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学位の種類	博士（医学）
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学位論文題目	Proteome profiling in the hippocampus, medial prefrontal cortex, and striatum of aging rat （老齢ラットの海馬、内側前頭前皮質および線条体におけるプロテオームプロファイリング）
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別紙様式３（課程博士・論文博士共用）

論　文　内　容　要　旨

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学位論文題目	Proteome profiling in the hippocampus, medial prefrontal cortex, and striatum of aging rat （老齢ラットの海馬、内側前頭前皮質および線条体におけるプロテオームプロファイリング）		

Background

Aging is a biological process considered a significant risk factor for neurodegenerative diseases. Decrease in multiple functions occurs in the brain with aging, all of which can contribute to age-related cognitive and locomotor impairments. Our recent study reported that increased brain atrophy in the hippocampus, medial prefrontal cortex (mPFC), and striatum occurred between middle- to late-aged in rats, which was accompanied by impairment in learning, memory, and locomotor activity. We also have conducted a metabolomic analysis using liquid chromatography tandem mass spectrometry (LC-MS/MS) on these rats, which demonstrated significant metabolic changes with age in these rats hippocampus, mPFC, and striatum. Hence, we carried out proteomics study on these rats to further our understanding of proteome changes in the brain of these four age groups. The current study was conducted to identify proteins related biomarkers in the aging brain using LC-MS/MS-based platform, in hoped to understand molecular events of aging.

Purpose

To investigate proteome profiles changes in middle- to late-aged rats hippocampus, medial prefrontal cortex (mPFC), and striatum.

Method

We studied the proteome profiling in the hippocampus, mPFC, and striatum of rats aged 14 (n=14), 18 (n=13), 23 (n=7), and 27 (n=10) months. All experimental procedures were carried out in accordance with the regulations of the Animal Care and Use Committee of Shiga University of Medical Science (2013-2-8H). The rats used in this study were subjected to magnetic resonance imaging measurements, behavioural tests (open field, object recognition, and Morris water maze), and metabolomic analysis as we have reported previously.

（備考）１．論文内容要旨は、研究の目的・方法・結果・考察・結論の順に記載し、２千字程度でタイプ等を用いて印字すること。
２．※印の欄には記入しないこと。

For proteomics analysis, proteins from the rat brain tissue were extracted and separated by SDS-PAGE, followed by in-gel digestion protocol. Mass spectrometry (MS) analysis was performed using a Q Exactive HF Orbitrap MS coupled to a Dionex Ultimate 3000 UHPLC instrument. Chromatographic separations were performed using a reversed-phase column with the electrospray ionization positive mode. The acquired MS data were processed using MaxQuant software. Perseus software was used to perform bioinformatics and statistical analysis. The proteins were subjected to pathway search against KEGG database. The proteins were validated by Western blotting.

Result and discussion:

The present study provides initial insights into understanding protein changes with aging in specific brain regions. We found that the proteins were not consistently changed between the three brain regions (hippocampus, mPFC, and striatum). The altered proteins with age were mostly categorized in oxidative phosphorylation, glutathione metabolism, and calcium signaling pathway. The disturbance in oxidative phosphorylation metabolism may increased oxidative stress in aging. With age, several proteins involved in glutathione metabolism were increased in the late aged rats including glutathione S-transferase, peroxiredoxins, and superoxide dismutase, suggesting a protective mechanism in the late-aged rat brain. Calcium signaling perturbation in aging brain can also greatly contribute to neurodegeneration. In hippocampus, proteins related with this pathway such as neurogranin and calmodulin were increased in aging rats. Age-related decline of glutamate receptors in the hippocampus may lead to reduced calcium influx, which can contribute to impairments in cognitive function. Overall, these changes were mostly observed in the late-aged (27 months old) rats when compared with the middle-aged (14 months old) animals. This finding is supported by our previous study that demonstrated brain atrophy, as well as cognitive and locomotor deficits which were marked in the 27 months old rats compared with the 14 months.

Conclusion:

The present study identified 97, 25, and 5 proteins as age-related proteins in the hippocampus, mPFC, and striatum, respectively. These altered proteins participated in oxidative phosphorylation, glutathione metabolism, and calcium signaling pathway, with the most prominent changes being observed in the oldest group of animals. These results suggest that alterations in oxidative phosphorylation, glutathione metabolism, and calcium signaling pathway may be involved in cognitive and locomotor impairments in aged rats.

別紙様式８（課程博士・論文博士共用）

学位論文審査の結果の要旨

整理番号	８３２	氏　　名	Hamizah Shahirah Binti Hamezah
論文審査委員			

（学位論文審査の結果の要旨）※明朝体１１ポイント、６００字以内で作成のこと

本論文では、14、18、23、および27ヶ月齢のラットの海馬、内側前頭前皮質、および線条体のプロテオーム解析を行い、老化におけるタンパク質プロファイル変化に関して、以下の点を明らかにした。

1） タンパク質プロファイルの変化は、海馬、内側前頭前皮質、線条体間で一致しなかった。
2） 酸化的リン酸化、グルタチオン代謝、およびカルシウムシグナリング経路に分類されるタンパク質の発現プロファイルが変化した。
3） 海馬での酸化的リン酸化酵素の発現亢進は加齢による酸化ストレスの増加を示し、海馬や線条体でのグルタチオン代謝酵素の発現増加は、酸化に対する防御機構の亢進の結果と考えられる。
4） 海馬において、カルシウムシグナリング経路に関連するカルモジュリン、ニューログラニン、Ca2+カルモジュリン依存性キナーゼ、およびグルタミン酸受容体などの発現異常が認められた。また、神経細胞死を引き起こすS100Bの発現増加が認められた。
5） これらの結果はラットの老化による脳萎縮や認知機能低下、自発運動量低下と関連していると考えられる。

本論文は、老化による脳のタンパク質プロファイル変化と、脳萎縮・脳機能低下との関連について新たな知見を与えたものであり、また最終試験として論文内容に関連した試問を実施したところ合格と判断されたので、博士（医学）の学位論文に値するものと認められた。

（総字数５７６字）

（平成３１年　１月３０日）