ASK1 is a novel master molecular switch controlling microglia/macrophage reactions that impact short- and long-term stroke outcomes

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Presenter Disclosure Information

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ASK1 is a novel master molecular switch controlling microglia/macrophage reactions that impact short- and long-term stroke outcomes

FINANCIAL DISCLOSURE: Research Grant; Significant; AHA

UNLABELED/UNAPPROVED USES DISCLOSURE: None
Molecular mechanisms regulating microglia/macrophage (Mi/MΦ) phenotype are unknown

Hypothesis: **Apoptosis Signal-regulating Kinase 1 promotes neurotoxic Mi/MΦ responses**

Gaps of knowledge:

- Cell type-specific roles of ASK1 after ischemic stroke
- ASK1 as a modulator of Mi/MΦ phenotype
- The impact of ASK1 on long-term stroke outcome
Selective deletion of ASK1 in neurons or Mi/MΦ

STAIR guidelines were followed throughout the experiments.
MCAO induces **transient** activation of ASK1-MKK4-JNK3 in neurons.
MCAO induces persistent activation of ASK1-MKK3-p38/STAT1 in Mi/MΦ

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>MCAO 3 days</th>
<th>MCAO 5 days</th>
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<tbody>
<tr>
<td>WT</td>
<td>lba1</td>
<td>p-p38</td>
<td>lba1</td>
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<td>ASK1 mKO</td>
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<td>WT</td>
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Graph showing the expression levels of lba1, p-p38, and ASK1 in WT and ASK1 mKO conditions under sham and MCAO conditions over 3 and 5 days.

n=6 (sham) or n=8 (MCAO)
MCAO induces **persistent** activation of **ASK1-MKK3-p38/STAT1** in **Mi/MΦ**

5 days after MCAO  
*n=4 (sham) or n=6 (MCAO)*
Neuron-specific ASK1 deletion elicits transient neuroprotection against ischemia/reperfusion brain injury

ASK1 → MKK4/7 → JNK → c-Jun → Apoptosis

48 h after MCAO, n=10

**Neuron-specific ASK1 deletion elicits transient neuroprotection against ischemia/reperfusion brain injury**

**Graphs:**
- **Corner test**
  - Days after MCAO: Pre, 1, 3, 5, 7, 10, 14, 21, 28
  - Graph shows changes in left turns/10 trials.
  - Key: Sham (pooled, n=18), MCAO WT (n=16), MCAO ASK1 nKO (n=12)
- **Foot fault test (forelimb)**
  - Days after MCAO: Pre, 3, 5, 7, 10, 14, 21, 28
  - Graph shows changes in forelimb fault (%).
- **Rotarod test**
  - Days after MCAO: Pre, 3, 5, 7, 10, 14, 21, 28
  - Graph shows latency to fall (s).
- **Foot fault test (hindlimb)**
  - Days after MCAO: Pre, 3, 5, 7, 10, 14, 21, 28
  - Graph shows forelimb fault (%).
Neuronal ASK1 knockout does not alter DAMP production after ischemia/reperfusion


n=5-6 mice per group
Mi/MΦ-specific ASK1 deletion improves long-term stroke outcomes
ASK1 primes Mi/MΦ towards a pro-inflammatory and neurotoxic phenotype after ischemia

3 days after MCAO

![Flow cytometry analysis](image)

MCAO WT vs MCAO mKO:
- CD16
- CD32
- CD86
- iNOS
- IL-1α
- TNF-α
- IL-6
- CCL2
- CCL3
- CCL4
- CCL5
- CXCL13
- CD206
- IL-10
- YM1/2
- Arg1
- TGFβ

qRT-PCR results:

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* NS
** Statistical significance
ASK1 mKO mitigates neuroinflammation after ischemia/reperfusion

3 days after MCAO ELISA Array
Mi/MΦ-specific p38α knockout improves cognitive but not sensorimotor functions after MCAO.
Mi/MΦ-specific STAT1 knockout improves sensorimotor but not cognitive functions after MCAO.
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