博士学位論文審査要旨

２０１９年2月６日

論 文 題 目 : Analysis of non-coding RNA expression in medium spiny neurons of Huntington disease model mice
ハンチントン病モデルマウスの中型有棘神経細胞におけるノンコーディングRNAの発現変化

学位申請者: 朴 洪宣

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要 旨 :

この学位論文は、ハンチントン病モデルマウスの線条体中型有棘神経細胞において、野生型マウスに比べて発現量が変化しているノンコーディングRNAを網羅的手法で検出し、複数の候補について定量的リアルタイムPCR、*in situ* hybridization、蛍光免疫組織学などを駆使して検証した論文である。特に*in situ* hybridizationに関しては、通常の方法では感度が低かったため、View RNA ISHという単一分子および単一細胞レベルの検出感度を持つ新しい解析手法に挑戦している。

候補遺伝子のうち、特に*Neat1*遺伝子に関しては、線条体中型有棘神経細胞における発現がモデルマウスの生後４週齢で減少すること、およびparaspeckleという核内構造体の構造変化についても多重免疫染色を用いて可視化し、新しい所見として報告している。この遺伝子がハンチントン病の発症、経過に及ぼす影響については今後の研究に委ねられるところであるが、ハンチントン病の決め手となる治療法がない現状を考えると、ノンコーディングRNAの発現変化という斬新な着眼点で野生型との相違点を見出した意義は大きいと考えられる。

口頭試問では、英語による適切な研究発表を行った。質疑応答では論文についての問題点がいくつか指摘されたが、それに対して概ね満足のできる回答・討論を行い、学位論文の**defense**を行うことができたと判断できる。

よって、本論文は、博士（理学）（同志社大学）の学位を授与するにふさわしいものであると認める。

総合試験結果の要旨

２０１９年2月６日

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要 旨：

博士学位候補者の朴洪宣氏に対して、2019年1月30日午後11時より総合試験を行った。

朴氏の学位研究は、神経変性疾患であるハンチントン病モデルマウスの線条体中型有棘神経細胞におけるノンコーディングRNAの解析である。そこで総合試験では主に分子生物学、神経病理学の基礎および専門的な知識についての試問を行った。なお、語学試験については、総合試験の前に行った公開口頭試問を英語で行ったため、総合試験では免除とした。

朴氏は、審査委員の質問について適切に答え、不明な点に関しては十分に論理的な考察を行った。関連分野の基礎知識は十分に備わっていると判断した。専門的な知識については、まだまだ不十分な点も認められるが、概して満足のできるレベルにあると判断した。

以上を踏まえて、審査員一同の協議の結果、総合試験の結果は合格であると認める。

博 士 学 位 論 文 要 旨

論 文 題 目： Analysis of non-coding RNA expression in medium spiny neurons of Huntington disease model mice（ハンチントン病モデルマウスの中型有棘神経細胞におけるノンコーディングRNAの発現変化）

氏　　名： 朴　洪宣

要　　旨：

Huntington disease (HD) is a dominantly-inherited neurodegenerative disorder with symptoms of movement disorders, psychiatric disturbances and dementia. In the brains of HD patients, neurodegeneration is most pronounced in the striatum, and medium spiny neurons (MSN) are lost. The HD gene was identified as huntingtin (*HTT*) by the Huntington's Disease Collaborative Research Group in 1993. The gene contains expanded CAG repeats in its exon1. While the normal range of the CAG repeat is about 6-35, but in HD, it exceeds from 36 to more than 120. HD is also known as a polyglutamine disease as the multiple (poly) CAG repeats encode polyglutamine. The mutant huntingtin gene product affects the transcriptional profile of neurons by disrupting the activities of transcriptional machinery such as transcriptional factors. For example, CREB-binding protein (CBP) is present in the polyglutamine nuclear aggregates in HD cell culture models, transgenic mice, and human HD postmortem brains, suggesting that mutant huntingtin protein specifically interferes with CBP-mediated transcription through the interaction with polyglutamine aggregates. Another example is NF-Y, which also colocalizes with ubiquitin-positive nuclear inclusions in cortex and striatum of HD model mouse. This abnormal localization of NF-Y is believed to affect the activities and expression of HSP70. Taking these together, mutant *HTT* may disrupt the activities of transcriptional factors and alter the expression of many genes, suggesting that the alterations of gene expression might be one of the crucial pathological changes in HD.

Through large-scale microarray analyses, previous studies have revealed that human HD patients and mouse models show significant alterations in brain mRNA levels. For example, mRNA levels of β4, the voltage-gated sodium channel, was found to be reduced significantly in the striatum of HD model mouse, R6/2, at an early stage of development. To investigate the alteration of gene expression in MSN directly, we generated Venus expressed R6/2 HD model mouse by crossing R6/2 with transgenic mice expressing the fluorescent marker, Venus, under the control of the *Scn4b* promoter which visualizes the axons of striatal projection neurons. Based on the subsequent FACS-array analysis, many coding and non-coding RNAs (ncRNAs)

showed dysregulation in MSN of R6/2 brains. However, the dysregulations found in the striatum and that of MSN did not always correspond to each other during early stages of development.

Until now, the alteration of ncRNA expression levels in the brains of either HD patients or model mice have not been fully investigated. In this study, from the candidate ncRNAs suggested by microarray, we identified mouse ncRNAs which both have homology with human ncRNAs and are believed to be highly implicated to HD. Also, we observed the dysregulations in their expression levels and determined their localization of those ncRNAs in HD model mouse brains.

In order to select candidate ncRNAs, we analyzed the gene expression profiling data which included a set of ncRNAs dysregulated in the MSN of 4-week-old R6/2 HD model mice. 4-week-old mice were used to exclude secondary effects of early dysregulated genes, since the alteration of gene expression was observed in the early stages of the disease. Due to the low expression levels, ncRNAs with fold-change (> 1.5), p-value (< 0.05) and raw signal value (> 500) were considered as dysregulated ncRNA. Among these genes, we found 12 ncRNAs which both are dysregulated in MSN of 4-week-old HD model mouse brains and have homology with human ncRNAs.

To investigate the expression levels of those ncRNAs in the striatum and MSNs of R6/2 and control mice, qRT-PCR was performed, followed by *in situ* hybridization (ISH) to examine the localizations of these ncRNAs in the R6/2 brain sections. Despite ISH being known as a useful method to observe precise localization of a specific segments of DNA or RNA within a histologic section, it was hard to detect signals of ncRNAs with low expression levels and determine any changes. Thus, we instead performed ViewRNA ISH for these poorly expressed ncRNAs.

ViewRNA ISH is a method that provides highly specific signals and ultra-sensitive single-molecule detection of RNA, where each signal dot corresponds to one target molecule. In practice, ViewRNA ISH was able to detect the signals of those poorly expressed ncRNAs such as *Abhd11os* which could not be detected by conventional ISH. By using ViewRNA ISH, *Abhd11os* signals were detected in both control and R6/2 brains. Also, *Scn4b*, a coding RNA which is downregulated in HD brains, was detected not only in the MSN of striatum and the Purkinje cells of cerebellum strongly, but also in the hippocampal neurons, in which *Scn4b* was not detected by conventional ISH. Expression levels of *Scn4b* were also shown to be markedly decreased in the striatum of 4-week-old R6/2 HD mouse brains. These results suggest that ViewRNA ISH has a higher sensitivity compared to conventional ISH.

As stated above, many ncRNAs were dysregulated in 4-week-old R6/2 HD model mouse brains. Among them, *Abhd11os* and *Neat1* were ncRNAs whose expression levels were significantly downregulated both in the R6/2 striatum and

MSN. Using ViewRNA ISH, dotted *Abhd11os* and *Neat1* signals were detected both using bright field and fluorescence imaging, and these signals seemed to decrease in the striatum of R6/2. We also confirmed that the number of signals of *Abhd11os* and *Neat1* were reduced in the striatum of R6/2.

Next, intracellular localizations of *Abhd11os* and *Neat1* were investigated by performing combined staining with a marker for MSN, DARPP32, after ViewRNA ISH, *Abhd11os* and *Neat1* were downregulated and found in the DARPP32 positive cells, indicating they indeed localize in MSN. Since *Neat1* is known to associate to form paraspeckles, a form of nuclear compartment in the interchromatin space, and maintain their integrity, we hypothesized that dysregulation of *Neat1* might affect the morphology of paraspeckles. By performing combined staining with an anti-PSF antibody which detects paraspeckles specifically, we found that *Neat1* signals were downregulated in PSF positive cells and that PSF positive cells were more dispersed in R6/2 compared to the control.

Taking all these together, this study revealed that the expression levels of *Abhd11os* and *Neat1* were dysregulated in MSN of presymptomatic R6/2 mice, and the disruption of *Neat1* expression might affect paraspeckle formation, which might be implicated to HD pathogenesis. The roles of ncRNA in neurodegenerative disease have not been fully understood, and further research regarding it may be challenging. This study also suggests that ViewRNA ISH is a useful method to detect the dysregulation of ncRNAs with low expression levels and can be used for the further studies about ncRNAs and related human diseases.