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The ubiquitin editing enzyme A20 (TNFAIP3) is upregulated during permanent middle cerebral artery occlusion but does not influence disease outcome

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Dear Editor,

Cerebral ischemia is characterized by the activation of glial cells, causing a rapid and massive local inflammatory reaction that leads to tissue damage and neuronal cell death.[1] Over the past decade, it has become increasingly clear that the transcription factor nuclear factor κB (NF-κB) has a central role in the pathogenesis of cerebral ischemia. The NF-κB family consists of five members, RelA (p65), RelB, c-Rel, p50 and p52, all of which are activated in the ischemic hemisphere of mice shortly after permanent middle cerebral artery occlusion (pMCAO), a well-established rodent model of focal cerebral ischemia.[2] Furthermore, p50-deficient mice show a reduction in ischemic damage after pMCAO and transient MCAO followed by reperfusion, suggesting a cell death

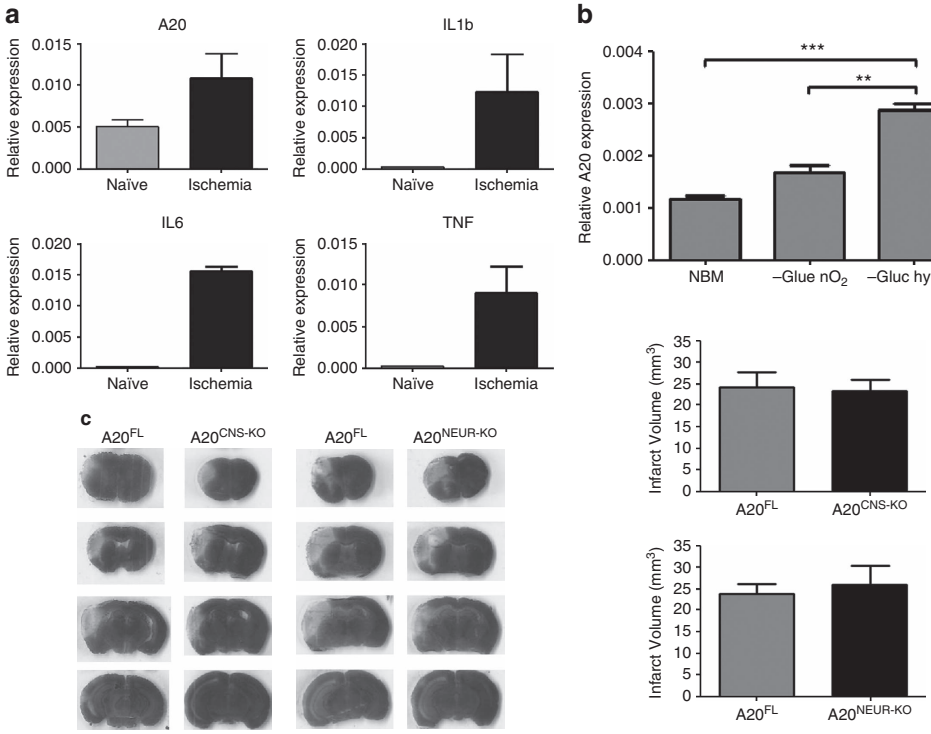

Figure 1 (**a**) pMCAO was induced in wild-type C57BL/6 mice and gene expression in the infarcted brain area was assessed by quantitative real-time PCR 24 h after pMCAO. (**b**) A20 expression in primary cortical neurons, as assessed by quantitative real-time PCR. NBM, neurobasal medium supplemented with B27; -Gluc nO_2, DMEM without glucose in normoxic conditions; -Gluc $hypoO_2$, DMEM without glucose in hypoxic conditions. $^{**}P<0.01$; $^{***}P<0.001$ (**c**) pMCAO was induced in CNS- ($A20^{CNS\text{-}KO}$; $n=12$) or neuron-specific ($A20^{NEUR\text{-}KO}$, $n=6$) A20-deficient mice and their control $A20^{FL}$ littermates ($n=9$ and $n=12$, respectively), as previously described.[2] Representative silver-stained brain sections (left panel) and evaluation of infarcts volume (right panel) 24 h after pMCAO. Values are means ± S.E.M.

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promoting role for NF-κB in this model.[3,4] In addition, mice lacking RelA in the central nervous system (CNS) also show reduced infarct size after 48 h of pMCOA, whereas germline deletion of the p52 or c-Rel subunits did not affect infarct volume.[2] In line with findings that NF-κB has a detrimental role during ischemia, mice lacking IKK2 in all neuroectodermal cells or specifically in neurons show a decreased infarct volume 48 h after pMCAO, whereas constitutive activation of IKK2 increased infarct size.[5] However, NF-κB may also act beneficial in conditions of ischemic preconditioning, which was shown to protect against a subsequent prolonged ischemic insult through transcriptional activation of NF-κB.[6]

The ubiquitin editing enzyme A20 is a key negative regulator of NF-κB signaling. Moreover, A20 can also act as a strong antiapoptotic protein in specific cell types.[7] Because of the central role of A20 in controlling NF-κB and apoptotic responses, we sought to further clarify its *in vivo* role in the pMCAO mouse model of brain ischemia.

We demonstrate here that NF-κB driven genes such as *A20, TNF* and *IL-6* are upregulated in the infarcted area 24 h post pMCAO (Figure 1a). We further questioned whether A20 would be differentially regulated in primary murine cortical neurons after glucose oxygen deprivation, an *in vitro* model mimicking pMCAO. In agreement with our *in vivo* data, A20 mRNA was upregulated in primary murine cortical neurons when placed in a glucose deprived 0.1% O_2 hypoxic environment for 4 h (Figure 1b). As A20 is upregulated both *in vivo* and *in vitro* during ischemic conditions, we questioned whether mice lacking A20 specifically in the CNS ($A20^{CNS\text{-}KO}$) or exclusively in neurons ($A20^{NEUR\text{-}KO}$) are affected differently after pMCAO when compared with wild-type littermates. $A20^{CNS\text{-}KO}$ and $A20^{NEUR\text{-}KO}$ mice were generated by crossing mice carrying a floxed *A20* allele[8] to mice expressing cre-recombinase under control of the nestin or thy1.2 promoter, respectively. These $A20^{CNS\text{-}KO}$ or $A20^{NEUR\text{-}KO}$ mice, together with control littermate mice, were subjected to pMCAO for 24 h after which the infarct size was estimated by means of a silver staining technique. To our surprise the infarct volume did not differ between $A20^{CNS\text{-}KO}$ or $A20^{NEUR\text{-}KO}$ and their respective wild-type control littermates (Figure 1c). Collectively, these results clearly demonstrate that, although A20 is upregulated in conditions of pMCAO, A20 deficiency in either all cells of neuroectodermal origin, or more specifically in neurons, does not influence the outcome of pMCAO in mice.

Conflict of Interest

The authors declare no conflict of interest.

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