Introduction Session 10
"Neurodegenerative diseases and ubiquitin-proteasome system"

The UPS and Pathogenesis of Neurodegeneration

The figure describes four different aspects related to protein misfolding and neurodegenerative diseases:

(1) *Triggers* that can cause the accumulation of misfolded proteins—these include both mutations and epigenetic factors.

(2) The *primary responses* to accumulating misfolded proteins—these are related to the reduced capacity of the UPS that is the consequence of protein overload/inhibition by aggregated substrates. The misfolded proteins that accumulate may be refolded by chaperones or accumulate in aggregates in the cytoplasm, nucleus, or extracellular space. The aggregates may sequester additional proteins.

(3) Types of *neuropathological* extracellular and intracellular protein deposits that can be found in the central nervous system of patients with PD, AD, Prion disease, ALS, and polyglutamine (PolyQ) disorders.

Finally, (4) four *secondary disease targets*, i.e., the different cell functions that are affected by the protein conformational disease. In different protein conformation disorders, it has been shown that gene transcription can be markedly disturbed at the levels of histone regulation and individual transcription factors. There is evidence for disrupted axonal transport of different cell constituents. At the presynaptic level, neurotransmitter synthesis can be impaired; vesicular storage is sometimes disrupted; proteins involved in vesicle cycling are altered. At the postsynaptic level, the misfolded proteins can change receptor densities and downstream signaling transduction pathways. Finally, the diseases can cause neuronal death by several mechanisms, including impairment of mitochondrial function and mitochondrial release of cytochrome c with subsequent caspase activation. It has also been suggested that aberrant protein folding can lead to pore formation in cell membranes and loss of ionic homeostasis.

Parkinson's disease (PD)

In PD, the main neuropathological feature is the **progressive death of neurons in the substantia nigra pars compacta with resulting loss of dopaminergic innervation of the striatum**. This causes a gradual development of **akinesia, rigidity, and tremor**. In the vast majority of patients, some of the remaining nigral dopaminergic neurons exhibit aggregated proteins in the form of cytoplasmic **LB (Lewy Body) inclusions**. Several apparently independent aberrations linked to defects in the UPS have been described in various rare forms of hereditary PD that shed light on basic pathogenic mechanisms that maybe relevant also to the most prevalent late-onset and sporadic cases of PD.

An important player in the pathogenesis of PD is **Parkin (*PARK2*)**. Parkin is a protein with a UBL domain at the N-terminal region and two RING finger motifs at the carboxy-terminal region, identified as an **ubiquitin- E3 ligase** that acts along with the ubiquitin-conjugating enzymes UbcH7 and UbcH8. Various deletion and point mutations in the gene have been

found in around 50% of the patients with **AR-JP (Juvenile or early-onset Parkinsonism)**, also known as Autosomal Recessive Parkinson's Disease [**ARPD**]), one of the most common familial forms of PD. Interestingly, with a few exceptions, AR-JP is characterized by a lack of LBs.

The RING finger domain is involved, most probably, in recruitment of the E2 component of the ubiquitination machinery, while the UBL serves as a proteasome binding thus facilitating transfer of the polyubiquitinated substrate(s) to the degrading machinery. It appears that Parkin serves as a common, RING finger-containing **component shared by a group** of putative, yet to be discovered, **SCF-like complexes**. Most of the point mutations described in Parkin reside in its **RING-IBR-(In Between-RINGS)-RING domain** and result in its inactivation. Some mutations may reduce the activity of the enzyme (the autocatalytic one and/or the one displayed toward the exogenous substrates) whereas others may affect other mechanisms, such as interaction of the E3 with essential partners.

A crucially important development in our understanding of Parkin function is the **identification of its native cellular substrates**. The overriding hypothesis is that a defect in Parkin will result in accumulation of this protein(s), which is toxic to the dopaminergic neurons. Several substrates have been identified that are ubiquitinated by Parkin, yet it is not clear whether is the accumulation of one or several of these proteins that underlies the pathogenesis of this familial form of PD.

Identified substrates for Parkin:

- **Cell Division Control related protein (CDCrel-1)**, very likely involved in the regulation of transmitter release via its role in regulating synaptic vesicle dynamics. Parkin also has other potential substrates related to synaptic transmission, such as **synaptotagmin IX** and **actin filaments.**

- **Parkin-associated endothelial-like receptor (PaeI) receptor**, putative G protein-coupled transmembrane polypeptide. When overexpressed, aggregates and parkin can ubiquitinate and target it for its degradation. Its accumulation elicits **cell-death promoted by the activation of the Unfolded Protein Response (UPR)** in the ER (a stress-responsive pathway that increases biosynthesis of chaperones in response to accumulation of misfolded/denatured/mutated proteins in this organelle).
  > **(overexpression of Parkin can rescue cells from the UPR elicited by a variety of stresses (H2O2, UV, osmolarity, heat shock, etc), fact that also suggests its role in clearing up cells from certain proteins that are toxic when accumulate).

- **α-synuclein (αSYN) and its associated protein Synphilin-1**, proteins of yet unclear role but found in protein inclusions (LBs) in patients with sporadic, late-onset PD.
  - αSYN is thought to regulate/participate in dopamine neurotransmission/release via effects on vesicular storage. Wild-type αSYN is monomeric but at high concentrations, it oligomerizes to β-pleated sheets known as **protofibrils**. These can further aggregate and precipitate as **amyloid fibrils** that are present in the Lewy Bodies (hallmark of sporadic, late-onset PD).

Mutations in the nonglycosilated 14 kDa αSYN are related to the pathogenesis of PD but, in particular, a novel 22 kDa form of **O-glycosilated αSYN (αSp22)** has been found to be a direct substrate for parkin. Since αSp22 accumulates in a

nonubiquitinated form **only** in the brains of patients with AR-JP, this accumulation may be toxic. This along with the lack of LBs in this kind of PD has led to the hypothesis that **ubiquitination of proteins may play a role in their aggregation and precipitation into inclusion bodies** (the inclusions may even have a primary **protective role** to momentarily 'get out of the way' protein aggregates of insoluble, potentially toxic effect, if for any reason the proteasome is "overwhelmed" and cannot immediately act on their removal – and at this regard it is noteworthy that both monomeric and aggregated αSYN can bind to the proteasome and inhibit its function if in excess-). In AR-JP, parkin is usually inactivated and cannot act in the ubiquitination of all its substrates. Therefore the cells cannot accumulate them in LBs.

- **Cyclin E, p38 subunit of the aminoacyl-tRNA complex,** are yet recently- found substrates for parkin whose accumulation leads also to neuronal apoptosis.

Besides parkin and its substrates, there are yet other recently-discovered proteins playing roles in both protein degradation by the UPS (the Ub C-terminal hydrolase **UCH-L1**, which displays both deubiquitinating and polyubiquitinating activities depending on its oligomeric state) and in protection from oxidative damage (**DJ-1 protein**), with mutations associated to PD.

Summarizing, in cases with non-apparent familial genetic defects associated, along with an **age-related tendency to accumulate oxidized damaged proteins, failure of the UPS to adequately remove misfolded/abnormal proteins may underlie the degeneration of nigral cells in sporadic disease.** Proteolytic stress with an accompanying defect in protein handling is crucial and the substantia nigra neurons are particularly vulnerable due to their high content of dopamine. And this vulnerability is very likely due to the **high basal rate of protein oxidation which is due to the enzymatic and autooxidative machineries involved in dopamine oxidation.**

Alzheimer's disease (AD)

In AD, the dominant symptom is **dementia,** initially characterized by a **loss of short-term memory which gradually develops into a loss of most higher faculties**. Patients with AD display **two types of protein deposits: extracellular amyloid plaques** and **intracellular neurofibrillary tangles**. The latter neuropathological change is also observed in a series of other neurological conditions that have been collectively named *tauopathies* and include some forms of Parkinsonism, Pick's disease, and boxing-induced dementia (Dementia pugilistica).

The **plaques** in AD are rich in **amyloid β peptides (Aβ) that are produced by proteolytic cleavage of the amyloid precursor peptide (APP)**, a glycolipid located in the outer cell membrane. Three different proteases, called **α, β, and γ secretases**, can cleave APP at specific sites. Concomitant cleavage of APP by β and γ secretase at specific sites can result in fragments that can **misfold** and form **extracellular fibrils**. These fibrils consist of β **sheets**. It is debated whether the fibrils are toxic through formation of pores or whether the extracellular amyloid deposits are the main culprits. The Aβ in plaques arises from specific

processing of APP that is positively regulated by the **presenilins PS1 and PS2**. Some patients express mutant presenilin forms that can change the processing of APP by altering γ secretase activity, thereby promoting the generation of amyloidogenic Aβ.

The **neurofibrillary tangles are intracellular and are rich in tau**, a structural protein that is normally associated with microtubuli. In conjunction with the formation of neurofibrillary tangles, synthesis of the tau protein increases, undergoing an abnormal posttranslational modification characterized by hyperphosphorylation. Proteolytic processing/degradation of tau is believed to be important for the formation of the neurofibrillary tangles. Mutations in the *tau* gene can cause the tauopathy Pick's disease but **mutant tau alone does not cause AD**, favoring that accumulation of **erroneously processed Aβ a key event** in AD pathogenesis.

Familial cases of AD with an autosomal-dominant inheritance constitute only 5% of the cases but as with PD studies, they have been instructive in unraveling possible pathogenic mechanisms involved in the sporadic, aging-related, cases. They have demonstrated that AD can be caused by mutations in the *APP* gene, causing abnormal APP processing, or giving rise to a peptide that is more likely to self-aggregate.

Several lines of evidence point to a **reduced UPS function in AD** and suggest that **both Aβ and tau in their abnormal forms** are important players in the pathogenesis of AD by ultimately **causing inhibition of proteasome function.**

Does a reduction in the activity of the UPS lead to a greater tendency Aβ to accumulate and generate amyloid plaques, or do conformational changes in Aβ or tau cause proteasome inhibition? One characteristic of AD is that it shows a higher prevalence with age. The **ability to increase ubiquitin conjugation in response to stressors decreases in ageing tissue**. The **proteasome activity in the mammalian brain decreases with increasing age** (Keller et al., 2002), suggesting that the aged brain is less able the aberrantly folded Aβ. That doesn't exclude that simultaneous abnormal increased levels of the peptide may start accumulating by additional reasons.

Perhaps the most compelling evidence for the involvement of the UPS in AD pathogenesis comes from the discovery that the **mutant Ub^{+1} ubiquitin form**. Ub^{+1} has been found not only in neurons of patients with AD, Down's syndrome (which develop AD-like brain pathology when middle-aged) but also in brains of elderly patients with mild cognitive impairment (without any other known predisposing factors) and with other neurodegenerative disease.

A transcriptional misreading can cause the deletion of two nucleotides in the mRNA coding for ubiquitin, which causes the **replacement of the C-terminal residue of Ub (G76) with a 20-residue (+1) extension** (Ub^{+1}). This mutant form cannot be attached directly to protein substrates but wild-type ubiquitin simultaneously produced from correctly generated transcripts can bind to its intact internal lysines. When a mutant Ub^{+1} is attached this way to the last wild-type ubiquitin added to a polyubiquitin chain in a substrate no more ubiquitins can subsequently be attached since Ub^{+1} is missing the C-terminal G76. These **Ub^{+1}-capped polyubiquitin** chains cannot be deubiquitinated and their **slow-rate accumulation** ends up causing a **"dominant-negative" inhibition of the proteasome.**

Therefore, recent reports showing that Ub^{+1} can inhibit the proteasome suggests that it may contribute to the pathogenesis of neurodegenerative diseases.