Supplement Material

Supplemental Methods:

Laser Capture Microdissection (LCM). Briefly, frozen brain coronal sections (8 μm) were immersed in acetone for 2 min of fixation and air-dried for 30s. After a brief rinse with 0.1% diethylpyrocarbonate-treated phosphate buffered saline (PBS), sections were stained with an antibody against von Willebrand factor (vWF, 1:50 dilution) for 10 min followed by a Cy3-conjugated secondary antibody (DAKO, 1:50 dilution) for 10 min. After a brief rinse with PBS, sections were air-dried under laminar flow for 10 min and immediately used for LCM. vWF-positive cells at the ischemic boundary area and homologous area of the contralateral hemisphere were excised with a Leica AS LMD System (Leica Microsystems Inc). Approximately 1000 endothelial cells from each animal were collected into a lysis buffer containing QIAzol reagent (Qiagen) in Eppendorf tubes and were stored at -80°C before miRNA isolation.

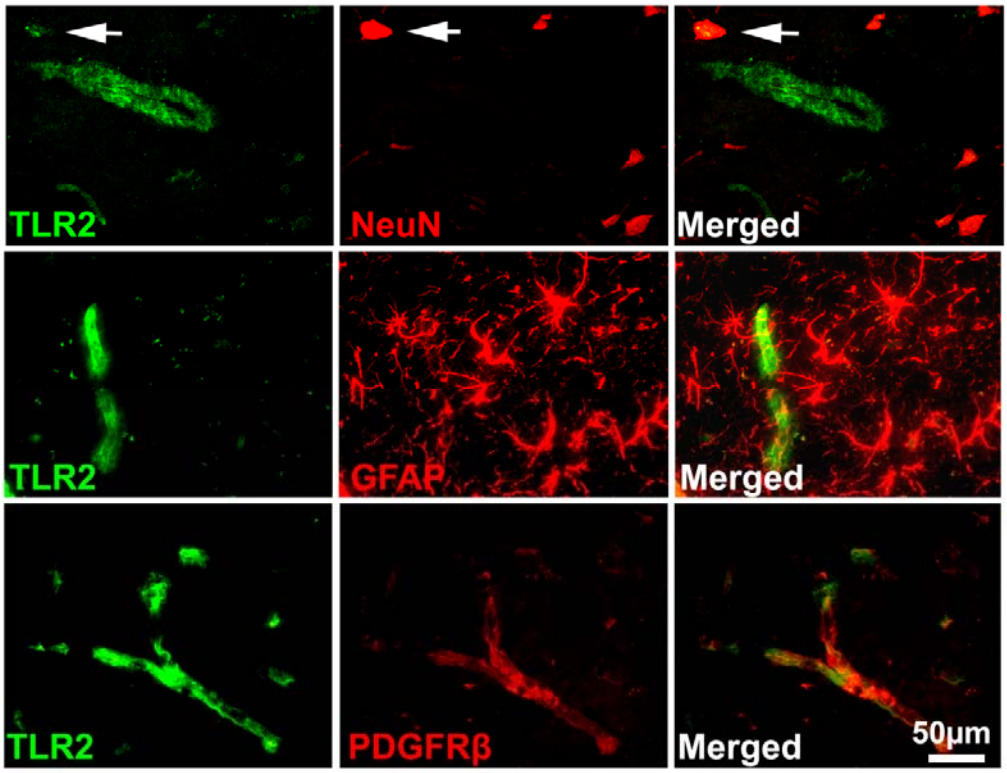
Quantification of mature and primary miRNAs by real-time RT-PCR. For evaluation of miR-146a levels, total RNAs from HBECs or endothelial cells isolated by LCM were extracted using miRNeasy Mini Kit (Qiagen). 15 μL Reverse transcription reactions consisted of 1-10 ng total RNA, 1x stem-loop RT specific primer, 1x reaction buffer, 0.25 mM each of dNTPs, 3.33 U/μl Multiscribe RT enzyme, 0.25 U/μl RNase inhibitor, and RNase-free water were incubated at 16°C for 30min, 42°C for 30min, 85°C for 5 min, and then held at 4°C until use in TaqMan real-time PCR. TaqMan real-time PCR reactions of miR-146a consisted of 1× TaqMan Universal PCR Master Mix No AmpErase UNG, 1× TaqMan miRNA assay, 1.33 μL of undiluted Reverse transcription product (cDNA), and nuclease free water at a total volume of 20 μL.

For evaluation of pri-miR-146a levels, RNAs extracted from the endothelial cells were reverse-transcribed using High Capacity RNA-To-cDNA Kit (Applied Biosystem). TaqMan® Pri-miRNA assays were performed. 20 μL Reverse transcription reactions consisted of 10-100 ng Total RNA, 1 μL of 20× RT Enzyme Mix and excessive RNAase free water. Reverse transcription reactions were incubated at 37°C for 60min, 94°C for 5min and then stored at 4°C until use in TaqMan Pri-miRNA assays. 20 μL TaqMan real-time PCR reactions were composed of 10 μL of 2× TaqMan Universal PCR Master Mix No AmpErase UNG, 1μL of 20× TaqMan Pri-miRNA assay, 1.33 μL of undiluted cDNA, and extra nuclease free water.

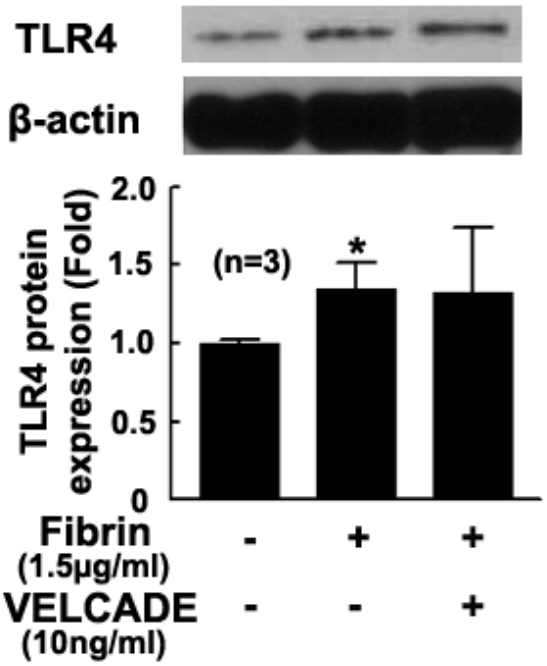
Each TaqMan assay was done in triplicate for each sample tested. Relative quantities were calculated using the $2^{-\Delta\Delta CT}$ method with U6 snRNA TaqMan miRNA control assay (Applied Biosystem) as the endogenous normalization control. Representative results are shown as fold expression relative to contralateral homologues area on the endothelial cells isolated by LCM, or to the control experimental conditions on primary human brain microvascular endothelial cells (HBECs). Reactions were run with the Standard 7000 default cycling protocol without the 50°C incubation stage, with reactions incubated at 95°C 10 min, followed by 40 cycles of 95°C 15 sec, 60°C 1 min. Fluorescence readings were collected during the 60°C step.

Supplemental Figures:

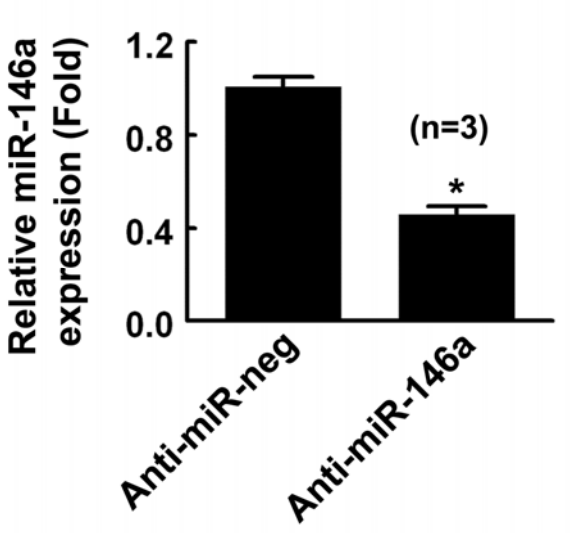
I. Co-localization of TLR2 with specific markers for neurons (NeuN), astrocytes (GFAP), and pericytes (PDGFRβ).



II. Western blot analysis and quantitative data of TLR4 protein levels on HBECs treated with fibrin in the presence and absence of VELCADE.



III. Real-time RT-PC analysis of miR-146a expression in HBECs transfected with miR-146a inhibitor (Anti-miR-146a) and a negative control miRNA inhibitor (Anti-miR-neg).



IV. RT-PCR amplification plots of PECAM-1 (A) and GFAP (B) in cells isolated by laser capture microdissection (LCM). Panel C shows the quantitative data of PECAM-1 mRNA levels on cells isolated from ipsilateral and contralateral hemisphere by LCM.