

## **The Kim Lab: Assessing the mechanism of neuropathology in Parkinson's disease and developing potential therapeutic applications**

Parkinson's disease (PD) is the most common neurodegenerative motor disease, affecting more than 1% of the population above the age of 60 in the US. 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.



### **Current Research Projects in the Kim Lab: Assessing the mechanism of neuropathology in Parkinson's disease and developing novel therapeutics for the disease:**

Parkinson's disease is the most common neurodegenerative motor disease, affecting more than 1% of the population above the age of 60 in the US. The disorder is primarily characterized by dopaminergic neuronal loss in the substantia nigra pars compacta (SNpc) in the midbrain and the 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.

**Project 1: Targeting senescent astrocytes/microglia for therapeutic intervention in PD pathology:** In collaboration with Dr. Julie Andersen at Buck Institute for aging research and HanSeok Ko at Johns Hopkins medicine, we have assessed the roles of cellular senescence processes in Parkinson's disease pathology. Using cellular senescence markers, such as p16, p21, HMGB1, Lamin B1, and SATB1, we found that external stress including alpha-synuclein preformed fibrils (PFF) reduces the senescence markers such as Lamin B1 and HMGB1, but enhanced the level of cell-cycle arrester, p21 in *in vitro*, *ex vivo* and mouse brains (Verma et al., Cells, 2021). Using Western blot and immunohistochemistry, we also found that these cellular senescence markers within reactive astrocytes as indicated by enlarged cell bodies within GFAP-positive cells and Iba1-activated microglia were responsive in  $\alpha$ -syn PFF injected mouse brains. These results indicate that PFF-induced pathology could lead to astrocyte and/or microglia senescence in PD brains, which may contribute to neuropathology in this model (Verma et al., 2021). Targeting senescent cells using senolytics could therefore constitute a viable therapeutic option for the treatment of PD. As a follow-up study, we test a hypothesis that the senescence processes result in the failure of maintaining the homeostasis in dopamine neurons or surrounding astrocytes/microglia, which is associated with PD pathology. With measuring the levels of senescence markers in the PD-related regions including the striatum and Substantia nigra from PFF-injected PD mouse model or human PD brains, we will determine the effects of cellular senescence in inducing dopaminergic neuronal loss and PD pathology and verify the validity of using senolytics in halting PD pathology. This study will allow us to understand the senescence aspects of neuropathology of PD, which may reveal potentially new therapeutic targets using senolytics for preventing neurodegeneration including PD. Currently we are testing senolytics in the PFF-injected PD mice and further developing a nanoparticle for a potential therapeutic application with a senolytic and ROS scavengers to target senescent and/or reactive damaged astrocytes (or microglia) in PD models.

### **Project 2: Oxidative stress increases the levels of deSUMOylation in PD related proteins for inducing PD pathology:**

Our preliminary data demonstrated that MPTP-induced toxicity reduces the SUMO conjugation from  $\alpha$ -synuclein in the striatum. Then, we have been intrigued to assess the mechanisms of deSUMOylation by MPTP (MPP<sup>+</sup>)-mediated oxidative stress in PD pathology. Using collected mouse striatum and brainstem samples after MPTP- or PFF-treatment, we measured the levels of SUMO proteases (SENPs) conjugated to  $\alpha$ -synuclein. This approach has provided insight into the role of SUMOylation in protecting dopaminergic neurons from oxidative insults and verified a potential mechanism, underlying this neuroprotection. There are at least 6 isoforms of SENPs that have been identified in mammalian cells and SUMO1 was detected in the halo of Lewy bodies (Kim et al., 2011). However, the level of deSUMOylation has never been reported in the PD brains, thus we turned our efforts to determine if the levels of SENPs are higher in the striatum and brainstem from human PD tissues than those in age-matched normal brains. We found that the level of SENP1 in human PD patient brains was higher than that in age-matched controls (a manuscript in prep). Thus, we set up a hypothesis that SENP1 level and/or activity is stimulated by oxidative stress, which is a part of pathological mechanisms of PD. Our preliminary results demonstrated that MPTP- and PFF-induced oxidative stress removes SUMO1 from  $\alpha$ -synuclein in mouse striatum and midbrain, while Ubc9 overexpression-mediated SUMOylation protects the dopaminergic neurons in the striatum and Substantia Nigra against the toxicities (Verma et al., eNeuro, 2020). Therefore, we are investigating whether SENP1 inhibitions (Momordin or S2A) protect dopaminergic neurons in the striatum and SNc in PFF-injected mouse model. We also expect to see that higher levels of SENP1 in the Lewy bodies than those in normal brainstem tissues. This approach will help us determine if stimulating SENP1 is related to induce the PD pathology in the human and mouse brains, and blocking SUMO1 removal by SENP1 inhibition can be a novel therapeutic target in PD pathology. Therefore, this approach will help identify how deSUMOylation could be targeted for potentially preventing or slowing pathological progress.

### **Project 3: Assessing regulatory roles 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 characterized in Parkinson's disease 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. In the lab, we have been assessing the role of SUMO conjugase, Ubc9 as a critical post-translational modifier to regulate the solubility, stability, and function of dopamine transporter (DAT, published in 2019),  $\alpha$ -synuclein (published at eNeuro in 2020), and LRRK2 in dopaminergic neurons *in vitro* and *in vivo*. The objectives of the work are to elucidate the mechanisms of SUMOylation in preventing  $\alpha$ -synuclein mediated protein aggregation and regulating LRRK2 kinase activity in dopaminergic neurons. This implies that pathological changes in the SUMOylation of  $\alpha$ -synuclein and LRRK2 may lead to alteration in acute regulation of protein (mis)folding or aggregation, which is related to the neuropathology of PD. Moreover, Ubc9-mediated SUMOylation increases the surface level of DAT in plasma membrane and further its action enhances DAT functional expression in the plasma membrane, triggering an increase in dopamine uptake capacity (Cartier et al., Frontiers Cell Neurosci, 2019). In the MPTP-lesioned mice, the chronic treatment substantially reduces the level of SUMO1 conjugated to  $\alpha$ -synuclein in the mouse striatum. This suggests that pathological changes in the SUMOylation of  $\alpha$ -synuclein result in significant alteration in protein (mis)folding or aggregation. Lately, we have injected the preformed fibrils (**PFF**) of  $\alpha$ -synuclein in the striatum to induce protein aggregation and dopaminergic neuronal loss as a better mouse model and identified that SUMOylated  $\alpha$ -synuclein is refractory to lysosomal degradation. However, the aberrant degradation was not detrimental due to the increased solubility. We also found that the overexpression of Ubc9 protects rat dopaminergic N27 cells against MPP+ induced oxidative stress and PFF injection, via reduced ROS generation and protein aggregation (Verma et al., eNeuro, 2020). Therefore, SUMOylation of  $\alpha$ -synuclein can be a potential therapeutic target for Parkinson's disease. Currently we focus on assessing the role of SUMOylation on LRRK2 and the mechanisms of aberrant protein degradation by SUMOylation on  $\alpha$ -synuclein.

### **Project 4: Developing neuroprotective compounds as potential therapeutics in Parkinson's disease mouse models:**

Most available PD drugs are designed to alleviate the PD motor symptoms, however, they cause side-effects after long-term use. Thus, we focus on identifying potential neuroprotective/recovery compounds to halt or slow the neuropathology, in addition to alleviate motor- and non-motor symptoms. In collaboration with AurimMed Pharma (Park City, Utah) and AptaBio Therapeutics (Kiheung, Korea), we focus on identifying novel small compounds to develop potential therapeutic drugs in Parkinson's disease. After our initial *in vitro* screening using cell viability (MTT) or cytotoxicity (LDH) assays, we narrowed a few target compounds for assessing neuro-recovery effects in PFF-induced, alpha-synuclein A53T mutant or Thy1-WT-a-syn (Line61) overexpressing mouse models. Our recent mouse studies suggest that oral treatment of novel compounds 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 a novel AptaBio compound provided the neuroprotective/recovery effects from the PFF-induced toxicity in mouse brains. Our study will provide prerequisites for developing a therapeutic application and launching an Investigational New Drug study. Since those novel compounds are safe and orally administrable for penetrating the blood brain barrier, a few lead compounds 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 5: Developing a combination therapy for halting PD pathology and identifying a biomarker from human PD**

**patients' saliva:** 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. Our group demonstrated that lithium has neuroprotective effects in Parkinson's disease *in vitro* and mouse models. We confirmed that the potential application of lithium in combination with L-Dopa/Carbidopa, not only reduces MPTP-induced abnormal involuntary movements (AIMs), but also protects against cell death in MPTP-lesioned mice (Lazzara et al., Brain Res, 2015). In collaborations with Dr. Brian Polster at U. of Maryland, medicine and Dr. Ruda Lee at Kumamoto University, Japan, we are developing a potential combination therapy of lithium with safe compounds, such as Idebenone, curcumin, and 7,8-Dihydroxyflavone (7,8-DHF) in nanoparticles for alleviating PD symptoms as well as providing neuroprotective effects in PD mouse models. This approach can be an excellent therapeutic option for neurodegenerative diseases due to low cost and low potential side-effects. The use of low dose of lithium in combination with other potential or pre-existing therapeutic compounds may be a promising approach for alleviating symptoms as well as providing neuroprotective effects to halt the disease progression of PD and other neurodegenerative diseases. In addition, currently we are assessing numerous saliva samples from human PD patients, in comparison with age- and gender-matched healthy control samples, for identifying a potential biomarker to detect or diagnose early stage of PD pathology from non-invasive and easy-to-collect samples like saliva.

### **Ongoing Research Support**

1R25GM122722	Kim & Harrington (PIs)	05/01/17 - 03/31/22	NIH-RISE
A graduate training program to increase diversity in biomedical Science.			
Private Fund	Kim (role: PI)	02/01/19 - 02/28/22	AptaBio therapeutics Inc.
Developing a novel anti-oxidant compound as a therapeutic for Parkinson's disease.			
1R15NS121784	Kim (PI)	10/01/21 - 09/30/24	NIH
Prevention of alpha-synuclein deSUMOylation as a Parkinson's therapeutic target.			

**Current Lab members:** Hwan Kim (PI); **Post-doc:** Dinesh Verma, Ph.D.; **Graduate:** Anurupa Ghosh (Neuroscience PhD); **Undergraduate researchers:** Karina Hernandez, Gabriela Cabrera, Adiah Janvier and Sydney Watson.

**Lab Alumni:** Etienne Cartier, Ph.D. (Post-doc), a senior researcher at U of Maryland, Baltimore, MD.

Juan Viana (MS, Neuroscience): Research Associate at GeneWiz, NJ.  
Lindsey Ruggiero (Neuroscience): Neuroscience PhD candidate at DSU.  
Dionne Williams (MS, Neuroscience): Neuroscience PhD candidate at DSU.  
Carol Lazzara (MS, Neuroscience): Realtor, Lowes beach, DE.  
Eric Janezic (MS, Neuroscience): Sr Scientist at Genentech, CA, after PhD at U of Washington, Seattle.  
Janae Caviness (MS, Neuroscience): Research scientist at Astellas Pharma, PA.  
Alex Burris (Undergrad researcher): MPH student at U of Delaware, Newark, DE.  
Austin Jackson (Undergrad researcher): Research Associate at Christiana HealthCare, DE.  
Suhyun Nam (Undergrad researcher): Research Associate II at GeneWiz, NJ.  
Sundus Ahmed (Undergrad researcher): Eurofins Lancaster Lab, West point, PA.  
Thaddeus Lehman (Undergrad researcher): Researcher at Siemens, Milford, DE.  
Sambee G Kanda (Undergrad researcher): Researcher at Christiana hospital, DE/Applying Med Sch.  
Margaret Steward (Undergrad researcher): Ph.D. student at the Ohio State University.  
Xenia Davis (Undergrad researcher): Ph.D. student at the University of Cincinnati.  
TaeHo Cho (Undergrad researcher): Ph.D. student at DGIST in S. Korea.  
Cassio Noso (Undergrad researcher): Ph.D. student at Univ. of Sao Paulo in Brazil.  
Nicole Brown (Undergrad researcher): Science teacher in DE.  
Kinjal Patel (former lab manager).  
Udeerna Tippabhatla (HS): BS/MD program at Howard University, DC.  
Shriya Boyapati (HS): BS in Neuroscience at U. Penn, PA.

**Former Undergraduate interns:** Chandana Elavarthi (UD), Elena Rangel (senior at DSU), Tahlia Casey (DSU), 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), Summer Stone (Wesley College), Alexis Neuer (DSU), and April Roeper (lab tech at DSU).

**Common Lab techniques:** Western blot, qRT-PCR, cell viability/cytotoxicity assays (MTT & LDH), ELISA, protein activity assays (including DAT, HAT & HDAC), ROS measurements, Protein aggregation (Thioflavin T) assay, primary neuron/astrocytes/microglia culture, microarray, Immunoprecipitation, immunohistochemistry, confocal microscopy, stereology, and Mass Spectrometry & MS imaging (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), CA	Ph.D.	Neuroscience
Johns Hopkins Medical Institution, Baltimore, MD	Post-doctoral fellow	Neuropathology & Neurodegeneration
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 Publication**

- Verma DK, Ghosh A, Seo BA, Ma SX, Cabrera G, Hernandez-Quijada K, Ko HS and **Kim YH**. The inhibition of SUMO-protease 1 (SENPI) prevents and reverses Parkinson's disease pathology in PFF-injected mice. (In preparation).
- Verma DK, Seo BA, Ghosh A, Ma SX, Hernandez-Quijada K, Andersen JK, Ko HS and **Kim YH**. Alpha-synuclein preformed fibrils induce cellular senescence in Parkinson's disease models. *Cells*. 2021 Jul 5; 10(7):1694. [PMID: 34359864](#)
- Verma DK, Ghosh A, Ruggiero L, Cartier E, Janezic EM, Williams D, Jung EJ, Moore M, Seo JB, and **Kim YH**. The SUMO conjugase Ubc9 protects Dopaminergic cells from cytotoxicity and enhances the stability of  $\alpha$ -synuclein in Parkinson's disease models. *eNeuro*. 2020 Sept 4, 0134-20. [PMID: 32887693](#)
- Choi SM, Chung JH, Nam MH, Bang EJ, Moon HY, Hong KS, **Kim YH**, Seo JB, and Chi SC. Elevated Aldolase 1A, retrogene 1 expression induces cardiac apoptosis in rat experimental autoimmune myocarditis model. *Canadian Journal of Physiol & Pharmacol*. 2020 Jun;98(6):373-382. [PMID: 31999472](#)
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- Chae KS and **Kim YH**. Potential impact of geomagnetic field in transcranial magnetic stimulation for the treatment of neurodegenerative diseases. *Frontiers in Human Neuroscience*. 2017 Sep, 478(11):1-5. Review. [PMID: 29021752](#)
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- 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. [PMID: 26119916](#)
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