論 文 の 内 容 の 要 旨

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論文題目　　Studies on the Neuroprotective Action of Progranulin
（プログラニュリンの神経保護作用に関する研究）

Progranulin (PGRN) is a 68.5-kDa glycoprotein containing 7.5 tandem repeats of a cysteine-rich motif, and also known as granulin, epithelin precursor, PC cell-derived growth factor, proepithelin, and acrogranin. PGRN is involved in multiple physiological functions and various disease processes, including autoimmune disorders, tumorigenesis, and insulin resistance. In the human brain, heterozygous mutation of the *GRN* gene is one of the major factors causing frontotemporal lobar degeneration (FTLD), which is characterized by ubiquitinated cytoplasmic inclusions containing trans-activation responsive region DNA binding protein of 43 kDa (TDP-43). Additionally, patients with the homozygous mutation present with adult onset neuronal ceroid lipofuscinosis (NCL) in humans. PGRN deficiency also increases the risk of developing Alzheimer's disease (AD), and modifies the course of amyotrophic lateral sclerosis (ALS). However, the relation between PGRN deficiency and pathogenesis of these neurodegenerative diseases was poorly understood. The present study was undertaken to elucidate the possible pathophysiological roles of PGRN in protecting the brain from neurodegeneration.

The neurodegenerative diseases as a result of PGRN deficiency are generally accompanied by neuroinflammation. Neuroinflammation is characterized by activated

microglia and astrocytes. Microglia, the resident innate immune cell in the brain, has been implicated as an active contributor to neuronal damage in neurodegenerative diseases. Interestingly, it has been shown that PGRN levels increase after traumatic brain injury (TBI), and activated microglia predominantly express PGRN. Macrophages from PGRN-deficient (KO) mice secrete higher levels of proinflammatory cytokines than those from wild-type (WT) mice. Furthermore, gliosis in aged mice was facilitated by PGRN deficiency. These observations suggest that PGRN is involved in the activation of microglia. In Chapter 1, to investigate the role of PGRN in inflammatory responses related to activated microglia, mice (8- to 9-week-old) were subjected to experimental TBI, which is accompanied by neuroinflammation. PGRN expression was increased in association with neuroinflammation after TBI. Further, The study by using double-immunostaining showed that PGRN-immunoreactive (IR) cells were mainly overlapped with CD68-IR cells, suggesting that the main source of PGRN was CD68-positive activated microglia. Next, immunoreactivity and expression of Iba1, CD68, and CD11b as markers for activated microglia were compared between WT and KO mice. The number of Iba1- and CD11b-IR cells and gene expression of Iba1 and CD11b were not significantly different between WT and KO mice, while the number of CD68-IR cells and CD68 expression in KO mice were significantly greater than those in WT mice. Further, double-immunohistochemical study showed that CD68-positive microglia expressed TGFβ1, and TGFβ1 expression and Smad3 phosphorylation in KO mice were elevated compared to WT mice. Double-immunostaining between pSmad3 and GFAP revealed that TGFβ1-Smad3 signal mainly increased in astrocytes. In addition, the levels of protein carbonyl groups, which reflect protein oxidation, and laminin immunoreactivity, which is associated with angiogenesis, were also significantly increased in KO mice compared to WT mice. These results suggest that PGRN is produced in CD68-positive microglia after TBI and suppresses excessive inflammatory responses related to activated microglia including astrogliosis, oxidative stress, and angiogenesis, which are at least partially mediated by TGFβ1.

As mentioned above, it has been demonstrated that patients with the homozygous mutation in PGRN gene present with NCL, and there is growing evidence that PGRN is related to lysosomal function. CD68 is a member of the lysosome-associated membrane protein (Lamp) family that is restrictedly expressed in cells of the monocyte/macrophage lineage, and predominately localize to endosomal and lysosomal compartments with a modest level of cell surface expression. It has been shown that a profusion of CD68-positive microglia appears in the cortex of model mice of mucopolysaccharidosis I

and IIIB, a type of lysosomal storage disease, and PGRN expression are up-regulated as well. The major contributor in the regulation of lysosomal gene expression is transcription factor EB (TFEB), and lysosomal gene expressions are up-regulated by TFEB translocation from the cytoplasm to the nucleus, and the translocation of TFEB is controlled by the mammalian target of rapamycin complex 1 (mTORC1). The translocated TFEB binds to the coordinated lysosomal expression and regulation (CLEAR) consensus sequence, which leads to an increase in the expression of lysosomal genes. Therefore, in Chapter 2, the possible role of PGRN in regulating lysosomes of activated microglia in the cerebral cortex after TBI was investigated using 8 to 10-week-old mice. First, the author found that the mouse *Grn* gene has two possible CLEAR sequences in the promoter region. After TBI, PGRN was colocalized with Lamp1, a lysosomal marker, and Lamp1-positive areas in KO mice were significantly expanded compared with WT mice. Expression of all the lysosome-related genes examined in KO mice was also significantly higher than that in WT mice. The number of activated microglia with TFEB localized to the nucleus was also significantly increased in KO as compared with WT mice. Since the TFEB translocation is regulated by mTORC1 activity in the lysosome, ribosomal S6 kinase 1 (S6K1) phosphorylation that reflects mTORC1 activity was compared between WT and KO mice. S6K1 phosphorylation in KO mice was significantly lower than that in WT mice. In addition, the number of nissl-positive living neurons and fluoro-jade B-positive degenerating neurons around the injury was significantly decreased and increased, respectively, in KO as compared with WT mice. These results suggest that PGRN localized in the lysosome is involved in the activation of mTORC1, and its deficiency leads to increased TFEB nuclear translocation with a resultant increase in lysosomal biogenesis in activated microglia and exacerbated neuronal damage in the cerebral cortex after TBI.

The results of Chapter 1 and 2 suggested that PGRN is involved in regulating neuroinflammation by decreasing lysosomal biogenesis. As mentioned above, haploinsufficiency of *GRN* gene in human is one of the major factors causing FTLD, which is characterized by ubiquitinated cytoplasmic inclusions containing TDP-43. However, the mechanism by which impaired production of PGRN causes the formation of TDP-43 inclusions in the cytoplasm remains unclear. In addition, although patients with a homozygous *GRN* mutation present with NCL, characterization of the brain pathology regarding NCL in PGRN-deficient mice has yet to be performed. Therefore, in Chapter 3, the relationship between lysosomes and disorders resulting from PGRN deficiency was investigated using 10- and 90-week-old WT and KO mice. Lysosomal

biogenesis and gliosis in KO mice were exacerbated with aging as compared with WT mice in the ventral posteromedial nucleus/ventral posterolateral nucleus (VPM/VPL), where reported NCL model animals show a particular vulnerability early in disease progression. The aggregates of p62, which is selectively degraded by autophagy-lysosomal system, were observed in neuronal and glial cells in the VPM/VPL of aged KO mice. TDP-43 aggregates in the cytoplasm of neurons were also observed in the VPM/VPL related to the disrupted autophagy-lysosomal pathway in aged KO mice. The NCLs display a relatively uniform phenotype in the central nervous system characterized by pronounced gliosis, a dramatic neuronal loss, lipofuscinosis, and impaired myelination. Neuronal loss was observed in the VPM/VPL of aged KO mice. Additionally, expressions of glial cell-derived cytotoxic factors, i.e., macrophage expressed gene 1, cytochrome b-245 light chain, cytochrome b-245 heavy chain, complement C4, tumor necrosis factor-α, and lipocalin 2, were increased in aged KO mice. Lipofuscinosis in the VPM/VPL and impaired myelination in the cerebral cortex were also observed in aged KO mice. These results suggest that PGRN deficiency causes exacerbated neuroinflammation including up-regulation of cytotoxic factors, increased TDP-43 aggregation in the cytoplasm of neurons and NCL-like pathology with aging, and such changes are probably due to lysosomal dysfunction.

The present study suggested that PGRN is involved in modulating lysosomal function in activated microglia, and its deficiency causes exacerbated neuroinflammation, increased lysosomal biogenesis, gliosis and neuronal loss associated with lysosomal dysfunction after TBI as well as aging. Additionally, aged PGRN deficient mice accumulated TDP-43 aggreagates in the cytoplasm of neurons and exhibited NCL-like pathology, and thus could be useful experimental models for both FTLD and NCL caused by PGRN deficiency in humans. Previous studies have shown that PGRN plays an essential role in the pathoetiology of neurodegenerative diseases such as FTLD, NCL, AD, and ALS. It has been also shown that impaired lysosomal functions are involved in the pathogenesis of these neurodegenerative diseases. Taken together, lysosomal dysfunction might be a common process in the pathogenic mechanisms of such neurodegenerative diseases resulting from PGRN deficiency. Further studies on the detailed mechanisms by which PGRN regulates lysosomal function may contribute a great deal to develop therapeutic methods for neurodegenerative diseases related to lysosomal dysfunction.