OXR1 Regulates Cellular Senescence and Neuronal Aging Through Retromer-Mediated Actin Branching

Kenneth A Wilson[1], Tyne LM McHugh[1,2], Sydney Alderfer[1], Anna L Girtle[1], Cristian Geronimo-Olvera[1], Harrison Baker[1], Anshuman Das[1], Jacob Rose[1], Jordan Burton[1], Carlos Galicia Aguirre[1,2], Elena Battistoni[1], Mikayla Hady[1], Catherine Cai[1,2], Indra Heckenbach[1,3], Alexandra Afenjar[4], Philippe M Campeau[5], Peter L Oliver[6], Akos A Gerencser[1], Pankaj Kapahi[1], Birgit Schilling[1], Lisa M Ellerby[1]*

*[1]The Buck Institute for Research on Aging, Novato, California, USA. [2]University of Southern California, Los Angeles, California, USA. [3]Center for Healthy Aging, Department of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen, Denmark. [4]Hôpital Armand Trousseau, Groupe Hospitalier Universitaire Paris, France. [5]CHU Sainte-Justine, Montreal, Canada. [6]MRC Harwell Institute, Didcot, United Kingdom. *Correspondence.*

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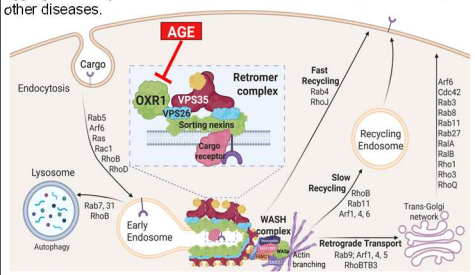
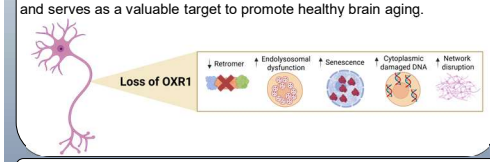
Introduction

The accumulation of senescent cells in the aging brain is associated with numerous age-related disorders but its link to the retromer complex and endolysosomal trafficking are unknown. The neuronal retromer traffics endocytosed lipids and proteins and loss of retromer function contributes to neuron decline. We previously found that the neuronal protein Oxidation Resistance 1 (OXR1) maintains retromer function. The retromer complex is essential to promote endolysosomal health and prevent the aggregation of proteins associated with Alzheimer's, Parkinson's, and other diseases.



We now find that fibroblasts from patients with loss of function *OXR1* mutations become senescent, marked by p21, p16, cytoplasmic DNA, loss of Lamin-B1, and release of the senescence-associated secretory phenotype (SASP). Retromer interaction with the WASH protein complex stimulates F-actin polymerization, but loss of OXR1 leads to retromer-WASH aggregates, which we call WASH protein Nucleus-Eroding Senescence Tangles (WASp-NESTs). These inhibit F-actin branching and cause nuclear membrane destabilization. *OXR1* knockdown in induced pluripotent stem cell (iPSC)-derived neurons recapitulates these markers and inhibits neuronal network formation. Pharmacological stabilization of the retromer with the compound R55 rescues these phenotypes. Further, we find that neuronal overexpression of OXR1 improves spatial learning and memory in mice. In all, we find that endolysosomal function through OXR1 and retromer function is essential to prevent cellular senescence and serves as a valuable target to promote healthy brain aging.

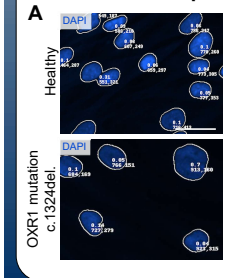


Question

How does OXR1 and the retromer regulate neuronal senescence?

Results

Fibroblasts from patients with mutations in *OXR1* are predicted to be senescent



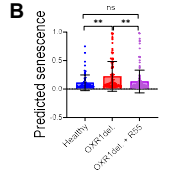


Figure 1. (A) Senescence prediction pipeline based on nuclear size and shape predicts fibroblasts from patients with *OXR1* mutation to be senescent. Treatment with R55 retromer stabilization compound rescues non-senescent prediction. (B) Quantification with p value determined by t-test. ** is p < 0.005. Scale bar is 50 μm.

Results

Retromer stabilization rescues *OXR1* mutation-induced senescence

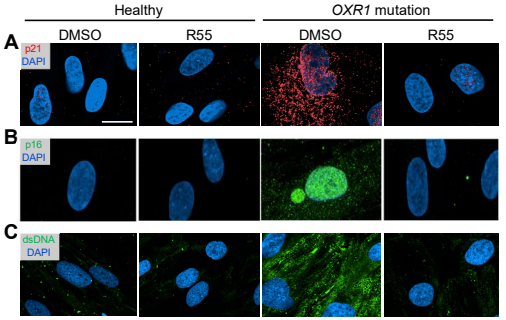


Figure 2. Immunohistochemistry in fibroblasts from human patients with *OXR1* mutations and control, treated with or without R55 retromer stabilization compound. (A,B) p21 and p16 senescence-associated proteins are upregulated in mutant cells, which is rescued by R55 treatment. (C) Cytoplasmic DNA is increased in *OXR1* mutant fibroblasts, which is rescued by R55 treatment. Scale bar is 20 μm.

OXR1 mutation induces secretomic changes that enrich to cytoskeleton

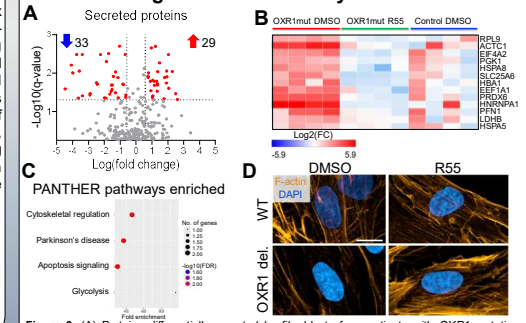


Figure 3. (A) Proteins differentially secreted by fibroblasts from patients with *OXR1* mutations relative to healthy controls. (B) Heatmap of proteins rescued by R55 changed in OXR1 mutation fibroblasts. (C) PANTHER pathways for proteins rescued by R55 enrich to cytoskeleton. (D) F-actin staining via Phalloidin shows reduced levels around nucleus of fibroblasts with *OXR1* mutation. Scale bar is 20 μm.

Loss of OXR1 generates WASH protein Nucleus-Eroding Senescence Tangles (WASp-NESTs)

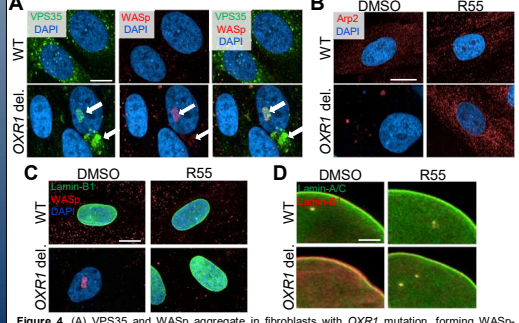


Figure 4. (A) VPS35 and WASp aggregate in fibroblasts with *OXR1* mutation, forming WASp-NESTs. (B) Actin-related protein 2 does not distribute throughout the cell in *OXR1* mutants. (C) Fibroblasts with WASp-NESTs lost Lamin-B1. (D) Loss of OXR1 induces separation of Lamin-A/C and Lamin-B1 layers in the nuclear membrane. Scale bar is 20 μm for A-C. Scale bar is 5 μm for D.

Results

Retromer loss induces senescence and WASp-NESTs in iPSC-derived neurons

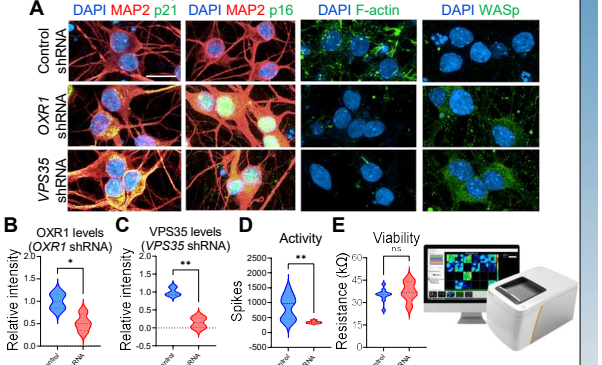


Figure 5. (A) Human iPSC-derived glutamatergic neurons with shRNA targeting *OXR1* (middle) or *VPS35* (bottom) show increased markers of senescence and WASp-NESTs. (B,C) Validation of shRNA targeting OXR1 or VPS35 expression. (D) Glutamatergic neurons with loss of OXR1 show reduced synaptic spiking. (E) Glutamatergic neurons with loss of OXR1 are viable, indicating loss of synaptic spiking signal is not due to cell death. Firing signals and neuron viability were measured with the use of Maestro Edge MEA System (Axion BioSystems). Scale bar is 15 μm.

Oxr1 overexpression improves spatial learning and memory in aged mice

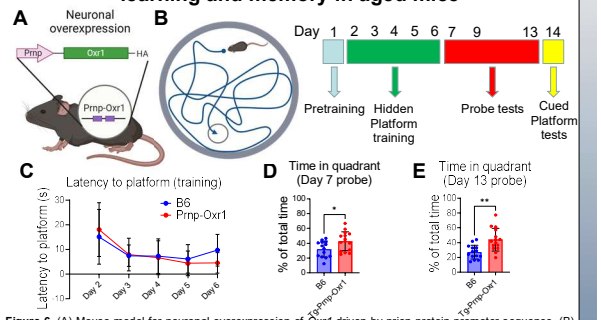


Figure 6. (A) Mouse model for neuronal overexpression of *Oxr1* driven by prion protein promoter sequence. (B) Schematic for Morris water maze training and probing. (C) Hidden platform training curve. (D) Short-term memory (Day 7, 24 hours after training) is improved with *Oxr1* overexpression. (E) Long-term memory (Day 13, 7 days after training) is improved with *Oxr1* overexpression.

Conclusions

- Stabilization of the retromer complex rescues DNA damage and cellular senescence-associated phenotypes in fibroblasts from patients with *OXR1* mutations.
- OXR1 loss impairs F-actin production via WASH complex signaling.
- Retromer loss induces senescence and reduces synaptic spiking in iPSC-derived glutamatergic neurons.
- *Oxr1* overexpression improves memory in mice.

References and Acknowledgements

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