How inactivation of mitochondrial calcium uniporter protects dopaminergic neurons

Jacek Kuźnicki

Parkinson's disease (PD) is an incurable neurodegenerative disease that usually affects the elderly. The remaining ~10% of patients have mutations of various genes like those which encode PINK1 or LRRK2 kinases or are poisoned with such substances as rotenone, which is found in some plants, or MPTP, which is a rare pollutant of some drugs. The cause of the disease is the disappearance of neurons in the brain, initially dopaminergic neurons that produce the dopamine neurotransmitter. This causes movement symptoms, such as uncontrolled tremor. Some patients experience problems with smell, sleep, depressed mood, and dementia. Dopamine receptor agonists and L-DOPA (the dopamine precursor) are the most commonly used drugs to treat only PD symptoms, but do not delay the disease. Since 1960s when L-DOPA was introduced there has been a continuous yet unsuccessful search for drugs that can delay or stop the development of PD.

Our project is a part of a series of basic research that seeks to identify diagnostic markers for pre-symptomatic PD and novel therapeutic targets for PD treatment. Disturbances of calcium ion homeostasis have been found in all PD models and cells from patients with this disease. Metabolism of iron is also changed in PD patients and its increase correlate with the decrease in dopamine production. MPTP causes iron accumulation paralleling neuronal loss. These perturbations of iron homeostasis are reminiscent of ferroptosis, and iron-dependent regulated cell death pathway characterized by excessive lipid peroxidation. Our published data show that the disappearance of dopaminergic neurons as a result of a mutation of the *pink1* gene or of MPTP toxicity can be inhibited by inactivation of the mitochondrial calcium uniporter in zebrafish (Mcu). Others showed that MCU inhibition rescues neurons also with LRRK2 mutations. MCU silencing reduces cell death and mitochondrial respiratory deficits in cortical neurons, lessens iron accumulation and the associated brain injury, and completely protects cardiac mitochondrial dysfunction caused by iron overload. MCU inactivation prevents both calcium and iron accumulation, decreases level of oxidative stress in models of traumatic brain injury. Based on these data, I hypothesize that PINK1 and LRRK2-mediated pathology is in part caused by ferroptosis and that MCU inactivation protects dopaminergic neurons by limiting not only calcium but also iron influx into mitochondria thereby mitigating ferroptosis

The aim of this project is to identify ferroptosis associated genes (FAGs) that are turned on or off in LRRK2 and PINK1 PD models when MCU inactivation rescues dopaminergic neurons, and to understand the mechanism of protection. It was suggested that inhibition of ferroptosis can be achieved through regulating neuronal Ca^{2+} homeostasis, thus FAGs might be involved in iron but also in Ca^{2+} homeostasis.

Mutations of LRRK2 represent the most common genetic cause of inherited PD and often occur in sporadic PD. Mutations in PINK1 are responsible for rare cases of inherited PD. We will use zebrafish PD models and dopaminergic neurons differentiated from induced Pluripotent Stem Cells (iPSCs) of PD patients. Between zebrafish and humans there are fundamental similarities of neuroanatomical and neurochemical pathways, including mechanisms of neuronal degeneration and the activation of glial cells.

We will establish conditions to induce ferroptosis in zebrafish lines and dopaminergic neurons with PINK1 or LRRK2 mutations and characterize features of both types of models. We will next identify genes, which change expression level because of treatment with MCU inhibitors in ferroptotic neurons obtained from human iPSCs. These genes will be referred to human “MCU-sensitive genes.” Among them we hope to find a novel potential FAG(s) in additions to known ones. In parallel, we will identify brain cells in which orthologs of those FAGs are down or upregulated by Mcu inactivation in *lrrk2* and *pink1* zebrafish mutants. Based on the similarities between human and zebrafish we assume that proteins encoded by zebrafish genes share key properties of human proteins. Finally, out of identified FAGs we will select and characterize human protein(s) encoded by “protective” MCU-sensitive gene(s) using differentiated dopaminergic neurons co-cultured with other types of cells such as for example microglia. We will manipulate the level of the selected protein by either overexpression or knockout of the individual gene to revert MCU-dependent protection. The positive result of this manipulation will support the role of a protein in the protection against ferroptosis. The last stage of the project will involve the characterization of proteins that are encoded by these "protective" genes. We will determine the intracellular location of "protective" proteins using antibodies.

We expect to identify a set of genes that change activity when calcium and iron ions cannot penetrate into mitochondria and identify cells in which proteins that are encoded by these genes operate. These genes and proteins may have diagnostic value before the appearance of PD symptoms, thereby providing a basis for new anti-PD therapies.