Potential roles of ROR-α in cardiovascular endocrinology

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Atherosclerosis is a chronic disease of the arteries whose development involves a local inflammatory response characterized by the activation of different cells such as macrophages, T-lymphocytes, smooth muscle cells (SMCs) and endothelial cells (ECs). This review will summarize recent evidence for a modulatory role of the nuclear receptor ROR-α in cardiovascular disease.

Received August 18th, 2003; Accepted October 15th, 2003; Published October 25th, 2003 | **Abbreviations:** **CaMKIV:** calcium/calmodulin-independent protein kinase; **CRP:** C-reactive protein; **CVD:** cardiovascular disease; **HDL:** high density lipoprotein; **HNF6:** hepatocyte nuclear factor 6; **IL-6:** interleukin 6; **SMCs:** smooth muscle cells; **TG:** triglyceride

Cite this article: Nuclear Receptor Signaling (2003) **1**, e011

Introduction

Cardiovascular disease (CVD) is a major cause of death in western societies. Atherosclerosis is an important cause of CVD and its clinical outcomes, myocardial infarcts, stroke and angina pectoris. Atherosclerosis is a chronic disease of the arteries. Its development involves a local inflammatory response characterized by the activation of different cells such as macrophages, T-lymphocytes, smooth muscle cells (SMCs) and endothelial cells (ECs) [Besnard et al., 2002; Ross, 1990]. Dyslipidemia is among the most important risk factors for CVD. There is convincing evidence from epidemiological and intervention studies that elevated low-density lipoprotein, reduced high-density lipoprotein cholesterol and elevated triglycerides in plasma are positively correlated to the progression of atherosclerosis and CVD [Assmann et al., 1998; Sprecher, 1998].

Inflammation, induced by locally elevated levels of atherogenic lipoproteins, is now considered as an important factor in the initiation of the lesions and their progression to the final stages leading to acute thrombotic complications and subsequent clinical events. Elevated circulating levels of inflammatory markers, such as CRP and IL6, are associated with an increased cardiovascular risk [Blake and Ridker, 2003]. Activated cells in the lesions, including ECs, SMCs and macrophages, produce an inflammatory response to these inflammatory stimuli via the activation of transcription factors, such as nuclear factor kappa-B (NFB), a redox-sensitive transcription factor regulating a battery of inflammatory genes. Activation of NFB induces gene programs leading to transcription of factors that promote local inflammation, such as leukocyte adhesion molecules, cytokines, and chemokines [Valen et al., 2001]. Rupture of advanced, unstable plaques provokes an atherosclerotic event leading to clinical sequelae. In the advanced plaque, neovascularisation occurs via angiogenesis that may influence plaque stability. Angiogenesis occurs under various pathological situations with an ischemic component [Carmeliet, 2000].

ROR-α (NR1F1)

RAR-related orphan receptors (RORs) constitute a subfamily of the nuclear receptors that includes three members: ROR-α, RORβ and RORγ. ROR-α is expressed in several organs and tissues, especially in skeletal muscle, fat tissue, retina, spleen, testis and the Purkinje cells in cerebellum [Giguere, 1999; Jetten et al., 2001; Lau et al., 1999]. RORβ, is highly expressed in different parts of the neurophotoendocrine system, the pineal gland, the retina, and suprachiasmatic nuclei, suggesting a role in the control of circadian rhythm. RORγ, is most highly expressed in the thymus and is shown to play an important role in thymopoiesis [Giguere, 1999; Jetten et al., 2001]. As most nuclear receptors, ROR-α is structured in a series of domains termed, from N- to C-terminus, A through F (see Figure 1). Four different isoforms are generated from the ROR-α gene, which differ in the first two domains, A and B. These splice variants are termed 1 through 4 (ROR-α4 has also been termed RZR). The C region contains the DNA binding domain (DBD), with two zinc finger motifs and a C-terminal extension of this region, called the T/A box, which assures contact to DNA and confers recognition specificity of the response element 5' nucleotides.

ROR-α binds either as a monomer to a ROR response element (RORE) composed of a 6 bp AT-rich sequence 5' to the consensus half-site AGGTCA core or as a homodimer to a direct repeat of the AGGTCA core separated by two base pairs (DR2 sites). Interestingly, the transcriptional repressor Rev-erbα, another nuclear receptor, binds to the same response elements [Harding et al., 1997; Raspe et al., 2002]. As a means to identify ROR-α target genes, RORE sites have been identified by homology searches in many gene promoters [Schrader et al., 1996]. Among the functionally characterised ROR-α target genes are apoC-III , Rev-erbα , and PPAR γ [Sundvold and Lien, 2001], which are especially induced by ROR-α1. Moreover, ROR-α4, in concert with HNF6, activates the α-fetoprotein gene in liver cells [Nacer-Cherif et al., 2003].

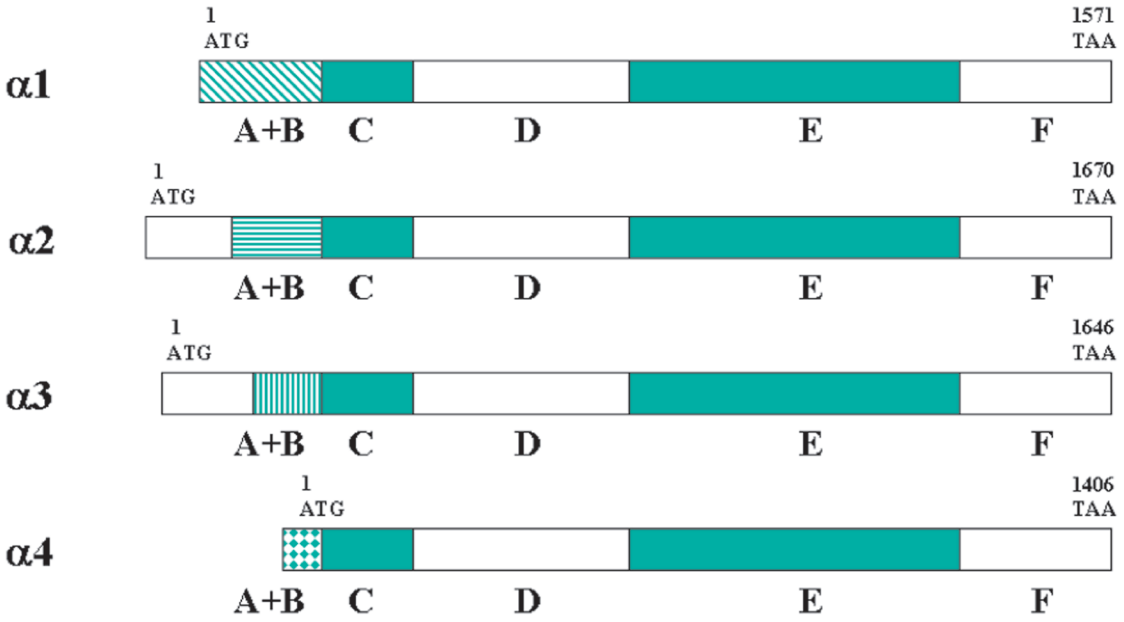

Figure 1. Alignment of different human ROR-α isoforms. Similar sequences are shown in white and green. Distinct sequences in hatched boxes. The coding sequence from ATG to TAA and the corresponding numbers for nucleotides are shown, respectively.

Ligands

Kallen *et al.* have determined the crystal structure of the ROR-α ligand binding domain (LBD). Structure analysis revealed the presence of a ligand in the binding pocket, which was identified as cholesterol. ROR-α transcriptional activity could be modulated by changes in intracellular cholesterol levels or mutation of ROR-α amino acid residues involved in cholesterol binding. Among cholesterol derivatives capable of activating ROR-α, the most active form identified was cholesterol-sulfate. This suggests that ROR-α could play a key role in the regulation of cholesterol homeostasis and may thus be a potential drug target for cholesterol-related diseases [Kallen et al., 2002]. Other ligands suggested to bind ROR-α are melatonin [Missbach et al., 1996] and synthetic compounds like certain thiazolidinediones [Wiesenberg et al., 1998], but these observations remain unclear.

Coregulators

So far, only a limited number of co-activators like, GRIP-1 and PBP, have been shown to interact with ROR-α [Atkins et al., 1999]. Mutational analyses have also revealed that the hinge and ligand binding domains of ROR-α may interact with the nuclear co-repressors N-CoR and SMRT [Harding et al., 1997]. Tissue-specific interactions with specific co-factors may constitute the molecular basis for distinct physiological activities of ROR-α [Harding et al., 1997].

Intracellular signalling pathways driven by changes in calcium levels also modulate ROR-α activity and a calcium/calmodulin-independent protein kinase, CaMKIV, potentiates ROR-α transcriptional activity. However, no direct phosphorylation of the ROR-α protein has been shown *in vitro* [Kane and Means, 2000].

The ROR-α-deficient staggerer mouse

The staggerer mouse that carries a deletion within the ROR-α gene, has been instrumental in most studies on the physiological function of ROR-α. This model mouse is characterized by severe neuronal and immune abnormalities [Hamilton et al., 1996; Herrup and Mullen, 1979; Trenkner and Hoffmann, 1986].

Control of inflammation

Staggerer mice have defects in thymus development and display a prolonged humoral response [Trenkner and Hoffmann, 1986]. At the cellular level, an overproduction of inflammatory cytokines has been observed in macrophages from *staggerer* mice [Kopmels et al., 1992]. This inflammatory and immunomodulatory role of ROR-α has since been linked to a direct action of ROR-α on the NF-B system [Delerive et al., 2001]. Ectopic expression of ROR-α1 in human primary SMCs inhibits TNFα-induced IL-6, IL-8 and COX-2 expression. ROR-α1 negatively interferes with the NF-B signalling pathway by reducing p65 translocation. This action of ROR-α1 on NF-B is associated with the transcriptional induction of IBα, the major inhibitory protein of the NF-B signalling pathway [Besnard et al., 2001; Delerive et al., 2001].

Role in vascular function

These observations along with the recognition of atherosclerosis as an inflammatory disease of the vessel wall, has prompted to analyse the susceptibility of the *staggerer* mice to atherosclerosis. When maintained on an atherogenetic diet, *sg/sg* mice develop a severe atherosclerosis and hypoalphalipoproteinemia. This decrease in HDL level is associated with lowered apoA-I expression in the intestine but not in the liver [Mamontova et al., 1998]. During recent years additional physiological roles have been identified for ROR. These include a role

in vascular tone, since *staggerer* mice display lower blood pressure as a consequence of altered control of vasomotor tone in small resistance arteries [Besnard et al., 2002]. Ischemia-induced angiogenesis is also enhanced in *staggerer* mice [Besnard et al., 2001] and expression of endothelial NO synthase (eNOS) protein is increased in ischemic tissues of *staggerer* mice [Besnard et al., 2001].

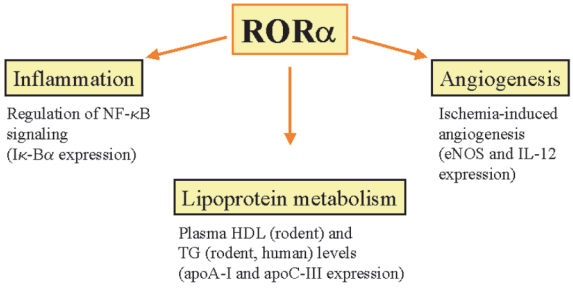

Figure 2. Metabolic and cardiovascular functions of ROR-α. See text for more details.

Lipoprotein metabolism

Staggerer mice display lowered plasma HDL cholesterol levels associated with decreased plasma apoA-I and apoA-II concentration. Expression of the murine apoA-I gene is lowered in the intestine, suggesting a physiological role for ROR-α in the intestine [Vu-Dac et al., 1997]. However, the regulation of apoA-I gene expression and, as a consequence, HDL metabolism, appears to species-specific. Indeed the RORE in the rodent apoA-I promoter is not conserved in the human gene [Vu-Dac et al., 1997]. However, ROR-α may control plasma TG metabolism both in rodents and humans. ROR-α positively regulates expression of the mouse and human apoC-III genes and in human HepG2 cells ROR-α1 activates the apo C-III gene promoter. Moreover, *sg/sg* mice have reduced plasma triglyceride and apo C-III levels [Raspe et al., 2001].

Conclusion

Altogether, these observations suggest a modulatory role for ROR-α in the control of lipid metabolism and inflammation related to CVD (Figure 2). The finding that cholesterol and/or its derivatives could be ligands for ROR-α opens the possibility to screen for synthetic agonists that may be useful to treat and prevent CVD. However, it will be necessary to develop compounds with dissociated activities, since a full agonist, while potentially exerting anti-inflammatory activities, may be expected to induce apoC-III expression and increase TG concentration. Such action on TG levels may increase the atherosclerotic risk profile. A possible application of ROR-α agonists could be in the treatment of acute inflammatory diseases.

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