**Seung-Jae Lee, Konkuk University**

*Review of exocytosis, extracellular forms and extracellular interactions critical for α-synuclein pathology*

Dr. Lee described synuclein exocytosis as not a robust event—only small amounts are released, but they occur without membrane damage and are temperature dependent, and therefore result from active processes. The presence of α-synuclein in CSF and plasma suggests that the processes are physiological. The mechanism is unknown; the process is not inhibited by brefeldin A, thus it is not through the conventional ER-Golgi pathway. Secreted synuclein co-fractionates with exosome markers. Treatments that favor accumulations of misfolded proteins, such as inhibitors of proteosomal, lysosomal, and mitochondrial function, favor release. Synuclein accumulates in multi-vesicular bodies and autophagosomes, and fusion of these with the plasma membrane has been demonstrated. Ultimately, establishing whether exosomes are involved in the pathway will require studies with cleaner exosome preparations, and manipulations of exosome formation and release.

Endocytosis also occurs. The specific aggregation state of the endocytosed synuclein is not clear, but it is sensitive to proteinase K, so it is probably not fibrillar. This raises the possibility of cell to cell transmission of α-synuclein pathology, as demonstrated in primary neuronal culture by Danzer et al. (2011; FASEB J 25(1): 326-36). Further support comes from animal models in which CNS pathology is caused by intragastric administration of proteosome inhibitors or rotenone. Exocytosed α-synuclein may be involved in propagation through neuro-inflammation. Uptake of α-synuclein more rapid in glial cells than neurons, and they find that aggregated or nitrated synuclein, or synuclein collected from neuron-conditioned medium, is capable of activating microglia. Antibodies to synuclein decrease levels of inflammatory activation by increasing clearance. Interestingly, the activation seems to be mediated by TLR2, since it is blocked by knockout of TLR2, but not TLR3 or -4.

**Patrick Brundin, Lund University**

*Update on Transplant Data*

This presentation summarized the recent findings on the fate of fetal mesencephalic neurons grafted in host Parkinson’s disease brain tissue. Studies in post-mortem cases from PD patients grafted with fetal nigral tissue found α-synuclein-containing Lewy bodies in a subset of grafted neurons more than a decade after transplantation. The accumulation of intracytoplasmic α-synuclein was time-dependent with ~40% of 11 year-old grafted neurons up to ~80% of 16 year-old grafted neurons. These observations suggest that the host environment may transfer aspects of the disease to healthy neurons. Several hypotheses have been postulated to explain the propagation of PD pathology from diseased to healthy transplanted neurons (Brundin et al., 2008, Nat Rev Neurosci). Among
these is the loss of neurotrophic support, inflammation, oxidative stress, excitotoxicity and cell-cell α-synuclein transmission. The cell transmission hypothesis was further tested in mice overexpressing human α-synuclein 6 months after intrastratal injection of wild-type mouse embryonic mesencephalic neurons. Results showed that approximately 5% of wild-type grafted neurons contained small human α-synuclein immunoreactive punctae. Is there any other pathology associated with grafted neurons? In long-term transplant cases, a reduction of dopamine transporter (DAT) and tyrosine hydroxylase (TH) staining in grafted tissue was observed. In particular, 22 years after transplant in a human PD case, loss of tyrosine hydroxylase (TH) staining was observed in grafted neurons. The TH negative neurons were however positive for neuromelanin suggesting that these neurons had been TH productive at one time after grafting but that they no longer were. These observations suggest that PD pathogenesis occurred in these cells.

Richard Smeyne, St-Jude Children’s Research Hospital

Viral Infection and Braak Progression of α-Synuclein in an Animal Model

The etiology of Parkinson’s disease is still unknown but genetic susceptibility to various environmental agents may be an important factor. This presentation focused on virus infection as a potential causal factor for Parkinson’s disease. Viruses can enter the nervous system and induce encephalopathies. In turn, this can lead to parkinsonism. There are several PD associated viruses including influenza, herpes, H5N1 to name a few. In the CNS, viruses have been shown to infect mostly neurons and microglia. In mice infected with the H5N1 virus, the progression of infection was found to mimic Braak staging as it occurs first in the mesenteric neurons of the enteric nervous system, then the dorsal vagus nerve and other brainstem nuclei, the midbrain substantia nigra and locus ceruleus, then the cortex and olfactory bulb. Does viral infection affect α-synuclein pathology? Quantitative immunohistochemistry found aggregated and Ser129 phosphorylated α-synuclein (P-Syn) everywhere the flu infection was detected whereas it was never present in regions with no flu infection such as the cerebellum, which never gets infected by flu viruses. In this mouse model, the virus was cleared from the nervous system ~21 days after infection whereas P-Syn aggregates were found later at ~50 days post-infection. Early microglial activation preceded the α-synucleopathy and was maintained up to 90 days, a time point well beyond the presence of detectable viral infection. In addition, variable patterns of cytokines were observed with early rise followed by down regulation then back to normal baseline followed by another activation at 48-50 days which resolved at ~60-90 days. Although not shown during the presentation, 32 cytokines were reportedly measured in 8 brain regions with the complete data set already submitted for publication. T-cell migration was also detected in the substantia nigra. Both T-cell migration and P-Syn aggregates occurred after the activation of the immune system. This is a clear case where immune system activation occurs well before synuclein phosphorylation and deposition. Although these 2 observations could be independent mechanisms, it is possible that
activation of cytokine release and the resulting inflammatory cascade causes P-
Syn accumulation because it occurs after infection.

**Jing Zhang, University of Washington**

**Glia Synuclein: Internalization of α-Synulcein in Glia and the Consequences to Neurons**

This presentation focused on the role of glial activation in synuclein toxicity. Multiple system atrophy (MSA) is associated with glial α-synuclein positive inclusions. In contrast, Parkinson’s disease (PD) brain is characterized with more abundant and readily detectable α-synuclein in neurons. Although α-synuclein has also been reported in glia under pathological situations like PD, its role in glial pathology during PD is still unclear. Whereas α-synuclein mRNA is difficult to detect in oligodendrocytes and microglia, it can be detected at low level in astrocytes and can be enhanced by LPS in both microglia and astrocytes. Therefore when you see α-synuclein in glia, does it come from an endogenous or exogenous source? Exogenous α-synuclein is likely derived from neurons through a mechanism that involves exocytosis and recycling. In synuclein deficient (Syn-KO) mice, whereas microglia are devoided of endogenous synuclein, they look more activated, have more inflammatory cytokines but cannot phagocytose α-synuclein or Aβ as effectively as the wild-type animals. In addition, astrocytes from synuclein knockout mice show alteration in glutamine-glutamate metabolism and/or homeostasis. What can we learn from the exogenous (i.e. non-glial) α-synuclein which is also the predominant form? The levels of α-synuclein mRNA is at least 10-fold higher in neurons compared to glia. Damaged neurons could release aggregated α-synuclein which can activate microglia. So how does α-synuclein enter the glia? Do microglia have to synthesize α-synuclein to be activated? The answer is no. Microglial MAC-1 (a cell surface receptor that can interact with α-synuclein) and PHOX (a membrane-associated NADPH oxidase that catalyzes the production of superoxide) seems to play an important role in the enhanced dopaminergic neurodegeneration produced by mutant/aggregated α-synuclein. In a series of cell-based studies, when internalization of α-synuclein into microglia was blocked by MAC-1 (but not scavenger receptor) depletion, it reduced superoxide production. In addition, PHOX depleted microglia also produced less superoxide. How can cells deal with increased exogenous α-synuclein? One key cell type that can take care of the toxic species of α-synuclein is the microglia. One way to approach this is by modulating the EP2 receptor. EP2 deficient mice (EP2-KO) produce super microglia that can phagocytose aggregated α-synuclein more aggressively without giving rise to inflammatory cytokine/chemokines which is likely via attenuation of PHOX activation. This is an important avenue that needs further investigation. Finally, investigating the early endosomal and late lysosomal pathway may also help us define the role of α-synuclein trafficking into cells.

**Pamela McLean, Massachusetts General Hospital**

**Extracellular Tissue Culture Model**
This presentation focused on novel cellular models to detect and directly visualize intracellular synuclein. Bioluminescent or fluorescent protein complementation assays are cellular culture models that can monitor intracellular α-synuclein oligomers (see Outeiro et al., PLoSOne, 2008). They take advantage of synuclein-synuclein interactions and can determine synuclein cellular localization in living cells. These assays use a cell permeable substrate. Whereas the fluorescent assay is irreversible and potentially locks the proteins into one oligomer conformation, the bioluminescent assay is fully reversible and can detect multiple interactions. This assay could potentially be used for the study of aggregation of α-synuclein ex-vivo, neuron-to-neuron transmission of α-synuclein (α-synuclein is secreted from axon terminals in culture), and/or bidirectional transmission of α-synuclein oligomers from neurons to neuroglioma cells.

Discussion
If microglia take up α-synuclein, are there microglial inclusions? Patrick Brundin commented that they have only looked in tyrosine hydroxylase positive cells (i.e. neurons). For other cells, it would be difficult to distinguish host from graft. The t1/2 of synuclein in microglia is about a tenth as long as in other cells; the rapid degradation may account for the lack of accumulation.

Is it possible that synuclein is transferred from glia to neurons? Nadia Stephanova pointed out that in MSA, it is possible that p25 α translocates from the oligodendroglia to neurons, with possible seeding of synuclein accumulation. However, they haven’t seen translocation of α-synuclein from oligodendroglia to neurons in their cell culture model, arguing that this either doesn’t happen or it is not recapitulated in their model.

How does synuclein pathology spread through the brain? Prion-like translocation from neuron to neuron may not completely explain the proposed progression of pathology; for example, connections between the dorsal motor nucleus of the vagus and the locus coeruleus are not particularly strong. Other proposed mechanisms include transmission of synuclein aggregates or infectious particles through the interstitial fluid, or transport by microglia and/or astrocytes, either by movement of cells or translocation between them. Richard Smyne suggested that pathology could be a response to cytokines, with propagation being a response to their spread.

Several people commented that toxicity may not depend on synuclein uptake, as they don’t always correlate. For example, differentiated neuroblastoma cells don’t take up synuclein well, but are susceptible to toxicity of oligomers. In contrast, Sung Jae Lee finds that uptake and cytokine release correlate well, and blockage of lysosomal function increases cytotoxicity (in astrocytes, not microglia).

If synuclein is released in exosomes, how does it get into the cytoplasm to form Lewy bodies? Several people said they see α-synuclein in exosomes, but also free in solution. The situation is complicated by leakage and dissociation of synuclein form the exosomes. Some synuclein can be removed by washing with sodium.
carbonate, but some cannot, arguing for both peripherally-associated and vesicle-enclosed synuclein. Proposed mechanisms include trafficking to the lysosome, trafficking to the ER by some “salmon-like” reverse transport, and direct transport, presumably of monomer, into the cytoplasm. Jing Zhang estimated, based on their confocal results, that about 2/3 of the endocytosed synuclein goes to lysosomes, 1/3 to the ER.