SYMPOSIUM PRESENTATION 3: PHYSICAL AND MENTAL HEALTH

Protein dynamics-based control of proteotoxicity in neurodegenerative diseases
S. B. Lee (Convener)

Purpose With increasing life expectancy, neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), Amyotrophic Lateral Sclerosis (ALS) and Huntington's disease (HD), have become one of the major threats to humans. Although the symptoms vary depending on the type of disease, these neurodegenerative diseases share protein toxicity as one of their key pathogenic mechanisms. Herein protein toxicity is defined as all the pathological changes that ensue from accumulation, mis-localization, and/or oligomerization of disease-associated toxic proteins such as α-synuclein in PD, polyglutamine (polyQ)-containing proteins in polyQ diseases (e.g., HD), and dipeptide repeat proteins and TDP-43 in ALS. Conventional understanding of protein toxicity is that protein toxicity simply reflects the amount of accumulated toxic proteins. For this reason, our challenges done so far against protein toxicity have been based on a simple strategy of reducing the amount of toxic proteins. However, the exact nature of protein toxicity appears to be much more complex than we have conceived, and thus new paradigm for understanding protein toxicity is highly demanded. In this talk, I will present our current efforts to understand exact nature of proteotoxicity in neurodegenerative diseases and potential solutions that can effectively control proteotoxicity, named as protein dynamics-based control of proteotoxicity. I will tell you about our recent study unveiling cellular intrinsic mechanisms regulating nucleocytoplasmic transport of TDP-43 in neurons. **Method** We used *Drosophila* as a primary model to study intrinsic regulatory mechanisms underlying nucleocytoplasmic transport of TDP-43. We used various experimental techniques, such as genetic analyses, immunohistochemistry, behavioral analyses, neuronal imaging, and FRAP. **Results and discussion** Dysregulation of protein localization, often observed in various neurodegenerative diseases, impairs functionality of the protein, alters the pool of its interactors, or both, thereby leading to cellular toxicity. TDP-43, one of well-characterized disease-associated proteins in Lou Gehrig's disease, are known to translocate from the nucleus to the cytoplasm in the disease condition, which is considered as a hallmark of the disease. Thus, unveiling the regulatory mechanism of intracellular localization of TDP-43 is very important to better understand the pathogenesis of Lou Gehrig's disease. However, still our understanding on the neuronal intrinsic program regulating the intracellular localization of TDP-43 remains mostly unclear. Interestingly, we observed that the intracellular localization of TDP-43 dynamically changes even in normal condition of a specific neuronal cell type, named *Drosophila* classIV da sensory neurons, along development. We first identified intracellular Ca2+ level to be critical for the translocation of TDP-43 between the nucleus and the cytoplasm. Additionally, through fluorescence recovery after photobleaching (FRAP) imaging analyses, we found that the nuclear entry of TDP-43 is critically controlled by intracellular Ca2+ level. Further genetic analyses identified Calpain and Importin a3 as mediators of Ca2+-dependent control of TDP-43 translocation in classIV da sensory neurons. Finally, by modulating Ca2+-Calpain-Importing a3 pathway, we could significantly modify the locomotive phenotypes shown in animal models for Lou Gehrig's disease. Even though we know well that aberrant translocation of TDP-43 is closely associated with Lou Gehrig's disease, we have only limited knowledge to date particularly on how its aberrant translocation initiates at the early stages of the disease. Our findings provide invaluable clues for the neuronal intrinsic program regulating the intracellular localization of TDP-43, of which changes may lead to the initiation of the pathogenic translocation of TDP-43. This study enables other researchers to consider protein dynamics-based control of proteotoxicity as a novel strategy against neurodegenerative diseases, in addition to their conventional approach (quantitative control of toxic proteins).

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Address: E4-413, DGIST, 333 Techno-jungang-daero, Dalseong-gun, Daegu, Korea
E-Mail: sblee@dgist.ac.kr

Targeting cellular senescence in adipose tissue: a potential treatment for type 2 diabetes

H. B. Jung, E. C. Kim, H. K. Sung*, J. R. Kim, S. Y. Park

Purpose Cellular senescence refers to the cessation of cell proliferation that can be triggered by endogenous and exogenous stimuli, such as telomere dysfunction, DNA damage, and oncogenic gene expression (Di Micco et al, 2021). Senescent cells release senescent-associated secretory phenotype (SASP), which turns neighboring normal cells into senescent cells. As the accumulation of senescent cells compromises tissue repair and function, cellular senescence eventually leads to tissue aging and aging-related chronic diseases, including type 2 diabetes. Therefore, killing the senescent cells (senolytics) or reversing the senescent cells to young cells (senomorphics) can prevent or alleviate aging and aging-associated diseases (Niedernhofer and Robbins 2018). Recently, previous studies showed the reduction in senescent cells in adipose tissue attenuates insulin resistance in high-fat diet (HFD)-fed obese mice, suggesting that targeting cellular senescence in adipose tissue could be the potential treatment for type 2 diabetes (Smith et al 2021). Currently, however, there is no clinically available effective senotherapy. In our study, we aimed to find a novel senotherapy for adipose tissue aging and insulin resistance. **Method** We examined 2,150 clinically available compounds for their senolytic or senomorphic effect in human dermal fibroblast (HDF) by using cell toxicity or senescent-associated beta-galactosidase staining, respectively. Among the 10 compounds which were found to have a senolytic or senomorphic effect in HDF, one compound also attenuated senescence in human preadipocytes and 3T3-L1 adipocytes. The effects of the new senolytic agent (HT) on adipose tissue aging and insulin sensitivity were examined in high-fat diet-fed obese mice and aged mice. **Results and Discussion** HT attenuated weight gain and reduced fat mass in obese mice even though it did not affect food intake. It reduced adipose tissue aging both in obese mice and aged mice, which was followed by a reduction in large-sized adipocytes, the number of crown-like structures, and the levels of inflammatory cytokines. HT improved glycemic control and insulin resistance. It also reduced adipose tissue aging in human subcutaneous adipose tissue ex vivo. Thus, these results suggest that we have found a novel senolytic agent that may be a potential therapeutic for type 2 diabetes.

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Address: Senotherapy-based Metabolic Disease Control Research Center, College of Medicine, Yeungnam University, Daegu, South Korea. *University of Toronto, Toronto, Ontario, Canada

E-mail: sypark@med.yu.ac.kr

Drug repositioning for a potential drug to prevent muscle wasting in aged mice

Y.-I. Lee (DGIST)

Purpose Muscle wasting, resulting from aging or pathological conditions, leads to reduced quality of life, increased morbidity, and increased mortality. Much research effort has been focused on the development of exercise mimetics to prevent muscle atrophy and weakness. In this study, we identified indoprofen from a screen for peroxisome proliferator-activated receptor γ coactivator α (PGC-1α) inducers and report its potential as a drug for muscle wasting. **Methods** The effects of indoprofen treatment on dexamethasone-induced atrophy in mice and in 3-phosphoinositide-dependent protein kinase-1 (PDK1)-deleted C2C12 myotubes were evaluated by immunoblotting to determine the expression levels of myosin heavy chain and anabolic-related and oxidative metabolism-related proteins. Young, old, and disuse-induced muscle atrophic mice were administered indoprofen (2 mg/kg body weight) by gavage. Body weight, muscle weight, grip strength, isometric force, and muscle histology were assessed. The expression levels of muscle mass-related and function-related proteins were analyzed by immunoblotting or immunostaining. **Results** In young (3-month-old) and aged (22-month-old) mice, indoprofen treatment activated oxidative metabolism-related enzymes and led to increased muscle mass. Mechanistic analysis using animal models and muscle cells revealed that indoprofen treatment induced the sequential activation of AKT/p70S6 kinase (S6K) and AMP-activated protein kinase (AMPK), which in turn can augment protein synthesis and PGC-1α induction, respectively. Structural prediction analysis identified PDK1 as a target of indoprofen and, indeed, short-term treatment with indoprofen activated the PDK1/AKT/S6K pathway in muscle cells. Consistent with this finding, PDK1 inhibition abrogated indoprofen-induced AKT/S6K activation and hypertrophic response. **Conclusion** Our findings demonstrate the effects of indoprofen in boosting skeletal muscle mass through the sequential activation of PDK1/AKT/S6K and AMPK/PGC-1α. Taken together, our results suggest that indoprofen represents a potential drug to prevent muscle wasting and weakness related to aging or muscle diseases.

Keywords: exercise mimetic, hypertrophic response, indoprofen, muscle wasting, muscle weakness, PDK1
Address: Daegu Gyeongbuk Institute of Science and Technology (DGIST), Daegu, South Korea
Email: ylee56@dgist.ac.kr