Silencing of the Activated Protein-1 Transcription Factor JunD Exacerbates Ischemia/Reperfusion-induced Cerebral Injury

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Background - Stroke

- **Stroke**
  sudden, non-convulsive **loss of neurological function** due to **brain ischemia (80%)** or **intracranial hemorrhages (20%)**

- **Epidemiology**
  - 200-250/100 000 per year

- **Disease burden**
  - 40% mortality
  - survivors: 70% reduced work capacity, 30% assisted self-care

- **Pathophysiology - vasculature**
  - apoptosis/cell death
  - inflammation
  - reactive oxygen species (ROS)

- **Treatment of ischemic stroke**
  - thrombolysis within a time-window of 4.5 h to allow reperfusion of the brain
Background - JunD

- DNA-binding protein and part of AP-1 transcription factor complex
  - AP-1: dimeric complex mainly of the Jun and Fos subfamilies
  - Jun subfamily: JunD, c-Jun, JunB,

- JunD regulates cell growth, survival and protects against oxidative stress

- Implication in cardio- and cerebrovascular disease
  - JunD decreases age-induced endothelial dysfunction
  - Lack of JunD promotes cardiac hypertrophy and LV dysfunction
JunD prevents cerebral ischemia/reperfusion injury

- by decreasing oxidative stress
- reducing apoptosis and
- increasing NO bioavailability
Vascular JunD responsiveness to cerebral ischemia/reperfusion

WT sham → ischemia → reperfusion

WT stroke → ischemia → reperfusion

45 min

24 hours

Transient middle cerebral artery occlusion (tMCAO) surgery

Middle cerebral artery

$\Delta$Ct (JunD/GAPDH) [AU]

WT sham

WT stroke

n=4

*
Experimental setup: JunD silencing in tMCAO

Stroke Size
- 2,3,5-triphenyltetrazolium chloride (TTC) staining

Neuromotor Function
- Bederson - Neurological score
- Rotarod - Performance test
**in vivo JunD silencing**

JunD protein expression in murine aortae, 72 hours after siJunD injection

![Graph showing JunD protein expression](image)

- **siScrub**
- **siJunD**

- **JunD-fl**: 40 kDa
- **Δ JunD**: 35 kDa
- **α-tubulin**: 51 kDa

n = 8/7
Stroke size and neuromotor deficit

Stroke volume [mm³]

N = 8/10

Neurological score [0-4]

RotaRod latency to fall [sec]

n = 13/15

pre-MCAO 2 hours 24 hours

0
1
2
3

*
Experimental setup *in vitro*

Human Brain Microvascular Endothelial Cells (HBMVECs) - scramble (siScr) vs JunD silencing (siJunD)

- **Normoxia (Nx) (8h)**
- **Hypoxia (4h)/Reoxygenation (4h)**

**Molecular analysis in HBMVECs**
- JunD responsiveness
- Cytotoxicity - LDH assay
- Oxidative stress - mitochondrial ROS
JunD responsiveness to H/R in HBMVECs

![Graph showing JunD protein expression](graph.png)

- **JunD protein expression [% ctrl (Nx)]**
- **n = 6**
- **Δ JunD**
  - 40 kDa
- **α-Tubulin**
  - 35 kDa
  - 51 kDa
JunD silencing and cell death in HBMVECs

**JunD silencing**

- JunD protein expression [% of siScr]
- siScr: 100
- siJunD: 50
- n = 6

**Endothelial cell death**

- LDH release [% of siScr]
- siScr: 100
- siJunD: 150
- n = 12

**Images**

- Western blot for JunD, Δ JunD, α-Tubulin
  - JunD-fl: 40 kDa
  - Δ JunD: 35 kDa
  - α-Tubulin: 51 kDa
Effects of JunD silencing on cytotoxicity and oxidative stress in HBMVECs exposed to H/R

**Endothelial cell death**

LDH release [% of siScr (H/R)]

- **siScr**: 100
- **siJunD**: 150

* p < 0.05

n = 12

**Oxidative stress**

Mitochondrial ROS [% of siScr (H/R)]

- **siScr**: 100
- **siJunD**: 150

n = 15
JunD responsiveness to ischemia/reperfusion in peripheral blood monocytes (PBMCs) of stroke patients

<table>
<thead>
<tr>
<th>Study population</th>
<th>Controls (n=14)</th>
<th>Stroke patients (n=20)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (range)</td>
<td>70.6 (63-82)</td>
<td>74.1 (52-90)</td>
<td>0.275</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>5 (35.7%)</td>
<td>10 (50%)</td>
<td>0.495</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>0 (0%)</td>
<td>5 (25%)</td>
<td>0.063</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>6 (42.9%)</td>
<td>9 (45%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Dyslipidemia, n (%)</td>
<td>1 (7.1%)</td>
<td>2 (10%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Atrial fibrillation, n (%)</td>
<td>0 (0%)</td>
<td>6 (30 %)</td>
<td>0.031</td>
</tr>
<tr>
<td>Coronary artery disease, n (%)</td>
<td>0 (0%)</td>
<td>4 (20%)</td>
<td>0.126</td>
</tr>
<tr>
<td>Previous TIA/stroke, n (%)</td>
<td>0 (0%)</td>
<td>2 (10%)</td>
<td>0.501</td>
</tr>
<tr>
<td>Peripheral artery disease, n (%)</td>
<td>0 (0%)</td>
<td>3 (15%)</td>
<td>0.251</td>
</tr>
</tbody>
</table>

With TPA

Without TPA
• Ischemia/reperfusion reduces vascular JunD expression in WT mice and in primary HBMVECs

• Likewise, JunD expression is decreased in PBMs of patients with ischemic stroke undergoing reperfusion therapy

• Vascular JunD silencing increases stroke size and neuromotor deficit in mice after transient MCAO.

• JunD silencing increases endothelial cytotoxicity in HBMVECs after hypoxia/reoxygenation.

• These findings strongly suggests the involvement of JunD in the pathogenesis of ischemia/reperfusion-induced brain damage.
Thank you for your attention!