Role of Sequestosome1 Variants in ALS Pathogenesis and its Mitigation using Flavonoid Compounds

ABSTRACT

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease (NDD) which came to light when the first case was diagnosed, and its pathophysiology was established by the French neurologist Jean-Martin Charcot in 1869. Later in 1993, the first ALS associated gene *SOD1* was discovered and since then many ALS related genes such a*s OPTN, FUS, TDP-43, TBK1, ERBB4, CHMP2B, C9orf72, DAO* and *SQSTM1* have been known and studied so far. Our group worked on next generation sequencing of DNA samples in a cohort of Indian ALS patients and identified two rare missense variants namely G262R (G>A) and P438L (C>T) in Sequestosome 1 (*SQSTM1*). *SQSTM1* codes for p62, is an autophagy adaptor protein involved in multiple signaling pathways such as apoptosis, inflammation, oxidative stress response and autophagy pathway. The protein p62 consist of eight domains through which it interacts with different signaling proteins and dysfunction in any of the *SQSTM1* domain due to mutation affects its functionality. This subsequently, led to association of *SQSTM1* with various diseases like Paget's disease of bone, Alzheimer's, Parkinson's, Frontotemporal dementia, ALS and many others. In this study, we investigated how these *SQSTM1* mutations affect p62 linked pathways, autophagy and the oxidative stress response pathway in SH-SY5Y neuronal cells through quantitative RT-PCR, immunoblotting and confocal microscopy. We further examined how the changes in the downstream signaling pathways alters nuclear-cytoplasmic localization of TDP-43 protein, a marker protein usually found in cytoplasmic inclusions in ALS patients. We observed upregulation of autophagy and downregulation of oxidative stress response due to the effect of mutation. We also observed a disbalance in nuclear to cytoplasmic localization of TDP-43 protein. In addition, we studied the neuroprotective effect of flavonoid compounds: Quercetin (QR) and Fisetin (FS), known for their pharmacological benefits in amelioration of neurodegeneration. We observed FS exerts more potential neuroprotective effects in comparison to QR by studying cell death, reactive oxygen species formation and elevating Nrf2 levels in oxidative stress pathway. FS also corrected TDP-43 nuclear-cytoplasmic protein levels in *SQSTM1* variants and was effective in down regulating late apoptosis in comparison to wild type in neuronal cells. Thus, with this study we infer that *SQSTM1* mutations (G262R and P438L) are pathogenic in nature and the flavonoids are helpful in mitigation of their pathogenicity by different cellular mechanisms.