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The University of Osaka

Abstract of Thesis

Name （ Sheng Kit Yeng ）	
Title	Establishment and applications of a novel dopa-decarboxylase gene reporter mouse model (ドーパ脱炭酸化酵素遺伝子レポーターマウスの樹立)

The study of molecular mechanism in neurons has been a great challenge in theneuroscience field due to the lack of an efficient neuron purification protocol from the brain. In this study, I established a Ddc knock-in reporter mouse model where endogenous Ddc expression is visualized by the red fluorescent protein hKO1 expression. I further established an efficient protocol for the collection of homogenous Ddc expressing cells using a fluorescent activated cell sorter for RNA-sequencing. Then, I applied them in the studies of genomic imprinting, and Parkinson's disease.

Genomic imprinting is monoallelic gene expression in a parent-of-origin manner and play an important role in the development and homeostasis of the brain. Although the number of imprinted gene is small (<1%), many of them were reported to regulate growth, development and social behaviours. However, the molecular mechanism and physiological function of genomic imprinting in the adult tissues are predominantly unknown. In particular, imprinting studies in the brain is challenging due to its complexity and heterogeneity in cell populations. Ddc was reported as an imprinted gene in the brain, and its expression pattern in previous studies were inconsistent and controversial. One of the reasons is that most studies uses whole brain or dissected regions for the analysis. Here, I took the advantage of a knock-in mouse model and compared the expression of maternally and paternally inherited Ddc-hKO1 in different brain regions. Ddc showed paternal biased expression in the arcuate nucleus, and showed maternal biased expression in the ventral tegmented area, substantia nigra pars compacta, retrorubral field and locus coerulus. In contrast, Ddc was biallelically expressed in the dorsal raphe nucleus region. These results demonstrated that Ddc expression is regulated in a region-specific manner.

Parkinson's disease is a progressive neurodegenerative disease that often characterized by rigidity and tremor. The pathological hallmarks are presence of Lewy body inclusions and loss of dopaminergic (DA) neurons. Lewy bodies are made up of misfolded a-synuclein, but the causal relationship between the accumulation of a-synuclein and DA neurodegeneration has remained unclear. We used the established Ddc reporter mice to reveal the early effect of α-synuclein accumulation in DA neuron. G51D mutated α-synuclein fibrils were injected intranigrally to induce the accumulation and Ddc-hKO1-positive DA neurons were collected at 7- and 12 weeks post-injection, prior to the occurrence of neurodegeneration. RNA-sequencing and gene ontology analyses demonstrated that the up-regulation genes related to lipid- and protein-associated biological processes were significant. Among them, fatty acid binding protein gene, up-regulation of Fabp1 in DA neuron was confirmed by immunohistochemistry.

In conclusion, Ddc-hKO1 reporter mice that I have established in this study showed to be a versatile mouse model with its application in two different fields, genomic imprinting and Parkinson's disease studies.

様式７

論文審査の結果の要旨及び担当者

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論文審査担当者	（職）　氏　名 主査　教授　仲野　徹 副査　教授　八木　健 副査　教授　山本　亘彦

論文審査の結果の要旨

申請者は、脳においてドーパミンなどの神経伝達物質の合成に必須の働きを果たしている、ドーパ脱炭酸酵素（Ddc）遺伝子に赤色蛍光タンパク質遺伝子hKO1を挿入したレポーターマウスを樹立し、２つの研究テーマを展開した。

一つは、アレル特異的発現であるゲノムインプリンティングの研究である。Ddcは脳の領域によって父方アレルが優先的に発現する領域と、母方アレルが優先的に発現する領域が存在することを見出した。もう一つは、パーキンソン病に関連する研究である。Ddc-hKO1マウスに変異型シヌクレインを注入し、セルソーターを用いてドーパミンニューロンを回収、転写プロファイルの変化を解析した。

本研究は新規レポーターマウスの樹立からその応用研究まで独自性の高いものであり、博士号に十分な成果であるといえる。特にドーパミンニューロンを効率的に回収、解析できる技術は汎用性が高く、今後の神経科学分野への応用も期待できる。

よって、博士の学位を授与するに値するものと認める。