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Short Heterodimer Partner as a Regulator in OxLDL-induced Signaling Pathway

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Abstract

Oxidized low-density lipoprotein (oxLDL) has been shown to modulate transactivations by the peroxisome proliferator activated receptor (PPAR)-γ and nuclear factor-kappa B (NF-κB). In this study, the oxLDL signaling pathways involved with the NF-κB transactivation were investigated by utilizing a reporter construct driven by three upstream NF-κB binding sites, and various pharmacological inhibitors. OxLDL and its constituent lysophophatidylcholine (lysoPC) induced a rapid and transient increase of intracellular calcium and stimulated the NF-κB transactivation in resting RAW264.7 macrophage cells in an oxidation-dependent manner. The NF-κB activation by oxLDL or lysoPC was inhibited by protein kinase C inhibitors or an intracellular calcium chelator. Tyrosine kinase or PI3 kinase inhibitors did not block the NF-κB transactivation. Furthermore, the oxLDL-induced NF-κB activity was abolished by the PPAR-γ ligands. When the endocytosis of oxLDL was blocked by cytochalasin B, the NF-κB transactivation by oxLDL was synergistically increased, while PPAR transactivation was blocked. These results suggest that oxLDL activates NF-κB in resting macrophages via protein kinase C- and/or calcium-dependent pathways, which does not involve the endocytic processing of oxLDL. The endocytosis-dependent PPAR-γ activation by oxLDL may function as an inactivation route of the oxLDL-induced NF-κB signal.

Short heterodimer partner (SHP), specifically expressed in liver and a limited number of other tissues, is an unusual orphan nuclear receptor that lacks the conventional DNA-binding domain. In this work, we found that SHP expression is abundant in murine macrophage cell line RAW 264.7 but suppressed by oxLDL and its constituent 13-HODE, a ligand for peroxisome proliferator-activated receptor γ. Furthermore, SHP acted as a transcription coactivator of nuclear factor-κB (NFκB) and was essential for the previously described NFκB transactivation by lysoPC, one of the

oxLDL constituents. Accordingly, NFκB, transcriptionally active in the beginning, became progressively inert in oxLDL-treated RAW 264.7 cells, as oxLDL decreased the SHP expression. Thus, SHP appears to be an important modulatory component to regulate the transcriptional activities of NFκB in oxLDL-treated, resting macrophage cells.

1. Roles of endocytosis in the nuclear factor-kappa B transactivation by oxidized low-density lipoprotein

Formation of atherosclerotic lesion is a complex process involving intracellular lipid accumulation and various signal transduction pathways in vascular cells, such as endothelial cells, smooth muscle cells, and monocyte/macrophages. Each vascular cell is capable of oxidatively modifying low-density lipoprotein (LDL) to generate oxidized LDL (oxLDL), which is taken up by macrophages through scavenger receptor (SR). The oxidative modification results in a number of important biological activities of LDL, including the induction of a number of genes encoding cytokines or growth factors. Among growth factors and cytokines that are secreted from the vascular cells, a regulatory network may be present to produce the lesion collectively.

Unlike the LDL receptor whose expression is finely regulated by intracellular cholesterol level, the expression of SR is stimulated by internalized oxLDL. Recently, it was reported that ligands for peroxisome proliferator activated receptor-γ (PPAR-γ), 9- and 13-hydroxyoctadecadienoic acids (9- and 13-HODE) were generated from oxLDL through the SR-mediated endocytosis followed by intracellular processing. The endocytosis- and ligand-dependent activation of PPAR-γ by oxLDL was responsible for regulating expressions of various macrophage genes, including scavenger receptors.

Several reports indicate that oxLDL activates signaling pathway(s) upon ligation to the SR. Nuclear factor-kappa B (NF-κB) has been suggested to be one of the possible mediators involved in the oxLDL-induced signaling. Supportive evidences include the finding of an activated p65 component of NF-κB in an atherosclerotic lesion and the observation of a retarded band on a NF-κB gel shift assay. In contrast, Ohlsson et al. demonstrated that oxLDL did not induce the binding of NF-κB while it suppressed lipopolysaccharide (LPS)-induced response to NF-κB. Many other investigators also showed that oxLDL blocked the LPS-induced gene expressions.

The RAW264.7 macrophage cells were stably transfected with the luciferase construct containing three upstream NF-κB binding sites of E-selectin to quantify the oxLDL effects on NF-κB activation. This assay system allows us to investigate the

regulatory connection between NF-κB and other possible signaling mediators, such as intracellular calcium or protein kinase C in resting macrophages. Using pharmacological inhibitors we show in this study that oxLDL activates NF-κB via protein kinase C- and/or calcium-dependent pathway. This process does not require the endocytic processing of oxLDL, unlike the oxLDL-induced PPAR-γ pathway. Furthermore, a specific ligand for PPAR-γ suppresses the oxLDL-induced NF-κB transcriptional activity. This study demonstrates for the first time the NF-κB regulatory network by oxLDL in resting macrophages.

2. The Orphan Nuclear Receptor SHP, as a Novel Coregulator of Nuclear Factor-κB in oxLDL-Treated Macrophage Cell RAW 264.7

Short heterodimer partner (SHP) is an orphan nuclear receptor, specifically expressed in liver and a limited number of other tissues, and its activities are in some ways opposite to those of RXR. SHP, like the orphan nuclear receptor DAX-1, lacks the conventional DBD. Both direct biochemical and the yeast two hybrid results demonstrated that SHP interacts with many members of the receptor superfamily. As expected from its lack of a DBD, addition of SHP inhibited in vitro DNA binding by nuclear receptors with which it interacted and, in mammalian cell cotransfections, SHP repressed their transactivation. In addition, the SHP sequences required for interaction with other superfamily members have recently been localized to the central portion of SHP, distinct from the I-box.

Cross-talk between transcription factors of distinct families is an important phenomenon in regulating gene transcription and has recently become the subject of intensive investigation. In particular, the transcription factor nuclear factor-κB (NFκB) has been shown to functionally interact with numerous other transcription factors, including members of the nuclear receptor superfamily, resulting in mutually repressed biological activity of these transcription factors.

A. SHP as a Novel Transcription Coactivator of NFκB

Many nuclear receptors have been shown to functionally interact with NFκB. Using GST-pull down assay, we showed that SHP interacted with the NFκB components p65 but not with luciferase or p50. Similar results were also obtained with the mammalian two hybrid-based tests. The SHP interaction interface was further mapped to the N-terminal 283 residues of p65. Confirming the functionality of these

interactions, cotransfected SHP enhanced transactivation by NFκB in a dose-dependent manner. In contrast, SHP efficiently suppressed transactivation by RAR, and had no significant effect on transactivation directed by Gal4 fusion to VP16. Interestingly, SHP synergized with SRC-1, a transcription coactivator of nuclear receptors and other transcription factors, including NFκB. Accordingly, GST-fusion to SHP specifically interacted with the radiolabeled C-terminal subregions of SRC-1. These results clearly demonstrate that SHP directly interacts with p65 and is a positive transcriptional coregulator of NFκB. It is intriguing that the positive regulatory role of SHP is in sharp contrast to its previously reported inhibitory role on a variety of receptor-dependent signaling pathways.

B. Down-Regulation of SHP by oxLDL in RAW 264.7

SHP was previously demonstrated to specifically express in liver and a limited number of tissues. SHP mRNA is also abundant in mouse macrophage cell line RAW 264.7. Surprisingly, this expression was significantly repressed by oxLDL, but not by native LDL (nLDL), in a time-dependent manner. However, the repression was not observed in the presence of endocytosis blocker cytochalasin B, suggesting that the inhibitory effect of oxLDL likely involve endocytosis of oxLDL. Troglitazone and 13-HODE was responsible for the inhibitory effect of oxLDL.. In contrast, lysoPC, another constituent of oxLDL that transactivates NFκB, was without any effect. However, the oxLDL-mediated repressive effects were not recapitulated with the previously described SHP promoter construct in cotransfections of CV1 and RAW 264.7 cells. SHP mRNA from oxLDL-treated cells degraded much faster than nLDL-treated cells in the presence of transcription blocker actinomycin D. The half-life of SHP mRNA in the presence of oxLDL and actinomycin D was approximately 1 hr, relative to 2 hr with nLDL and actinomycin D. Interestingly, cycloheximide, protein synthesis blocker, had no effect on the inhibitory action of oxLDL, suggesting that new protein synthesis is not required for the repression. Overall, these results strongly suggest that oxLDL suppresses SHP expression in resting macrophage cells, likely through affecting its mRNA stability.

C. SHP as a Modulator of the NFκB Activities in oxLDL-Treated RAW 264.7

SHP also interacted with PPARγ as well as NFκB in the yeast two hybrid tests. Thus, we examined the effects of cotransfected SHP on either oxLDL-mediated transactivation of NFκB or PPARγ in CV1 cells. Surprisingly, oxLDL did not readily enhance the NFκB transactivation unless SHP is coexpressed, suggesting the essentiality of SHP in the NFκB transactivation by oxLDL. Furthermore, SHP was an activator of both the basal and ligand-induced levels of PPARγ transactivation in CV-1

cells, as opposed to its previously described inhibitory effects with other nuclear receptors. However, it is not currently known if SHP could still be an activator of the PPARγ transactivation in RAW 264.7 cells. Consistent with an idea that SHP alone may not effectively stimulate the PPARγ transactivation in RAW 264.7 cells, the PPARγ transactivation level was relatively low during the early phase of oxLDL treatment in which SHP expression is still abundant. A putative repressor molecule of PPARγ, which is dominant over the stimulatory effect of SHP and suppressible by oxLDL, may also exist in the resting RAW 264.7cells. Interestingly, treatment of RAW 264.7 cells with oxLDL did not lead to significant changes in PPARγ expression, in contrast to the previously described results with THP-1 monocytic leukemia cells. In addition, the previously described 13-HODE/PPARγ-mediated repression of the NFκB transactivation was impaired with the increasing amount of SHP-expression vector. The 13-HODE-dependent repression was not observed in the absence of cotransfected PPARγ. Taken all of these results together, the NFκB transactivation was expected to gradually diminish in oxLDL-treated RAW 264.7 cells, as the SHP expression becomes repressed. Indeed, stimulation of the NFκB transactivation was readily observed with oxLDL or lysoPC for the initial 4-8 hrs after treatment, whereas lysoPC was much less effective and oxLDL and 13-HODE were rather repressive with the NFκB transactivation for the 12-24 hrs post treatment. In particular, the latter results likely result from the previously described PPARγ-mediated repression of the NFκB transactivation, which doesn't seem to operate when SHP level is relatively high. Currently, it is not clear why the PPARγ transcriptional activities were much higher for the 12-24 hrs post treatment generating a biphasic transition from the NFκB- to PPARγ-transactivation during oxLDL treatment, although the putative, oxLDL-suppressible repressor may exist in the resting RAW 264.7 cells as mentioned already. Overall, these results suggest that the oxLDL-mediated down-regulation of SHP, coupled with the PPARγ-mediated repressive actions, may result in efficient shut-down of the NFκB transcriptional activities in oxLDL-treated RAW 264.7 cells.

In summary, we identified SHP as a novel transcription coactivator of NFκB and further presented the experimental results indicating that targeted expression of SHP appears to function as a distinct regulatory component of the transcriptional activities of NFκB in oxLDL-treated, resting macrophage cells.