.E. (2015). Transgenerational effects from developmental exposures to bisphenol A or 17α-ethinylestradiol in medaka, *Oryzias latipes*. *Scientific Reports.* 5: 9303. [PMID: 25790734]
2. Wang, X., Bhandari, R.K. (2019). DNA methylation dynamics during epigenetic reprogramming of medaka embryo. *Epigenetics*,14:611-622. [PMID: 31010368]
3. Wang, X. and Bhandari, R.K. (2019). The dynamics of DNA methylation during epigenetic reprogramming of medaka primordial germ cells. *Epigenetics*. published online. [PMID: 31851575]
4. Wang, X. Bhandari, R.K. Incorporation of DNA methylation into hypomethylated genomic locus by CRISPR-dCas9. (*in preparation*)

B. Positions and Honors

Positions and Employment

2002-2003 Lecturer, University of the Ryukyus, Okinawa, Japan
2003-2006 Postdoctoral Fellow with Dr. Yoshitaka Nagahama, National Institute for Basic Biology, Japan

2006-2011 Postdoctoral Research Associate with Dr. Michael Skinner, Washington State University, Pullman, WA.
2011- 2012 Assistant Research Professor, Department of Physiological Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL.
2012- 2016 Assistant Research Professor, Division of Biological Sciences, University of Missouri-Columbia, Columbia, MO.
2012- Visiting Scientist, USGS Columbia Environmental Research Center, Columbia, MO.
2016 - Assistant Professor, Biology Department, University of North Carolina at Greensboro, NC.
2019- Adjunct Associate Professor, Applied Science & Technology PhD Program at North Carolina A&T State University

Other Experience and Professional Memberships

2008- Member, Society for Study of Reproduction
2012- Member, Society for Developmental Biology
2014- Member, Society of Environmental Toxicology and Chemistry
2018- Member, Society of Toxicology

Honors

2007 Young Researcher Award | Zoological Society of Japan
2008 Lalor Foundation Merit Award | Society for Study of Reproduction
2011 Outstanding Postdoctoral Presentation Award | CRB | Washington State University
2011 Lalor Foundation Merit Award | Society for Study of Reproduction
2018 Bernard and Glickman Dean's Professorship Award for Teaching & Research Excellence, UNCG
2019 Thomas Undergraduate Research Mentor Award 2019, UNCG

C. Contribution to Science

1. **Epigenetic programming of medaka embryo and its developing germ cells**: My laboratory is studying epigenetic programming of medaka embryo, especially DNA methylation dynamics, expression profile of DNA methyltransferases (*DNMTs*), *TET* genes, and miRNAs during early embryogenesis. We have identified DNA methylation reprogramming events in medaka embryo during early cleavage stage and in primordial germ cells during embryogenesis and post-embryonic stages. Findings suggest that DNA methylation dynamics during reprogramming of somatic cells in postfertilization embryo and in primordial germ cells (PGCs) are highly conserved between fish and mammals (humans & mice). This finding has allowed us to design studies to address mechanisms underlying stressor-induced epigenetic effects in primordial germ cells (PGCs) and their transgenerational inheritance by future generations. So far, we have found specific epigenetic signatures that are established by BPA exposure on PGCs which can survive epigenetic reprogramming events and are inherited as are by sperm. Also, we are profiling circulating miRNAs to identify biomarkers of the past BPA exposure and of current reproductive impairment in adult males. Our ultimate aim from this project is to correlate transgenerational epimutations/miRNAs profiles with the history of past exposure to BPA and with associated phenotypes. We have developed CRISPR-dCas9 mediated epigenome editing method to correct transgenerational epimutations in our animal model. Preliminary studies suggest that this method can precisely remove or add DNA methylation from/onto a genomic locus in embryos undergoing cleavage.
    a. Wang, X., Bhandari, R.K. (2019). DNA methylation dynamics during epigenetic reprogramming of medaka embryo. *Epigenetics*,14:611-622.
    b. Wang, X. and Bhandari, R.K. The dynamics of DNA methylation during epigenetic reprogramming of medaka primordial germ cells. *Epigenetics*. published online. [PMID: 31851575]
    c. Wang, X., vom Saal, F.S., Tillitt, D.E., Bhandari, R.K. Dynamics of BPA-induced epigenetic memories during reprogramming in primordial germ cells and their inheritance by sperm. (*In preparation)*
    d. Wang, X., Bhandari, R.K. Epigenetic reprogramming of somatic cells in post-fertilization embryo and primordial germ cells in fish- a review. *Submitted.*

2. **Transgenerational inheritance of environmentally induced phenotypes**: Gene environment interactions lead to development of phenotypes with possibility for transgenerational transmission of

phenotypic traits to offspring in the subsequent generations. Mechanisms underlying such a vertical transmission of environmentally induced adverse health phenotypes to offspring are enigmatic, despite several studies demonstrate epigenetic marks in the germline of descendants associated with ancestral exposure. Our laboratory is trying to understand how environmental stressors (chemical or non-chemical) induce epigenetic changes that result in adverse health outcomes or disease. Utilizing fish and rodents as models, we are trying to pinpoint the epigenetic changes that lead to harmful health effects because of direct exposure to stressors or due to ancestral exposure.

a. Cleary, J.C., Tillitt, D.E., vom Saal, F.S., Bhandari, RK. (2019). Transgenerational effects of developmental atrazine exposure on reproduction of male medaka fish. *Environmental Pollution,* 251: 639-650.

b. Bhandari R.K., vom Saal, F.S., Tillitt, D. E. (2015). Transgenerational effects from early developmental exposures to bisphenol A or 17α-ethinylestradiol in medaka, *Oryzias latipes*. *Scientific Reports, 5: 9303.*

c. Skinner M.K., Haque M.M., Nilsson E., Bhandari R.K., McCarrey J.R. (2013). Environmentally induced transgenerational epigenetic reprogramming of primordial germ cells and the subsequent germ line. *PLoS One. 15;8(7):e66318* . PMID: 23869203

d. Thayil, A., Wang, X. Bhandari P., vom Saal, F.S., Tillitt, D.E., Bhandari, R.K. Bisphenol A-induced transgenerational differences in expression of genes on reproductive axis of the male medaka. *Biology of Reproduction (Under review)*

3. **Molecular mechanism of sex determination in fish and mammals:** As a postdoctoral fellow, I studied molecular mechanisms of gonad development in fish and mammals. Studies in fish focused on role of endogenous estrogens in maintenance of germ cells' sexual fate. Studies in mammals focused on molecular pathways downstream of sex determination and environmental influence on male sex determination. Taking a ChIP-on-chip promoter tiling array approach, I identified downstream targets of sex determining factor proteins in fetal rat Sertoli cells, including some bHLH transcription factors and studied their role in Sertoli cell differentiation in the testis. Following are representative findings among the major findings:

   a. Bhandari R.K, Haque M.M. & Skinner M.K. (2012). Global genome analysis of the downstream binding targets of testis determining factor SRY and SOX9. *PLoS One. 2012;7(9):e43380*
   b. Bhandari, R.K., Schinke, E.N., Haque, M.M., & Skinner, M.K. (2012). SRY-induced TCF21 genome- wide targets and cascade of bHLH factors during Sertoli cell differentiation and male sex determination in the rat. *Biology of Reproduction, 87: 131-136.*
   c. Bhandari, R.K., Clement, T., Sadler-Riggleman, I. & Skinner, M.K. (2011). Basic helix-loop- helix transcription factor Tcf21 is a downstream target of male sex determining gene Sry. *PLoS One,* 6(5): e19935. pp1-11.
   d. Skinner, M.K., Bhandari, R.K., Haque, M.M., Nilsson, E.E. (2015). Environmentally induced epigenetic transgenerational inheritance of altered SRY genomic binding during gonadal sex determination. Environmental Epigenetics 1: 1-10. doi: 10.1093/eep/evv004

Complete List of My Published Work
http://www.ncbi.nlm.nih.gov/pubmed/?term=Ramji+Bhandari

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

<u>Ongoing Research Support</u>

R21 HD 098621 Bhandari (PI) 12/01/2019-11/30/2021
Correction of endocrine disruptor-induced transgenerational epimutations by CRISPR-dCas9
This research will develop CRISPR-dCas9 epigenome editing tool for precise insertion or deletion of DNA methylation marks into or from the epigenome. Further, it will be used to correct EE2-induced transgenerational epimutations on estrogen receptor CpG island #3 in medaka fish.

R21 ES027123 Bhandari (PI) 04/01/2017-03/31/2020
Germline transmission of epigenetic alterations to offspring induced by bisphenol A exposure
This research is aimed to characterize epigenetic reprogramming in medaka and identify heritable epigenetic memories in primordial germ cells established by developmental exposure to BPA.

R21 ES027123-02S1 Bhandari (PI) 04/01/2019-03/31/2020
Germline transmission of epigenetic alterations to offspring induced by bisphenol A exposure
This research concerns establishment of reprogramming-resistant epigenetic memory in the germ cells established by developmental exposure to BPA and their transmission to germ and soma in the third generation.

Faculty First Grant. Bhandari (PI) 04/01/2019- 03/31/2020
Development of CRISPR-dCas9 epigenome editing method to correct transgenerational epimutations in medaka.
The faculty first grant is an internal grant that supports studies to generate preliminary data for extramural grant application.

Completed Research Support

USGS G15AS00092 Bhandari (PI) 08/15/2015-12/31/2018
Transgenerational effects of atrazine exposure in medaka, *Oryzias latipes*
This research examined the long-term, transgenerational effects of developmental exposure to atrazine (broadleaf herbicide) on reproduction, epigenetic modifications (histone profiles, DNA methylation, and miRNA expression) in medaka germ cells across four generations.

Mizzou Advantage Grant 04/01/2014-3/31/2019
Endocrine disruption of the epigenome across taxa
This research examined effects of bisphenol A on brain and spermatogonial stem cell transcriptome and epigenome in three classes of vertebrates: fish, turtles, and mice.
Role: Co-PI (Dr. Cheryl Rosenfeld, PI)

University of North Carolina Greensboro Bhandari (PI) 08/01/2016-07/31/2019
Faculty Start up fund (Assistant Professor)
This fund was utilized to set up the laboratory and support supplies and salaries for student/postdoctoral fellows.