# Transcription factors in response to oxidative stress: implications for atherosclerosis-related inflammation.

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## Summary

It is well recognized that inflammation plays a crucial role in the initiation and progression of atherosclerosis. Many biological effects of reactive oxygen species and oxidized-low density lipoprotein during atherogenesis are mediated through signaling pathways, especially via the activation of transcription factors, which in turn stimulate the expression of genes involved in the inflammatory and oxidative stress response or in cell cycle regulation. In this review, we will examine the various transcription factors activated during atherosclerosis-related inflammation. Identification of transcription factors and some of the downstream genes regulated by oxidative stress has a major importance in relation to the understanding of molecular mechanism involved in atherosclerotic lesion formation.

**Key words**: Atherosclerosis, oxidative stress, inflammation, transcription factors.

## Introduction

An imbalance between oxidants and antioxidants resulting from increased production of oxidants and/or reduction in the amounts of antioxidants generates a state of stress in the cell termed oxidative stress (OS). Clearly, OS encompasses a wide variety of physiological and pathophysiological processes that directly or indirectly affect the cellular redox state (1). These responses ultimately modulate transcriptional outputs to influence cell functions. In the past two decades, a number of transcription factors and signaling pathways have been identified and delineated to mediate critical transcriptional responses to OS. These facts demonstrate the importance as well as the complexity of how alterations in intracellular reactive oxygen species (ROS) are converted into discrete and reproducible alterations in gene expression, and ultimately disease outcomes (2).

Regardless of the origin of ROS, high levels have two consequences mainly: activation of specific signal transduction pathways and damage to cellular components, both of which significantly impact on physiology and the development of disease. Mechanistically, many of these effects involve activation of specific transcription factors to control the transcription of a range of target genes. These genes encode specific proteins/enzymes to mediate biological responses to OS (3). In recent years, significant advances have been made in understanding the interaction between OS and the transcriptional machinery, in particular, the molecular mechanism of such interaction and the implication of the interaction in human disease.

It is well recognized that oxidized low-density lipoprotein (oxLDL) plays a crucial role in the initiation and progression of atherosclerosis, which can be considered a chronic inflammatory disease (4).

OxLDL presents many deleterious effects such as production of inflammatory cytokines and chemotactic factors from numerous cell types of the arterial wall (5). OxLDL might also lead to a procoagulant activity (6) of the vascular cell surface, whereas the local synthesis of adhesion molecules induces the recruitment of mononuclear cells to the endothelium. These cells are then transformed to macrophages and finally to the lipid-loaded foam cells. The oxLDL particle also has cytotoxic properties toward various cell types, and this toxicity has been attributed to the modified lipid moiety of the particle (reviewed in 7).

The purpose of this review is to examine major transcription factors that mediate gene regulation in response to OS and their relation with inflammation during atherogenesis.

**Atherosclerosis as a result of a chronic inflammation**

The inflammatory response is commonly thought to operate during severe disturbances of homeostasis, such as infection, tissue injury and the presence of foreign bodies or irritants. Whatever the cause of the inflammatory response its 'purpose' is to remove or sequester the source of the disturbance to allow the host to adapt to the abnormal conditions and, ultimately, to restore functionality and homeostasis to the tissue. If the abnormal conditions are transient, then a successful acute inflammatory response returns the system to the basal homeostatic set points. If, by contrast, the abnormal conditions are sustained, then an ongoing inflammatory state shifts the system to different set points, as occurs during chronic inflammation (8).

Recent investigations of atherosclerosis have focused on inflammation, providing new insight into mechanisms of disease (9). Inflammatory cytokines involved in vascular inflammation stimulate the generation of endothelial adhesion molecules, proteases, and other mediators, which may enter the circulation in soluble form. Endothelial cells normally resist leukocyte adhesion. Proinflammatory stimuli, including a diet high in saturated fat, hypercholesterolemia, obesity, hyperglycemia, insulin resistance, hypertension, and smoking, trigger the endothelial expression of adhesion molecules such as P-selectin and vascular cell adhesion molecule-1 (VCAM-1), which mediate the attachment of circulating monocytes and lymphocytes (10). Interestingly, atherosclerotic lesions often form at bifurcations of arteries, regions characterized by disturbed blood flow, which reduces the activity of endothelial atheroprotective molecules such as nitric oxide and favors regional proinflammatory substances expression (11).

The inflammatory processes of atherosclerosis require both innate and adaptive immunity. Macrophages play a central role among immune cells involved in this pathology, since they take part in both the lipid core formation and the production of inflammatory mediators. In addition, accumulating evidence supports the important role of T-cells in the first steps of atherosclerosis, whereas B-cells are poorly represented in the intimal plaque and their role is limited to antigen presentation (12,13).

Chemoattractant factors, which include monocyte chemoattractant protein-1 (MCP-1) produced by vascular wall cells in response to modified lipoproteins, direct the migration and diapedesis of adherent monocytes (8). Monocytes directly interacting with human endothelial cells increase monocyte matrix metalloproteinase 9 (MMP-9) production several fold, allowing for the subsequent infiltration of leukocytes through the endothelial layer and its associated basement membrane (14). Within the intima, monocytes mature into macrophages under the influence of macrophage colony stimulating factor, which is overexpressed in the inflamed intima (15). Then, an unregulated uptake of ox-LDL by macrophage leads to foam cells formation (16). The non-resolution of inflammation and the perpetuation of these events can results in chronic inflammation that leads to atherosclerosis development.

**Proinflammatory transcription factors**

*Nuclear factor κ of B cells (NF- κB)*

The NF-κB transcription factor family includes p65, p50, p52, c-Rel, and rel-B. The most abundant combination is the p65-p50 heterodimer. NF-κB has often been referred to as a central mediator of the immune response because of its regulation of the expression of inflammatory cytokines, chemokines, immune receptors, and cell surface adhesion molecules (17). Also, NF-κB has been involved in multiple steps in the progression of atherosclerosis, including initiation of monocyte adhesion, foam cell formation, and inflammatory reactions (18).

The involvement of NF-κB in atherosclerosis is finally demonstrated by the presence of the activated form in human atherosclerotic plaques (19) and by the fact that selective inhibition of NF-κB reduces foam cell formation (18).

ROS have been shown to mediate NF-κB activation leading to the expression of vascular cell adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1) which stimulate leukocytes recruitment to the damaged site. These events lead to inflammation and atherosclerosis development (20,21).

On the other hand, activation of NF-κB by minimally modified LDL in endothelial cells, and by $Cu^{2+}$-or cell-oxLDL in fibroblasts, endothelial cells, and smooth muscle cells have been reported (7). Finally, it is of note that high-density lipoprotein (HDL) prevents the intracellular OS and the inflammatory response elicited by oxLDL by inhibiting the NF-κB signaling pathway (22).

*Hypoxia-inducible factor-1 (HIF-1)*

Cells subjected to hypoxia respond by transcriptional changes that promote increased anaerobic metabolism, erythropoiesis, angiogenesis and other adaptive responses. The key mediator of these changes is the transcription factor HIF-1, a heterodimer of the protein subunits HIF-1α, which is induced and accumulated by hypoxia and HIF-1β or aryl hydrocarbon receptor nuclear translocator (ARNT), which is constitutively expressed (23,24).

The role of HIF1 in atherosclerotic plaque rupture was suggested by the fact that apoptosis and angiogenesis are induced in the unstable coronary plaque, probably by an increase in VEGF demonstrated by immunostaining (25). Blocking HIF1 activation inhibits lesion formation by inhibition of angiogenesis in injured vessels (26). HIF1α in turn stimulates the expression of atherosclerosis-related genes such as cyclooxygenase 2 (COX2), VCAM1, and IL1β. Also, RNA interference of HIF1α inhibits oxLDL-induced foam cell formation (27).

***Specific protein 1 (Sp1)***

Sp1 is a human transcription factor involved in early development. Sp1 seems to be also involved in the inflammatory response and, together with NF-κB and AP1, upregulates the expression of VCAM1 and ICAM1 adhesion molecules, tumor growth factor (TGFβ) and platelet-derived growth factor (PDGFβ), and, finally, monocytes chemotactic protein-1 (MCP1) and osteopontin cytokines (28). In addition, Sp1 and AP1 have been shown to protect against angiotensin II-induced inflammation in aorta and heart in rats (7).

In endothelial cells, the threonine phosphorylation and nuclear localization of Sp1 is under the control of protein kinase A, and Sp1 increases the expression of VEGF (29). Sp1 binding sites are also found in the promoter of the VEGF receptor 2 gene, which is critical for angiogenic responses during chronic inflammation. In these cells, Sp1 also regulates the expression of the antioxidant enzyme manganese-Superoxide dismutase (Mn-SOD) and the expression of adhesion molecules such as VCAM1 and ICAM1. In monocytes, an Sp1 binding site was characterized in the 5-lipoxygenase gene promoter (reviewed in 7).

The activation of Sp1 by native and OxLDL was reported in VSMC, in which Sp1 controls the tissue factor gene expression. Sp1 is also activated by reactive oxygen species generated under OxLDL in rat mesangial cells and promotes the expression of fibronectin (30).

***Signal transducer and activator of transcription (STAT1/3)***

The transcription factors from the STAT family, mediating the effects of cytokines and growth factors, were first described for their role in the control of hematopoietic cell proliferation (31).

Concerning the role of STAT factors in inflammation, it was reported that in human arterial SMC, the secretory phospholipase A2 gene is under the control of STAT3 (32). Furthermore, the cytosolic form of phospholipase A2, which controls vascular SMC motility, is also STAT3 dependent (33). Another gene regulated by STAT3 is the IL18 inflammatory gene (34). In macrophages, STAT1 controls monocyte chemoattractant protein 1 (MCP1) (35), a key gene involved in atherosclerosis. In this case, the activation by STAT1 has been demonstrated to be mediated by JAK1 and JAK2. Recently, it was demonstrated that actin-binding protein profiling 1, another proatherogenic gene, is regulated by the JAK2/STAT3 signaling pathway. The role of this pathway in atherogenesis in vivo was reported by Gharavi et al. (36), who found the activated form of STAT3 in the inflammatory regions of human atherosclerotic lesions.

## Conclusions

It has been recognized that inflammation is a key factor during atherosclerotic development. However, it can be noted that in vascular cells and macrophages, activated proinflammatory transcription factors have a preponderant role and are considered as effectors of pathogenic events which take place in atherosclerosis.

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