TLR2 promotes VSMC chondrogenic differentiation and consequent calcification via the concerted actions of OPG suppression and IL-6-mediated RANKL induction

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Abstract

Objective—Vascular smooth muscle cell (VSMC) transformation to an osteochondrogenic phenotype is an initial step toward arterial calcification, which is highly correlated with cardiovascular disease-related morbidity and mortality. Toll-like receptor 2 (TLR2) plays a pathogenic role in the development of vascular diseases, but its regulation in calcification of arteries and VSMCs remains unclear. We postulate that TLR2-mediated inflammation participates in mediating atherosclerotic arterial calcification and VSMC calcification.

Approach and Results—We found that ApoE^{-/-} Tlr2^{-/-} genotype in mice suppressed high-fat diet (HFD)-induced atherosclerotic plaques formation during initiation, but progressively lost its preventative capacity, compared with ApoE^{-/-} mice. However, TLR2 deficiency prohibited HFD-induced advanced atherosclerotic calcification, chondrogenic metaplasia, and osteoprotegerin (OPG) downregulation in the calcified lesions. Incubation of VSMCs in a calcifying medium revealed that TLR2 agonists significantly increased VSMC calcification and chondrogenic differentiation. Further, TLR2 deficiency suppressed TLR2 agonist-mediated VSMC chondrogenic differentiation and consequent calcification, which were triggered via the concerted actions of IL-6-mediated receptor activator of nuclear factor kappa-B ligand (RANKL) induction and OPG suppression. Inhibition experiments with pharmacological inhibitors demonstrated that IL-6-mediated RANKL induction is signaled by p38 and ERK1/2 pathways, whereas the OPG is suppressed via NF-κB dependent signaling mediated by ERK1/2.

Conclusions—We concluded that upon ligand binding, TLR2 activates p38 and ERK1/2 signaling to selectively modulate the upregulation of IL-6-mediated RANKL and downregulation of OPG. These signaling pathways act in concert to induce chondrogenic transdifferentiation of VSMCs, which in turn leads to vascular calcification during the pathogenesis of atherosclerosis. (*Arterioscler Thromb Vasc Biol.* 2019;39:432–445)

Results:

Fig. 1. TLR2 deficiency prevents HFD-induced atherosclerotic calcification. HFD-induced atherosclerotic calcification and plasma IL-6 levels were suppressed in ApoE^{-/-} Tlr2^{-/-} mice (Fig. 1A-C). HFD-mediated downregulation of OPG expression in the intima and media was reduced in ApoE^{-/-} Tlr2^{-/-} mice compared with that in ApoE^{-/-} mice (Fig. 1D-E). These results suggest that TLR2 participates in HFD-induced atherosclerotic calcification by increasing IL-6 production and suppressing OPG expression.

Fig. 2. TLR2 activation by agonists promotes VSMC chondrogenic differentiation and consequent calcification, which is mediated via the concerted actions of IL-6-mediated RANKL induction and OPG suppression.

Fig. 3. 5-MTP suppresses TLR2-mediated responses and protects against HFD-induced vascular calcification in ApoE^{-/-} mice

Highlights

- TLR2 signaling has a detrimental effect on vascular calcification.
- 5-MTP prevents vascular calcification via inhibiting TLR2 signaling.