THE OHIO STATE UNIVERSITY
BIOMEDICAL SCIENCES
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THE OHIO STATE UNIVERSITY
COLLEGE OF MEDICINE

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PhD Candidate

“Hit the Brake Pedal for Multiple Human Diseases:
Roles of Slit-Robo Signaling in Pathogenesis of HIV-1
Infection, Vascular Endothelial Inflammation and
Breast Cancer”

April 2nd, 2015
BRT 115
9:00am-10:00am
VITA

2010..................................................................B.E. of Biomedical Engineering, Beijing University of Technology
2010 to present ..............................................Graduate Research Associate, Department of Pathology, College of Medicine, The Ohio State University
2013 to present ..............................................Pelotonia Fellow, The Ohio State University

COMMITTEE MEMBERS

Ramesh K. Ganju, PhD, Advisor
Li Wu, PhD, Committee Chair
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ABSTRACT

Discovered as the neuronal migration repelling mechanism during animal development, Slit-Robo signaling has been widely accepted to be the chemotactic inhibitory regulator of various types of cells. If one were to compare a cell to a vehicle, chemokine signaling would be the forward gear and gas pedal of the vehicle, and Slit-Robo signaling would be the reverse gear and brake pedal.

The signal molecule, Slit, is a family of secreted glycoprotein, which contains 3 isoforms, Slit1-3. The cellular surface receptor for Slit is Robo (Roundabout), which contains 4 isoforms, Robo1-4. It is now clear that, Slit and Robo are expressed and functional in a variety of tissues besides the neuronal system. And Slit-Robo signaling also plays important roles in regulating cell functions that are not directly related to cell migration. Thus, Slit-Robo signaling is proposed to regulate the post-development pathogenesis of multiple human diseases.

**HIV-1 infection:** Slit2 has been shown to regulate immune functions. However, not much is known about its role in HIV-1 pathogenesis. In our study, we show that the N-terminal fragment of Slit2 (Slit2-N) inhibits replication of HIV-1 virus in T-cell lines and peripheral blood T cells. Slit2-N inhibits HIV-1 infection by binding to Robo1 receptor and blocking viral entry into T cells. Slit2-N abrogates HIV-1 envelope-induced actin cytoskeletal dynamics in both T cell lines and primary T cells. Taken together, Slit2-N inhibits HIV-1 replication through novel mechanisms involving modulation of cytoskeletal dynamics.

**Vascular endothelial inflammation:** Slit2 and its receptors Robo1 and Robo4 are considered to regulate mobility and permeability of endothelial cells and other cell types. However, the roles of Slit2 and its two receptors in endothelial inflammatory responses remain to be clarified. In our study, we show that, in primary HUVECs, Slit2 represses LPS-induced secretion of certain inflammatory cytokines/chemokines, cell adhesion molecule ICAM-1 upregulation, and monocyte adhesion. Slit2’s anti-inflammatory effect is mediated by its dominant endothelial-
specific receptor Robo4. However, the minor receptor Robo1 has pro-inflammatory properties and is downregulated by Slit2 via targeting of miR-218. Slit2 represses inflammatory responses by inhibiting the Pyk2–NF-κB pathway downstream of LPS–TLR4. LPS enhances endothelial inflammation by downregulating the anti-inflammatory Slit2 and Robo4 in vitro as well as in vivo during endotoxemia. These results suggest that Slit2–Robo4 signaling is important in regulating LPS-induced endothelial inflammation.

**Breast cancer I:** Slit2-Robo4 signaling has been shown to protect endothelial integrity during sepsis shock and arthritis. In our study, we show that endothelial Robo4 is important for suppressing breast cancer growth and metastasis in vivo. Robo4 inhibits breast cancer growth and metastasis by regulating tumor angiogenesis, endothelial leakage and tight junction protein ZO-1 downregulation. Treatment of SecinH3, a small molecule drug which deactivates ARF6 downstream of Robo4, can enhance Robo4 signaling and thus inhibit breast cancer growth and metastasis by reducing tumor angiogenesis. In conclusion, endothelial Robo4 signaling is important for suppressing breast cancer growth and metastasis.

**Breast cancer II:** S100A7 is an inflammatory protein known to be broadly upregulated in breast cancer. However, the role of S100A7 in breast cancer has been elusive, since both pro- and anti-proliferative roles have been reported in different types of breast cancer cells and animal models. In our study, we show that S100A7 differentially regulates the expression of miR-29b in Estrogen Receptor (ER)-positive (MCF7) and ER-negative breast cancer cells (MDA-MB-231) through distinct modulation of NF-κB activation. The differential regulation of miR-29b by S100A7 further leads to differential regulation of PI3K p85α/CDC42 - p53 pathways. Reversing the S100A7-caused changes of miR-29b expression can inhibit the effects of S100A7 on cell proliferation and tumor growth in nude mice. In conclusion, the distinct modulations of the NF-κB – miR-29b – p53 pathway make S100A7 an oncogene in ER-negative and a cancer-suppressing gene in ER-positive breast cancer cells, with miR-29b being the determining regulatory factor.
All in all, Slit acts on different Robo receptors on various cell types, which modulates the cell behaviors during post-development pathological events. Upon comparing these regulatory effects of Slit-Robo in different diseases, there appears a common feature: “slowing the cells down as a brake pedal”. Slit-Robo slows down the T cell cytoskeletal dynamics during HIV-1 infection; Slit-Robo slows down the endothelial inflammatory responses to bacterial infection; Slit-Robo slows down the tumor blood vessel generation in breast cancer. Hence, it seems promising to target the Slit-Robo signaling for novel therapeutic methodologies of multiple human diseases.

RECENT ABSTRACTS AND PRESENTATION

- Breast cancer tissue context determines whether inflammation with bacterial/Psoriasin signature is pro- or anti-carcinogenesis. AACR San Antonio Breast Cancer Symposium, Texas. 12/2014.
- Regulation of Tetherin/BST-2 in Human T cells. Center for Retrovirus Research Meeting, OSU. 12/2013.
- S100A7/Psoriasin differentially modulates breast cancer growth through distinct regulations of the NF-kB – miR-29b – p53 pathway. Biomedical Sciences Graduate Program Annual Retreat, OSU. 12/2013. (Travel Award Winning)
- Slit-Robo Signaling Regulates Lipopolysaccharide-induced Endothelial Inflammation and Expression of Slit/Robo is Dysregulated During Endotoxemia. American Heart Association - Basic Cardiovascular Sciences Conference, Nevada. 07/2013.
RECENT PUBLICATIONS

AWARDS AND HONORS

- **Graduate Student of the Year**, Department of Pathology, College of Medicine, OSU. 05/2014
- **Biomedical Sciences Graduate Program Travel Award**. 12/2013.
- **Awarded the American Association for the Advancement of Science (AAAS) membership**. Nominated through the Program for Excellence in Science by Dr. Joanna Groden (Vice Dean, College of Medicine, OSU). 09/2013.

FUTURE PLANS

Helong is moving to Univ. of Utah for his post-doctoral training. And he is planning to pursue a career of biomedical research.