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

Laiba Iqbal, Uma Priya Mohan, Eric Lazartigues

Cardiovascular Center of Excellence, Louisiana State University Health Sciences Center, School of Medicine, New Orleans, LA

Introduction

ADAM17 (aka TNFα converting enzyme, TACE) is a protease that sheds membrane-anchored proteins in response to activation by angiotensin-II (Ang-II) type 1 receptor (AT_1R) signaling pathways. 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. Accordingly, **we hypothesize that ADAM17-mediated shedding of CX3CL1 from neurons will promote CX3CR1 activation in microglia leading to TNF-α and IL-1β secretion.**

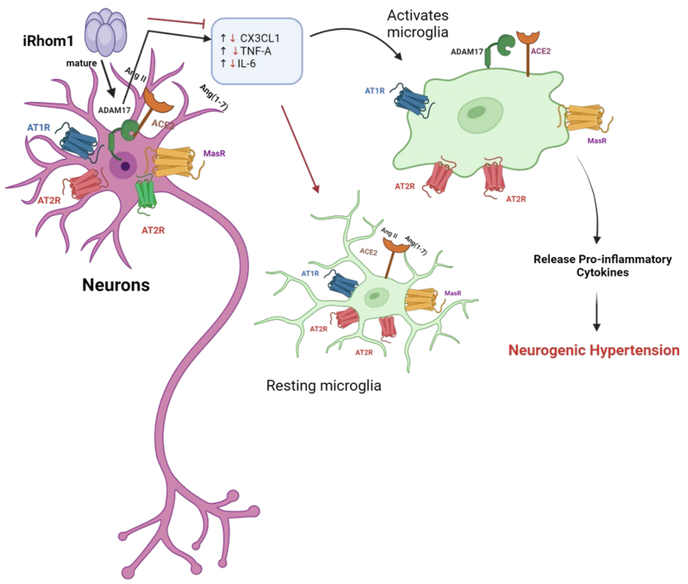

Figure 1: ADAM17 maturation is controlled by iRhom1. iRhom1 is expressed in neurons and regulates ADAM17. Mature ADAM17 releases CX3CL1 and TNF-α, which bind to their respective receptors on microglia (CX3CR1 and TNFα-R1), thereby activating microglia. Activated microglia releases pro-inflammatory cytokines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

Experimental Design

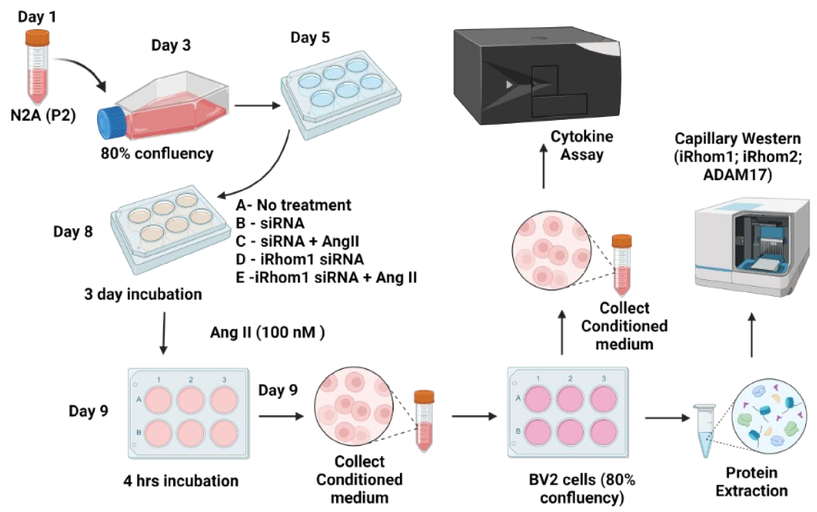

Figure 2. Diagram representation of experimental protocol using neuronal Neuro-2a (N2A) and microglial (BV2) cells.

Validation of iRhom1 siRNA

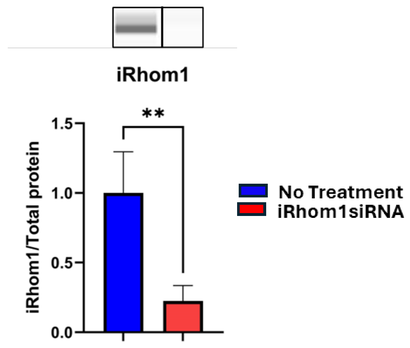

Figure 3. (A) Validation of efficacy of iRhom1 siRNA (20 nM) using Lipofectamine 2000 (Invitrogen). Student t-test (n=3, **P<0.001).

iRhom1 maturation of ADAM17

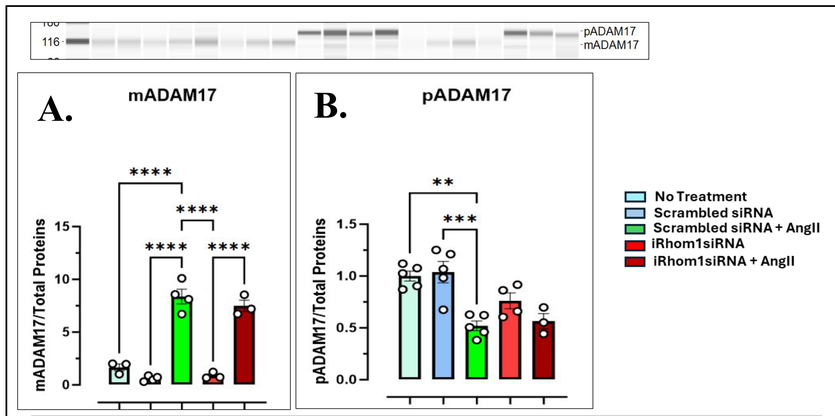

Figure 4. Capillary western data of mature (A) and pro-ADAM17 (B) expression in N2A cell cultures across the five test groups. All data are mean ± SEM with one-way ANOVA analysis. (***P<0.0001,** P<0.001, *P<0.04)

Results

Mature neuronal ADAM17 is required for microglial activation

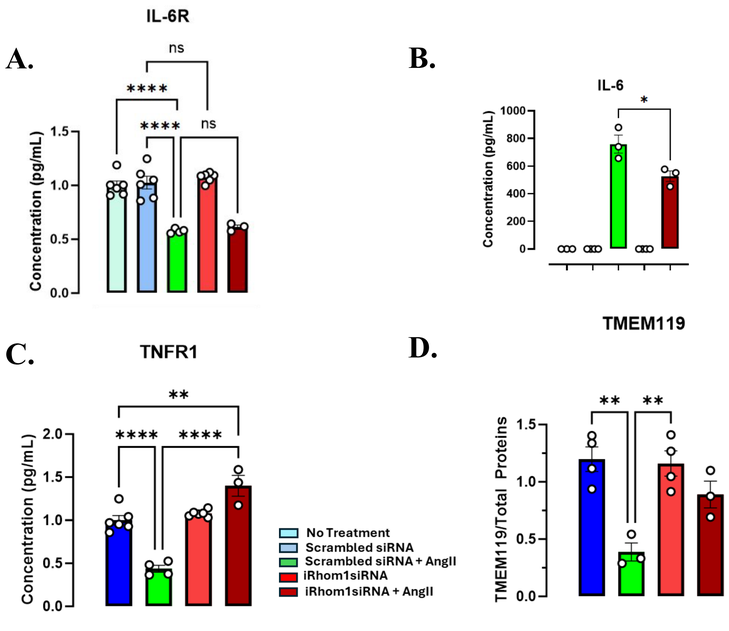

Figure 5. Neurons treated with iRhom1 siRNA + Ang-II showed a reduced level of proinflammatory cytokines, including **(B)** IL-6 (Scramble siRNA + Ang-II: 759±65 pg/mL, iRhom1 siRNA + Ang-II: 523±38 pg/mL, compared to scramble siRNA + Ang-II: 759±65 pg/mL, pg/mL, P<0.05), and increased **(C)** TNFα-R1 (Scramble siRNA + Ang-II: 0.43±0.1 pg/mL vs iRhom1 siRNA + Ang-II: 1.4±0.1 pg/mL vs Control: 1±0.1 pg/mL and scramble siRNA: 0.9±0.2 pg/mL), respectively (P<0.05 n=6). Microglia activation was assessed by the expression of **(D)** TMEM119 using capillary western. (Scramble siRNA + Ang-II: 0.38±0.1, iRhom1 siRNA + Ang-II: 1.0±0.2 vs. Control: 1.1±0.2 and scramble siRNA: 0.9±0.2, P<0.05).

Conclusion

- Together, our data suggest that activation of microglia (BV2) is secondary to increased maturation of ADAM17, mediated by iRhom1 in neurons (N2A).
- Our data show that iRhom1 siRNA + Ang II treatment of N2A led to a reduced level of IL-6 and increased TNFα-R1 (i.e. shed TNF receptor) compared to the scramble group.
- We conclude that neuronal ADAM17 maturation is required for microglial activation following Ang-II treatment.

Acknowledgments

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