様式第１９号

学位論文審査の結果の要旨

審査区分 ㊐・論	第706号	氏名	Magdeline Elizabeth Carrasco Apolinario
審査委員会委員		主査氏名	井原健二
		副査氏名	濱田文彦
		副査氏名	秦暢宏

論文題目

Behavioral and neurological effects of Vrk1 deficiency in zebrafish
（VRK1 遺伝子欠損ゼブラフィッシュにおける行動学的・神経学的解析）

論文掲載雑誌名

Biochemical and Biophysical Research Communications (BBRC)

論文要旨

Vaccinia-related kinase 1 (VRK1) is a serine/threonine kinase, for which mutations have been reported cause to neurodegenerative diseases, including spinal muscular atrophy, characterized by microcephaly, motor dysfunction, and impaired cognitive function, in humans. Partial Vrk1 knockdown in mice has been associated with microcephaly and impaired motor function. However, the pathophysiological relationship between VRK1 and neurodegenerative disorders and the precise mechanism of VRK1-related microcephaly and motor function deficits have not been fully investigated. To address this, in this study, we established vrk1-deficient (vrk1−/−) zebrafish and found that they show mild microcephaly and impaired motor function with a low brain dopamine content. Furthermore, vrk1−/− zebrafish exhibited decreased cell proliferation, defects in nuclear envelope formation, and heterochromatin formation in the brain. To our knowledge, this is the first report demonstrating the important role of VRK1 in microcephaly and motor dysfunction in vivo using vrk1−/− zebrafish. These findings contribute to elucidating the pathophysiological mechanisms underlying VRK1-mediated neurodegenerative diseases associated with microcephaly.

本研究では、VRK1 欠損ゼブラフィッシュを用いて VRK1 の小頭症および運動機能障害における役割を初めて実証し、関連するヒトの神経変性疾患の病態を明らかにするモデルを提供した。今後の臨床医学への応用と展開を期待させる重要な研究であり，審査員の合議により本論文は学位論文に値するものと判定した。

様式第２０号

最終試験
の結果の要旨
~~学力の確認~~

審査区分 ㊟・論	第706号	氏名	Magdeline Elizabeth Carrasco Apolinario
審査委員会委員		主査氏名	井原健二
		副査氏名	濡田文秀
		副査氏名	秦暢弘

学位申請者は本論文の公開発表を行い，各審査委員から研究の目的，方法，結果，考察について以下の質問を受けた。

1. VRK1は他の組織でも発現していますが、ノックアウトにより中枢神経系の機能不全のみが起きているのはなぜでしょうか。他の組織の機能には影響がありましたか。
2. ノックアウトを作成する際、Exon 2をターゲットとした理由は何ですか？
3. ノックアウトによりVRK1のmRNA発現レベルは正常と比較して半分程度に減少しますが、完全には消失しないのはなぜでしょうか。ヘテロのノックアウトモデル（VRK+/-）の場合、この発現レベルはどのようになっていますか？
4. 症例で確認されるVrk1遺伝子の変異は、Vrk1のキナーゼ活性と関係していますか？
5. Fig. 2で用いられている抗HuC/HuD抗体が反応する蛋白質は何ですか？
6. Fig. 4Bでは、何個の細胞をカウントしていますか？増殖中の細胞の比率はどのように計算しましたか？
7. Fig. 4Cの図の説明には、白の矢印はヘテロクロマチンを、赤の矢印は核膜の欠損とヘテロクロマチンの形成を示すと記載されています。これは正確ですか？
8. Fig. 4Dはヘテロクロマチン陽性細胞の比率を表示していますが、すべての細胞がヘテロクロマチンを持っているのではないですか？
9. VrkファミリーのVrk1遺伝子とVrk2遺伝子について、機能や構造の違いは何ですか？
10. Vrk2ノックアウトでは体型が小さくなるようですが、ヒトの患者でも同様の表現型が見られますか？また、Vrk2ノックアウトで脳のサイズが小さいとされていますが、体格が小さいことから相対的な差異は認めないのではないですか？
11. Vrk2ノックアウトで体型が小さいため、泳ぐ距離やパターンが変化したのですか？また、この小さな体型の原因として、筋肉や骨の変化は確認されましたか？
12. ノックアウトの脳のドーパミンが低下したことに対して、治療研究への展開はどのように考えていますか？

これらの質疑に対して，申請者は概ね適切に回答した。よって審査委員の合議の結果，申請者は学位取得有資格者と認定した。

（注）不要の文字は２本線で抹消すること。

様式第８号 No. 1

学　位　論　文　要　旨

氏名　Magdeline Elizabeth Carrasco Apolinario

論　文　題　目

Behavioral and neurological effects of Vrk1 deficiency in zebrafish

(VRK1 遺伝子欠損ゼブラフィッシュにおける行動学的・神経学的解析)

要　旨

【Introduction】

Vaccinia-related kinase 1 (VRK1) is a serine/threonine kinase whose mutations have been reported to cause neurodegenerative diseases, including spinal muscular atrophy (SMA), characterized by microcephaly, motor dysfunction, and impaired cognitive function in humans. However, the pathophysiological mechanisms of VRK1-related microcephaly and motor function deficits have not been fully investigated. The aim of this study is to address the precise pathophysiological phenotype and mechanisms of microcephaly associated to VRK1 using an *in vivo* zebrafish model.

【Material and Methods】

We established vrk1-deficient (*vrk1*$^{-/-}$) zebrafish using CRISPR-Cas9 technology and evaluated the morphological phenotype by analysis of body length, head size, and brain size. We examined the detailed morphological features of the zebrafish brain using hematoxylin and eosin staining and evaluated the number of mature neurons by immunohistochemistry with anti-HuC/D antibody. Next, to investigate the behavioral phenotype of *vrk1*$^{-/-}$ zebrafish, we assessed locomotor activity and anxiety with the novel tank diving test (NTD). Furthermore, we measured the content of a series of neurotransmitters including acetylcholine, dopamine, norepinephrine, and serotonin through LC-MS/MS. In the end, to understand the mechanisms involved in these phenomena, we examined the proliferation of radial glial progenitor cells in the brain using immunohistochemistry and the detailed features of the neurons using scanning electron microscopy (SEM).

【Results】

The *vrk1*$^{-/-}$ zebrafish clearly showed microcephaly and a significant reduction in the number of mature neuron cells in the brain. In the behavioral study using the NTD, the *vrk1*$^{-/-}$ zebrafish showed a marked decrease in locomotor activity, indicating impaired motor function. Additionally, the latency in the top area of the tank was increased for *vrk1*$^{-/-}$ zebrafish, implying resistance to anxiety due to cognitive decline. In the analysis of neurotransmitters, dopamine content in the brain was significantly lower, which might be related to the abnormal motor function in *vrk1*$^{-/-}$ zebrafish. Furthermore, *vrk1*$^{-/-}$ zebrafish exhibited decreased radial glial progenitor cell proliferation, defects in nuclear envelope formation, and heterochromatin formation in the brain.

【Discussion】

The microcephaly and motor impairment in *vrk1*$^{-/-}$ zebrafish may be due to defects in brain development caused by reduced VRK1. The reduced number of mature neurons in the brain and the reduced number of radial glial progenitor cells suggest that VRK1 plays an essential role in brain development. Previously, Vrk1 has been associated with microcephaly due to the loss of neuroblasts in flies and was found to be required for nuclear membrane disassembly and assembly during mitosis. As expected, in the present study, abnormal nuclear membrane assembly and increased heterochromatin formation in *vrk1*$^{-/-}$ zebrafish neurons were observed, indicating that VRK1 contributes to zebrafish neuronal development. In addition, neurotransmitter kinetic analysis showed that *vrk1*$^{-/-}$ zebrafish had decreased dopamine content in the brain, and the behavioral study showed motor dysfunction. This suggests that VRK1 may contribute, not only to microcephaly and motor dysfunction, but also to the development of neurodegenerative diseases such as Parkinson's disease.

【Conclusion】

This is the first report demonstrating the important role of VRK1 in microcephaly and motor dysfunction *in vivo* using *vrk1*$^{-/-}$ zebrafish. These findings contribute to elucidating the pathophysiological mechanisms underlying VRK1-mediated neurodegenerative diseases associated with microcephaly.