Rage-tnip Axis Drives Inflammation in Alzheimer's by Targeting $\alpha\beta$ to Mitochondria in Microglia

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Short report

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Abstract

Alzheimer's disease (AD) is the most common form of dementia characterized by progressive memory loss and cognitive decline. Although neuroinflammation and oxidative stress are well-recognized features of AD, their correlations with the early molecular events characterizing the pathology are not yet well clarified. Here we demonstrated the role of RAGE-TXNIP axis in neuroinflammation in relation to amyloid-beta ($A\beta$) burden in both *in vivo* and *in vitro* models. In the hippocampus of 5xFAD mice, microglial activation correlates with increased TXNIP expression, and TXNIP silencing or its pharmacological inhibition prevented the neuroinflammation in those mice. TXNIP increased expression has been found also in human subjects affected by AD and other dementia when plasma samples were analyzed, suggesting TXNIP as a putative biomarker of neurodegeneration and cognitive decline. To better elucidate whether a mechanistic link between RAGE-TXNIP axis and AD exist, we investigated how TXNIP is involved in $A\beta$ intracellular trafficking downstream RAGE. Indeed, TXNIP is an α -arrestin protein involved in endocytosis. We found TXNIP associated to RAGE and $A\beta$. RAGE-TXNIP axis was required for targeting $A\beta$ into mitochondria, as shown in primed primary microglia exposed to $A\beta$ dimers. Silencing of TXNIP or inhibition of RAGE activation reduced $A\beta$ transport from the cellular surface to mitochondria. Detrimental effect of $A\beta$ in mitochondria has been also confirmed by evaluating increased mitochondrial- and intracellular- oxidative stress. When RAGE-TXNIP axis is down-regulated, mitochondrial functionality was restored and $A\beta$ toxicity was mitigated. Furthermore, $A\beta$ transport into mitochondria and subsequent mitochondrial dysfunction drove inflammation in microglia cells via NLRP3 inflammasome activation, and down-regulation of RAGE-TXNIP axis was able to efficiently mitigate $A\beta$ proinflammatory effect. Overall this data suggest RAGE-TXNIP axis is required for NLRP3 inflammasome activation induced by $A\beta$ in microglia.

Our work shed light on novel mechanism of action of RAGE-TXNIP axis in microglia, which is intertwined with $A\beta$ and ultimately causes inflammation, suggesting TXNIP as a druggable target to be better deepened for AD.

Full Text

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Figures

Fig. 1

Figure 1

TXNIP increased expression in 5XFAD AD mice model and human plasma. A. Immunofluorescence (IF) analysis of TXNIP expression (red) in 4m old control and 5XFAD mice. DAPI staining is reported in blue. B. ELISA analysis of TXNIP plasma levels in healthy human control (20), AD (15), and other dementia (9) patients (**p<0.001 versus control, *p<0.05 versus control). C. IF analysis of TXNIP (green) and GFAP (red) in 4m 5xFAD and control (WT) mice hippocampus treated with lentiviral shRNA targeting TXNIP

(siTXNIP) control shRNA scramble (siScramble), and Verapamil as reported in the figure. DAPI staining is reported in blue. These data are representative of 4 independent experiments (n=4). D. ELISA analysis of hippocampal TXNIP in control (WT) and 5xFAD (Tg) mice treated with siScramble, siTXNIP and Verapamil as indicated in the figure. These data are the average of 4 independent experiments (n=4, *p<0.001 versus WT mice, #p<0.001 versus 5xFAD mice treated with siScramble). Results are reported as percentage (%) over control. E, F. Western blot analysis (E) and quantification (F) of GFAP protein content in control and 5xFAD mice treated with siScramble, siTXNIP and Verapamil. In E the data are representative of 3 independent experiments (n=3). In F the data are the average of 3 independent experiments (n=3, *p<0.001 versus WT mice, #p<0.001 versus 5xFAD mice treated with siScramble). Tubulin was used as load control. G. ELISA analysis of secreted hippocampal IL-1 β in control (WT) and 5xFAD (Tg) mice treated with siScramble, siTXNIP and Verapamil. These data are derived from 4 independent experiments (n=4, *p<0.001 versus WT mice, #p<0.001 versus 5xFAD mice treated with siScramble).

Fig. 2

Figure 2

RAGE-TXNIP axis is implicated in A β transport to mitochondria in microglia both in in-vivo and in-vitro. A. IF analysis of TXNIP (green) and Iba1 (red) in 4m old control (WT) and 5x*FAD* mice hippocampus treated with lentiviral shRNA targeting TXNIP (siTXNIP) control shRNA scramble (siScramble), and Verapamil as reported in the figure. These data are representative of 4 independent experiments (n=4). B. Quantification of microglial Iba1+ cells, expressed as cells per field of view (FOV) in control (WT) and 5x*FAD* (Tg) mice

treated with siScramble, siTXNIP and Verapamil as indicated in the figure. These data are the average of 4 independent experiments (n=4, *p<0.001 versus WT mice, #p<0.001 versus 5xFAD mice treated with scramble shRNA). C. IF analysis of TXNIP (green) and mitochondria labeled with red mitotracker (red) in LPS primed microglia cultures treated for 4h with A β in the presence or absence of anti RAGE blocking antibody as indicated in the figure. These data are representative of 5 independent experiments (n=5). D. Western blot analysis of TXNIP in LPS primed microglia cultures both untreated and treated with A β in the absence or presence of anti RAGE blocking antibody after subcellular fractionation, as indicated in the figure. These data are representative of 5 independent experiments (n=5). Quantification derived from 5 independent experiments is shown in Supplementary figure 2. We analyzed the presence of specific markers of subcellular fractions: cytoplasmic (GAPDH); nuclear (histone H3) and mitochondrial (Cox IV) fractions, which showed the appropriate subcellular localization as reported in the figure. E. IF analysis of A β (green) and mitochondria (red) in LPS primed microglia cultures treated for 4h with A β dimers alone or challenged with siScramble, anti-RAGE blocking antibody, or siTXNIP and Verapamil as indicated in the figure. These data are representative of 5 independent experiments (n=5). F. IF analysis of A β (green) and TXNIP (red) in LPS primed microglia treated with A β 2h both in the presence and absence of anti RAGE blocking antibody as indicated in the figure. These data are representative of 5 independent experiments (n=5). G. IF analysis of LPS primed microglia cells treated for 30 min with A β dimers TOP: A β (green) and RAGE (red); BOTTOM: TXNIP (green) and RAGE (red). These data are representative of 5 independent experiments (n=5). H. Subcellular fractionation and western blot analysis of A β oligomers in LPS primed microglia cells treated for 4h with A β dimers (A β) both in the absence and the presence of anti-RAGE blocking antibody (A β), siTXNIP (A β siTXNIP) or Verapamil (A β Ver) as indicated in the figure. These data are representative of 4 independent experiments (n=4). CoxIV is used as marker for mitochondrial fraction. I. Immunoprecipitation (IP) with an anti-Drp1 antibody in LPS-primed microglia treated for 4h with A β dimers (A β) in the absence or presence of anti-RAGE blocking antibody (anti-RAGE), siTXNIP, siScramble or Verapamil as 22 indicated in the figure. We indicated the western blot analysis of A β oligomers (A β), TXNIP and Drp1. These data are representative of 4 independent experiments (n=4).

Fig. 3

Figure 3

RAGE-TXNIP axis is implicated in Aβ-induced changes in mitochondrial membrane potential, ROS formation, and activation of the NLRP-3 inflammasome complex in microglia. A IF analysis of Aβ (green) and Drp1 (red) in LPS primed microglia cells treated for 4h with Aβ dimers. These data are representative of 5 independent experiments (n=5). B. TMRM fluorescence staining of LPS primed microglia cells untreated and treated for 4h with Aβ dimers in the presence of siScramble (control, Aβ + siScramble),

siTXNIP (A β + siTXNIP), anti-RAGE blocking antibody (A β + anti-RAGE), or Verapamil (A β + Verapamil) as indicated in the figure. These data are representative of 4 independent experiments (n=4). Quantification of the data is shown in supplementary figure 3. C. Quantification of JC-1 fluorescence ratio (520/590 nm) to measure mitochondrial membrane potential. LPS-primed cells treated for 4h with A β dimers treated with control siScramble (siScramble +A β), siTXNIP (siTXNIP +A β), anti-RAGE blocking antibody (RAGEblock +A β), Verapamil (Verapamil+A β). We also analyzed the effect on mitochondrial membrane potential of anti-RAGE blocking antibody alone (RAGE block) and Verapamil alone (Verapamil) as indicated in the figure. These data are the average of 4 independent experiments (n=4, *p<0.001 versus control cells, #p<0.001 versus cells treated with A β dimers together with control siScramble). D. Analysis of cytoplasmic ROS production measured by CM-H2DCFDA fluorescence fold increase versus control untreated with A β dimers in LPS primed microglia untreated and treated for 4h with A β dimers both in the absence or presence of the following treatments: control siScramble, anti-RAGE blocking antibody (anti-RAGE), siTXNIP or Verapamil as indicated in the figure. These data are the average of 4 independent experiments analyzed in triplicate (n=12, *p<0.001 versus control cells, #p<0.001 versus cells treated with A β dimers together with control siScramble). The effect of Verapamil alone was also analyzed, showing no changes compared to control cells. E. Quantification of mitochondrial ROS production by MytoSOX fluorescence (n=6) in LPS primed microglia untreated and treated for 4h with A β dimers both in the absence or presence of the following treatments: control siScramble, anti-RAGE blocking antibody (anti-RAGE), siTXNIP or Verapamil as indicated in the figure. These data are the average of 3 independent experiments analyzed in duplicate (n=6, *p<0.001 versus untreated control cells, #p<0.001 versus cells treated with A β dimers together with control siScramble). The effect of Verapamil alone was also analyzed, showing no changes compared to control cells. F-G. Western blot 23 analysis (F) and quantification (G) of TXNIP, NLRP3, Cleaved caspase-1 and caspase-1 protein levels in LPS primed microglial cells untreated and treated for 4h with A β dimers in the presence of siScramble, anti-RAGE blocking antibody (anti-RAGE), siTXNIP or Verapamil as indicated in the figure. The western blot analysis (F) is representative of 4 independent experiments (n=4). The quantitative analysis (G) is expressed as fold change over control in (G) and is the average of 4 independent experiments (n=4, *p<0.001 versus untreated control cells, #p<0.001 versus cells treated with A β dimers together with control siScramble). Actin was used as loading control. H. Quantification of caspase-1 activity in LPS primed microglia untreated and treated for 4h with A β dimers in the presence of control siScramble, anti-RAGE blocking antibody (anti-RAGE), siTXNIP or Verapamil. These results are the average of 5 independent experiments (n=5, *p<0.001 versus untreated control cells, #p<0.001 versus cells treated with A β dimers together with control siScramble). Treatment with only Verapamil has been also included, which has no effect. I. Analysis by ELISA of secreted IL-1 β in the medium of microglia untreated and treated for 4h with A β dimers in the presence of siScramble as control of treatment with lentivirus, anti-RAGE blocking antibody (anti-RAGE), siTXNIP or Verapamil. These results are the average of 4 independent experiments in triplicates (n=12, *p<0.001 versus untreated control cells, #p<0.001 versus cells treated with A β dimers together with control siScramble). Treatment with only Verapamil has been also included, showing that Verapamil alone has no any effect compared to control cells.

Supplementary Files

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