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β-Catenin Pathway Is Involved in TREM2-Mediated Microglial Survival

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Review of Zheng et al.

Amyloid plaques and neurofibrillary tangles are prominent lesions that are commonly found in the brains of Alzheimer's disease (AD) patients. Hence, faulty amyloid processing, deposition, and clearance have been postulated as the central event in disease pathogenesis, which along with abnormal tau hyperphosphorylation leads to synaptic and neuronal loss (Hardy and Selkoe, 2002). Although neuroinflammation has been associated with plaques and tangles (Rogers et al., 1992; Duong et al., 1997), it was considered a passive process that arose in the terminal stages of AD. However, the last few years have seen a resurgence of interest in the role of neuroinflammation in AD. This is partly due to a series of genetic studies that identified microglial immune receptors, such as TREM2, CD33, and CR1 as risk factors (Lambert et al., 2009; Naj et al., 2011; Guerreiro et al., 2013a; Jonsson et al., 2013).

TREM2 is an innate immune receptor and a Type I transmembrane protein. TREM2 mediates signaling through the phosphorylation of the ITAM motif of its partnering adaptor protein DNAX-activating protein of 12 kDa (DAP12) (Bouchon et al., 2001). This further recruits the tyrosine kinase Syk, which activates downstream targets. In the CNS, it is expressed exclusively in microglia, and it has mainly been shown to be involved in phagocytosis (Xiang et al., 2016), promoting microglial survival (Otero et al., 2009), and in regulating inflammation in the CNS. A loss-of-function mutation in the TREM2 gene gives rise to Nasu-Hakola disease, which manifests as dementia and bone cysts. Furthermore, recent genome-wide association studies have shown that an arginine to histidine (R47H) coding variant in TREM2 triples the AD risk of the carrier, having an effect size comparable with that of the well-established late-onset AD risk gene, *APOE4* (Neumann and Daly, 2013; Guerreiro et al., 2013b). The R47H mutation has also been found to be associated with frontotemporal dementia, amyotrophic lateral sclerosis, and Parkinson's disease (Cady et al., 2014; Rayaprolu et al., 2013). Although this mutation is rare and found in <1% of the population, the study of TREM2 offers new insights into the role of neuroinflammation in AD and could lead to developing potential novel therapeutics.

The role of TREM2 in imparting vulnerability to AD, and its key downstream players are yet to be thoroughly investigated. Thus, in a recent study published in *The Journal of Neuroscience*, Zheng et al. (2017) investigated the molecular pathways underlying TREM2-mediated proliferation and survival of microglia. Using microglia derived from TREM2 knockout (KO) mice, the authors demonstrated a reduction in microglial cell proliferation as well as survival. TUNEL staining indicated that a larger percentage of microglia were apoptotic in KO mice than in wild-type (WT), and this was confirmed by the increase in proapoptotic cleaved Caspase-3 and reduction of antiapoptotic protein Bcl-2. Notably, proliferation and survival were also reduced in primary microglia from WT mice after TREM2 was knocked down using siRNAs targeting different domains. The two siRNAs reduced TREM2 mRNA levels by ∼50 and 70%, respectively, thus possibly simulating a heterozygous phenotype. These findings dovetailed with those of Wang et al. (2015), who also observed that microglia from TREM2-deficient animals were more likely to undergo apoptosis.

Using intraperitoneal injection of kainic acid to induce glutamate excitotoxicity in WT and TREM2 KO mice, Zheng et al. (2017) studied TREM2-dependent microglial activation *in vivo*. Using Iba1 as a microglial marker, they observed a marked difference between KO and WT in the number and morphology of microglia in both cortical and hippocampal regions. Specifically, WT microglia were more numerous, had greater area, and had shorter processes than TREM2-deficient microglia. These results suggest that

knocking out TREM2 reduced microglial activation.

After demonstrating TREM2 involvement in cell proliferation and apoptosis, Zheng et al. (2017) investigated the role of TREM2 in cell cycle progression and control. TREM2 KO resulted in an increased percentage of microglia in the G_1/G_0 phase compared with WT microglial cells. Further confirming this finding, levels of Cyclin D1 (a protein essential for progression through G_1 phase) and c-Myc (a direct regulator of cell cycle machinery) were reduced in TREM2 KO microglia.

Because β-catenin initiates transcriptional activation of cyclin D1 and c-Myc, Zheng et al. (2017) hypothesized that this protein might be a downstream effector of TREM2. First, they asked whether TREM2 regulates β-catenin transcription by measuring mRNA content. They found no difference between the WT and KO cells. Therefore, they asked whether TREM2 regulates proteasomal degradation of β-catenin by measuring protein levels in the presence of a protein synthesis inhibitor. β-Catenin protein levels showed a faster reduction over time in the KO cells, suggesting that TREM2 suppresses the proteasomal degradation of β-catenin. This was further confirmed by treatment of the KO cells with a proteasomal inhibitor, MG132, which rescued the degradation effect.

Under canonical Wnt signaling, β-catenin accumulates in the cytoplasm before eventually translocating to the nucleus, but in the absence of Wnt, β-catenin is targeted for proteasomal degradation by GSK3β (Clevers and Nusse, 2012). GSK3β can, in turn, be inactivated upon phosphorylation by activated AKT. To investigate how TREM2 might stabilize β-catenin, Zheng et al. (2017) measured the phosphorylation status of Akt and GSK3β. Phosphorylation levels of Akt and GSK3β were significantly lower in TREM2 knockdown and KO microglia than in controls, confirming the role of Akt/Gsk3β pathway activation in TREM2-mediated β-catenin stabilization.

Next, Zheng et al. (2017) asked whether stabilizing β-catenin would rescue the effects of TREM2 loss. Indeed, activating the canonical Wnt signaling pathway with Wnt3a treatment partially restored microglial survival and proliferation of TREM2 KO microglia. In addition, the GSK3β inhibitor lithium chloride (LiCl) inhibited Gsk3β-induced β-catenin degradation, increased β-catenin levels, and moderately increased microglia viability *in vitro*. These results were replicated *in vivo* by LiCl treatment, although the rescue of

c-Myc and cyclin D1 levels was partial, suggesting that this is not the sole pathway through which TREM2 influences microglial survival and proliferation.

In summary, Zheng et al. (2017) demonstrated that TREM2 mediates its effects on microglial proliferation through a β-catenin pathway. These findings are in agreement with recent studies showing that TREM2-DAP12, upon activation by macrophage colony stimulating factor, promotes macrophage survival in a β-catenin-dependent manner (Otero et al., 2009). These results also align with another study showing that soluble TREM2 (sTREM2), arising from proteolytic cleavage of TREM2, may complement TREM2 activity of enhancing microglial survival and suppressing apoptosis, in a PI3K-Akt-dependent manner (Zhong et al., 2017). Additionally, studies using TREM2-deficient mice have confirmed poor activation of microglia (Kawabori et al., 2015).

Observations from Zheng et al. (2017), along with other studies, thus hint at a possible mechanism by which TREM2 mutation imparts vulnerability to AD. Amyloid deposition is an important pathological hallmark in AD, and previous studies have suggested that TREM2 aids in compacting amyloid plaques (Yuan et al., 2016). A loss-of-function mutation in TREM2 would result in decreased surviving and dividing microglia as well as an increased area occupied by plaques. This would expose more neurons to amyloid plaques, which might result in greater dystrophy. Components of damaged and dead neurons, such as sphingolipids and nucleic acids (Kawabori et al., 2015; Wang et al., 2015), serve as potential ligands to TREM2, but as Zheng et al. (2017) suggest, a loss-of-function mutation would compromise microglial activation. Furthermore, TREM2-deficient animals have been shown to have impaired phagocytosis and clearance (Kawabori et al., 2015), leading to more uncleared cell debris. These events may put the system in a vicious neurotoxic cycle.

Zheng et al. (2017) provide a therapeutic direction of enhancing Wnt/β-catenin signaling to push microglia to a healthier state. Indeed, converging lines of evidence suggest that the Wnt pathway is dysregulated in AD, and Wnt3a and LiCl have been used to rescue β-amyloid-induced neurodegeneration in rats (De Ferrari et al., 2003). However, Wnt3a stimulation has also been shown to exacerbate the proinflammatory immune response of microglia (Halleskog et al., 2011). Although this could be beneficial in early stages, aiding in clearing amyloid and de-

bris; with aging and disease progression, chronic activation of microglia could be counterproductive, leading to neurotoxicity. Hence, a proper titration of dosage to tightly regulate the immune response is important.

In conclusion, Zheng et al. (2017) have reported new insights into TREM2-mediated microglial survival and identified β-catenin as an important role player in TREM2-initiated signaling pathways. Therefore, it offers the promise of developing effective therapeutics to overcome microglial dysfunction in neurodegeneration.

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