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Review

Roles for lipid-activated transcription factors in atherosclerosis

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The initial cellular event in atherosclerosis is the recruitment of monocytes to the vessel wall, and the formation of foam cells by the uptake of modified lipoproteins. The role of macrophages in this process is the uptake and processing of lipoproteins ultimately leading to foam cell formation. These cells also sustain a chronic inflammatory reaction believed to participate in disease progression. We have been interested in identifying regulatory processes contributing to these events. Some members of a distinct class of transcription factors, nuclear hormone receptors, are expressed in macrophages and are likely to have roles in the initiation of atherosclerosis. We review here the identification of interrelated nuclear receptor-regulated pathways involving peroxisome proliferator-activated receptor, liver X receptor, and retinoid receptors, and contributing to lipid uptake and efflux in macrophages.

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Steroid hormone receptors can be grouped into three general categories. There are classical steroid hormone receptors such as the estrogen receptor, glucocorticoid receptor, progesterone receptor and others, which act as endocrine receptors and have high affinity hormonal ligands. Most other receptors have been cloned by sequence homology to these receptors and were identified as receptors without known endogenous activators, and hence termed orphan receptors [1]. A subset of these receptors was adopted by the identification of endogenous ligands/activators. Interestingly, most of the identified lipids turned out to be intermediary metabolites (fatty acids, cholesterol metabolites, bile acids), which bind to the respective nuclear receptors with relatively low affinity. These findings brought about the concept of metabolic sensors.

The metabolic sensors such as the peroxisome proliferator-activated receptors (PPARs) for modified fatty acids, the liver X receptors (LXRs) for oxidized cholesterol, and the bile acid receptor (BAR) for bile acids have more than one metabolite of the respective metabolic pathway that activates them. Therefore, these receptors connect the metabolic state of the cell to its expression profile by inducing or repressing genes/gene networks.

The PPARs are the fatty acid sensors of the cells [2]. This family of receptors has three members PPARα, γ, and β/δ [3]. PPARα is expressed in the liver and brown adipose tissue, and regulates fatty acid oxidation. PPARγ is expressed in adipose tissue and in macrophages and dendritic cells, and regulates differentiation as well as lipid uptake and storage [4–6]. PPARβ/δ is ubiquitously expressed and also regulates lipid handling. All three receptors have been linked to disease states and therefore synthetic ligands are available to probe their biological roles. PPARα is the target for fibrates, used in the treatment of hyperlipidaemia, PPARγ is the pharmacophore of the thiazolidinedione type insulin sensitizers, used in the treatment of type II diabetes, and there are efforts to identify the role of PPARβ/δ in high-density lipoprotein (HDL) metabolism. Strikingly, besides lipid metabolism, all three receptors have been linked to inflammation control. These findings suggested a nuclear receptor-mediated correlation of these seemingly unrelated fields: lipid metabolism and inflammation control. A particular cell type "active" on both of these fields is the macrophage. Macrophages are linked to lipid uptake and handling, and are also a key cell type in innate immunity. Several

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Abbreviations: ABC, ATP-binding cassette transporter; **LDL**, low-density lipoprotein; **LXR**, liver X receptor; **PPAR**, peroxisome proliferator-activated receptor

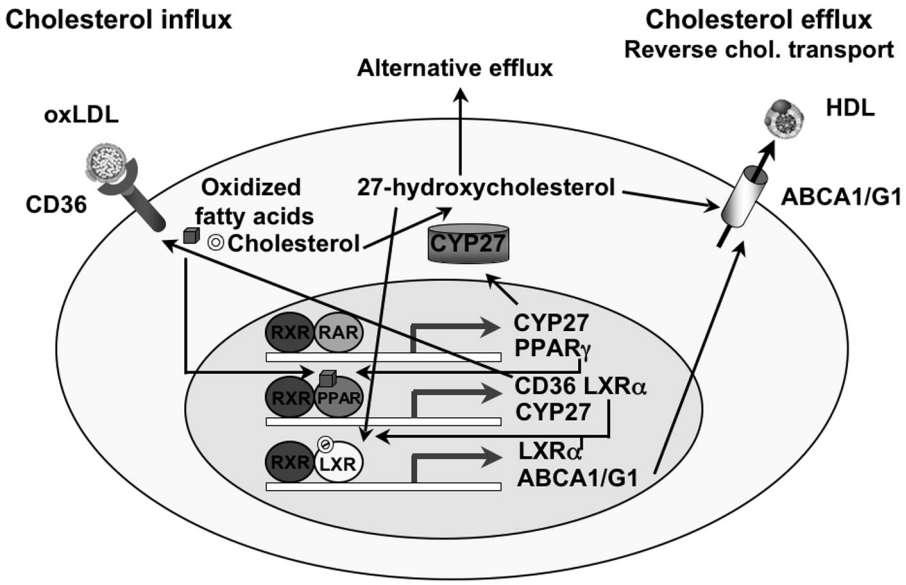

Figure 1. Elements of the nuclear receptor regulatory network that coordinate lipid metabolism and transport in macrophages.

years ago we decided to examine the role of these receptors in macrophages in a disease state, atherosclerosis, linking lipid metabolism, and inflammation control.

We and others [4, 5, 7–9] have been able to show that PPARγ is expressed in atherosclerotic lesions in humans and also in murine models of the disease and regulates lipoprotein uptake.

We have been investigating the PPARγ-regulated lipid metabolic processes and identified an important role for PPARγ in regulating cholesterol uptake, and efflux in macrophages. PPARγ promoted uptake of oxidized and not native low-density lipoprotein (LDL), and subsequent differentiation of the monocytes to foam cells [5]. Synthetic PPARγ agonists caused similar differentiation both in myelomonocytic cell lines and in primary human monocytes. It was shown that PPARγ was expressed in foam cells of atherosclerotic lesion. Its expression could further increase with oxLDL. It was proven that scavenger receptor CD36 could be regulated by PPARγ as a direct target gene of the receptor. Two components from the lipids in oxLDL, 9-hydroxy octadecadienoic acid (9-HODE), and 13-HODE were identified as endogenous activators and *bone fide* ligands for PPARγ [4]. These results suggested a model of macrophage lipid metabolism. Macrophages internalize modified LDL *via* scavenger receptors (*i.e.*, CD36), which unlike LDL receptor are not down-regulated by high cholesterol levels. On the contrary, oxLDL increases the expression of a scavenger receptor CD36 by PPARγ and potentiates its own uptake. These findings provided explanation for the formation of lipid-loaded macrophages (foam cells) and suggested the existence of a vicious cycle leading to atherosclerosis. But the question, how PPARγ regulates atherosclerosis, remained unclear. Using a genetic approach, PPARγ null mutant embryonic stem (ES) cells were created by homologous recombination and it was shown that PPARγ was not essential for the development of the macrophage lineage *in vitro* and *in vivo*, but was an important regulator of macrophage gene expression. PPARγ deficient ES cells gave rise to macrophages in an *in vitro* differentiation assay and in chimeric mice generated with mutant ES cells. PPARγ deficient cells were also able to contribute to the macrophage lineage *in vivo* [10, 11]. In these studies it was also proven that the antiinflammatory effects of PPARγ activators were receptor-independent. The expression of proinflammatory genes was also inhibited by natural and synthetic ligands in both wild type and PPARγ deficient macrophages. Next PPARγ was shown not only to regulate lipid uptake, but also to have a central role in cholesterol efflux from cells. PPARγ induces ATP-binding cassette transporter (ABC) A1 expression and cholesterol removal from macrophages through a transcriptional cascade driven by PPARγ and LXRα. ABCA1 and ABCG1 are members of the ATP-binding cassette family of transporter proteins. Several studies [12–15] reported that LXRs mediate cholesterol efflux by inducing cholesterol transporters ABCA1, ABCG1 and later ABCG5, and ABCG8. Then LXRα was identified as a PPARγ target gene [16]. Since both PPARγ and LXR could be activated by lipid components of oxLDL, it was hypothesized that these nuclear receptors composed a transcriptional cascade that regulated macrophage response to oxLDL. Chawla *et al*. analyzed the promoter of the ABCA1 gene and showed that LXR:RXR could activate it but PPARγ:RXR heterodimer could not [16]. These results were incorporated into our model which now involves elements of the first cycle and the new findings as well (Fig. 1): oxLDL induces PPARγ and CD36 levels that stimulates further oxLDL uptake. PPARγ also induces the expression of LXRα, which activates transcrip-

tion of cholesterol transporters *e.g.* ABCA1, and these lead to increased cholesterol efflux to ApoAI from macrophages. Linking of the two receptor systems (PPARγ and LXRα) provides an attractive but still not well understood model to explain lipid/cholesterol uptake and efflux from macrophages.

Identification of PPARγ-regulated pathways in macrophages: the CYP27 pathway [17].

The issue how the activation of the two receptors may be coupled has not been addressed. It was assumed that lipid content of lipoproteins may act as activators or ligands for both PPARγ [4] and LXR [18]. The fact that LXR signaling is activated in macrophages exposed to acetylated LDL [18], which does not contain oxidized cholesterol suggests that there must be other ways to activate/produce ligand for this receptor. PPAR-related induction of LXRα is not enough for getting an activated LXR that induces cholesterol efflux. It requires an endogenous ligand. Production of endogenous LXR activator that itself induces changes through LXR has not been reported yet. A number of oxysterols have been identified as potential endogenous ligands for LXR [19–22]. One of these compounds, 27-hydroxycholesterol is produced by a p450 enzyme CYP27. It has been also associated with atherosclerotic lesions [23, 24]. The enzymes product 27-hydroxycholesterol has been shown to activate LXR [25, 20]. These data raised the possibility that CYP27 might serve as a regulator of LXR activity by generating ligand to it. We have identified a p450 enzyme CYP27 as a gene commonly induced during monocyte-macrophage transition and as a PPARγ:RXR and RAR:RXR-regulated gene in myeloid cells. Promoter analysis revealed complex regulation by retinoid receptors and PPARs *via* a response element on the promoter of human CYP27 further underscoring the interrelatedness of these pathways. These findings tie retinoid, PPAR, and LXR signaling into one regulatory network (Fig. 1) requiring natural ligands: retinoids and modified fatty acids or prostanoids to activate an entire metabolic pathway and leading to coordinate regulation of lipid/cholesterol uptake, metabolism and efflux. Furthermore, we provided evidence that all components of the described pathways exist in human atherosclerotic lesions [17].

In summary we can say that the identification of nuclear receptor-mediated processes in macrophages revealed that a transcriptional regulatory network exists for the uptake and processing of lipids in macrophages, and this can be potentially exploited in the treatment of diseases such as atherosclerosis. It remains to be seen how amenable these pathways are for pharmacological intervention.

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