**Transmembrane BAX Inhibitor-1 Motif-containing 4：A Novel Therapeutic Target for Preeclampsia via Inhibition of Trophoblastic NLRP3 Inflammasome Activation and Pyroptosis**

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**Background and aims.** Preeclampsia is a leading cause of death in pregnancy, yet it is a condition without a cure. Activation of the NLRP3 inflammasome–pyroptosis axis mediates inflammatory responses in key placental cells, trophoblasts, representing a crucial pathogenic mechanism in preeclampsia. Inhibiting NLRP3 inflammasome activity in trophoblasts is a promising therapeutic strategy for preeclampsia, however, molecular mechanisms governing NLRP3 inflammasome degradation are poorly understood. Based on our finding that transmembrane BAX inhibitor-1 motif-containing 4 (TMBIM4) inhibits NLRP3 inflammasome activation in trophoblast [1], we aim to demonstrate that harnessing TMBIM4 function can alleviate preeclampsia symptoms and features.

**Methods.** Using immunohistochemistry, TMBIM4 expression/localization was quantified in placental tissues, following preeclampsia and healthy pregnancies. In in vitro models, TMBIM4 was knocked out using CRISPR/Cas9 in HTR-8/SVneo first-trimester trophoblast cells, and its impact on cell function and NLRP3 inflammasome activity was determined with or without NLRP3 inflammasome activation by Lipopolysaccharide(LPS)/ATP treatment Adenovirus-coated TMBIM4 recombinant protein was delivered via intravenous injection in an *in vivo* inflammation/LPS-induced model of preeclampsia and the preeclampsia-like phenotype was assessed, including blood pressure, proteinuria, and placental morphology.

**Results.** Our findings confirmed that TMBIM4 is highly expressed in all trophoblast subtypes in early pregnancy, and significantly reduced in preeclampsia cases. TMBIM4 knockout in the HTR-8/SVneo cell line led to reduced cell viability, impaired migration, and invasion, which were further exacerbated by LPS/ATP treatment. Furthermore, TMBIM4 deficiency heightened NLRP3 inflammasome activity, inducing pyroptosis irrespective of LPS/ATP treatment. TMBIM4 induces significant degradation of exogenous NLRP3 protein, a process that relies on the autophagolysosome pathway. Compared with wild-type pregnant mice, *Tmbim4*-/- mice display a pronounced preeclampsia-like phenotype such as higher blood pressure, higher urine protein levels and reduced fetal weight, and heightened activation of placental NLRP3 inflammasomes following LPS induction. Treatments with TMBIM4 overexpression can alleviate the preeclampsia-like phenotype in pregnant mice.

**Conclusion/Discussion.** This study identifies TMBIM4 as a novel therapeutic target for preeclampsia, demonstrating and provide new technologies targeting NLRP3 degradation through the autophagic-lysosomal pathway for preeclampsia prevention and treatment.

**References.** Chen Y, Xiao L, Sun G, Li M, Yang H, Ming Z, Zhao K, Shang X, Zhang H, **Liu C**. TMBIM4 Deficiency Facilitates NLRP3 Inflammasome Activation-Induced Pyroptosis of Trophoblasts: A Potential Pathogenesis of Preeclampsia. Biology (Basel). 2023 Jan 29;12(2):208. doi: 10.3390/biology12020208.

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