**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 65. 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:**

This work will advance our understanding in the regulation of dopamine transporter (DAT) expression and functionality on the plasma membrane by SUMO conjugation, a critical posttranslational modification. In addition, this research will determine how SUMO modification regulates DAT association with alpha-synuclein, an important protein involved in the pathology of Parkinson’s disease (PD). DAT SUMOylation would be a novel regulatory mechanism for dopaminergic transmission, uptake and degradation. This implies that pathological changes in the SUMOylation of DAT may lead to alteration in acute regulation of dopamine signaling as well as protein aggregation-mediated neurodegenerative pathologies including Parkinson’s disease. This study will provide insights into understanding of how SUMO modification is able to regulate DAT association with alpha-synuclein and the mechanisms by which SUMOylation of DAT and alpha-synuclein could prevent protein aggregation and protect dopaminergic neurons against oxidative stress in PD pathology. Therefore, understanding the mechanisms of SUMO conjugation and DAT/alpha-synuclein regulation may be critical to regulate SUMOylation in alpha-synuclein to prevent Lewy-body formation in PD pathology.

**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. 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 dopamine synthesis/cytoskeleton.
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.

Project 4: Synergistic Damage of Commercially Available Environmental Toxins in Parkinson’s Disease Models:
The vast majority of PD cases are considered to be sporadic, or due to multifactorial reasons. Interestingly, there have been epidemiological studies that have shown that PD is more prevalent amongst farmers and rural populations. It has been suggested that exposure to pesticides and other environmental toxins may increase the risk of PD. In support of this notion, the pesticide rotenone is known to cause PD symptoms in flies and mice. Furthermore, it has been shown that the herbicide, paraquat, and the fungicide, maneb, can cause motor deficits individually, but cause synergistic damage when used together. Since this discovery, these pesticides have been banned in the US and EU. Here we propose that commercially available pesticides, when used together, can cause additive or synergistic damage in in vitro and mouse models of PD. We exposed rat dopaminergic N27 cells to commercially used pesticides, such as acephate, alachlor, atrazine, chlorothalonil, diuron, glyphosate, imazethapyr, MCPA, and mecoprop, at varying concentrations and measured cell viability using the MTT assay. Following single pesticide treatments, we measured cell viability when exposed to different combinations of these pesticides. Our results showed that only high concentrations of single pesticide treatments (e.g. ≥ 62.5 μM of chlorothalonil) caused a significant decrease in cell viability. However, when we examined the effect of the combined pesticides (starting with 14 combinations) at concentrations that did not show damage individually, we identified eight combinations that caused additive damage and three that displayed synergistic damage. Our results suggest that exposure to multiple combinations of pesticides may cause dopaminergic toxicity and further lead to the PD pathology. Currently we are assessing the mechanism of action of toxicity in vitro, and analyzing the mouse brain, especially the striatum and the midbrain, after IP injections of a combination of pesticides for two weeks. The objectives of this study are to warn the public of the potential danger of using numerous combinations of pesticides and to impact on changes in policy for approval of pesticides. The results from this study may reveal potential causes for PD and reduce the prevalence of PD.

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, Assistant Professor)
Etienne Cartier, Ph.D. (Post-doc),
Carol Lazzara, MS (Ph.D. student),
Eric Janezic (MS student),
Janae Caviness (MS student),
Undergraduate researchers: Margaret Steward, Xenia Davis, Sundus Ahmed and Taeho Cho.
Undergraduate alumni: Nicole Brown, Sambee G Kanda, Cassio Noso, Doug Mullen, and Young Lee.
Common Lab techniques: Western-blot, qRT-PCR, cell viability assays (MTT & LDH), ELISA, protein activity assays (including HAT & HDAC), microarray, Immunoprecipitation, immunohistochemistry and Mass Spectrometry (collaboration).

Education & Training (Y. Hwan Kim)

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<tr>
<th>University/Institution</th>
<th>Degree</th>
<th>Field of Study</th>
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<tbody>
<tr>
<td>Korea University, Seoul, S. Korea</td>
<td>BA</td>
<td>Plant Physiology/Genetic Engineering</td>
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<tr>
<td>Korea University, Seoul, S. Korea</td>
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<tr>
<td>University of California, Los Angeles (UCLA)</td>
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<tr>
<td>Johns Hopkins Medical Institution, Baltimore, MD</td>
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<td>Neuropathology in School of Medicine</td>
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<tr>
<td>Buck Institute for Research on Aging, Novato, CA</td>
<td>Sr. Post-Doctor</td>
<td>Parkinson’s Disease</td>
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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

- Cartier E, Garcia-Oliveares J, Janezic EM, Li M, Torres G, Amara S and Kim YH. The Ubc9 SUMO conjugase enhances the function of the dopamine transporter by decreasing its ubiquitination and further degradation. Journal of Neuroscience. (In the process of submission).

(May, 2015)