Association of Korean Neuroscientists

1st AKN Faculty Symposium

June 9, 2018

Burke Neurological Institute

Weill Cornell Medicine

Association of Korean Neuroscientists

1st AKN Faculty Symposium

Hotel: **Residence Inn Marriott**
5 Barker Avenue
White Plains, New York
(Phone, 914-761-7700)

Venue: **Burke Neurological Institute**
785 Mamaroneck Ave
White Plains, NY 10605
(Dr. Sunghee Cho, 203-912-0819)

Welcome
Rajiv R. Ratan MD. PhD, Executive Director, BNI

Opening Remarks
Prof. Jin Mo Chung, Ph.D.

Honorary Speaker
Prof. Tong H. Joh, Ph.D.

"Fortuitos moments in tortoise's stroll"

Registration and Breakfast: **8:30 – 9:10 am**
Opening and Greetings: **9:10 – 9:40 am**

Session One
Chaired by Dr. Un Jung Kang **9:40 – 12:00 pm**

Jae Lee, Ph.D. **9:40 – 10:00 am**
The Miami Project to Cure Paralysis, University of Miami School of Medicine
"Targeting the wound healing response to treat spinal cord injury"

Shin Kang, Ph.D. **10:00 – 10:20 am**
Anatomy and Cell Biology at Temple University
"OPC control for improved neural repair"

Sunghee Cho, Ph.D. **10:20 – 10:40 am**
Burke Neurological Institute, Weill Cornell Medical College
"From stroke pathology to recovery"

Coffee Break **10:40 – 10:55 am**

Andrew Yoo, Ph.D. **10:55 – 11:15 am**
Washington University School of Medicine
"Generation of human neurons via neuronal reprogramming of somatic cells and disease modeling"

In-Hyun Park, Ph.D. **11:15 – 11:35 am**
Yale School of Medicine
"Reprogramming and Rett syndrome"

Young-Jin Son, Ph.D. **11:35 – 11:55 pm**
Shriner Hospitals Pediatric Research Center and Center for Neural Repair
Lewis Katz School of Medicine, Temple University
"Repairing peripheral nerve injury"

Lunch **12:00 – 1:00 pm**

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Session Two

Chaired by Dr. Young-Jin Son — **1:00 – 4:10 pm**

Un Jung Kang, M.D. — **1:00 – 1:20 pm**
H Houston Merritt Professor of Neurology,
Chief of Movement Disorders Division, Columbia University
"Basal ganglia plasticity in PD and therapy"

Daewoo Lee, Ph.D. — **1:20 – 1:40 pm**
Biological Sciences, Ohio University
"Protein kinases as modifiers of tau toxicity and release"

Hanseok Ko, Ph.D. — **1:40 – 2:00 pm**
Johns Hopkins University
"New targets for Parkinson's disease"

Kwang-Soo Kim, Ph.D. — **2:00 – 2:20 pm**
Director of Molecular Neurobiology Lab at McLean Hospital
Harvard Medical School
"Personalized cell therapy for PD: Hype or Reality?"

Coffee Break — **2:20 – 2:35 pm**

Young Hwan Kim, Ph.D. — **2:35 – 2:55 pm**
Biological Sciences, Delaware State University
"SUMOylation of alpha-synuclein as a regulatory target in
Parkinson's disease pathology"

Yoon-Seong Kim, M.D., Ph.D. — **2:55 – 3:15 pm**
Burnett School of Biological Sciences, UCF
"Multiple layers of alpha-synuclein regulation and Parkinson's disease pathogenesis"

Jin Mo Chung, Ph.D. — **3:15 – 3:35 pm**
Cecil H. and Ida M. Green Distinguished University Endowed Chair
in Neurocience and Cell Biology, University of Texas Medical Branch
"Mechanisms of Acute and Chronic Pain"

Discussion Led by Dr. Kwang-Soo Kim — **3:40 – 5:00 pm**

Back to Hotel for Rest — **5:00 – 6:00 pm**

Dinner Dr. Sunghee Cho's House — **6:30 pm**
10 old Jackson Ave., Unit# 5
Hastings on Hudson, NY 10706
Home: 914-725-2943
Cell: 203-912-0819

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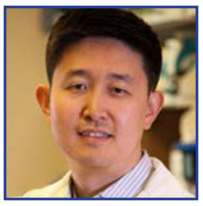

Dr. Jae Lee

University of Miami
Miller School of Medicine

The major research interest of my lab is to understand the wound healing response after spinal cord injury, which is comprised of inflammation, cell proliferation, and tissue remodeling. In particular, we are interested in the fibrotic scar and how it affects the regenerative capacity of the spinal cord. Our goal is to identify novel therapeutic targets that limit cellular damage and/or promote regeneration after injury to the spinal cord. Towards this goal, there are currently four major research projects in our lab.

1) Mechanisms of fibrotic scar formation after CNS injury: We have identified perivascular fibroblasts that infiltrate the lesion and form a fibrotic scar after experimental models of spinal cord injury and multiple sclerosis. This fibrotic scar has beneficial effects in closing the wound, but leads to an environment that is inhibitory to axon and oligodendrocyte regeneration. We are investigating mechanisms that can be targeted to limit this inhibitory effect.

2) Macrophage function after spinal cord injury: We previously found that hematogenous macrophages are an important mediator of fibrosis after spinal cord injury. Thus, we performed a macrophage-specific transcriptional profiling and discovered that the most highly expressed genes in macrophages at the injury site pertain to lipid metabolism, and that they have a very similar transcriptional profile as “foamy macrophages” that reside in atherosclerotic plaques. We are currently pursuing this surprising finding by investigating the mechanisms of lipid-laden macrophage formation, and the effect of lipid uptake on macrophage function at the injury site.

3) Oligodendrocyte progenitor cell response after spinal cord injury: A major roadblock in successful regeneration in the CNS is the lack of appropriate progenitor pools. However, there is a population of progenitors called the oligodendrocyte progenitor cells (OPCs) that are spread throughout the CNS. Although OPCs typically differentiate only into oligodendrocytes under physiological conditions, they display lineage plasticity after CNS injury by differentiating into astrocytes and even Schwann cells. We are investigating the mechanisms behind this lineage plasticity, and whether this process can be targeted to promote spinal cord repair.

4) Epigenetic regulation of inflammation after CNS injury: In spite of a large number of antiinflammatory agents in the market, none have passed clinical trials and received FDA approval for spinal cord injury. Thus, to identify novel anti-neuroinflammatory targets, we are currently investigating epigenetic regulation of neuroinflammation. In particular, we are interested in bromodomain and extra-terminal domain (BET) proteins, which are epigenetic readers that bind to acetylated histones to recruit transcriptional complexes. We found that pharmacological inhibition of BETs significantly reduces the expression of a wide range of pro-inflammatory cytokines after spinal cord injury. We are currently investigating cellspecific effects of BET inhibition, and identifying small molecule BET inhibitors that are best suited for treating spinal cord injury.

Novel methods used in Jae Lee’s lab:

- Tissue clearing using 3DISCO technique, and imaging using light sheet fluorescent microscopy.
- RiboTag method to obtain cell-specific mRNA that can be used with RNA-sequencing.

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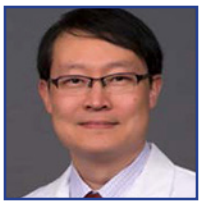

Dr. Shin Kang

Temple University

Our primary research interest is to understand molecular bases of oligodendrocyte (OL) dysfunctions and impaired OL regeneration, both of which profoundly impact pathogenic progression in many CNS disorders. Specifically, we ask why OLs are so vulnerable to disease-related stresses in a specific disease or injury contexts, and investigate the critical regulatory mechanisms for the remarkable regenerative behaviors of adult OL progenitors (OPCs, also known NG2+ cells) in the healthy CNS. We also seek to identify novel molecular targets for an improved OL regeneration for the timely functional myelin repair after injury or in diseases.

1. ***Cell-intrinsic mechanisms for OPC density control and cell mobilization toward OL differentiation:*** Adult OPCs are abundant and widespread throughout adult life, and these cells serve as the cellular reservoir for remyelination upon myelin damage or OL loss. Despite dynamic cell motility and proliferative nature, they maintain their cell density constant and exhibit a unique tiled distribution. Upon demyelinating injury, some OPCs proliferate more actively, while others exit from the cell cycle and undergo maturation processes to OL. Using OPC-specific conditional KO mice, we dissect and examine major signaling cascades to determine which signaling pathway regulates what aspect of the OPC behaviors.

2. ***Role of myelin turnover in ALS:*** The non-cell autonomous (i.e., glial-cell-derived) mechanisms contributing to motor neuron (MN) toxicity during the disease progression of ALS have been well documented. I have reported a massive gray matter OL degeneration in rodents and patients of ALS in parallel with MN injury, and an abnormal cell turnover of OL lineage cells in the spinal cord
of ALS mice. The OL-lineage-specific mutant SOD1 transgene gene deletion markedly delayed the disease onset and prolonged lifespan of the ALS mice. Using the genetic and pharmacological tools for myelin level control, we study the role of myelin integrity in the disease progression of ALS, and the relevant OL-specific disease related mechanisms.

3. ***Cell-specific expression profile of OLs and OPCs:*** We use BAC-TRAP approach and subsequent RNA-seq to profile genome-wide protein translation in the contexts of OL aging, disease-related OL degeneration, and GM vs. WM difference.

4. ***Role of glutamate receptor-mediated Ca2+ signaling in OL regeneration:*** OPCs and OLs express NMDA- or AMPA-type glutamate receptors, and their roles are not clearly understood. Especially, because the ionotropic glutamate receptors on OPC membrane form neuron-OPC synapses, it has been hypothesized that OPC-resident glutamatergic synapses may underlie neuronal activity-dependent myelination regulation. However, earlier studies suggested that OLlineage specific glutamate receptors mediate excitotoxic stress in injury. Thus, we are currently studying the cell-specific, stage-specific roles of NMDAR- or AMPAR-mediated Ca2+ signaling in injury, and post injury regeneration using mouse systems for conditional KO of NR1 (Grin1) subunit and OPC-specific overexpression of GluR2 (Gria2) in an injury mouse model.

Techniques that are currently used in the lab: Adult OPC fate tracking, OL linage-, stage-specific genetic manipulations (gene cKO, cell ablation, molecular overexpression, SOD1 (G93A) expression, myelin formation blockade etc.), BAC transgenesis, OL- or OPC-specific RNAseq in the physiological or pathological contexts, primary OPC and OL culture, a mouse model of perinatal hypoxic-ischemic injury.

Dr. Sunghee Cho

Burke Neurological institute/Weill Cornell Medicine

My lab investigates the pathology and repair/recovery mechanisms in chronic stroke with a focus on translational studies. We specifically address on the role of immune receptor CD36 that is highly expressed in monotypes/macrophages. Their role on stroke-induced inflammation and injury and how these immune cells interact with a neuronal system to influences stroke outcome and functional benefits are the main subjects.

1. ***The role of CD36 in stroke-induced inflammation/injury and recovery***
Since CD36 is expressed in many different tissues and cell types, including peripheral monocytes/macrophages, we investigate the effect of CD36 expressed in the peripheral organs including bone marrow, spleen and blood. The major questions to address in this study are the recognition of peripheral immunity on CNS injury, validating CD36 as a target in acute pathology, and characterizing a pharmacological agent that modulate CD36 pathways.

2. ***Comorbid-modified inflammation and brain injury in stroke***
The recurring failure to translate neuroprotective strategies in animal models into clinical settings prompted us to reevaluate existing preclinical stroke models. One major issue in preclinical studies has been the lack of inclusion of prevalent risk factors in animal models of stroke. We have been addressing this issue by including hyperlipidemia and diabetes, prevalent co-morbid conditions, in our experimental model of stroke. These studies will define if and how these risk factors modify peripheral immunity and influence stroke outcome and functional recovery and provide an importance of the inclusion of comorbidities in animal models of stroke.

3. ***Stroke recovery mechanism/Genetics***
Because strategies that reduce acute stroke-induced injury and inflammation in preclinical studies have not successfully translated into clinical practice, studies to understand repair/recovery mechanisms that promote functional recovery have been emerged. Genetics is among several factors that influence stroke recovery. We address a role of the BDNF single nucleotide polymorphism (SNP) on stroke recovery, which is common in humans. Using mice that contain the human BDNF SNP variant, we investigate the impact of the BDNF SNP on stroke recovery and underlying event for functional recovery with BDNF SNP carriers, by dissecting structural and molecular plasticity in the brain in chronic stroke.

The followings are major techniques established in my lab.

1. ***Animal model of stroke***
We generate animal model of stroke using an intraluminal thread method to occlude middle cerebral artery (MCAO). This is the most widely used animal model of stroke that produce injury in the striatum and part of cortex. The model has been used to study the pathology on acute (hours to days) and subacute (days to weeks) period and long-term recovery mechanisms (weeks and up o 6p month).

2. ***Behavior testing on motor/gait function and cognition.***
We have established comprehensive behavior testing modules for motor and cognitive function. The behavior tests include rotarod and gait functions by digitized Nordus Catwalk analyses system for stride length, walk speed, interlimb coordination, swing speed. For cognition, we use an automated system to record noble object recognition, Y-maze, elevated platform for anxiety test, and water maze for hippocampal memory.

3. ***Flow cytometer determination of immune cell.***
We have established a protocol to isolate immune cells from CNS and periphery in normal and stroked animals. Flow cytometeric measurement of immune cell population using 3-laser, 8-channel flow cytometer, this technique has been used to identify specific immune cell populations and subpopulations in the brain and peripheral organs.

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Dr. Andrew Yoo

Washington University School of Medicine

The primary goal of my research is to understand genetic pathways underlying neurogenesis and develop experimental methods to generate human neurons through direct conversion of human somatic cells. We model adult-onset neurodegenerative disorders by directly converting patient fibroblasts to discrete neuronal subtypes.

MicroRNAs and neuronal reprogramming: We study how microRNAs and chromatin remodeling complexes contribute to the adoption of neuronal identity and leverage this information to develop cellular reprogramming approaches to generate human neurons by directly converting (reprogramming) non-neural somatic cells such as dermal fibroblasts. We focus on miR-9/9* and miR-124, microRNAs which we discovered to be potent cell fate regulators that when ectopically expressed in human fibroblasts, could induce direct fate conversion to neurons through extensive reconfiguration of chromatin accessibilities. We found that the miRNAinduced neuronal state then permits the input of additional transcription factors enriched in distinct brain regions to generate discrete subtypes of human neurons. Based on these findings, we currently investigate which transcripts microRNAs target to affect the chromatin state and whether the genetic pathways defined in neuronal reprogramming is conserved during neurogenesis in vivo.

Modeling late-onset neurodegenerative disorders: Importantly, the converted neurons were found to retain the cellular age stored in the primary fibroblasts, allowing the generation of neurons that reflect the age of fibroblast donors, a feature beneficial for modeling adult-onset disorders. Leveraging these findings, we apply the neuronal reprogramming techniques in fibroblast samples derived from the patients of inherited neurodegenerative disorders to model and study the pathogenesis of the disease. Indeed, we recently established striatal medium spiny neurons (MSNs) generated through neuronal conversion of fibroblasts from Huntington's disease (HD) patients (HD-MSNs) as a cellular model of HD. HD-MSNs robustly display many hallmarks of HD pathology including HTT protein aggregation, increased DNA damage, mitochondrial dysfunction and neuronal death. We currently utilize HD-MSNs as a patient cell- based model to investigate genetic and epigenetic effectors in HD-MSNs underlying neurodegeneration in HD. In addition to HD, we leverage our recent discovery that when human adult fibroblasts undergo neuronal conversion, the converted neurons express transcripts of tau (MAPT) isoforms characteristic of adult human neurons, and use the converted neurons to delineate molecular mechanisms underlying tau isoform regulation in human adult neurons.

Experimental methods used in the lab: neuronal reprogramming protocols, lentiviral transduction, various genomics approaches (including HITS-CLIP (for Ago proteins), ATAC-seq, RNA-seq, single cell RNA-seq, slice culture, mouse embryo electroporation, standard molecular biology and biochemistry techniques.

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Dr. In-Hyun Park

Yale University

My research interest is to use human pluripotent stem cells (hPSCs) to investigate the human brain development and diseases.

1. Generation of human brain oragnoids.
We established methods to generate human dorsal or ventral cortical forebrain regions. These methods are useful to study human cortical development and developmental disorders.

2. Investigate the function of MeCP2
We engineered human embryonic stem cells (hESC) to introduce mutations in MeCP2 gene. These hESCs are useful to study Rett syndrome.

3. Investigate the X chromosome status in human pluripotent stem cells.
MeCP2 is present on X chromosome. In hESC, X chromosome status is unstable. My lab investigates X chromosome status in hESC and iPSCs.

4. Study the mechanism of epigenetic reprogramming
My lab also investigates how human or murine somatic cell reprogramming is established at epigenetic level.

The followings are major techniques established in my lab.

1. Differentiation of hESCs into neuronal cells and brain organoids
We use hESC and can differentiate neuronal lineages.

2. Genomics tools.
We perform and analyze RNA-seq, ChIP-seq, ATAC-seq, RRBS, and HiC, and use the data to study transcriptome, epigenome, and global chromatin status.

3. scRNA-seq
My lab can perform and analyze the scRNA-seq using 10XGenomics of Chromium.

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Dr. Young-Jin Son

Temple University

Repairing Motor and Sensory connections between the Spinal cord and Muscle

The long-term goals of my research are to elucidate the mechanisms that govern maintenance and regeneration of synaptic connections in the spinal cord and muscle, particularly those associated with glial cells, and to use this knowledge to promote repair of spinal cord-muscle connections in adults with injury, disease, disuse or aging.

Research projects (Current)

1. Oligodendrocyte progenitor cells as a novel inhibitor of CNS regeneration.
We are testing if Dorsal root axons fail to regenerate into the spinal cord by forming aberrant synapses with OPCs (or NG2 glia).

2. Coactivation of BRAF and mTOR signaling to promote spinal cord regeneration.
We have found that concurrent activation of BRAF and inhibition of PTEN induces unprecedented robust regeneration of dorsal root axons into the spinal cord after dorsal root injury. We are extending the finding to test further the strategy can lead to robust regeneration of primary sensory axons after direct SCI.

3. Novel Spinal cord ischemia induced by spinal root injury.
We are studying unexpectedly robust damage dorsal root injury can elicit in the spinal cord and related mechanisms.

4. Enhancing peripheral nerve regeneration.
We are manipulating BRAF, PTEN, ErbB2 signaling in motor and sensory axons, and/or Schwann cells to facilitate nerve regeneration after peripheral nerve injury (proximal and chronic injury models).

5. Role of YAP and TAZ in Schwann cell myelination and nerve repair.
We are studying the roles of Hippo signaling and the oncoproteins, YAP/TAZ in the development and maintenance of peripheral nerve myelination, Schwann cell plasticity and nerve repair.

Research methods

We primarily use mouse to study these projects. Our techniques include transgenic and knockout mice, cell type specific conditional and inducible gene or cell manipulation, in vivo time-lapse imaging, mouse microsurgery for spinal cord and DRG neurons, virus injection and tranduction of DRG and sciatic nerve (AAV and lentivirus), tissue clearing, in vitro co-culture of DRG neurons, OPCs, and/or Schwann cells, and other standard molecular, cellular and behavioral analyses.

Dr. Un Jung Kang

Columbia University

My current experimental research focus is on understanding basal ganglia plasticity in PD and explore neurorestorative therapy. In PD, degeneration of dopaminergic (DA) neurons leads to profound motor impairment. Although motor symptom is initially treatable by the DA drugs including the precursor levodopa (L-DOPA), patients experience disabling motor fluctuations, only partially treated with medications and deep brain stimulation (DBS). The loss of DA and replacement therapy leads to plasticity in the basal ganglia that may not correct by replacement of dopamine regardless of the mode of delivery including gene therapy (which was the focus of my earlier studies). Therefore, we study the neuronal circuitry changes that occurs in PD models. We are pursuing the idea that understanding the changes in specific cell types with each structure and connectivity between the basal ganglia structures will help us to design new approaches that overcome the limitation of current pharmacological therapy that targets the whole brain and the current DBS that targets anatomical structures and passing fibers without discriminating circuit and cell type specificity.

We focus on a component of L-DOPA's antiparkinsonian response, known as the long-duration response (LDR). I believe LDR is the key factor in the emergence of disabling motor fluctuation whereas the current PD therapy is focused on prolonging the short-duration response that occurs in the time scale of hours after the administration of dopaminergic drugs. We developed the first animal of model of LDR and proposed mechanism involving motor learning based on basal ganglia plasticity. We are testing the hypothesis that gradual motor impairment during LDR decay is task specific and results from aberrant LTP in specific ensembles of indirect pathway medium spiny neurons (iMSNs) that are normally suppressed during normal movement by D2R stimulation, but become pathologically active during task exposure if DA is depleted. Understanding molecular and circuitry mechanisms of LDR may provide novel nondopaminergic approaches to PD therapy.

Another aspect of motor fluctuation is excessive abnormal involuntary movements (L-DOPAinduced dyskinesia [LID]) that develops over several years of treatment. We are looking for differential pathways and mechanisms that produce LID vs. antiparkinsonian therapeutic effects. We have recently reported that elevated cholinergic signaling in the striatum may be a major and selective contributor to LID. We utilize chemogenetic and optogenetic modulation of the striatal ChI to study their functional effects. We employ transcriptomic analysis of ChI to find potential mechanisms of altered ChI activity and therapeutic targets. We are also studying role of the basal ganglia output structures such as the substantia nigra pars reticulata (SNr) - the target of current DBS - and their connectivity in antiparkinsonian effect and production of LID.

I am also interested in neurodegenerative mechanisms using rodent models, which have been challenging to translate to effective therapy. My current focus is on biomarker development for PD that is based on the understanding of pathogenesis since I believe that understanding the pathogenic heterogeneity of PD is critical for translation. I lead a multicenter biomarker cohort, BioFIND which serves as an open resource for bioassay development and is involved in biomarker review panels for PD cohorts to coordinate the international biomarker studies.

I have a limited but active clinical practice in movement disorders and lead a team on translational research programs involving genetics and biomarkers.

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Dr. Daewoo Lee

Ohio University

My current research is to understand pathogenic mechanisms underlying neurodegenerative diseases. In particular, we are interested in prion-like propagation of pathogenic proteins such as α-Synuclein and tau.

1. ***Cell-to-cell propagation of α-Syn:*** Abundant neuronal protein α-Syn is a pathogenic protein to form abnormal accumulation of protein aggregates, called Lewy body (LB) and cause several neurodegenerative diseases such as Parkinson's disease. This prion-like spreading of α-Syn is an exciting new discovery in the progression of neurodegenerative diseases. Nonetheless, there are critical gaps in our understanding of α-Syn spreading. We aim to explore how α-Syn spreads between cells in the nervous system. Specifically, we are interested in studying how neuronal subtype, α-Syn form (wildtype, mutants), and functional/molecular factors affect pathological transmission of α-Syn.

2. ***Protein kinases as modifiers of tau propagation:*** Neurofibrillary tangle (NFT) is a hallmark of Alzheimer's disease (AD) and related disorders. The tangle is composed of microtubule associated protein tau (MAPT), which can be hyper-phosphorylated and aggregated. Indeed, tau protein purified from the brains of AD patients is hyper-phosphorylated, that has led to investigate the role of tau phosphorylation in mediating its toxicity. A number of kinases such as GSK3 have been studied for their role in tauopathy. However, a whole range of kinases selected by genome-wide screening need to be examined in order to have a comprehensive understanding on the role of tau phosphorylation in AD because tau protein has over 80 putative phosphorylation sites. Unbiased genetic screens using transgenic Drosophila lines have identified enhancers and suppressors of tau toxicity, including 15 human kinase homologs. We plan to examine all of these identified kinases for tau toxicity and release in Drosophila neuronal culture system that expresses human tau (hTau).

Other research projects:

3. ***Dopamine signaling and Parkinson's disease:*** We have studied neurodegenerative and neuroprotective role of dopamine signaling in PD. Dysregulation of dopamine homeostasis causes selective neurodegeneration while activation of D2 receptors is neuroprotective.

4. ***Biogenic amine signaling and olfactory learning.*** The main goal of this project is to investigate functional roles of dopamine and serotonin receptors in synaptic plasticity and olfactory learning. We also study their downstream G-protein signaling mechanisms. It is of our particular interest to understand the role of DA autoreceptors in modulating excitability, synaptic inputs and neuroprotection in DA neurons.

Technical expertise in my lab:

Electrophysiology (patch clamp, amperometry), Primary neuronal culture, Cellular imaging/analysis, Western blot. Confocal microscopy, Optogenetics & chemogenetics, Drosophila genetics (mutant & transgenic approaches), Behavioral assays (learning & locomotion)

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Dr. Hanseok Ko

Johns Hopkins University

My work has been focused on the elucidation of the molecular mechanisms of neurodegeneration and other discoveries that have led to innovative approaches and enhanced the development of new agents to treat Parkinson disease and other neurodegenerative disorders. In addition, I have been contributing to establish PD mouse models that lead to progressive age dependent loss of dopamine neurons.

New therapeutic targets for PD:
1. c-Abl: We have defined a critical role of c-Abl in α-synuclein-induced neurodegeneration and demonstrated that selective inhibition of c-Abl is sufficient for neuroprotection, and that phosphotyrosine 39 α-synuclein is a potential disease indicator for Parkinson's disease (PD) and related α-synucleinopathies. We have been investigating potentially safer and more effective c-Abl inhibitor drug options in mouse models of PD.

2. Lymphocyte-Activation Gene 3 (LAG3): We have elucidated the molecular mechanisms of prion-like cell-to-cell transmission of misfolded α-synuclein in the brain of Parkinson's disease. We have identified lymphocyte-activation gene 3 (LAG3) as a misfolded α-synuclein preformed fibrils (PFF) transmission receptor.

3. Graphene Quantum Dots (GQDs): We have found that graphene quantum dots (GQDs) have notable potency in not only inhibiting fibrillization of α-synuclein (α-syn) but also disaggregating mature fibrils mainly by virtue of their amphiphilic nature via direct interaction with α-syn. In addition, for the first time, we have found that treatment of GQDs rescue a cardinal Parkinson's disease phenotypes induced by α-syn preformed fibrils (PFF) in vitro and in vivo through the penetration of the blood-brain barrier (BBB). Our research team is investigating whether GQDs' strong anti-amyloid effect on Alzheimer's Aβ, tau and TDP-43 fibrils.

4. New Glucagon-like peptide-1 (GLP-1) agonist: We have shown that a potent, brain penetrant long acting GLP-1R agonist NLY01 protects against the loss of dopamine neurons and behavioral deficits in the α-synuclein preformed fibril (α-syn PFF) model of sporadic PD. NLY01 also prolongs the life and reduces the behavioral deficits and neuropathological abnormalities in the human A53T α-synuclein (hA53T) transgenic (Tg) model of α-synucleinopathy induced neurodegeneration. We have found that NLY01 is a potent GLP-1R agonist with favorable properties that is neuroprotective via the direct prevention of microglial mediated conversion of astrocytes to an A1 neurotoxic phenotype. Our research team is investigating whether NLY01 possesses strong neuroprotective effect in Alzheimer's and ALS mouse models.

5. Glucocerebrosidase 1 (GBA1): Currently our research team is investigating on how dysfunction of mutated GBA1 leads to PD or how GBA1 becomes dysfunctional in the absence of mutations in sporadic PD.

PD mouse models:
1. Gut PD mouse model: We have developed a method where PFFs are injected into the muscle layer of the pylorus and duodenum, mimicking the spread of pSer129-α-syn accumulation observed in Parkinson's disease.

2. Conditional VPS35 transgenic PD mouse model: To better understand the pathogenic involvement of VPS35 mutations in PD, we have generated a tetracycline conditional human VPS35 transgenic (Tg) mouse where expression of mutant human D620N VSP35 or wild-type (WT) human VPS35 proteins is achieved in the nigrostriatal dopaminergic pathway, under the control of the dopamine pathway-specific tyrosine hydroxylase (TH)-tTA driver.

Technology: We have various iPS cells and mouse models related to Parkinson's disease.
1. Differentiation of PD-iPSCs into human dopaminergic neurons: Triplication α-SNCA, GBA1-N370S and E326K along with control iPSC lines
2. Knockout (KO) and Knockin (KI) mouse lines: c-Abl cKO, Trip12 cKO, NOD2 KO, DJ-1 KO, RIPK2 KO, GBA1 KO, GBA1 E326K cKO, GBA1-L444P KI, and GBA1-D409H KI. GLP-1R KO.
3. Transgenic (Tg) mouse lines: TetP-WT, A53T, and E46K α-synuclein, hA53T α-synuclein G2 mice, TetP-WT and D409H VPS35, C9-500-FTD, RPL-Tg, SODG93A, 3xTg-AD, 5xFAD-Tg.
4. Driver mouse lines: TH-tTA, CamkIIα-tTA, Nestin-Cre, CMV-Cre, DAT-Cre, CX3CR1-Cre

Dr. Kwang-Soo Kim

McLean Hospital/Harvard Medical School

My research interest has been in molecular genetic mechanisms underlying the development and fate specification of midbrain dopamine neurons, focusing on identification and characterization of the transcriptional regulatory cascade. Based on these studies, we are identifying potential drug target(s) to develop mechanism-based neuroprotective therapeutics for neurodegenerative disorders such as PD and AD. In addition, my laboratory is interested in developing stem cell technology for better understanding and treatment of neurodegenerative disorders.

1. ***Transcriptional regulatory cascade of midbrain dopamine neurons:*** My laboratory has identified and/or characterized several fate-determining transcription factors that play key roles for the development and survival of midbrain dopamine neurons. Genetic and functional network between signaling molecules (e.g., Wnt1 and Shh) and key transcription factors (e.g., Nurr1, Lmx1a, Pitx3) have been elucidated. Based on these molecular and developmental studies, my laboratory is interested in translational research with potential preclinical and clinical applications.

2. ***Nurr1 as a potential drug target and identification of synthetic and natural ligands of Nurr1 as potential small molecule drug candidates:*** Our research has recently been focusing on a potential drug target, the orphan nuclear receptor Nurr1, which is critical not only for the development and long-term maintenance of midbrain dopamine neurons, but also for their protection from inflammation-induced cell death by suppressing pro-inflammatory gene expression. Toward this, we established efficient high throughput screening assays for small molecules that can boost the transcriptional activity of Nurr1 Although Nurr1 is known to be a ligandindependent nuclear receptor, we identified both synthetic and natural ligands that activate Nurr1 through binding to its ligand binding domain.

3. ***Molecular mechanisms underlying metabolic reprogramming:*** During the reprogramming process, cell fate changes from somatic tissues to iPS cells are accompanied by dramatic metabolic changes, which is so called metabolic reprogramming. We are investigating molecular mechanisms underlying this metabolic reprogramming.

4. ***Personalized cell therapy for Parkinson's disease:*** During our stem cell research, I had keen interest in the development of clinically feasible and safe induced pluripotent stem (iPS) cell technology. The iPS cell research was pioneered by Prof. Shinya Yamanaka in 2006, igniting explosive interests from scientific and non-scientific communities because iPS cells may be used as ideal cell source to avoid ethical and scientific issues by providing unlimited patient-specific stem cells. However, there are many issues to overcome to realize iPSC-based personalized cell therapy. To address these, we are currently investigating molecular mechanisms underlying the reprogramming process, and developing efficient and safe reprogramming methods. In addition, we developed a chemical method that can eliminate remaining undifferentiated stem cells with a potential of tumor formation. We also optimized in vitro differentiation protocol to generate unlimited dopamine progenitor cells to treat and study Parkinson's disease.

Major research techniques in the lab:

- High throughput screening of Nurr1-activating small molecules
- Optimal generation of clinical grade human iPSCs
- Metabolic analyses of different cell fates
- Optimal in vitro differentiation of hESCs or hiPSCs to neuronal lineages
- Animal models of Parkinson's disease

Dr. Y. Hwan Kim

Delaware State University

Studying the neuropathology of Parkinson's disease & developing potential combination therapies

Assessing mechanisms of SUMOylation in DAT, alpha-synuclein, and LRRK2 in Parkinson's disease pathology: Post-translational modification (PTM) has been addressed as a key regulatory mechanism for modulating protein aggregation/degradation in neurodegeneration. However, a form of PTM, Small Ubiquitin-like Modifier (SUMO) has not been well studied in Parkinson's disease (PD) pathology. Although SUMOylation may increase the solubility of alpha-synuclein, SUMOylated proteins including alpha-synuclein have been detected in the halo of Lewy bodies. Thus it is still unclear in understanding the role of SUMOylation in dopaminergic neurons. Here, we assess the role of SUMO conjugase, Ubc9 as a critical post-translational modifier to regulate the solubility, stability, and function of dopamine transporter (DAT), alpha-synuclein, and LRRK2 in dopaminergic neurons in vitro and Ubc9 over-expressing transgenic mice. The objectives of this work is to elucidate the mechanisms of SUMOylation in preventing alpha-synuclein mediated protein aggregation and enhancing dopamine uptake via DAT and kinase activity from LRRK2 in dopaminergic neurons. This implies that pathological changes in the SUMOylation of DAT and/or alpha-synuclein may lead to alteration in dopamine reuptake and acute regulation of protein (mis)folding or aggregation, which is related to the neuropathology of PD. We identified that DAT, alpha-synuclein and LRRK2 are constitutively SUMOylated in mouse striatum and midbrain. Our in vitro preliminary results demonstrated that Ubc9 over-expression protects rat N27 dopaminergic cells against MPP+ induced oxidative stress and prevents DAT and alpha-synuclein degradation via inhibition of proteasome and lysosome (Cartier et al., under 3rd review). Moreover, Ubc9-mediated SUMOylation increases the surface level of DAT in the plasma membrane and further enhancing the dopamine uptake capacity. In the MPTP-lesioned mice, the chronic treatment substantially reduces the level of SUMO1- conjugated alpha-synuclein in the mouse striatum. This suggests that pathological changes in the SUMOylation of DAT and alpha-synuclein result in significant alteration in dopamine clearance/recycling and protein (mis)folding or aggregation, respectively. Therefore, SUMOylation of DAT, alpha-synuclein and LRRK2 can be potential therapeutic targets for neurological disorders such as ADHD, depression, and PD.

Developing neuroprotective compounds as potential therapeutics in Parkinson's disease mouse models: Most available PD drugs are designed to alleviate the PD motor symptoms and cause side effects after long-term use. Thus we focus on identifying potential neuroprotective compounds to halt or slow the neuropathology, in addition to alleviating motor- and non-motor symptoms. After our initial screening of over 100 novel compounds from our collaborator, AurimMed Pharma Inc (Park City, Utah), we identified that more than 20 compounds showed potent neuroprotective effects in dopaminergic cells. Further, the lead compound (AMP-X-0079) not only provided neuroprotective effects and higher survival rates from rotenone-induced toxicity, but also induced higher mobility in the fly and mouse models. Our recent mouse studies suggest that oral treatment of AMP-X-0079 for two weeks was sufficient to improve motor functions in behavioral tests such as pole, hindlimb clasping, cross-beam, rotarod, and open-field ambulatory mobility tests. Furthermore, we identified that the novel compound provided the neuroprotective/recovery effects from the MPTP-induced deficits in the mouse brain. Our study will provide prerequisites for developing a therapeutic application and launching an Investigational New Drug study. Since AurimMed's compounds are safe and orally administrable for penetrating the blood brain barrier, the lead compound can be quickly moved on to be tested in human subjects. The overall goal is to develop clinically safe, orally available anti-Parkinsonian drug candidates intended to significantly slow down the disease progression via the neuroprotective properties, in addition to relieving PD symptoms.

Main research techniques in the lab: Cell viability/cytotoxicity assay, Western blot, Confocal microscopy, stereology, Cycloheximide-protein stability assay, FRET, Mass Spectrometry, behavioral (mobility) tests, and mouse brain imaging (MS-imaging and fMRI).

Dr. Yoon-Seong Kim

Burnett School of Biomedical Sciences at UCF

Our studies mainly focus on oxidative stress, epigenetic regulation and their roles in the pathogenesis of Parkinson's disease (PD) with special emphasis on alpha-synuclein (a-SYN).

1. ***The role of NADPH oxidases (NOXs)-mediated oxidative stress in the pathogenesis of Parkinson's disease.*** In addition to mitochondria, we have identified NADPH oxidase 1 (NOX1) as a molecular source of ROS which is responsible for dopaminergic neuronal death. NOX1 is highly expressed in the intestinal epithelium, from where recent accumulating evidence suggests that a-SYN aggregates progressively propagate to the brain parenchyma. Using Nox1 null mice, we are investigating gut microbiome-Nox1 activation-a-SYN pathogenesis axis in PD.

2. ***Contribution of transcriptional mutagenesis of oxidative DNA lesions to generating new mutant α-SYN species and aggregation.*** We have recently discovered that 8-oxo-dG, the most frequent oxidative DNA lesion, can generate mutant a-SYN species by the intriguing mechanism called transcriptional mutagenesis. These mutant a-SYN mRNA species were more frequently observed in the substantia nigra of PD patients compared to normal subjects. We are investigating how these mutant species contribute to the alpha-synucleinopathy.

3. ***Pum2-mediated translational regulation of alpha-synuclein mRNA on the outer surface of mitochondria.*** Interplay between mitochondria and a-SYN has been widely documented yet without clear molecular mechanism. We have found that a-SYN mRNA is localized to the outer surface of mitochondria and its translation is initiated upon stimuli causing mitochondrial ROS. We have identified that Pum2, a translational repressor, binds to the 3'UTR of a-SYN mRNA and it is released upon mitochondrial ROS, allowing translational initiation of a-SYN near mitochondria. We are investigating the role of translational control of a-SYN near mitochondria.

4. ***Chromatin landscape and epigenetic regulation of a-SYN in PD.*** In human, the α-SYN gene (SNCA) contains high CpG rich region around transcription start site. We have found that CpGs in this region of dopaminergic neurons and human brain tissue are largely unmethylated in both control and PD conditions. Histone marks, however, demonstrate significant differences between them with for example much higher H3K4me3 levels in PD, supporting elevated a-SYN levels. To modulate epigenetic marks in a precise target-specific manner, a CRISPR/dCas9-Suntag technique has been developed.

The followings are major techniques established in my lab.

1. ***The CRIPSR/dCas9-Suntag based target-specific epigenetic modifiers.*** We recently established ten epigenetic modifying enzymes that modulate major histone marks including H3K4me3, H3K27me3, H3K9ac, H3K27ac and DNA methylation using CRISPR/dCas9-Suntag system. This epigenetic tool kit allows target-specific modulations of each genomic loci. In conjunction with sgRNA library spreading over the entire genome, this innovative technique can be applied to the identification of specific genes whose epigenetic modulations are critical for various disease conditions.

2. ***Single-molecule fluorescence in situ hybridization (smFISH) with human brain clearing technique.*** To overcome strong auto-fluorescence from human brain tissue, especially dopaminergic neurons due to neuromelanin, we have established the technique to clear proteins/lipids after RNA-anchoring/gel embedding, enabling clear visualization of a single RNA. Together with the expansion microscope technique, subcellular localization of single RNA molecule can be visualized.

3. ***Single-molecule pull down assay to count minute amounts of proteins from human brain.*** We established this technique to literally count small amount of mutant a-SYN protein contained in postmortem substantia nigra samples. This technique uses TIRF microscope with mathematical image analysis, allowing stoichiometric information of proteins, for example, oligomeric status of a-SYN.

Association of Korean Neuroscientists

Dr. Jin Mo Chung

University of Texas Medical Branch at Galveston

Title: Mechanisms of Acute and Chronic Pain

Since most of the audience are not pain researchers, I will avoid a heavy data presentation. Instead, I will give an introductory overview talk on the mechanisms of acute and chronic pain with a light mixture of experimental data.

The tentative table of contents is as follows:

- Pain – Incidence and costs
- Types of Pain: acute and chronic pain
- Mechanisms of acute pain
- Mechanisms of chronic pain
    - o Sensitization of spinal dorsal horn neurons
    - o Cause of central sensitization - Ectopic discharges
    - o Cause of ectopic discharges
    - o Nature of central sensitization – ROS involvement and spinal LTP
- Chronification of acute to chronic pain
- Summary

Techniques being used include:

- In-vivo rodent pain models
- Behavioral tests for pain
- Whole cell electrophysiology in spinal cord slices
- Optogenetic and chemogenetic application for selected afferent populations
- The most important “technique” I emphasize to my trainees, but they don’t do enough of is “thinking”