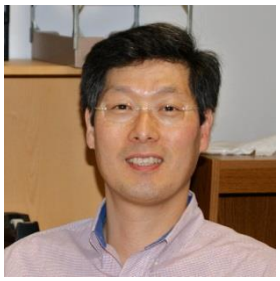
The Kim Lab: Assessing the neuropathology of Parkinson's disease and developing potential combination therapies



Parkinson's disease (PD) is the most common neurodegenerative motor disease, affecting more than 1% of the population above the age of 60. The disorder is primarily characterized by the selective loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) in the midbrain and formation of intraneuronal inclusions called "Lewy bodies" which contain alpha-synuclein as their major protein component. The pathological mechanisms involved in neuropathology associated with PD is largely unknown, however, the A53T mutation of alpha-synuclein causes Lewy body formation and is a well-known genetic PD model.

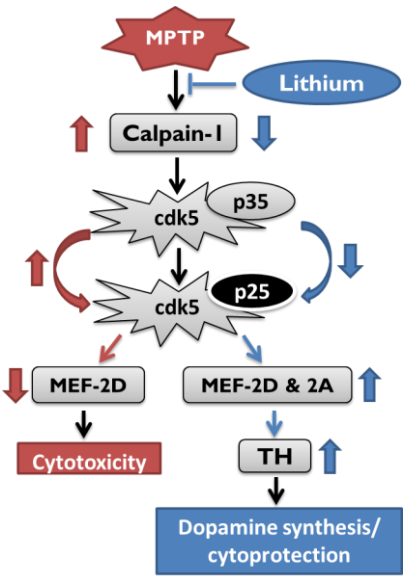
Project 1: **Developing neuroprotective compounds as potential therapeutics in Parkinson's disease mouse models:** Most available PD drugs are designed to alleviate the PD motor symptoms and cause side-effects after long-term use. Thus we focus on identifying potential neuroprotective compounds to halt or slow the neuropathology, in addition to alleviating motor- and non-motor symptoms. After our initial screening of over 90 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.*

Project 2: **Assessing mechanisms of SUMOylation of DAT and alpha-synuclein 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 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) and alpha-synuclein in dopaminergic neurons *in vitro* and *in vivo*. The objective of this work is to assess SUMOylation as a novel regulatory target for preventing alpha-synuclein mediated protein aggregation and regulating dopamine uptake via DAT 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 both DAT and alpha-synuclein 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. Moreover, Ubc9-mediated SUMOylation increases the surface level of DAT in the plasma membrane and further its action enhances DAT functional expression in the plasma membrane, triggering an increase in dopamine uptake capacity. In the MPTP-lesioned mice, the chronic treatment substantially reduces the level of SUMO1 conjugated to alpha-synuclein in the mouse striatum. This implies that pathological changes in the SUMOylation of DAT and alpha-synuclein may lead to alteration in acute regulation of dopamine clearance/recycling and protein (mis)folding or aggregation, respectively. Therefore, SUMOylation of DAT and alpha-synuclein can be a potential therapeutic target for neurological disorders such as ADHD, depression, and PD.

Project 3: **Lithium as a potential therapeutic in PD and its mechanisms:** Lithium has recently been suggested to have neuroprotective effects in several models of neurodegenerative disease including Parkinson's disease (PD). Levodopa (L-Dopa) replacement therapy remains the most common and effective treatment for PD, although it induces the complication of L-Dopa induced dyskinesia after years of use. Here we examined the potential use of lithium in combination with L-Dopa/Carbidopa for both reducing MPTP-induced abnormal involuntary movements (AIMs) as well as protecting against cell death in MPTP-lesioned mice. Chronic lithium administration (0.127% LiCl in the feed) in the presence of daily L-Dopa/Carbidopa injection for a period of 2 months was sufficient to effectively reduce MPTP-induced AIMs in mice. Mechanistically, lithium was found to suppress MPTP-induced calpain activities *in vivo* coinciding with down-regulation of calpain-1 but not calpain-2 expression in both the striatum (ST) and the brain stem (BS). Calpain

inhibition has previously been associated with increased levels of the rate-limiting enzyme in dopamine synthesis, tyrosine hydroxylase (TH), which is probably mediated by the up-regulation of the transcription factors MEF-2A and 2D. Lithium was found to induce up-regulation of TH expression in the ST and the BS, as well as in N27 rat dopaminergic cells. Further, histone acetyltransferase (HAT) expression was substantially up-regulated by lithium treatment *in vitro*. These results suggest the potential use of lithium in combination with L-Dopa/Carbidopa not only as a neuroprotectant, but also for reducing AIMs and possibly alleviating potential side-effects associated with the current treatment for PD.

In conclusion, lithium-only treatment may not be an excellent therapeutic option for neurodegenerative diseases due to inconsistent efficacy and potential side-effects, however, the use of low dose of lithium in combination with other potential or pre-existing therapeutic compounds may be a promising approach to reduce symptoms and disease progression in numerous neurodegenerative diseases.



A schematic view of putative mechanisms involved in lithium induced cytoprotection. Abb.: cdk5: cyclin-dependent kinase-5; MEF2: myocyte enhancer factor 2; TH: tyrosine hydroxylase.

Project 4: **Synergistic Damage of Commercially Available Environmental Toxins in Parkinson's Disease Models:**

Although several genetic mutations have been identified to cause Parkinson's disease (PD), the vast majority of cases are considered to be sporadic or multifactorial. Interestingly, epidemiological studies have shown that PD is more prevalent amongst farmers and rural populations. Thus, it has been suggested that exposure to pesticides and other environmental toxins may increase the risk of PD. In support of this notion, it has been shown that the herbicide, paraquat, and the fungicide, maneb, can cause motor deficits individually, as well as cause synergistic damage in mice when used together. Here we tested commercially available pesticides for causing synergistic or additive damage in an *in vitro* PD model, when used together. We exposed rat dopaminergic N27 cells to commercially-used pesticides such as acephate, alachlor, atrazine, diuron, 2-methyl-4-chlorophenoxyacetic acid (MCPA), and mecoprop at varying concentrations and measured cell viability using MTT assays. After identifying the non-toxic concentrations of single pesticide treatments, we measured the cell viability with the exposure of two different combinations of these pesticides. When tested individually, only high concentrations of diuron (14.4 and 28.8 µM) caused a significant decrease in cell viability. However, when we examined the effect of the combined pesticides at concentrations that did not show damage individually, we identified four combinations that cause synergistic loss, and five that cause additive loss in cell viability. Our results suggest that exposure to multiple combinations of pesticides may cause dopaminergic toxicity and further lead to sporadic PD pathology. Furthermore, we found that apoptosis was the mechanism of cell death in at least one of the pesticide combinations. Our results can bring more public awareness to the detrimental effects of combined pesticide usage in PD pathology. Currently we are testing those synergistic toxicities of pesticide combinations in PD fly (collaboration with Lawal Lab) and mouse models.

Project 5: Differential alpha-synuclein interactions mediate Parkinson's disease pathology: Using recently created alpha-synuclein-EGFP transfected N27 rat dopaminergic cell lines, we compare the nuclear and mitochondrial localization rate of wild type (WT) synuclein with that of various mutants of the protein (the familial mutant A53T and two mutants of non-phosphorylated (S129A) and phosphorylated mimic (S129D) at ser-129 to assess how these events may alter subcellular location and further dopaminergic cellular dysfunction. In our preliminary data, we found that serine-129 phosphorylation mimic (S129D) was significantly interrupted in the translocation to nucleus, compared with non-phosphorylation mimic (S129A). In addition, the histone acetyltransferase (HAT) activity in S129D was lower than that in S129A, while the histone deacetylase (HDAC) activity was not different.

We hypothesize that the mutation (A53T) and alteration at ser-129 phosphorylation may impact on rates of nuclear localization, thereby affecting histone binding/acetylation and subsequent transcriptional regulation. These mutation/alterations in alpha-synuclein may also induce different protein-protein interactions for mediating protein aggregation or degradation, in addition to impact on localization to the nucleus or the inner mitochondrial membrane and mitochondrial function. These proposed studies will give us insights of the mechanisms involved in subcellular localization and differential protein-protein interactions of different forms of alpha-synuclein (WT, A53T, S129D, and S129A) and how this impact on neuropathological processes associated with PD via alterations in protein degradation and gene expression or mitochondrial dysfunction. The Mass spectrometry analysis will be performed by a collaborator (Alex Cole Lab) at the Central Florida University.

Current Lab members: Y. Hwan Kim, Ph.D. (Principal Investigator, Associate Professor)
Etienne Cartier, Ph.D. (Post-doc),
Carol Lazzara, MS (Ph.D. student, Neuroscience),

Juan Viana (MS student, Neuroscience),
Undergraduate researchers: Sundus Ahmed, Thaddeus Lehman, Alexis Neuer, and David Dale.

Lab Alumni: Eric Janezic (MS, Neuroscience): a Ph.D. student at the University of Washington, Seattle, WA
Janae Caviness (MS, Neuroscience): Cell culture scientist, Eurofins Lancaster Lab, West point, PA
Sambee G Kanda (Undergrad researcher): a M.S. student at the University of Delaware.
Margaret Steward (Undergrad researcher): a Ph.D. student at the Ohio State University.
Xenia Davis (Undergrad researcher): a Ph.D. student at the University of Cincinnati.
TaeHo Cho (Undergrad researcher): a Ph.D. student at DGIST in S. Korea.
Cassio Noso (Undergrad researcher): a Ph.D. student at Univ. of Sao Paolo in Brazil.
Nicole Brown (Undergrad researcher): M.A. student in Science education.
Kinjal Patel (former lab manager).

Former Undergraduate interns: Doug Mullen (MS student, Liberty Univ), Young Lee (U. Delaware), JT Lee (Applying med school), Katrina Mitchell (Preparing for med school), Joseph Katz (U. Delaware), and Summer Stone (Wesley College).

Common Lab techniques: Western-blot, qRT-PCR, cell viability assays (MTT & LDH), ELISA, protein activity assays (including DAT, HAT & HDAC), microarray, Immunoprecipitation, immunohistochemistry, stereology, and Mass Spectrometry (collaboration).

Education & Training (Y. Hwan Kim)

Korea University, Seoul, S. Korea	BS	Plant Physiology/ Genetic Engineering
Korea University, Seoul, S. Korea	MS	Biotechnology
University of California, Los Angeles (UCLA)	Ph.D.	Neuroscience
Johns Hopkins Medical Institution, Baltimore, MD	Post-doctoral fellow	Neuropathology in School of Medicine
Buck Institute for Research on Aging, Novato, CA	Sr. Post-Doc	Parkinson's Disease

Memberships and Awards (Hwan Kim)

Jul, 2015 - present: Scientific Advisory Board member, AurimMed Pharma, Inc. Park City, UT.
Aug. 2016 - present: Editorial Board Member, Current Updates in Aging. OPR Science.
2016: Junior Faculty Research Award, College of Math, Natural Science and Tech (CMNST), DSU.
2002 - present: Member, Society for Neuroscience (SfN).
2004 - 2005: Graduate student Dissertation fellowship, UCLA. Los Angeles, CA.

Patent

Julie Andersen and **Kim YH.** Low dose Lithium in the treatment or Prophylaxis of Parkinson's disease. 2013 USPTO patent # US 20130017274 A1

Peer-Reviewed Research Publication

- Chae KS and Kim YH. A potential impact of geomagnetic field in transcranial magnetic stimulation therapy for neurodegenerative diseases. Frontiers in human neuroscience. (under review)
- Cartier E, Garcia-Olivares J, Lin ML, Janezic EM, Torres G, and Kim YH. The SUMO conjugase Ubc9 regulates the dopamine transporter stability, degradation, and enhances its functional expression. J. Biol. Chem. (in final revision).
- Janezic EM, Caviness J, Kanda GS, Davis XD, and Kim YH. Commercially available pesticides cause additive or synergistic damages in dopaminergic cells: Relevance for Parkinson's disease pathology. Annals of Neurodegenerative disorders. 2016 1(2):1010:1-9.
- Lazzara CL and Kim YH. Potential application of Lithium in Parkinson's and other neurodegenerative diseases. Frontiers Neuroscience. 2015 Oct 27:9:403. review.
- Lazzara CL, Riley RR, Rane A, Andersen JK and Kim YH. The combination of lithium and L-dopa/Carbidopa reduces MPTP-induced abnormal involuntary movements (AIMs) in mice: relevance for Parkinson's disease therapy. Brain Res. 2015 Jul, 1622:127-136.

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