論文の内容の要旨

論文題目　Elucidation of physiological function of SCAP, a crucial sterol-sensor molecule in astrocytes
（ステロールセンサー分子 SCAP のアストロサイトにおける生理的機能の解明）

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【Background】

The prevalence of diabetes mellitus (DM) in elder increases by year and has become not only a medical but also a socioeconomic problem all over the world. One of the serious diabetic complications is the progression of cognitive impairment and neurodegenerative diseases. Initially reported in Rotterdam Study and the Hisayama study, following meta-analysis have shown that diabetic patients have statistically significant associations for all-cause dementia, Alzheimer's diseases and vascular dementia at hazard ratio of 1.7, 1.6 and 2.2, respectively, compared with those without DM. Nowadays several hypotheses have been proposed and it is generally believed that abnormal processing of amyloid beta, tau overphosphorylation, oxidative stress, formation of advanced glycation end products, and inflammatory reactions, as well as impaired cholesterol synthesis due to insufficient insulin action in the brain could be possible mechanisms to explain the close interaction between diabetes and neurodegenerative diseases.

Sterol metabolism in the mammalian central nervous system (CNS) is highly conserved and self-sufficient due to the isolation with blood-brain-barrier and delicate ligand-transporter system. Dysfunctional sterol metabolism in CNS has been reported to relate to major depression, neurodegenerative diseases and other psychiatric disorders, however, the mechanisms are still largely unknown. Our previous studies have found that mRNA expressions of SREBP2 and its downstream genes were significantly downregulated in the mouse hypothalamus and cortex with insulin-deficient diabetes induced by injection of streptozotocin (STZ). The total cholesterol amount in synaptosomes was also found to be decreased, indicating that insulin signaling in the central nervous system may have an important role of fine-tuning the cholesterol homeostasis. Furthermore, successive experiments showed that in brains of diabetic mice, the amount of SREBP-cleavage activating protein (SCAP) decreased. Adult heterozygous *Scap* knockout mice with the nestin-Cre showed a decrease in cholesterol synthesis in the brain by 30 percent. Impaired in synapse function in the hippocampus and recognition memory was noted in this mouse model evidenced by neural electrophysiological studies and behavioral tests such as novel object recognition test.

Based on the findings of our previous studies, we continued this topic and focused on cell types which were responsible for sterol synthesis and the timing of cholesterol-synthesizing. Compared with neurons, astroglial cells were more likely to be responsible for neurotrophic support, the stability and maturation of synapses, synaptogenesis and cycle of neurotransmitters such as glutamate, other reports have found that SREBP transcription factors in astrocytes were of importance in lipid metabolism in remodeling synapses

and regulating neuronal functioning. Because our previous basic research studies used the nestin Cre-loxP system since embryo, the results of phenotypes and the molecular data would be influenced during the development period. In order to understand most of the acquired neurodegenerative diseases and psychologic disorders, it would be more physiological and pathological to use an animal model that deletion of ability in sterol synthesis during adulthood could be accessible.

The objectives of this study were to clarify the role of SCAP in GFAP-positive astrocytes by using an inducible approach to knockout SCAP expression in vitro and in vivo. We studied the impact of SCAP deficiency in primacy culture astrocytes, and examined phenotypes of a mouse model with inducible Scap knockout in the astrocytes, in order to elucidate the mechanisms and find clues for possible treatment of cognitive impairment in diabetes or other neurodegenerative diseases in the future.

【Method】

The hGFAP-CreERT2 transgenic mice were kindly provided by Dr. Frank Kirchhoff (Germany). The hGFAP-CreERT2 transgene was constructed and contained the human GFAP promoter, a tripartite intron, the open reading frame of CreERT2 and the human growth hormone poly A site as the same as previous studies. We mated the mice with Scap flox mice purchased from the Jackson Laboratory (USA) via CLEA, Japan. In this model, tamoxifen administration by intraperitoneal injection would lead to partial knockout of genomic SCAP by tamoxifen-inducible Cre recombinase and produce non-functional protein expression of SCAP in GFAP-positive cells, mostly astrocytes in the central nervous system.

After confirmation the creditability of hGFAP-CreERT2(Tg):: *Scap* flox mice, we studied the astrocytes by primary cell cultures. Not only traditional serum-based astroglial cultures but also a novel primary astrocyte culture by method of immunopanning were practiced. Relating gene expression evaluated by rt-PCR and western blotting were used.

Meanwhile, we performed several behavioral examinations testing short-term and long-term memory, learning ability, stress responses in a novel environment, and motor coordination on hGFAP-CreERT2(Tg):: *Scap* flox mice after tamoxifen injection intraperitoneally at 8 weeks of age. We also measured the stress-related hormonal patterns of daily rhythm and the responses under fasting and a period time of restraint to rule out the involvement of hormonal responses in hypothalamus-pituitary-adrenal axis in behavioral examinations.

【Results】

The new strain of hGFAP-CreERT2(Tg):: *Scap* flox mice was established successfully in our hand. After administration of tamoxifen intraperitoneally, the genome of SCAP in GFAP positive cells would be partially deleted and appearance of knockout band would be shown by DNA genotyping in the whole brain where astrocytes resided. In addition, these knockout bands existed at 45 weeks of age after tamoxifen induction at 8 weeks of age. No knockout bands were detected in Cre positive mice if no tamoxifen was

given, and were not detected in liver, heart, skeletal muscle or white adipose tissue after tamoxifen administrations. No significant differences in body weight changes between Cre positive and negative mice before and after tamoxifen administration from 5 to 20 weeks of age. No differences in brain size and structural abnormalities were found between these two groups. Genomic recombination of *Scap* in astrocytes did not cause obvious differences in numbers of GFAP-positive cells in areas of hippocampus evidenced by immunostaining of brain tissue.

In vitro primary astroglial cell culture study from P0-P1 brains of hGFAP-CreERT2(Tg):: *Scap* flox mice, we had confirmed that the inhibition of SCAP expression in transcription and translation levels after tamoxifen induction. This changes did not occur in the Cre negative control group. Moreover, inhibition of SCAP expression had led to affect triglycerides and sterol synthesis. As triglycerides synthesis was supposed to be inhibited as decreased expression of *SREBP1c* was noted. Decreased expression of cholesterol-synthesizing relating enzymes such as *Srebf2, Hmgcr, Ldlr, and Sqle* in transcriptional level were found in Cre positive cell cultures after tamoxifen induction. Similar results were noted in primary astrocyte culture by method of immunopanning.

There were no apparent morphologic changes in the traditional primary astroglial culture. However, compared with traditional primary astroglial culture, astrocytes purified by the method of immunopanning revealed that these cells had more process-like structures and ratio of GFAP-positive cells significantly increased. The number of surviving cells decreased dramatically in Cre positive astrocytes after tamoxifen induction by light microscopy in the method of immunopanning. Further evaluation had shown that by immunostaining of GFAP, the ratio of GFAP-positive surviving cells decreased significantly in Cre positive culture after tamoxifen induction. In transcriptional level, GFAP expression was also decreased in Cre positive cultures with addition of tamoxifen compared to those without 4-OH tamoxifen given. No significant changes of expression of interleukin 6 (IL-6), receptor alpha of interleukin 6 (IL-6RA), vascular endothelial growth factor-alpha (VEGF-A) and angiopoietin-1 (ANG-1) in both the traditional primary astroglial culture and the immunopanning astrocytes culture.

After administration of tamoxifen at 8 weeks of age, we performed several behavioral tests to determine the possible roles of SCAP in astrocytes in vivo. In elevated platform test, hGFAP-CreERT2(Tg):: *Scap* flox mice after tamoxifen injection (represented as GFAP-SCAP(-) in the following for abbreviations) showed less time of freezing behavior when standing on the transparent narrow cylinder compared with GFAP-CreERT2(-):: *Scap* flox mice after tamoxifen injection (represented as the control group in the following for easy-reading). Mice with same weeks of age with no tamoxifen injection showed no differences in time of freezing between two groups. In the elevated plus maze, GFAP-SCAP(-) mice also showed more exploration of open arms (thus less anxiety) significantly than the control mice. Although no statistical significance was reached, GFAP-SCAP(-) mice spent less time to firstly enter the unfamiliar arena and start eating after 24-hour-fasting when being put into a new environment in the test of novelty suppressed feeding. No differences in food intake between these two groups after 24-hour-fasting, ruling

out the possibility that drive of hunger were not the explanation.

Besides that, impaired consolidation of long term memory and social interaction were seem in GFAP-SCAP(-) mice but not short term memory and new object learning ability. It was found that GFAP-SCAP(-) mice would have impairment in social interaction and recognition according to the results of the social recognition tests. Defected consolidation of remote memory may also be presented in GFAP-SCAP(-) mice according to the results of passive avoidance tests compared with the control mice. Both groups of mice showed good performance in working memory and learning ability in Y maze and novel object recognition test, respectively. No differences of balance, motor coordination and learning, glucose intolerance, insulin resistance and stress-related hormones were noted between GFAP-SCAP(-) mice and the control mice.

【Conclusion】

Dysfunctional lipid metabolism in the mammalian nervous system would cause various metabolic disorders, including the cognitive impairment and neurodegenerative disorders. To understand the sterol regulations in the brain, we designed and created the transgenic mouse line of GFAP-CreERT2(Tg):: *Scap* flox, studied its roles of SCAP in GFAP positive cells, mostly astrocytes. In this study, we found that SCAP, a crucial sterol-sensor molecule, in astrocytes was proven to regulate of triglyceride and sterol synthesis via control of both SREBP1c and SREBP2, and the down-stream genes. In addition, by method of immunopanning, we disclosed that higher purification of primary astrocytes could be attained and SCAP might play an important role in survival and growth in the serum-deficient medium. In this mouse model, mice with inducible knockout of SCAP in GFAP positive cells (GFAP-SCAP(-) mice) showed impairment in consolidation of long-term memory and improper stress responses proven by correlative behavioral examinations. No influences of the growth, body weight changes, food intake, insulin resistance, glucose tolerance, stress hormonal variations or motor coordination were noted. Although exact molecular mechanisms had not been identified yet but we speculate that induction of Scap deletion in GFAP positive cells may have impacts on the integrity and maturation of synapses to affect the secretion of neurotransmitter, to change the pattern of adult neurogenesis, and to inhibit the active remodeling of neuron-to-neuron interactions in vivo. Once we could discover further clues for the link between lipid alteration in astrocytes impacting on neurons and the consequent change in behavioral patterns, they should have a great potential for finding a novel strategy to combat against the diabetes-related cognitive dysfunction and accompanying behavioral and psychological symptoms of dementia (BPSD), for cure or for prevention in advance.