



Title	MiRNA-132/212 regulates tight junction stabilization in blood-brain barrier after stroke
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Citation	大阪大学, 2022, 博士論文
Version Type	
URL	https://hdl.handle.net/11094/87878
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論文内容の要旨

Synopsis of Thesis

氏 名 Name	YAN HAOMIN
論文題名 Title	MiRNA-132/212 regulates tight junction stabilization in blood-brain barrier after stroke (MiRNA-132/212は脳卒中血液脳関門におけるTight Junctionの安定性を制御する)
論文内容の要旨 〔目 的(Purpose)〕 Stroke is a leading cause of mortality and long-term disability worldwide. Although intensive efforts have been made to develop new pharmacological therapies, tissue plasminogen activator (tPA) is still the only Food and Drug Administration (FDA)-approved medication for ischemic stroke, but its use is restricted to a narrow time window, with a high risk of hemorrhage. MicroRNAs (miRNAs), a group of endogenous non-coding RNAs composed of ~20 nucleotides, function as a critical role in post-transcriptional gene regulation via sequence-specifically binding to the 3'-untranslated region (UTR) of one or more messenger RNAs (mRNAs). Among the miRNAs involved in central nervous system diseases, miRNA-132/212 cluster is a highly brain-enriched microRNA cluster and has been well-studied as an upstream inhibitory regulator on mRNA expression in CNS and has been linked to a particularly wide variety of brain diseases, but the role of miR-132/212 in the neurovascular unit in ischemic stroke is still unclear. In this study, we sought to elucidate the role of miR-132/212 in the interaction among neurons and endothelial cells in ischemic stroke. 〔方法ならびに成績(Methods/Results)〕 As CREB-regulated transcription coactivator 1 (CRTC1) has been predicted to be an upstream regulator of miR-132/212 by in silico analysis, we generated CRTC1 KO mice using CRISPR/CAS9, then subjected both CRTC1 KO and wild-type (WT) mice to 1 h of middle cerebral artery occlusion. CRTC1 deletion led to a decrease in miR-132/212 expression, and significantly increased infarct volume and aggravated blood-brain barrier (BBB) dysfunction after ischemic stroke, with worsening neurological deficits. CRTC1 deletion also upregulated RNA-binding Fox-1 (RBFOX-1) protein in neurons, and consistent with this, transfection of miR-132/212 antagomir upregulated RBFOX-1 in neurons after ischemic stroke. Furthermore, CRTC1 deletion suppressed vascular bed and upregulated protein levels of tight junction associated protein-1 (TJAP-1) and Claudin-1 in mice brain. Dual luciferase assay demonstrated that miR-132/212 translationally suppresses RBFOX-1, TJAP-1 and Claudin-1 by directly binding to their respective 3'-untranslated regions. Moreover, human umbilical vein endothelial cells (HUVECs) co-cultured with CRTC1-deficient neurons exhibited increased susceptibility to hypoxia. To functionally specify the direct relationship between neuron-derived miR-132 and tight junction proteins, we transfected miR-132 mimic/antagomir/vehicle into neurons which were cocultured with HUVECs, and found HUVECs co-cultured with neurons which were transfected with miR-132 mimic gradually attenuated the TEER decline compared to vehicle group, suggesting that miR-132 upregulated ECs resistance to OGD by an external pathway. 〔総 括(Conclusion)〕 Our findings demonstrate that CRTC1 deficiency aggravates neurological deficits in mice in the early phase after stroke. This worsening is mediated by RBFOX1 via disruption of CRTC1-miR-132 signaling in neurons. The miR-132/212 dysregulation impairs BBB function, and is associated with perturbations in TJAP-1, Claudin-1 and Claudin-5. Further studies are needed to clarify the paracrine communication mechanisms by which miRNAs and non-coding RNAs affect CNS pathology after stroke. Investigation of the intercellular signaling mechanisms mediated by miRNAs may lead to novel approaches for the treatment of stroke.	

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨					
脳卒中後のNeurovascular Unit (NVU) におけるMicroRNA-132の役割及び上流の制御因子については不明である。ゲノム編集によりCRTC1ノックアウトマウスを作成し、CRTC1がmiRNA-132 / 212の直接的な上流の調節因子であることを示した。CRTC1の欠失は、脳梗塞後におけるmiRNA-132 / 212発現低下、梗塞体積の有意な増加、BBB透過性の亢進を伴う神経機能予後悪化を齎した。NVUにおいて、miRNA-132は、神経細胞におけるRBFOX-1、脳血管内皮細胞における密着結合におけるClaudin-1、TJAP-1を標的因子として制御していた。このように神経細胞から遊離されたmiR-132が、パラクライン機序を介して血管内皮細胞に作用し、血液脳関門のバリア機能に寄与することを証明した意義は大きく、脳梗塞に対するMicroRNA-132の新たな治療法開発に繋がる業績であり、本論文は学位の授与に値すると考える。					