ADAM17 mediated activation of microglia leads to neuronal excitation and inflammation in Ang-II induced hypertension

Laiba Iqbal[1], Uma Priya Mohan[1,2] and Eric Lazartigues [1,2]

[1]Cardiovascular Center of Excellence, Louisiana State University Health Sciences Center, New Orleans, LA 70112;
[2]Southeast Louisiana Veterans Health Care System, New Orleans, LA 70119, USA

Introduction

- ADAM17 sheds several cytokines and receptors (e.g. TNFα, CX3CL1 and IL-6R) which act on surrounding cells, including neurons and microglia, to modulate synaptic function.
- We recently observed that CX3CL1 is significantly elevated in neuronal cultures exposed to Ang-II and that ADAM17 on microglia is involved in the local reduction of ACE2 levels.
- The role of neuronal ADAM17 maturation in microglia activation is not well understood.

Hypothesis

ADAM17-mediated shedding of CX3CL1 from neurons will promote CX3CR1 activation in microglia leading to TNF-α and IL-1β secretion.

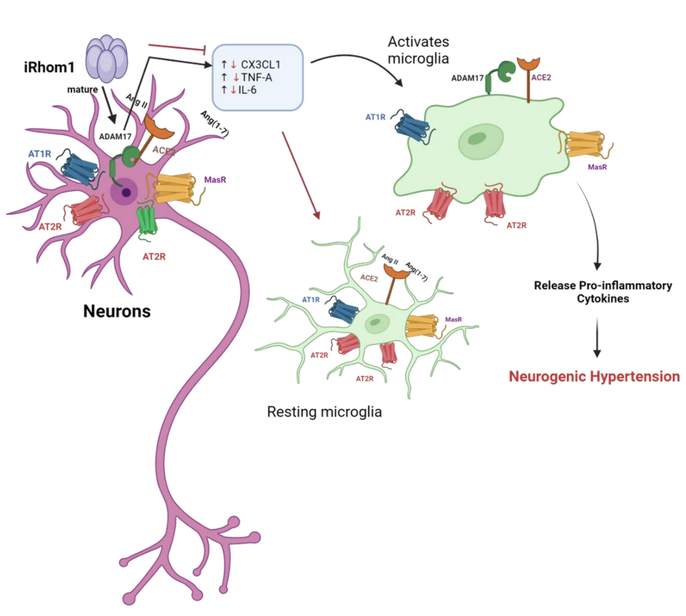

Figure 1: Hypothesis: ADAM17 maturation is controlled by iRhom1. iRhom1 is expressed in neurons and regulates ADAM17 maturation. Mature ADAM17 releases CX3CL1 and TNF-α, which bind to their respective receptors on microglia (CX3CR1 and TNFα-R1), thereby activating microglia. Activated microglia releases pro-inflammatory cytokines and possibly contributes to neuroinflammation and hypertension as a result of communication between CX3CL1 (on neurons) and CX3CR1 (on microglia).

Experimental Design

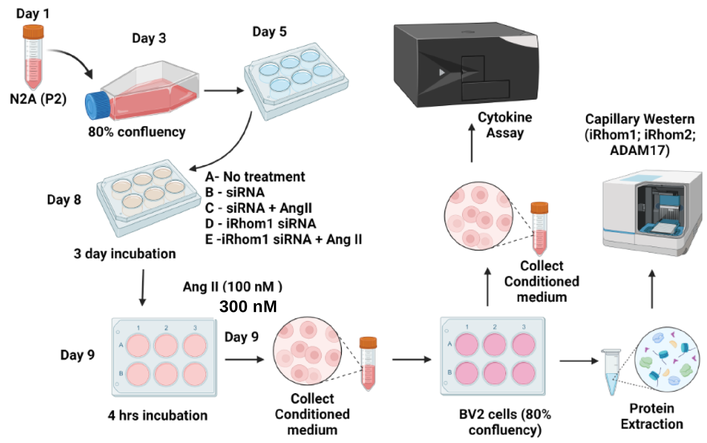

Figure 2. Diagram of experimental protocol using neuronal Neuro-2a (N2A) and microglial (BV2) cells. N2A and BV2 cell lines were grown to 80% confluency. Neurons were pre-treated with siRNA for iRhom1, a protein involved in ADAM17 maturation, or scrambled siRNA (20 nM) and after 48 h, the neurons were exposed to Ang-II (300 nM). After 24 h, the conditioned medium was collected for ACE2 activity and measurement of cytokines. Microglia cultures were exposed for 24 h to conditioned medium before the medium was assessed for cytokine levels. Proteins were also extracted from the cells for analysis of protein expression.

Validation of iRhom1 siRNA

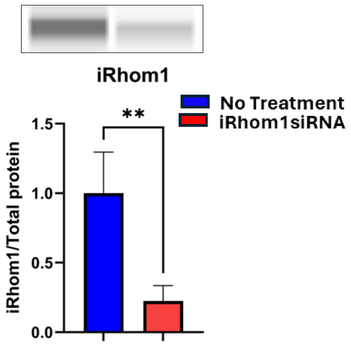

Figure 3. Validation of efficacy of iRhom1 siRNA (20 nM). Compared to the no treatment control, iRhom1 expression was decreased in the iRhom1 siRNA group, which confirms the inhibition of iRhom1. Analysis performed by capillary western (Student's t-test (n=3, **P<0.01).

iRhom1 act as a mediator for ADAM17 mediated shedding and activates microglia

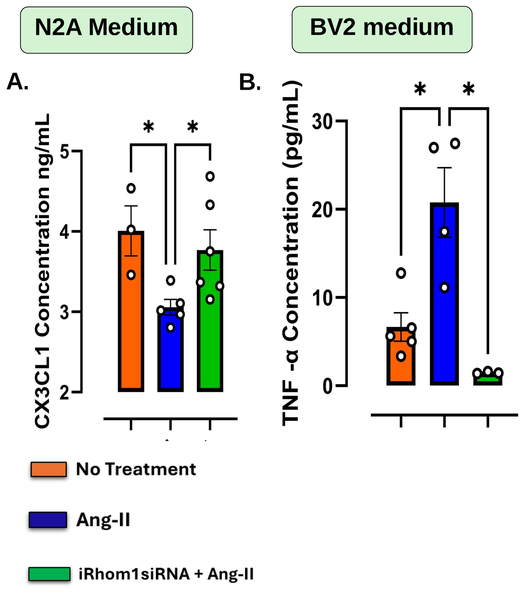

Figure 4: iRhom1 silencing in neurons altering the release of pro-inflammatory cytokines in microglia. **A.** N2A treated with Ang-II showing that the CX3CL1 level in the medium was decreased in Ang-II treated group compared to iRhom1siRNA+ Ang-II; B**.** BV2 treated with conditioned media from N2A showed an increase in the pro-inflammatory cytokines TNF-α (**D**) in the Ang-II treated group compared to iRhom1 siRNA treated group. (***P<0.001, **P<0.01, *P<0.05)

Results

iRhom1 silencing inhibits the maturation of ADAM17 and alters microglial activation

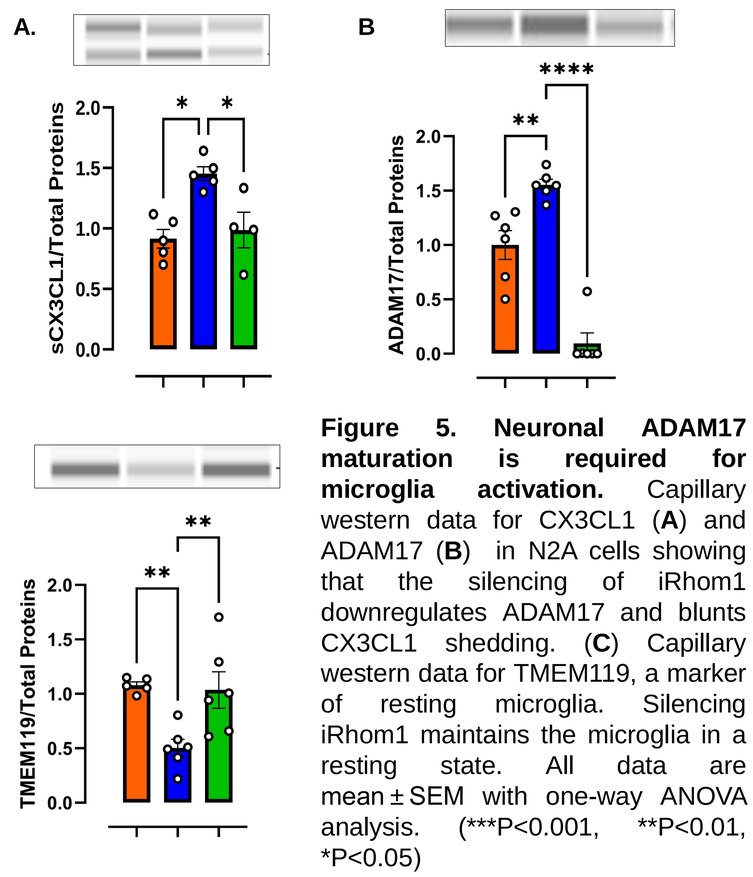

Figure 5. Neuronal ADAM17 maturation is required for microglia activation. Capillary western data for CX3CL1 (**A**) and ADAM17 (**B**) in N2A cells showing that the silencing of iRhom1 downregulates ADAM17 and blunts CX3CL1 shedding. (**C**) Capillary western data for TMEM119, a marker of resting microglia. Silencing iRhom1 maintains the microglia in a resting state. All data are mean ± SEM with one-way ANOVA analysis. (***P<0.001, **P<0.01, *P<0.05)

Conclusion

- Together, our data suggest that iRhom1 is necessary for the activation of ADAM17.
- Silencing iRhom1 in neurons inhibits ADAM17 maturation, thereby reducing the production of CX3CL1.
- Silencing of iRhom1 in neurons dampens microglia activation and reduces their release of pro-inflammatory cytokines (TNFα).
- We conclude that neuronal ADAM17 maturation is required for microglial activation.

Acknowledgments

This work was supported in part by a research grant from the National Institutes of Health (HL163588) EL.