

Novel therapeutic strategy for cerebral stroke using Npas4-related factors

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Project Outline

Cerebrovascular diseases are the fourth leading cause of death in Japan, with a high incidence. However, when the brain is damaged by cerebral stroke (ischemia), which accounts for the majority of cerebrovascular diseases, an effective treatment to replace the lost neurons and circuits has not yet been established. In addition, cerebrovascular diseases, along with dementia, are the biggest causes of people requiring nursing care. Therefore, "how to reconstruct and repair the brain structure and functions that have been disrupted by cerebral infarction?" is an extremely important question in our super-aging society.

It has been suggested that the expression of the transcription factor *Npas4* is strongly induced in neurons surrounding the infarct foci in the early stage after stroke and regulates the survival and death of neurons; yet the genes acting downstream of *Npas4* remain unknown.

We found that when mice were transferred from a home cage to expose them for a short time into a new cage containing play equipment (an enriched environment), and then subjected to stroke surgery, neuronal death in the mice was surprisingly reduced. This appeared to be because exposure of the mice to the enriched environment caused a marked increase of *Npas4* expression in cortical neurons. Furthermore, based on an *in vitro* ischemia-like stress model, we systematically searched for genes downstream of *Npas4* to successfully identify *Gem*, which encodes a Ras-like small GTPase directly involved in neuroprotection. In addition, when *Gem* was transiently expressed in the mouse brain, neuronal death after stroke was markedly reduced (**Fig. 1**). This reveals that *Gem*, downstream of *Npas4*, plays an important role in suppression of neuronal death (neuroprotection) via mis-localization of L-type voltage-gated Ca^{2+} channel.

Since an orthologue of *Gem* is also induced in human cortical organoids by ischemic-like stress, *Gem* is expected to be a novel drug target for the therapy of stroke (Takahashi *et al.*, *Proc Natl Acad Sci USA*, **118**: e2018850118, 2021).

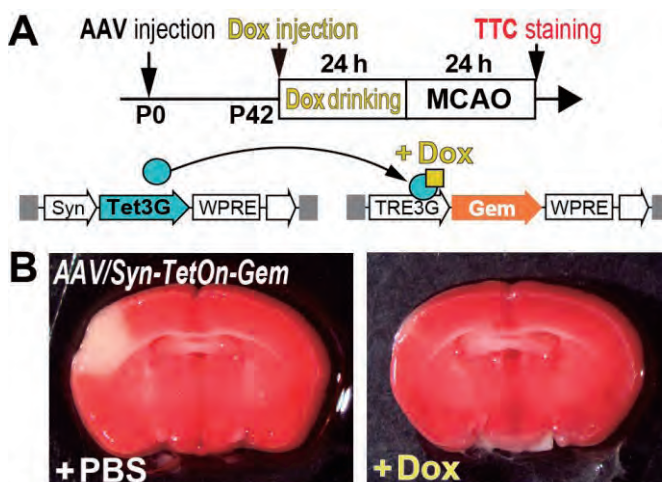


Figure 1. Transient expression of *Gem* in the mouse brain markedly reduced neuronal death after stroke.

(A) Schematic diagram of the experiment in which *Gem* was overexpressed using the adeno-associated virus AAV/Syn-TetOn system before stroke surgery. Two viruses (AAV/Syn-Tet3G and AAV/TRE3G-Gem) were simultaneously injected into the ventricles of pup mice at postnatal day 0 (P0). Forty-two days later, mice were treated with either PBS (phosphate-buffered saline) or Dox (doxycycline) and then subjected to stroke surgery. Twenty-four hours later, TTC staining was performed to measure the size of the infarct foci.

(B) Transient expression of *Gem* in the mouse brain markedly reduced neuronal cell death after stroke.

Patent information in addition to target diseases: cerebrovascular disorders, patent pending
 Characteristics of the technology, marketability, and issues in development: Search for target compounds
 Desired nature of corporate collaboration: Joint research
 Division of roles between companies and academia: Product development and basic research