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The NR4A subfamily of nuclear receptors: potential new therapeutic targets for the treatment of inflammatory diseases

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Keywords: chemokines, cytokines, inflammation, NF-κB, NR4A, transactivation, transrepression

Abbreviations

A2M; alpha-2 macroglobulin

AF-1; activating function-1

AP-1; activator protein-1

CoREST: Corepressor for Repressor Element 1 Silencing Transcription Factor

COX-2; cyclooxygenase-2DBD; DNA-binding domain

DR-5; Direct Repeat 5

EC; endothelial cells

ERK1/2; extracellular signal-regulated kinases 1/2

ETS; E26 transformation-specific sequence 1

GR¸Glucocorticoid Receptor

GSK3β; glycogen synthase kinase 3β

ICAM-1; intercellular adhesion molecule 1

IKK; IkB kinase

IL; interleukin

JNK; Jun N-terminal kinase

LBD; ligand binding domain

LPS; lipopolysaccharide

MAPK; mitogen-activated protein kinase

MCP-1; monocyte chemotactic protein 1

MMPs; matrix metalloproteinases

NBRE; NGFI-B-response element

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NCOR2: Nuclear Receptor Corepressor 2

NF-κB; nuclear factor κB

NGF; Nerve growth factor

NGFI-B; Nerve growth factor-induced clone B

NOR-1; Neuron-derive Orphan Receptor-1

NRs; Nuclear Receptors

Nurr1; Nurr-related factor 1

NurRE; Nur-response element

oxLDL; oxidized low-density lipoprotein

PAI-1; plasminogen activator inhibitor-1

PGE2; prostaglandin E2

PPARs; Peroxisome Proliferator-Activated Receptors

RAR; retinoic acid receptor

RXR; retinoid X receptor

SMC; smooth muscle cells

SRC-1; steroid receptor coactivator-1

TIMP-2, tissue inhibitor of metalloproteinases 2)

TNFα; tumor necrosis factor α

VCAM-1; vascular cell adhesion molecule 1

VSMC; vascular smooth muscle cells

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Abstract

Introduction

Prolonged inflammatory response contributes to the pathogenesis of chronic disease-related disturbances. Among nuclear receptors (NRs), the orphan NR4A subfamily, which includes Nur77 (NR4A1), Nurr1 (NR4A2) and NOR1 (NR4A3), has recently emerged as a therapeutic target for the treatment of inflammation.

Areas covered

This review focuses on the capacity of NR4A receptors to counter-regulate the development of the inflammatory response, with a special focus on the molecular transrepression mechanisms.

Expert opinion

Recent studies have highlighted the role of NR4A receptors as significant regulators of the inflammatory response. NR4A receptors are rapidly induced by inflammatory stimuli, thus suggesting that they are required for the initiation of inflammation. Nevertheless, NR4A anti-inflammatory properties indicate that this acute regulation could be a protective reaction aimed at resolving inflammation in the later stages. Therefore, NR4A receptors are involved in a negative feedback mechanism to maintain the inflammatory balance. However, the underlying mechanisms are not entirely clear. Only a small number of NR4A-target genes have been identified, and the transcriptional repression mechanisms are only beginning to emerge. Despite further research is needed

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to fully understand the role of NR4A receptors in inflammation, these NRs should be considered as targets for new therapeutic approaches to inflammatory diseases.

Article highlights

- Inflammation is an adaptive biological response to infection, injury or autoimmune processes that aims to restore tissue structure and function. While acute inflammation is considered to be a beneficial mechanism that returns tissue to homeostasis, prolonged inflammation is associated with the pathogenesis of chronic disease disturbances.

- NR4A orphan nuclear receptors are early-response genes recently involved in inflammatory processes. The three NR4A receptors are rapidly induced by pro-inflammatory stimuli and are all involved in the counter-regulation of the later stages of inflammation.

- Despite the role of the NR4A in the counter-regulation of the inflammatory response has been well stablished, the precise contribution of these molecules guiding inflammatory responses and outcomes could be cell specific, since in some cell types they show pro-inflammatory features.

- The molecular mechanisms through which NR4A receptors are involved in the counter-regulation of inflammation include the direct binding to NR4A-target gene promoters (transactivation) and mechanisms that are independent of their DNA-binding capacity (transrepression).

- The identification of structurally diverse compounds that regulate NR4A activity has opened the door to the potential exploitation of these NRs as a therapeutic approach to inflammatory diseases.

- Although the findings are promising, extrapolating them to a clinical setting remains elusive, since further development of new selective regulators for each NR4A is required. Additionally, assessment of the efficacy and safety of the new NR4A regulators is required before they can be considered appropriate for clinical use.

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1. Introduction

Inflammation is an adaptive biological response to an external challenge or cell injury and is characterized by the recruitment of immune cells to damaged regions. However, a prolonged inflammatory response contributes to the pathogenesis of chronic disease-related disturbances. Increasing evidence suggest that several nuclear receptors (NRs) play a protective role in the regulation of both acute and chronic inflammatory responses. Within the NR superfamily, the NR4A subfamily of orphan NRs has recently emerged as a new player in the counter-regulation of the inflammatory response in several cell types. The three NR4A receptors are rapidly induced by inflammatory insults via nuclear factor κB (NF-κB)-mediated transactivation of the NR4A promoters, and all three inhibit the activity of this transcription factor through several regulatory mechanisms. Therefore, the NR4A receptors are involved in a negative feedback mechanism that maintains the inflammatory balance.

2. General signaling pathways involved in inflammation: Role of NF-κB and AP-1

Inflammatory diseases represent one of the world's most significant health issues. Over the last decade, increasing evidence has shown a strong association between chronic low-level inflammation and a broad range of pathological conditions, including rheumatoid arthritis, inflammatory bowel disease, cancer, cardiovascular disease and metabolic disorder. Inflammation is an adaptive biological response to infection, injury or autoimmune processes and is characterized by the recruitment of immune cells to damaged regions to restore tissue structure and function [1, 2]. It is believed that acute

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inflammation is a beneficial mechanism that returns tissue to homeostasis. However, prolonged inflammation is often associated with more serious complications, so it is crucial to avoid the onset of chronic inflammation.

At the molecular level, inflammation is characterized by the activation of several pro-inflammatory pathways, thus leading to gene expression and the release of the cytokines and chemokines involved in the infiltration of leukocytes (macrophages, neutrophils, etc.) in inflamed regions [3, 4, 5]. Moreover, during the inflammatory process there is an increase in the production of many inflammatory mediators, including growth factors, lipid-derived mediators (prostanoids, leukotrienes, etc.), membrane receptors, adhesion molecules, matrix metalloproteinases (MMPs) and other enzymes involved in the progression of inflammation. Conversely, the production of anti-inflammatory cytokines is repressed during this process, in such way that inflammation can be defined as a mismatch in the balance between pro- and anti- inflammatory mediators. The rise in the production of pro-inflammatory molecules is due to the activation of several signal transduction systems that ultimately induce the activity of transcription factors such as NF-κB and the activator protein-1 (AP-1), which are the most important regulators of inflammation (Figure 1) [6, 7, 8, 9, 10, 11]. These two transcription factors coordinate the transcriptional reprogramming of immune cells, thus activating the expression of pro-inflammatory cytokines, chemokines, adhesion molecules, MMPs and other pro-inflammatory molecules.

NF-κB is a transcription factor composed of homo- and heterodimers of the Rel family of proteins (p65 (RelA), RelB, c-Rel, p105/p50 (NF-κB1) and p100/p52 (NF-κB2)), the most common of which is the heterodimer consisting of the p50 and p65 subunits [12,

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13]. In resting cells, NF-κB remains inactive in the cytoplasm complexed with a family of inhibitory proteins called IκBs [12]. After stimulation, the heterotrimeric IκB kinase (IKK) complex is activated, thus promoting phosphorylation, polyubiquitination and the subsequent proteasomal degradation of IκBs (Figure 1) [14, 15, 16, 17, 18, 19, 20]. Alternatively, NF-κB can also be complexed with the precursor Rel proteins (p105 and p100) in the non-canonical signaling pathway and rests inactive in the cytoplasm. IKK is also involved in the proteolytic processing of p100 into p52, which leads to NF-κB activation [7, 21]. Consequently, free NF-κB translocates into the nucleus to regulate the expression of several pro-inflammatory molecules involved in inflammation. Furthermore, post-translational modifications of the NF-κB proteins such as acetylation [22, 23, 24, 25], SUMOylation [26] and phosphorylation [23, 27] can also regulate their transcriptional activity.

The AP-1 family of transcription factors is composed of four subfamilies: Jun (c-Jun, v-Jun, Jun B and Jun D), Fos (c-Fos, Fos B, Fra-1 and Fra-2), MAF (MAFA, MAFB, c-MAF, NRL, MAFF, MAFG and MAFK) and activating transcription factor [ATF2, ATF3, B-ATF, Jun dimerization protein (JDP)-1, JDP-2] [28, 29, 30, 31]. The most common form of AP-1 is the Jun-Fos heterodimer [32]. AP-1 activity can be regulated at different stages, including changes in AP-1 subunit mRNA and protein levels. Additionally, the AP-1 activity can be further modified through the binding of specific proteins and other transcription factors and via post-translational regulation of the AP-1 proteins (for review see [2]).

3. NRs and inflammation. Transrepression mechanisms

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The NR superfamily is composed of a large number of transcription factors that act as intracellular receptors, thereby regulating the transcription of genes involved in nearly all aspects of development and adult physiology (Figure 2A) [33, 34, 35]. Furthermore, NRs crosstalk with several signal transduction systems and inhibit the activity of other transcription factors through a mechanism that is independent of their DNA-binding capacity (Figure 2B). This mechanism, termed transrepression, is responsible for most of the anti-inflammatory effects of NRs. Thus, through the transrepression mechanism, NRs suppress the activity of several transcription factors, including NF-κB and AP-1 (for review see [36]). There are three main transrepression strategies by which NRs regulate the activity of other transcription factors. In the first strategy, transrepression is the result of the competition for limiting amounts of shared co-regulatory proteins that makes them unavailable to other transcription factors [37, 38, 39]. Second, NRs block the activation of other transcription factors by establishing protein-protein interactions with them and blocking their transcriptional activity, thus acting themselves as co-regulators [38, 39, 40]. Finally, NRs are able to regulate the activity of certain transcription factors that are downstream of specific cellular signaling pathways, via inhition of the mitogen-activated protein kinase (MAPK) [41].

The anti-inflammatory properties of NRs make them interesting targets for the treatment of a broad range of inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease, cancer, cardiovascular disease and metabolic disorder. In recent years, there has been extensive evidence of the anti-inflammatory role of well-characterized ligand-activated NRs, including peroxisome proliferator-activated receptors (PPARs), the glucocorticoid receptor (GR) and retinoid receptors such as the retinoid X receptor (RXR) and the retinoic acid receptor (RAR). However, the ligands and physiological

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functions of many orphan NRs remain unknown [42]. Among these orphan NRs, the NR4A family has recently been associated with several inflammatory processes, and these orphan NRs therefore represent a promising area for research and development, since they are potential targets for new therapeutic strategies against inflammatory diseases.

4. Specific features of the NR4A family of nuclear receptors

The NR4A family consists of three members: Nur77 (NR4A1 according to the unified nomenclature system for the NR superfamily), Nurr-related factor (Nurr)1 (NR4A2) and the neuron-derived orphan receptor (NOR)-1 (NR4A3) [43, 44]. Nur77, also known as NGFI-B (nerve growth factor-induced clone B), was the first member of the NR4A family to be identified as a gene induced by NGF (nerve growth factor) in the rat pheochromocytoma cell line PC12 [45]. Nurr1 was characterized in dopaminergic neurons as a "brain-specific" transcription factor [46], and NOR-1 was first identified by Okura *et al*. in forebrain neural cells undergoing apoptosis [47].

The similarity of the genomic structures of the NR4A family members suggests that these receptors are derived from a common ancestral gene [48]. As with other NRs, NR4A receptors are composed of several functional domains, including an N-terminal activation function 1 (AF-1) domain, a central DNA-binding domain (DBD) with two zinc fingers that allow the DNA to interact and a C-terminal ligand binding domain (LBD) [33]. However, unlike other NRs, where the LBD is essential for the recruitment of small lipophilic molecules (ligands) that function as molecular switches in the transcriptional activity of these receptors, NR4A receptors encode an atypical LBD. X-ray crystallography studies reveal that the structure of the Nurr1 LBD contains tightly

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packed, bulky hydrophobic residues instead of the ligand-binding pocket found in other NRs [49]. Similar studies performed in DHR38, the Drosophila ortholog of the mammalian NR4A family, have shown the absence of a classic ligand-binding site [50]. Furthermore, molecular modeling studies have demonstrated that the LBDs of Nur77 and NOR-1 also encode unusual hydrophilic surfaces [51, 52]. Based on these studies, the possibility that the NR4A family of NRs is regulated by naturally occurring ligands was initially ruled out, and it was therefore suggested that they act as constitutively active and ligand-independent transcription factors [49, 53]. However, although endogenous ligands for these molecules have not yet been identified, there is increasing evidence that structurally diverse synthetic molecules interact directly with the LBD of the NR4A receptor, and that both act as agonists or antagonists (for review see [54]). In addition, a large number of extracellular stimuli regulate the activity of these NR4A receptors. Post-translational modifications such as phosphorylation and SUMOylation [55, 56, 57], or changes in their mRNA levels, are the most common mechanisms that regulate NR4A activity. Interestingly, the basal expression of these NRs is low, but it is rapidly induced in response to changes in environmental factors, and they act as early-response genes in the presence of extracellular cues (for review see [43, 58]). Once its expression is induced, NR4A receptors regulate transcription by binding to specific sequence sites in the promoter region of their target genes (Figure 3). Specifically, these NRs bind as monomers to a DNA-specific octamer sequence (AAAGGTCA) known as the NGFI-B-response element (NBRE) [59], and as homodimers or NR4A-heterodimers to NBRE palindromic sequences (TGATATTTn6AAATGCCA) called the Nur-response element (NurRE) [60]. Furthermore, Nur77 and Nurr1 (but not NOR-1) bind as heterodimers with the 9-cis retinoic acid receptor (RXR) to an imperfect direct repeat sequence separated by five nucleotides (GGTTCACCGAAAGGTCA) (direct repeat 5,

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DR-5) [61, 62]. While it was initially thought that these transcription factors only regulated gene expression through transactivation mechanisms by binding to these response elements, recent studies have provided evidence that NR4A receptors also influence gene transcription through transrepression mechanisms by crosstalking with several transduction systems. Through these two mechanisms, NR4A receptors are involved in the regulation of key cellular functions, including inflammation, proliferation and cell survival [44, 63].

5. Pharmacological regulation of the NR4A receptors

As mentioned above, the NR4A show an atypical LBD filled by tightly packed, bulky hydrophobic residues [49, 50, 51, 52], which hinders the binding of naturally occurring ligands. However, it have been identified diverse structural molecules able to modulate the NR4A activity (for review see [59]). Prostaglandin A2 has been reported as NOR-1 ligand [64], and the antimetabolite cancer drug 6-mercaptopurine has been identified as resgulator of Nurr1 and NOR-1, acting through their N-terminal AF-1 domain [65]. Additionally, several benzimidazole and isoxazolopyridinone compounds have shown high affinity for the Nurr1 [66]. Cytosporone B (CsnB) and related compounds, ethyl [2,3,4-trimethoxy-6-(i-octanoyl)phenyl]acetate (TMPA), and 1-(3,4,5-trihydroxyphenyl)nonan-1-one (THPN) bind to different amino acid residues within the LBD of Nur77, thus modulating the activity of this NR [67, 68, 69, 70]. However, whereas CsnB [69, 71, 72] and THPN [70] act as activators of this NR, TMPA shows antagonic properties [68]. Additonally, diindolylmethane compounds (C-DIM) are able to modulate the Nur77 transactivation (for review see [54]), through the direct binding of these compounds to specific sites in the LBD [73]. However, it is worth to note that most C-DIMs compounds show an inhibitory effect of the Nur77 transactivation [73].

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Since the LBD is highly conserved among the three NR4A, the C-DIMs compounds could potentially modulate the transcriptional activity to Nurr1 and NOR-1. Specifically, the Nurr1 regulation by the C-DIM12 compound has been already demonstrated [74].

6. Role of NR4A in inflammatory-related conditions

The NR4A receptors have been associated to a wide range of pathological inflammatory-related conditions, such as cancer, immune alterations and metabolic, cardiovascular or neurological diseases (for review see [54]). Following we briefly discuss the involvement of the NR4A in some of these pathologies, paying special attention to role of these NRs in the counter-regulation of the inflammatory response.

6.1. Role of the NR4A in cancer

In cancer, the NR4A family show a dual role, exhibiting both pro-oncogenic or tumor suppressor-like activies [75]. Nur77 and Nurr1 have been related to decrased survival. However, the function of NOR-1 in these pathologies has not been fully explored. Interestingly, several studies with C-DIMs compounds have identified these molecules as effective anticancer agents [54], proposing the NR4A as molecular targets for pharmacological intervention against these diseases. Members of the NR4A receptors have been found expressed in a wide range of solid tumors (for review see [54]), and are involved in cell proliferation, survival, cell cycle progression, migration and invasion (for review see [54]). The molecular mechanisms underlying the pro-oncogenic effects of NR4A involved nuclear export (for review see [75] and [54]) and the formation of a pro-apoptotic mitochondrial NR4A-bcl2 complex [76, 77]. It has been recently shown that Nurr1 nuclear exclusion in oxidative stress-induced necrosis is mediated by the

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Apoptosis Signal-reglating kinase 1 (ASK1)-p38 pathway [78]. Additionally, some NR4A, such as Nur77, inactivate p53 and AMPKα [79], and act as cofactor for regulating the expression of Specificity protein (Sp)-target genes with GC-rich promoters, thereby regulating the expression of several genes involved in cancer progression [73, 80, 81, 82].

6.2. Role of the NR4A in neuronal system

The NR4A receptors have been involved in relevant neuronal functions [83, 84, 85, 86], and mediate CREB-dependent neuroprotection [84, 87, 88]. The three NR4A are induced in mice hippocampus by induced learning by contextual fear conditioning or histone deacetylase inhibitor-induced (HDAC) enhanced memory [84]. However, they are differentially expressed according with the brain area [89, 90] showing differential functions. Nur77 is need for object location [85], and it has been related to synaptic remodeling, behavioral changes, dopaminergic loss and response to L-DOPA in animal models [86, 91, 92, 93]. Additionally, it has been proposed as a therapeutic target for the treatment of psychosis [91, 94] and strokes [95]. Nurr1 is involved in long term memory, object location and recognition [84]. Despite Nurr1 is expressed throughout the brain, its function has been mainly studied in dopaminergic neurons and Parkinson Disease [38, 54, 96, 97]. Finally, NOR-1 has been related to depressive behaviour [98] and nicotine addiction in patients with mental health disease [99]. Interestingly, NR4A regulators such as the C-DIMs compounds, have been also proposed for the treatment of Parkinson Disease, since these molecules prevent loss of dopaminergic neurons [96, 97, 100, 101, 102].

6.3. Role of the NR4A in metabolism

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Since the NR4A regulate the expression of a large number of genes involved in both glucose and fatty acid metabolism (for review see [66]), this family of NRs seems to play a pivotal role in the control of metabolic regulation. The three NR4A are induced in obese subjects and are reduced after fat loss [103]. In addition, they are induced in liver of a murine model of dietary restriction, positively correlating with improved glucose utilization an insulin sensitivity [104]. Further studies have highlighted the role of Nur77 [68, 69, 103, 105, 106] and Nurr1 [66, 104] in the control of glucose homeostasis and metabolic diseases such as type 2 diabetes mellitus (T2DM). Despite the role of NOR-1 in experimental models of obesity and T2DM have not been fully investigated, several studies also support a relevant role of this NR in the control of energy metabolism [54]. Interestingly, several experimental studies using both NR4A agonists (CsnB) [69] and antagonists (TMPA) [68] have highlighted the involvement of these NRs in the control of glucose homeostasis, proposing them as potential therapeutic targets for the treatment of metabolic diseases.

6.4. Role of the NR4A in cardiovascular disease

Increasing evidences have identified the NR4A receptors in cardiovascular disease (CVD) (for review see [43, 54, 107, 108]). The NR4A receptors are induced by a wide range of stimuli in vascular cells and have been found induced in atherosclerosis lessions in human subjects and experimental models [54]. However, while Nur77 and Nurr1 show a protective role, NOR-1 promotes the development of atherosclerotic lesions [108]. The opposite effect of the NR4A members on CVD is due to their different actions on vascular cells. Nur77 inhibits vascular smooth muscle cells (VSMC) proliferation [109, 110] and reduces neointimal hyperplasia induced by balloon injury [111]. Additionally, Nur77 controls endothelial cell proliferation and

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angiogenesis [112, 113, 114] and decreased atherosclerotic plaque formation [72, 115, 116]. Recently, Nur77 have been also involved in the attenuation of the cardiac hypertrophy in response to β-adrenergic stimuliation [117]. Nurr1 reduces SMC proliferation in in-stent restenosis [118], and inhibits oxidized low-density lipoprotein (oxLDL) uptake [119] and reduces pro-inflammatory gene expression in macrophages [38, 120]. Conversily, NOR-1 induces VSMC proliferation [54] and enhances neointintima hyperplasia after mechanical injury [121, 122]. Moreover, in endothelial cells NOR-1 mediates the VEGF-induced proliferation [123, 124] and the expression of cell adhesion molecules [125]. However, in VSMC NOR-1 shows anti-inflammatory properties [41], suggesting a dual role of this NR in the development of the atherosclerotic process. Despite further studies are need to fully characterize the role of NR4A in CVD, it is worth to note that treatment with NR4A agonists, such as Csn, show a protective cardiovascular role [72].

6.5. Role of the NR4A in immune response

The NR4A has been also involved in the regulation of the immune response. All three are crucial in the regulation of T reg cell [126, 127] and CD81 T cells [128] development, taking part in both autoimmunity and in resolution of infections. Furthemore, some NR4A members, such as Nur77, also take part in the development and maturation of both $Ly6C^{low}$ [129] and $Ly6C^{high}$ [130] monocytes. Specifically, Nur77 depletion has been related to macrophage differentiation toward as a pro-inflammatory M1 phenotype [116, 131] and Nurr1 controls the expression of genes characteristics of M2 phenotype and protects against endotoxin-induced sepsis [132].

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7. Role of the NR4A in the counter-regulation of the inflammation. Molecular mechanisms

The receptors of the NR4A family were initially described as pro-inflammatory factors [133, 134] when the three NR4A receptors were found to be aberrantly expressed in human inflamed synovial tissue, colorectal cancer cells, psoriasis, atherosclerotic lesions and multiple sclerosis [58, 71, 109, 119, 134, 135, 136, 137]. In fact, the three members of the NR4A family are strongly upregulated in activated primary cells by a huge range of pro-inflammatory stimuli, including lipopolysaccharide (LPS), cytokines and oxidized lipids, thereby suggesting that these receptors may play a role in the transcriptional regulation of inflammatory signaling [133, 135, 138, 139]. Pei *et al.* demonstrated that regulation of the NR4A family by inflammatory stimuli in macrophages is mediated by NF-κB activation (Figure 4) [133]. Two highly conserved NF-κB sites critical for LPS responsiveness were identified in the Nur77 promoter [133]. In addition, Nur77 overexpression in macrophages induces the expression of inducible kinase IKKi/IKKε [134], which promotes IκBs degradation by phosphorylation and NF-κB activation. Regulation of IKKi by NR4A receptors is dependent on their binding to a functional NR4A binding site in the IKKi promoter, which would suggest that IKKi is a direct NR4A-target gene [133, 134]. However, recent reports have suggested that members of the NR4A family can act as repressors of cell type-specific inflammatory responses, since they are involved in the counter-regulation of the inflammatory response in macrophages, endothelial cells (EC) and other cell types [38, 40, 41, 113, 116, 118, 131, 140]. For instance, lentiviral overexpression of the three NR4A receptors reduces the expression of pro-inflammatory cytokines and chemokines in human macrophages, possibly by inhibiting macrophage

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differentiation [119], and all of them are essential for thymic regulatory T cell development and immune homeostasis [126].

7.1. Anti-inflammatory role of Nur77

Several reports have demonstrated the anti-inflammatory properties of Nur77. Using a systematic, genome-wide survey, Nur77 was identified as the strongest NF-κB inhibitor in a monocyte-adherent model; it inhibited NF-κB activation by tumor necrosis factor α (TNFα) and interleukin (IL)-1β in similar levels to those reported for IκBα [141]. Shortly afterwards, Bonta *et al.* showed that Nur77 overexpression reduces mRNA levels and the production of pro-inflammatory cytokines and chemokines such as IL-1β, IL-6, IL-8 and monocyte chemotactic protein 1 (MCP-1) [119]. Furthermore, the authors described that Nur77 overexpression represses the activation of the IL-2 promoter in front of several stimuli, by preventing the NF-κB interaction with two low-affinity binding sites in its promoter [40]. However, Nur77 was unable to repress the NF-κB binding to high affinity elements. In addition, the authors identified that the regulation of NF-κB by Nur77 involved elements placed in the C-terminal sequence of p65. In fact, a direct interaction between p65 and the C-terminal region of Nur77 had been demonstrated previously (Figure 4) [39]. Such interaction prevents the binding of steroid receptor coactivator-1 (SRC-1) to this region, thereby blocking the SRC-1 enhancement of Nur77 transactivation and the suppression of steroidogenic genes. Nevertheless, both reports show a reciprocal response in which the crosstalk between these two proteins results in NF-κB inhibition, but also in the blockage of Nur77 transcriptional activation [39, 40]. However, while some authors underline the relevance of the Nur77 N-terminal region [40], others show that p65 binds to its C-terminal region [39], thereby suggesting that both domains are required for optimal crosstalk between these two proteins.

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Recently, it has been shown that Nur77 phosphorylation by p38α in response to LPS promotes the dissociation between this NR and p65, favouring the inflammatory response, and that treatment with n-pentyl 2-[3,5-dihydroxy-2-(1-nonanoyl)-phenyl]acetate (PDNPA), a chemical compound which impedes the binding between p38α and Nur77, inhibits Nur77 phosphorylation and NF-κB activation [142]. Additionally, it has been recently shown that Nur77 also prevents the LPS-induced sepsis and acute liver injury by direct binding to Tumor necrosis factor receptor associated factor 6 (TRAF6) [143]. Apart from direct protein-protein interaction, the crosstalk between Nur77 and the inflammatory response can be mediated through other transrepression mechanisms. For instance, Nur77 expression inhibits NF-κB nuclear translocation by inducing IκBα expression at transcriptional level (Figure 4), thereby blocking the expressions of vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) and reducing monocyte adhesion in both TNFα- and IL-1β-activated EC [113]. Moreover, this NR suppresses the oxLDL-induced inflammatory response in macrophages through a mechanism that involves the inhibition of cyclooxygenase-2 (COX-2) [140]. Nur77 is also involved in the regulation of Treg cell [126, 127] and CD81 T cells [128] development. In addition, Nur77 deficiency reduces the development of $Ly6C^{low}$ monocytes by impairing its differentiation from a myeloid dendritic precursor [129], and enhances the levels of CCR2 in the surface of $Ly6C^{high}$ monocytes, promoting myocardium infiltration and differentiation to inflammatory macrophages [130]. Interestingly, bone marrow-derived macrophages from $Nur77^{-/-}$ mice exhibit altered expression of M2-specific markers and an inflammatory M1-phenotype, and $Nur77^{-/-}$ bone-marrow transplantation in $Ldlr^{-/-}$ mice leads to the presence of larger atherosclerotic lesions than those observed in mice transplanted with bone marrow from wild-type mice [131]. The role of Nur77 depletion

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in the development of macrophage polarization towards a pro-inflammatory M1 phenotype was further confirmed by Hanna *et al*, who showed that Nur77 deficiency in monocytes and macrophages enhances toll-like receptor signaling and increases atherosclerosis in both $ApoE^{-/-}Nur77^{-/-}$ and $Ldlr^{-/-}Nur77^{-/-}$ mice fed with a western diet [116]. Additionally, Nur77 mediates the inhibition of the inflammatory signaling by the apoptotic cells once they have been phagocytized by macrophages [144].

Nur77 has also been involved in the regulation of MMP activity (Figure 4) [115, 145, 146, 147]. Transgenic mice overexpressing Nur77 in VSMC exhibit low MMP-2 levels in remodeled carotid arteries [115] and Nur77 overexpression reduces MMP-2 mRNA and protein levels in VSMC [147]. In addition, Nur77 may modulate the activity of other MMPs by modulating TIMP-2 (tissue inhibitor of metalloproteinases 2) protease expression in some tissues [145]. Nur77 further induces the expression of other MMP inhibitors in several cell types. For instance, in human endothelial cells Nur77 controls the expression of the serine protease inhibitor (serpin) E1 (plasminogen activator inhibitor-1, PAI-1) [146], in HepG2 it regulates the serpin A3 (alpha 1-antichymotrypsin) transcription [148] and it has been recently shown that in human VSMC this NR regulates the promoter activity of the antiproteinase alpha-2 macroglobulin (A2M) [147]. These data suggest that this NR modulates MMP activity by regulating the expression of these molecules in different tissues.

7.2. Anti-inflammatory role of Nurr1

Nurr1 has been also involved in the regulation of the inflammatory response. Interestingly, Nurr1 was found expressed in inflammatory-related conditions, such as in human in-stent restenosis and it was shown that endogenous Nurr1 protected against

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vascular lesion formation in the carotid arteries of $ApoE^{-/-}$ mice [118]. Such regulation is consistent with the inflammatory response associated with these alterations, since it has been found that Nurr1 expression is induced in cultured human (SMC) in response to serum or TNFα [118]. Interestingly, Nurr1 overexpression reduces TNFα-induced IL1β, TNFα and MCP-1 mRNA levels, and shRNA-mediated knockdown of endogenous Nurr1 strongly increases the expression of TNFα in these cells, thereby demonstrating the involvement of this NR in the regulation of both the basal- and TNFα-induced inflammatory response in vascular cells [118]. Indeed, the same authors had previously reported that Nurr1 also inhibits the inflammatory response in macrophages [119], which supported the data found in a genome-wide screen in monocytes and indicated that Nurr1 acts as a novel NF-κB inhibitor [141]. Specifically, it has been shown that Nurr1 depletion enhances NF-κB activation and promotes the expression of inflammatory markers in front of TLR/adenosine receptor stimulation [149]. Similar to other NR4A, Nurr1 also regulates T cell maturation and development [66, 126, 150] and takes part in maintaining the M2 macrophage phenotype to protect against endotoxin-induced sepsis [132].

Moreover, several reports have shown that Nurr1 regulates MMP activity in different tissues [147, 151, 152] through a wide range of mechanisms. Despite the fact that Nurr1 overexpression only reduces the MMP-2 mRNA levels in human VSMC, it reduces both MMP-2 and MMP-9 protein levels and gelatinolytic activities in these cells [147]. The molecular mechanism underlying such regulation involves the upregulation of the broad-spectrum antiprotease A2M through a transcriptional mechanism (Figure 4) [147]. In addition, Mix *et al.* demonstrated that Nurr1 represses MMP gene expression in cartilage, thus highlighting the protective role of Nurr1 in inflammatory joint disease

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[151]. Nurr1 expression is strongly induced by COX-2-derived prostaglandin E2 (PGE2) in chondrocytes and is associated with preventing the induction of MMP-1 levels found in IL-1β-stimulated cells. Indeed, the authors demonstrated that transient Nurr1 overexpression suppresses MMP-1 promoter activity and reduces both endogenous MMP-1 mRNA and secreted protein levels. Furthermore, Nurr1 overexpression also prevents the IL-1β-induced expression of MMP-1, 3 and 9 through a molecular mechanism that involves a transcriptional antagonism between this NR and ETS (E26 transformation-specific sequence) 1 [151]. Interestingly, the Nurr1-mediated repression of the MMP-1 promoter does not require NBRE sequences; likewise, the repression of aromatase transcription by Nurr1 had previously been described [153]. The Nurr1-responsive regions of the MMP-1 and aromatase promoters overlap with the positive regulatory sequences of the ETS1 and cyclic adenosine monophosphate (cAMP)-regulated regions, respectively, thereby suggesting that Nurr1 may confer transcriptional repression by interfering with these transcription factors. In addition to this transcriptional antagonism, Nurr1 may be involved in the control of inflammation in synovial tissue and cartilage through the transcriptional regulation of osteopontin [154], which may inhibit inflammation in cartilage by blocking some of the effects of IL-1β [155]. Conversely, other reports have attributed a pro-inflammatory role of Nurr1 in synoviocytes in arthritis [156], and Nurr1 can directly transactivates some MMP family members, such as MMP-13, in human synoviocytes [152].

Nurr1 has also been found to act as a key factor in suppressing the production of inflammatory neurotoxic mediators that trigger the death of dopaminergic neurons [38]. Nurr1 expression is induced by inflammatory stimuli, and the loss of this NR results in exaggerated and prolonged inflammatory responses in microglia and astrocytes, thereby

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enhancing the loss of tyrosine hydroxylase-expressing neurons in response to LPS. Interestingly, Nurr1 activators such as C-DIM compounds, suppress the expression of pro-inflammatory genes in primary astrocytes and prevent loss of dopaminergic neurons [101, 102], through inhibition of NF-κB-target neuroinflammatory gene expression and increase of proteins tyrosine hydroxylase and the dopamine transporter [97, 100]. Saijo *et al.* clearly demonstrated that attenuation of the inflammatory response in these cells is due to the recruitment of Nurr1 by inflammatory gene promoters through mechanisms other than direct binding to DNA-specific sequences [38]. These mechanisms involve the glycogen synthase kinase 3β (GSK3β)-mediated phosphorylation of Ser468 of p65 and SUMOylation of Nurr1 at key lysine residues, and lead to Nurr1-p65 docking (Figure 4). Nurr1 then recruits the Corepressor for Repressor Element 1 Silencing Transcription Factor (CoREST) and the Nuclear Receptor Corepressor 2 (NCOR2) complexes, which result in transcriptional repression [38, 96]. In addition, CoREST mediates p65 clearance from the target promoters and restores gene expression at basal state [38].

Additionallly, Nurr1 has been also involved in other inflammatory related processes. For instance, it has been shown that the Apoptosis Signal-reglating kinase 1 (ASK1)-p38 pathway promotes the Nurr1 nuclear exclusion in oxidative stress-induced necrosis [78] and it may prevent liver fibrosis reducing hepatic stellate cell proliferation and extracellular matrix by MAPK activation [157].

7.3. Anti-inflammatory role of NOR-1

In contrast to Nur77 and Nurr1, the function of NOR-1 in the counter-regulation of the inflammatory response could be cell-type specific. While NOR-1 overexpression

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reverses inflammatory response in THP-1 macrophages challenged to inflammatory stimuli [119], in NOR-1-deficient endothelial cells this NR shows a pro-inflammatory role [125]. In human endothelial cells NOR-1 overexpression mediates monocyte adhesion by increasing adhesion molecules. Conversely, NOR-1 deficiency decreases adhesion molecule expression in front of inflammatory stimuli and reduces the macrophage content in the lesion found in aorta of hypercholesterolemic aopoE$^{-/-}$ mice [125]. The role of NOR-1 in the development of vascular lesions has been studied in depth [121, 122, 158]. NOR-1 knockout mice present a decrease in neointima formation after femoral artery denudation injury [158], and NOR-1 overexpression in SMC exacerbates neointimal hyperplasia after permanent ligation of the left common carotid artery [121, 122]. These apparently contradictory results could be due to the fact that the atheroma produced by hypercholesterolemia in apoE$^{-/-}$ mice differs greatly from injuries caused by mechanical stimuli. While NOR-1 deficiency in endothelial cells reduces the development of diet-induced atherosclerotic lesion in apoE$^{-/-}$ mice by decreasing macrophage infiltration, lesions induced by mechanical insults are due mainly to SMC proliferation rather than inflammatory cell infiltration. Indeed, in both genetically modified NOR-1 models, macrophage infiltration is barely detected in the injured areas. It is worth noting that the role of NOR-1 in the inflammatory response of macrophages is the complete reverse of that found in endothelial cells. Bonta *et al.* showed that, as with Nur77 and Nurr1, NOR-1 lentiviral overexpression in macrophages reduces the inflammatory response and oxLDL uptake [119]. In addition, the authors demonstrated that NOR-1 knockdown results in enhanced cytokine and chemokine synthesis and increased lipid loading, thereby indicating that endogenous NR4A takes part in the inhibition of macrophage activation, foam-cell formation and differentiation [119]. The anti-inflammatory properties of NOR-1 were further demonstrated when this NR was

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also identified as a novel NF-κB inhibitor in a genome-wide survey assay performed in adherent monocytes [141]. Interestingly, NOR-1 also takes part in T cell regulation and development [66, 126]. However, the molecular mechanisms and signaling pathways underlying the NOR-1 anti-inflammatory response were not fully demonstrated in these works. A new piece was recently added to the puzzle when it was found that NOR-1 also plays an anti-inflammatory role in VSMC [41]. NOR-1 overexpression reduced cytokine expression in VSMC in response to inflammatory stimuli such as LPS, TNFα and oxLDL, whereas NOR-1 silencing increased basal mRNA cytokine levels. These data suggest that this NR is involved in both the basal and induced regulation of the inflammatory process. These findings were further confirmed in the aortas from transgenic mice that overexpress human NOR-1 in SMC, thereby showing attenuated upregulation of pro-inflammatory mediators in response to LPS treatment compared to wild-type littermates. The molecular mechanisms underlying such regulation involve reduced phosphorylation of cellular signaling pathways such as extracellular signal-regulated kinases 1/2 (ERK1/2), p38 mitogen-activated protein kinase and Jun N-terminal kinase (JNK) (Figure 4). In addition, NOR-1 overexpression further retards LPS-induced IκBα phosphorylation/degradation and attenuates p65 phosphorylation in Ser^{536} and p65 nuclear translocation (Figure 4). These findings provide an explanation for the reduction in the DNA-binding capacity/activation of NF-κB and the subsequent expression of inflammatory mediators.

NOR-1 is also involved in the regulation of MMP activity. In a similar way to other NR4A receptors, NOR-1 overexpression downregulates MMP-2 expression in human VSMC and reduces secreted protein levels and the gelatinolytic activities of both MMP-2 and MMP-9 [147]. In line with *in vitro* data, the expression of MMP-2 (but not MMP-

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9) was found to be reduced in aorta from transgenic mice overexpressing NOR-1 in SMC, compared to wild-type littermates. Further, *in vivo* NOR-1 overexpression attenuates both LPS-induced MMP-2 and MMP-9 mRNA levels [147]. Despite the protective role of NOR-1 that controls the MMP levels/activity observed in both models, the underlying mechanisms between humans and mice may differ. While the effect of the NOR-1 overexpression that reduces both MMP-2 and MMP-9 gelatinolytic activities in humans is at least partially mediated by direct NOR-1 transactivation of the A2M (Figure 4), the mouse A2M promoter is unresponsive to NOR-1 [147]. *In silico* analysis revealed that the NBRE site in the human A2M promoter, which is responsible for NR4A-mediated regulation, is not conserved in mice. Therefore, since MMP-2 and MMP-9 are regulated by NF-κB in VSMC [159], the anti-inflammatory activity of NOR-1 that inhibits this transcription factor could explain the observed modulation of these MMPs in mice.

8. Conclusion

Since the discovery of NRs as potential regulators of inflammatory processes, extensive research has been carried out in order to exploit this superfamily of transcription factors as therapeutic targets. Within the NR superfamily, the NR4A family of orphan NRs has recently been associated with several inflammatory processes. The three NR4A receptors are rapidly induced by inflammatory insults in several cell types, and all of them inhibit the inflammatory response through several regulatory mechanisms. Therefore, NR4A receptors are involved in a negative feedback response to maintain the inflammatory balance. These orphan NRs therefore constitute a promising area for research and development, since they could be considered as potential therapeutic targets for the treatment/prevention of inflammatory diseases.

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9. Expert opinion

Since inflammation is the common physiological factor in a wide range of acute and chronic diseases, extensive research has focused on identifying the molecular mechanisms that underlie this response and developing new therapeutic approaches aimed at regulating the factors that govern them. NRs have emerged as key players in the regulation of both acute and chronic inflammatory response, thus making them interesting targets for the treatment of a broad range of inflammatory diseases. PPARs, GR and other NRs have been studied extensively in a number of inflammatory settings. Interestingly, recent studies have identified an anti-inflammatory role for other members of the NR superfamily and have therefore highlighted the role of the NR4A family members as key regulators of the inflammatory response. The three NR4A receptors are rapidly induced by inflammatory stimuli in several cell types, thereby suggesting that they are necessary for the initiation and progression of the inflammatory response. Nevertheless, the anti-inflammatory properties described indicate that, due to their role as early-response genes, the acute regulation in the presence of inflammatory stimuli could be a protective reaction aimed at counter-regulating the inflammatory response and resolving inflammation in the later stages. NR4A expression is regulated by NF-κB at the onset of the inflammatory response, and all NR4A receptors inhibit the activity of this transcription factor through several mechanisms and are thus involved in a negative feedback mechanism responsible for maintaining the inflammatory balance. However, the molecular mechanisms involved in this regulation are not entirely clear. Only a small number of NR4A-target genes have been identified so far, and the transcriptional repression mechanisms are only beginning to emerge. Although further research is needed to fully understand the role of these NRs in inflammation, the evidence reviewed

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here supports the claim that NR4A receptors are potential targets for new therapeutic strategies against inflammatory diseases.

Despite the amount of experimental data that support the anti-inflammatory properties of NR4A receptors, we are still a long way from using these NRs modulation as a therapeutic approach to treating inflammatory diseases. Most of the data reviewed here have been obtained by means of molecular biology tools in order to overexpress or knockdown of these transcription factors, in both *in vitro* and *in vivo* experimental models. The naturally occurring ligands of these NRs have not yet been identified, and their nature makes searching for small lipophilic molecules that modulate NR4A activity difficult. However, the identification of structurally diverse compounds that activate or inactivate NR4A receptors (for review see [54]) has opened the door to future research aimed at pharmacologically modulating NR4A activity. To fully exploit the potential of NR4A receptors for therapeutic intervention, the pharmaceutical industry will have to target new drug-development strategies to selectively regulate the activity of each NR4A. NR4A drug discovery could therefore represent an interesting research area for drug development companies.

In summary, while NR4A receptors have been identified as potential inflammation modulators, there are still some concerns regarding the development of NR4A drugs that pharmacologically modulate the activity of these NRs selectively and safely. Although data from experimental models seem promising, the development of new drugs is required before NR4A modulation can be considered a realistic therapeutic approach for the clinical treatment of inflammatory diseases.

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Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Glass CK, Saijo K. Nuclear receptor transrepression pathways that regulate inflammation in macrophages and T cells. Nat Rev Immunol. 2010;10:365-76. Epub 2010/04/24.

2. Beck IM, Vanden Berghe W, Vermeulen L, Yamamoto KR, Haegeman G, De Bosscher K. Crosstalk in inflammation: the interplay of glucocorticoid receptor-based mechanisms and kinases and phosphatases. Endocr Rev. 2009;30:830-82. Epub 2009/11/06.

3. Standiford TJ, Kunkel SL, Basha MA, Chensue SW, Lynch JP, 3rd, Toews GB, Westwick J, Strieter RM. Interleukin-8 gene expression by a pulmonary epithelial cell

ACCEPTED MANUSCRIPT

line. A model for cytokine networks in the lung. J Clin Invest. 1990;86:1945-53. Epub 1990/12/01.

4. Polito AJ, Proud D. Epithelia cells as regulators of airway inflammation. J Allergy Clin Immunol. 1998;102:714-8. Epub 1998/12/29.

5. Ritchlin C. Fibroblast biology. Effector signals released by the synovial fibroblast in arthritis. Arthritis Res. 2000;2:356-60. Epub 2000/11/30.

6. Ghosh S, Karin M. Missing pieces in the NF-kappaB puzzle. Cell. 2002;109 Suppl:S81-96. Epub 2002/05/02.

7. Hayden MS, Ghosh S. Shared principles in NF-kappaB signaling. Cell. 2008;132:344-62. Epub 2008/02/13.

8. O'Neill LA. The interleukin-1 receptor/Toll-like receptor superfamily: 10 years of progress. Immunol Rev. 2008;226:10-8. Epub 2009/01/24.

9. Verstrepen L, Bekaert T, Chau TL, Tavernier J, Chariot A, Beyaert R. TLR-4, IL-1R and TNF-R signaling to NF-kappaB: variations on a common theme. Cell Mol Life Sci. 2008;65:2964-78. Epub 2008/06/07.

10. Karin M. How NF-kappaB is activated: the role of the IkappaB kinase (IKK) complex. Oncogene. 1999;18:6867-74. Epub 1999/12/22.

11. Wagner EF, Eferl R. Fos/AP-1 proteins in bone and the immune system. Immunol Rev. 2005;208:126-40. Epub 2005/11/30.

12. Oeckinghaus A, Ghosh S. The NF-kappaB family of transcription factors and its regulation. Cold Spring Harb Perspect Biol. 2009;1:a000034. Epub 2010/01/13.

13. Hayden MS, Ghosh S. Signaling to NF-kappaB. Genes Dev. 2004;18:2195-224. Epub 2004/09/17.

14. DiDonato J, Mercurio F, Rosette C, Wu-Li J, Suyang H, Ghosh S, Karin M. Mapping of the inducible IkappaB phosphorylation sites that signal its ubiquitination and degradation. Mol Cell Biol. 1996;16:1295-304. Epub 1996/04/01.

15. Rodriguez MS, Wright J, Thompson J, Thomas D, Baleux F, Virelizier JL, Hay RT, Arenzana-Seisdedos F. Identification of lysine residues required for signal-induced ubiquitination and degradation of I kappa B-alpha in vivo. Oncogene. 1996;12:2425-35. Epub 1996/06/06.

16. Roff M, Thompson J, Rodriguez MS, Jacque JM, Baleux F, Arenzana-Seisdedos F, Hay RT. Role of IkappaBalpha ubiquitination in signal-induced activation of NFkappaB in vivo. J Biol Chem. 1996;271:7844-50. Epub 1996/03/29.

ACCEPTED MANUSCRIPT

17. DiDonato JA, Hayakawa M, Rothwarf DM, Zandi E, Karin M. A cytokine-responsive IkappaB kinase that activates the transcription factor NF-kappaB. Nature. 1997;388:548-54. Epub 1997/08/07.

18. Mercurio F, Zhu H, Murray BW, Shevchenko A, Bennett BL, Li J, Young DB, Barbosa M, Mann M, Manning A, Rao A. IKK-1 and IKK-2: cytokine-activated IkappaB kinases essential for NF-kappaB activation. Science. 1997;278:860-6. Epub 1997/11/05.

19. Zandi E, Rothwarf DM, Delhase M, Hayakawa M, Karin M. The IkappaB kinase complex (IKK) contains two kinase subunits, IKKalpha and IKKbeta, necessary for IkappaB phosphorylation and NF-kappaB activation. Cell. 1997;91:243-52. Epub 1997/11/05.

20. Tanaka K, Kawakami T, Tateishi K, Yashiroda H, Chiba T. Control of IkappaBalpha proteolysis by the ubiquitin-proteasome pathway. Biochimie. 2001;83:351-6. Epub 2001/04/11.

21. Tokunaga F, Sakata S, Saeki Y, Satomi Y, Kirisako T, Kamei K, Nakagawa T, Kato M, Murata S, Yamaoka S, Yamamoto M, Akira S, Takao T, Tanaka K, Iwai K. Involvement of linear polyubiquitylation of NEMO in NF-kappaB activation. Nat Cell Biol. 2009;11:123-32. Epub 2009/01/13.

22. Chen LF, Williams SA, Mu Y, Nakano H, Duerr JM, Buckbinder L, Greene WC. NF-kappaB RelA phosphorylation regulates RelA acetylation. Mol Cell Biol. 2005;25:7966-75. Epub 2005/09/02.

23. Perkins ND. Post-translational modifications regulating the activity and function of the nuclear factor kappa B pathway. Oncogene. 2006;25:6717-30. Epub 2006/10/31.

24. Chen LF, Mu Y, Greene WC. Acetylation of RelA at discrete sites regulates distinct nuclear functions of NF-kappaB. EMBO J. 2002;21:6539-48. Epub 2002/11/29.

25. Kiernan R, Bres V, Ng RW, Coudart MP, El Messaoudi S, Sardet C, Jin DY, Emiliani S, Benkirane M. Post-activation turn-off of NF-kappa B-dependent transcription is regulated by acetylation of p65. J Biol Chem. 2003;278:2758-66. Epub 2002/11/07.

26. Mabb AM, Miyamoto S. SUMO and NF-kappaB ties. Cell Mol Life Sci. 2007;64:1979-96. Epub 2007/05/29.

27. Neumann M, Naumann M. Beyond IkappaBs: alternative regulation of NF-kappaB activity. FASEB J. 2007;21:2642-54. Epub 2007/04/14.

ACCEPTED MANUSCRIPT

28. Zenz R, Eferl R, Scheinecker C, Redlich K, Smolen J, Schonthaler HB, Kenner L, Tschachler E, Wagner EF. Activator protein 1 (Fos/Jun) functions in inflammatory bone and skin disease. Arthritis Res Ther. 2008;10:201. Epub 2008/01/30.

29. Hess J, Angel P, Schorpp-Kistner M. AP-1 subunits: quarrel and harmony among siblings. J Cell Sci. 2004;117:5965-73. Epub 2004/11/27.

30. Eychene A, Rocques N, Pouponnot C. A new MAFia in cancer. Nat Rev Cancer. 2008;8:683-93. Epub 2009/01/15.

31. Shaulian E, Karin M. AP-1 as a regulator of cell life and death. Nat Cell Biol. 2002;4:E131-6. Epub 2002/05/04.

32. van Dam H, Castellazzi M. Distinct roles of Jun : Fos and Jun : ATF dimers in oncogenesis. Oncogene. 2001;20:2453-64. Epub 2001/06/13.

33. Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P, Evans RM. The nuclear receptor superfamily: the second decade. Cell. 1995;83:835-9. Epub 1995/12/15.

34. Giguere V. Orphan nuclear receptors: from gene to function. Endocr Rev. 1999;20:689-725. Epub 1999/10/26.

35. Aranda A, Pascual A. Nuclear hormone receptors and gene expression. Physiol Rev. 2001;81:1269-304. Epub 2001/06/28.

36. Pascual G, Glass CK. Nuclear receptors versus inflammation: mechanisms of transrepression. Trends Endocrinol Metab. 2006;17:321-7. Epub 2006/09/01.

• An interesting review about the trans-repression mechanisms of Nuclear Receptors.

37. Kurakula K, Koenis DS, van Tiel CM, de Vries CJ. NR4A nuclear receptors are orphans but not lonesome. Biochim Biophys Acta. 2014;1843:2543-55. Epub 2014/07/01.

38. Saijo K, Winner B, Carson CT, Collier JG, Boyer L, Rosenfeld MG, Gage FH, Glass CK. A Nurr1/CoREST pathway in microglia and astrocytes protects dopaminergic neurons from inflammation-induced death. Cell. 2009;137:47-59. Epub 2009/04/07.

•• A recent study reporting that Nurr1 inhibits NF-κB by recruitment of CoREST co-repressor in dopaminergic neurons.

39. Hong CY, Park JH, Ahn RS, Im SY, Choi HS, Soh J, Mellon SH, Lee K. Molecular mechanism of suppression of testicular steroidogenesis by proinflammatory

ACCEPTED MANUSCRIPT

cytokine tumor necrosis factor alpha. Mol Cell Biol. 2004;24:2593-604. Epub 2004/03/17.

•• Key study demostrating that the molecular interaction between Nur77 and NF-κB supress testicular steroidogenesis.

40. Harant H, Lindley IJ. Negative cross-talk between the human orphan nuclear receptor Nur77/NAK-1/TR3 and nuclear factor-kappaB. Nucleic Acids Res. 2004;32:5280-90. Epub 2004/10/07.

•• Key study demostrating the molecular interaction between Nur77 and NF-□B the human T cell leukemia cell line Jurkat.

41. Calvayrac O, Rodriguez-Calvo R, Marti-Pamies I, Alonso J, Ferran B, Aguilo S, Crespo J, Rodriguez-Sinovas A, Rodriguez C, Martinez-Gonzalez J. NOR-1 modulates the inflammatory response of vascular smooth muscle cells by preventing NFkappaB activation. J Mol Cell Cardiol. 2015;80:34-44. Epub 2014/12/24.

•• A recent study demonstrating that NOR1 over-expression reduces the inflammatory response in VSMC by repressing NF-κB at several levels.

42. Chawla A, Repa JJ, Evans RM, Mangelsdorf DJ. Nuclear receptors and lipid physiology: opening the X-files. Science. 2001;294:1866-70. Epub 2001/12/01.

43. Martinez-Gonzalez J, Badimon L. The NR4A subfamily of nuclear receptors: new early genes regulated by growth factors in vascular cells. Cardiovasc Res. 2005;65:609-18. Epub 2005/01/25.

44. Pols TW, Bonta PI, de Vries CJ. NR4A nuclear orphan receptors: protective in vascular disease? Curr Opin Lipidol. 2007;18:515-20. Epub 2007/09/22.

45. Milbrandt J. Nerve growth factor induces a gene homologous to the glucocorticoid receptor gene. Neuron. 1988;1:183-8. Epub 1988/05/01.

46. Law SW, Conneely OM, DeMayo FJ, O'Malley BW. Identification of a new brain-specific transcription factor, NURR1. Mol Endocrinol. 1992;6:2129-35. Epub 1992/12/01.

47. Ohkura N, Hijikuro M, Yamamoto A, Miki K. Molecular cloning of a novel thyroid/steroid receptor superfamily gene from cultured rat neuronal cells. Biochem Biophys Res Commun. 1994;205:1959-65. Epub 1994/12/30.

48. Maruyama K, Tsukada T, Ohkura N, Bandoh S, Hosono T, Yamaguchi K. The NGFI-B subfamily of the nuclear receptor superfamily (review). Int J Oncol. 1998;12:1237-43. Epub 1998/06/04.

ACCEPTED MANUSCRIPT

49. Wang Z, Benoit G, Liu J, Prasad S, Aarnisalo P, Liu X, Xu H, Walker NP, Perlmann T. Structure and function of Nurr1 identifies a class of ligand-independent nuclear receptors. Nature. 2003;423:555-60. Epub 2003/05/30.

50. Baker KD, Shewchuk LM, Kozlova T, Makishima M, Hassell A, Wisely B, Caravella JA, Lambert MH, Reinking JL, Krause H, Thummel CS, Willson TM, Mangelsdorf DJ. The Drosophila orphan nuclear receptor DHR38 mediates an atypical ecdysteroid signaling pathway. Cell. 2003;113:731-42. Epub 2003/06/18.

51. Wansa KD, Harris JM, Muscat GE. The activation function-1 domain of Nur77/NR4A1 mediates trans-activation, cell specificity, and coactivator recruitment. J Biol Chem. 2002;277:33001-11. Epub 2002/06/26.

52. Wansa KD, Harris JM, Yan G, Ordentlich P, Muscat GE. The AF-1 domain of the orphan nuclear receptor NOR-1 mediates trans-activation, coactivator recruitment, and activation by the purine anti-metabolite 6-mercaptopurine. J Biol Chem. 2003;278:24776-90. Epub 2003/04/24.

53. Codina A, Benoit G, Gooch JT, Neuhaus D, Perlmann T, Schwabe JW. Identification of a novel co-regulator interaction surface on the ligand binding domain of Nurr1 using NMR footprinting. J Biol Chem. 2004;279:53338-45. Epub 2004/10/01.

54. Safe S, Jin UH, Morpurgo B, Abudayyeh A, Singh M, Tjalkens RB. Nuclear receptor 4A (NR4A) family - orphans no more. J Steroid Biochem Mol Biol. 2015. Epub 2015/04/29.

•• A recent review compiling the advances in the development of molecules capable of regulating the NR4A activity.

55. Pekarsky Y, Hallas C, Palamarchuk A, Koval A, Bullrich F, Hirata Y, Bichi R, Letofsky J, Croce CM. Akt phosphorylates and regulates the orphan nuclear receptor Nur77. Proc Natl Acad Sci U S A. 2001;98:3690-4. Epub 2001/03/29.

56. Masuyama N, Oishi K, Mori Y, Ueno T, Takahama Y, Gotoh Y. Akt inhibits the orphan nuclear receptor Nur77 and T-cell apoptosis. J Biol Chem. 2001;276:32799-805. Epub 2001/07/05.

57. Galleguillos D, Vecchiola A, Fuentealba JA, Ojeda V, Alvarez K, Gomez A, Andres ME. PIASgamma represses the transcriptional activation induced by the nuclear receptor Nurr1. J Biol Chem. 2004;279:2005-11. Epub 2003/10/16.

58. Zhao Y, Bruemmer D. NR4A Orphan Nuclear Receptors in Cardiovascular Biology. Drug Discov Today Dis Mech. 2009;6:e43-e8. Epub 2009/01/01.

ACCEPTED MANUSCRIPT

59. Wilson TE, Fahrner TJ, Johnston M, Milbrandt J. Identification of the DNA binding site for NGFI-B by genetic selection in yeast. Science. 1991;252:1296-300. Epub 1991/05/31.

60. Philips A, Lesage S, Gingras R, Maira MH, Gauthier Y, Hugo P, Drouin J. Novel dimeric Nur77 signaling mechanism in endocrine and lymphoid cells. Mol Cell Biol. 1997;17:5946-51. Epub 1997/10/07.

61. Perlmann T, Jansson L. A novel pathway for vitamin A signaling mediated by RXR heterodimerization with NGFI-B and NURR1. Genes Dev. 1995;9:769-82. Epub 1995/04/01.

62. Zetterstrom RH, Solomin L, Mitsiadis T, Olson L, Perlmann T. Retinoid X receptor heterodimerization and developmental expression distinguish the orphan nuclear receptors NGFI-B, Nurr1, and Nor1. Mol Endocrinol. 1996;10:1656-66. Epub 1996/12/01.

63. Maxwell MA, Muscat GE. The NR4A subgroup: immediate early response genes with pleiotropic physiological roles. Nucl Recept Signal. 2006;4:e002. Epub 2006/04/11.

64. Kagaya S, Ohkura N, Tsukada T, Miyagawa M, Sugita Y, Tsujimoto G, Matsumoto K, Saito H, Hashida R. Prostaglandin A2 acts as a transactivator for NOR1 (NR4A3) within the nuclear receptor superfamily. Biol Pharm Bull. 2005;28:1603-7. Epub 2005/09/06.

65. Ordentlich P, Yan Y, Zhou S, Heyman RA. Identification of the antineoplastic agent 6-mercaptopurine as an activator of the orphan nuclear hormone receptor Nurr1. J Biol Chem. 2003;278:24791-9. Epub 2003/04/24.

66. Pearen MA, Muscat GE. Minireview: Nuclear hormone receptor 4A signaling: implications for metabolic disease. Mol Endocrinol. 2010;24:1891-903. Epub 2010/04/16.

67. Liu JJ, Zeng HN, Zhang LR, Zhan YY, Chen Y, Wang Y, Wang J, Xiang SH, Liu WJ, Wang WJ, Chen HZ, Shen YM, Su WJ, Huang PQ, Zhang HK, Wu Q. A unique pharmacophore for activation of the nuclear orphan receptor Nur77 in vivo and in vitro. Cancer Res. 2010;70:3628-37. Epub 2010/04/15.

68. Zhan YY, Chen Y, Zhang Q, Zhuang JJ, Tian M, Chen HZ, Zhang LR, Zhang HK, He JP, Wang WJ, Wu R, Wang Y, Shi C, Yang K, Li AZ, Xin YZ, Li TY, Yang JY, Zheng ZH, Yu CD, Lin SC, Chang C, Huang PQ, Lin T, Wu Q. The orphan nuclear

ACCEPTED MANUSCRIPT

receptor Nur77 regulates LKB1 localization and activates AMPK. Nat Chem Biol. 2012;8:897-904. Epub 2012/09/18.

69. Zhan Y, Du X, Chen H, Liu J, Zhao B, Huang D, Li G, Xu Q, Zhang M, Weimer BC, Chen D, Cheng Z, Zhang L, Li Q, Li S, Zheng Z, Song S, Huang Y, Ye Z, Su W, Lin SC, Shen Y, Wu Q. Cytosporone B is an agonist for nuclear orphan receptor Nur77. Nat Chem Biol. 2008;4:548-56. Epub 2008/08/12.

70. Wang WJ, Wang Y, Chen HZ, Xing YZ, Li FW, Zhang Q, Zhou B, Zhang HK, Zhang J, Bian XL, Li L, Liu Y, Zhao BX, Chen Y, Wu R, Li AZ, Yao LM, Chen P, Zhang Y, Tian XY, Beermann F, Wu M, Han J, Huang PQ, Lin T, Wu Q. Orphan nuclear receptor TR3 acts in autophagic cell death via mitochondrial signaling pathway. Nat Chem Biol. 2014;10:133-40. Epub 2013/12/10.

71. de Waard V, Arkenbout EK, Vos M, Mocking AI, Niessen HW, Stooker W, de Mol BA, Quax PH, Bakker EN, VanBavel E, Pannekoek H, de Vries CJ. TR3 nuclear orphan receptor prevents cyclic stretch-induced proliferation of venous smooth muscle cells. Am J Pathol. 2006;168:2027-35. Epub 2006/05/26.

72. Hu YW, Zhang P, Yang JY, Huang JL, Ma X, Li SF, Zhao JY, Hu YR, Wang YC, Gao JJ, Sha YH, Zheng L, Wang Q. Nur77 decreases atherosclerosis progression in apoE(-/-) mice fed a high-fat/high-cholesterol diet. PLoS One. 2014;9:e87313. Epub 2014/02/06.

73. Lee SO, Li X, Hedrick E, Jin UH, Tjalkens RB, Backos DS, Li L, Zhang Y, Wu Q, Safe S. Diindolylmethane analogs bind NR4A1 and are NR4A1 antagonists in colon cancer cells. Mol Endocrinol. 2014;28:1729-39. Epub 2014/08/08.

74. Inamoto T, Papineni S, Chintharlapalli S, Cho SD, Safe S, Kamat AM. 1,1-Bis(3'-indolyl)-1-(p-chlorophenyl)methane activates the orphan nuclear receptor Nurr1 and inhibits bladder cancer growth. Mol Cancer Ther. 2008;7:3825-33. Epub 2008/12/17.

75. Safe S, Jin UH, Hedrick E, Reeder A, Lee SO. Minireview: role of orphan nuclear receptors in cancer and potential as drug targets. Mol Endocrinol. 2014;28:157-72. Epub 2013/12/04.

76. Kolluri SK, Zhu X, Zhou X, Lin B, Chen Y, Sun K, Tian X, Town J, Cao X, Lin F, Zhai D, Kitada S, Luciano F, O'Donnell E, Cao Y, He F, Lin J, Reed JC, Satterthwait AC, Zhang XK. A short Nur77-derived peptide converts Bcl-2 from a protector to a killer. Cancer Cell. 2008;14:285-98. Epub 2008/10/07.

ACCEPTED MANUSCRIPT

77. Ferlini C, Cicchillitti L, Raspaglio G, Bartollino S, Cimitan S, Bertucci C, Mozzetti S, Gallo D, Persico M, Fattorusso C, Campiani G, Scambia G. Paclitaxel directly binds to Bcl-2 and functionally mimics activity of Nur77. Cancer Res. 2009;69:6906-14. Epub 2009/08/13.

78. Watanabe T, Sekine S, Naguro I, Sekine Y, Ichijo H. Apoptosis Signal-regulating Kinase 1 (ASK1)-p38 Pathway-dependent Cytoplasmic Translocation of the Orphan Nuclear Receptor NR4A2 Is Required for Oxidative Stress-induced Necrosis. J Biol Chem. 2015;290:10791-803. Epub 2015/03/11.

79. Lee SO, Andey T, Jin UH, Kim K, Singh M, Safe S. The nuclear receptor TR3 regulates mTORC1 signaling in lung cancer cells expressing wild-type p53. Oncogene. 2012;31:3265-76. Epub 2011/11/15.

80. Lee SO, Abdelrahim M, Yoon K, Chintharlapalli S, Papineni S, Kim K, Wang H, Safe S. Inactivation of the orphan nuclear receptor TR3/Nur77 inhibits pancreatic cancer cell and tumor growth. Cancer Res. 2010;70:6824-36. Epub 2010/07/28.

81. Hedrick E, Lee SO, Doddapaneni R, Singh M, Safe S. NR4A1 Antagonists Inhibit beta1-Integrin-Dependent Breast Cancer Cell Migration. Mol Cell Biol. 2016;36:1383-94. Epub 2016/03/02.

82. Lee SO, Jin UH, Kang JH, Kim SB, Guthrie AS, Sreevalsan S, Lee JS, Safe S. The orphan nuclear receptor NR4A1 (Nur77) regulates oxidative and endoplasmic reticulum stress in pancreatic cancer cells. Mol Cancer Res. 2014;12:527-38. Epub 2014/02/12.

83. Hawk JD, Abel T. The role of NR4A transcription factors in memory formation. Brain Res Bull. 2011;85:21-9. Epub 2011/02/15.

84. Hawk JD, Bookout AL, Poplawski SG, Bridi M, Rao AJ, Sulewski ME, Kroener BT, Manglesdorf DJ, Abel T. NR4A nuclear receptors support memory enhancement by histone deacetylase inhibitors. J Clin Invest. 2012;122:3593-602. Epub 2012/09/22.

85. McNulty SE, Barrett RM, Vogel-Ciernia A, Malvaez M, Hernandez N, Davatolhagh MF, Matheos DP, Schiffman A, Wood MA. Differential roles for Nr4a1 and Nr4a2 in object location vs. object recognition long-term memory. Learn Mem. 2012;19:588-92. Epub 2012/11/20.

86. Mahmoudi S, Samadi P, Gilbert F, Ouattara B, Morissette M, Gregoire L, Rouillard C, Di Paolo T, Levesque D. Nur77 mRNA levels and L-Dopa-induced dyskinesias in MPTP monkeys treated with docosahexaenoic acid. Neurobiol Dis. 2009;36:213-22. Epub 2009/07/29.

ACCEPTED MANUSCRIPT

87. Volakakis N, Kadkhodaei B, Joodmardi E, Wallis K, Panman L, Silvaggi J, Spiegelman BM, Perlmann T. NR4A orphan nuclear receptors as mediators of CREB-dependent neuroprotection. Proc Natl Acad Sci U S A. 2010;107:12317-22. Epub 2010/06/23.

88. Colon-Cesario WI, Martinez-Montemayor MM, Morales S, Felix J, Cruz J, Adorno M, Pereira L, Colon N, Maldonado-Vlaar CS, Pena de Ortiz S. Knockdown of Nurr1 in the rat hippocampus: implications to spatial discrimination learning and memory. Learn Mem. 2006;13:734-44. Epub 2006/12/05.

89. Eells JB, Wilcots J, Sisk S, Guo-Ross SX. NR4A gene expression is dynamically regulated in the ventral tegmental area dopamine neurons and is related to expression of dopamine neurotransmission genes. J Mol Neurosci. 2012;46:545-53. Epub 2011/09/21.

90. Xiang B, Wang W, Li W, Li X, Li X, Li G. Differential expression of oxidored nitro domain containing protein 1 (NOR1), in mouse tissues and in normal and cancerous human tissues. Gene. 2012;493:18-26. Epub 2011/12/14.

91. Levesque D, Rouillard C. Nur77 and retinoid X receptors: crucial factors in dopamine-related neuroadaptation. Trends Neurosci. 2007;30:22-30. Epub 2006/12/01.

92. St-Hilaire M, Bourhis E, Levesque D, Rouillard C. Impaired behavioural and molecular adaptations to dopamine denervation and repeated L-DOPA treatment in Nur77-knockout mice. Eur J Neurosci. 2006;24:795-805. Epub 2006/08/26.

93. Mount MP, Zhang Y, Amini M, Callaghan S, Kulczycki J, Mao Z, Slack RS, Anisman H, Park DS. Perturbation of transcription factor Nur77 expression mediated by myocyte enhancer factor 2D (MEF2D) regulates dopaminergic neuron loss in response to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). J Biol Chem. 2013;288:14362-71. Epub 2013/03/29.

94. Mahmoudi S, Blanchet PJ, Levesque D. Haloperidol-induced striatal Nur77 expression in a non-human primate model of tardive dyskinesia. Eur J Neurosci. 2013;38:2192-8. Epub 2013/04/05.

95. Xiao G, Sun T, Songming C, Cao Y. NR4A1 enhances neural survival following oxygen and glucose deprivation: an in vitro study. J Neurol Sci. 2013;330:78-84. Epub 2013/05/15.

96. De Miranda BR, Popichak KA, Hammond SL, Jorgensen BA, Phillips AT, Safe S, Tjalkens RB. The Nurr1 Activator 1,1-Bis(3'-Indolyl)-1-(p-Chlorophenyl)Methane

ACCEPTED MANUSCRIPT

Blocks Inflammatory Gene Expression in BV-2 Microglial Cells by Inhibiting Nuclear Factor kappaB. Mol Pharmacol. 2015;87:1021-34. Epub 2015/04/11.

•• A recent study reporting that Nurr1 activation by C-DIMs compunds inhibits NF-κB by recruitment of CoREST and NCOR2 co-repressors.

97. Hammond SL, Safe S, Tjalkens RB. A novel synthetic activator of Nurr1 induces dopaminergic gene expression and protects against 6-hydroxydopamine neurotoxicity in vitro. Neurosci Lett. 2015;607:83-9. Epub 2015/09/19.

98. Schaffer DJ, Tunc-Ozcan E, Shukla PK, Volenec A, Redei EE. Nuclear orphan receptor Nor-1 contributes to depressive behavior in the Wistar-Kyoto rat model of depression. Brain Res. 2010;1362:32-9. Epub 2010/09/21.

99. Novak G, Zai CC, Mirkhani M, Shaikh S, Vincent JB, Meltzer H, Lieberman JA, Strauss J, Levesque D, Kennedy JL, Le Foll B. Replicated association of the NR4A3 gene with smoking behaviour in schizophrenia and in bipolar disorder. Genes Brain Behav. 2010;9:910-7. Epub 2010/07/28.

100. De Miranda BR, Popichak KA, Hammond SL, Miller JA, Safe S, Tjalkens RB. Novel para-phenyl substituted diindolylmethanes protect against MPTP neurotoxicity and suppress glial activation in a mouse model of Parkinson's disease. Toxicol Sci. 2015;143:360-73. Epub 2014/11/20.

101. Tjalkens RB, Liu X, Mohl B, Wright T, Moreno JA, Carbone DL, Safe S. The peroxisome proliferator-activated receptor-gamma agonist 1,1-bis(3'-indolyl)-1-(p-trifluoromethylphenyl)methane suppresses manganese-induced production of nitric oxide in astrocytes and inhibits apoptosis in cocultured PC12 cells. J Neurosci Res. 2008;86:618-29. Epub 2007/11/28.

102. Carbone DL, Popichak KA, Moreno JA, Safe S, Tjalkens RB. Suppression of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced nitric-oxide synthase 2 expression in astrocytes by a novel diindolylmethane analog protects striatal neurons against apoptosis. Mol Pharmacol. 2009;75:35-43. Epub 2008/10/09.

103. Pei L, Waki H, Vaitheesvaran B, Wilpitz DC, Kurland IJ, Tontonoz P. NR4A orphan nuclear receptors are transcriptional regulators of hepatic glucose metabolism. Nat Med. 2006;12:1048-55. Epub 2006/08/15.

104. Oita RC, Mazzatti DJ, Lim FL, Powell JR, Merry BJ. Whole-genome microarray analysis identifies up-regulation of Nr4a nuclear receptors in muscle and liver from diet-restricted rats. Mech Ageing Dev. 2009;130:240-7. Epub 2009/01/20.

ACCEPTED MANUSCRIPT

105. Chao LC, Wroblewski K, Zhang Z, Pei L, Vergnes L, Ilkayeva OR, Ding SY, Reue K, Watt MJ, Newgard CB, Pilch PF, Hevener AL, Tontonoz P. Insulin resistance and altered systemic glucose metabolism in mice lacking Nur77. Diabetes. 2009;58:2788-96. Epub 2009/09/11.

106. Kim YD, Kim SG, Hwang SL, Choi HS, Bae JH, Song DK, Im SS. B-cell translocation gene 2 regulates hepatic glucose homeostasis via induction of orphan nuclear receptor Nur77 in diabetic mouse model. Diabetes. 2014;63:1870-80. Epub 2014/03/22.

107. Bonta PI, Pols TW, de Vries CJ. NR4A nuclear receptors in atherosclerosis and vein-graft disease. Trends Cardiovasc Med. 2007;17:105-11. Epub 2007/04/10.

108. Ranhotra HS. The NR4A orphan nuclear receptors: mediators in metabolism and diseases. J Recept Signal Transduct Res. 2015;35:184-8. Epub 2014/08/05.

109. Arkenbout EK, de Waard V, van Bragt M, van Achterberg TA, Grimbergen JM, Pichon B, Pannekoek H, de Vries CJ. Protective function of transcription factor TR3 orphan receptor in atherogenesis: decreased lesion formation in carotid artery ligation model in TR3 transgenic mice. Circulation. 2002;106:1530-5. Epub 2002/09/18.

110. Pires NM, Pols TW, de Vries MR, van Tiel CM, Bonta PI, Vos M, Arkenbout EK, Pannekoek H, Jukema JW, Quax PH, de Vries CJ. Activation of nuclear receptor Nur77 by 6-mercaptopurine protects against neointima formation. Circulation. 2007;115:493-500. Epub 2007/01/24.

111. Kim HJ, Kim JY, Lee SJ, Kim HJ, Oh CJ, Choi YK, Lee HJ, Do JY, Kim SY, Kwon TK, Choi HS, Lee MO, Park IS, Park KG, Lee KU, Lee IK. alpha-Lipoic acid prevents neointimal hyperplasia via induction of p38 mitogen-activated protein kinase/Nur77-mediated apoptosis of vascular smooth muscle cells and accelerates postinjury reendothelialization. Arterioscler Thromb Vasc Biol. 2010;30:2164-72. Epub 2010/09/11.

112. Liu D, Jia H, Holmes DI, Stannard A, Zachary I. Vascular endothelial growth factor-regulated gene expression in endothelial cells: KDR-mediated induction of Egr3 and the related nuclear receptors Nur77, Nurr1, and Nor1. Arterioscler Thromb Vasc Biol. 2003;23:2002-7. Epub 2003/10/04.

113. You B, Jiang YY, Chen S, Yan G, Sun J. The orphan nuclear receptor Nur77 suppresses endothelial cell activation through induction of IkappaBalpha expression. Circ Res. 2009;104:742-9. Epub 2009/02/14.

ACCEPTED MANUSCRIPT

114. Zeng H, Qin L, Zhao D, Tan X, Manseau EJ, Van Hoang M, Senger DR, Brown LF, Nagy JA, Dvorak HF. Orphan nuclear receptor TR3/Nur77 regulates VEGF-A-induced angiogenesis through its transcriptional activity. J Exp Med. 2006;203:719-29. Epub 2006/03/08.

115. Bonta PI, Matlung HL, Vos M, Peters SL, Pannekoek H, Bakker EN, de Vries CJ. Nuclear receptor Nur77 inhibits vascular outward remodelling and reduces macrophage accumulation and matrix metalloproteinase levels. Cardiovasc Res. 2010;87:561-8. Epub 2010/03/02.

116. Hanna RN, Shaked I, Hubbeling HG, Punt JA, Wu R, Herrley E, Zaugg C, Pei H, Geissmann F, Ley K, Hedrick CC. NR4A1 (Nur77) deletion polarizes macrophages toward an inflammatory phenotype and increases atherosclerosis. Circ Res. 2012;110:416-27. Epub 2011/12/24.

• Key study reporting that Nur77 depletion induces polarization of macrophages toward a pro-inflammatory M1 phenotype.

117. Yan G, Zhu N, Huang S, Yi B, Shang X, Chen M, Wang N, Zhang GX, Talarico JA, Tilley DG, Gao E, Sun J. Orphan Nuclear Receptor Nur77 Inhibits Cardiac Hypertrophic Response to Beta-Adrenergic Stimulation. Mol Cell Biol. 2015;35:3312-23. Epub 2015/07/22.

118. Bonta PI, Pols TW, van Tiel CM, Vos M, Arkenbout EK, Rohlena J, Koch KT, de Maat MP, Tanck MW, de Winter RJ, Pannekoek H, Biessen EA, Bot I, de Vries CJ. Nuclear receptor Nurr1 is expressed in and is associated with human restenosis and inhibits vascular lesion formation in mice involving inhibition of smooth muscle cell proliferation and inflammation. Circulation. 2010;121:2023-32. Epub 2010/04/28.

119. Bonta PI, van Tiel CM, Vos M, Pols TW, van Thienen JV, Ferreira V, Arkenbout EK, Seppen J, Spek CA, van der Poll T, Pannekoek H, de Vries CJ. Nuclear receptors Nur77, Nurr1, and NOR-1 expressed in atherosclerotic lesion macrophages reduce lipid loading and inflammatory responses. Arterioscler Thromb Vasc Biol. 2006;26:2288-94. Epub 2006/07/29.

120. Hamers AA, Hanna RN, Nowyhed H, Hedrick CC, de Vries CJ. NR4A nuclear receptors in immunity and atherosclerosis. Curr Opin Lipidol. 2013;24:381-5. Epub 2013/09/06.

121. Rodriguez-Calvo R, Guadall A, Calvayrac O, Navarro MA, Alonso J, Ferran B, de Diego A, Muniesa P, Osada J, Rodriguez C, Martinez-Gonzalez J. Over-expression

ACCEPTED MANUSCRIPT

of neuron-derived orphan receptor-1 (NOR-1) exacerbates neointimal hyperplasia after vascular injury. Hum Mol Genet. 2013;22:1949-59. Epub 2013/02/08.

122. Rodriguez-Calvo R, Guadall A, Calvayrac O, Alonso J, Ferran B, Marti I, Navarro MA, de Diego A, Osada J, Rodriguez C, Martinez-Gonzalez J. [The nuclear receptor NOR-1 regulates the activation of vascular cells and vascular remodelling in response to hemodynamic stress]. Clin Investig Arterioscler. 2014;26:66-75. Epub 2014/03/19.

123. Martinez-Gonzalez J, Rius J, Castello A, Cases-Langhoff C, Badimon L. Neuron-derived orphan receptor-1 (NOR-1) modulates vascular smooth muscle cell proliferation. Circ Res. 2003;92:96-103. Epub 2003/01/11.

124. Rius J, Martinez-Gonzalez J, Crespo J, Badimon L. NOR-1 is involved in VEGF-induced endothelial cell growth. Atherosclerosis. 2006;184:276-82. Epub 2005/06/14.

125. Zhao Y, Howatt DA, Gizard F, Nomiyama T, Findeisen HM, Heywood EB, Jones KL, Conneely OM, Daugherty A, Bruemmer D. Deficiency of the NR4A orphan nuclear receptor NOR1 decreases monocyte adhesion and atherosclerosis. Circ Res. 2010;107:501-11. Epub 2010/06/19.

126. Sekiya T, Kashiwagi I, Yoshida R, Fukaya T, Morita R, Kimura A, Ichinose H, Metzger D, Chambon P, Yoshimura A. Nr4a receptors are essential for thymic regulatory T cell development and immune homeostasis. Nat Immunol. 2013;14:230-7. Epub 2013/01/22.

127. Fassett MS, Jiang W, D'Alise AM, Mathis D, Benoist C. Nuclear receptor Nr4a1 modulates both regulatory T-cell (Treg) differentiation and clonal deletion. Proc Natl Acad Sci U S A. 2012;109:3891-6. Epub 2012/02/22.

128. Nowyhed HN, Huynh TR, Blatchley A, Wu R, Thomas GD, Hedrick CC. The nuclear receptor nr4a1 controls CD8 T cell development through transcriptional suppression of runx3. Sci Rep. 2015;5:9059. Epub 2015/03/13.

129. Hanna RN, Carlin LM, Hubbeling HG, Nackiewicz D, Green AM, Punt JA, Geissmann F, Hedrick CC. The transcription factor NR4A1 (Nur77) controls bone marrow differentiation and the survival of Ly6C- monocytes. Nat Immunol. 2011;12:778-85. Epub 2011/07/05.

130. Hilgendorf I, Gerhardt LM, Tan TC, Winter C, Holderried TA, Chousterman BG, Iwamoto Y, Liao R, Zirlik A, Scherer-Crosbie M, Hedrick CC, Libby P, Nahrendorf M, Weissleder R, Swirski FK. Ly-6Chigh monocytes depend on Nr4a1 to

ACCEPTED MANUSCRIPT

balance both inflammatory and reparative phases in the infarcted myocardium. Circ Res. 2014;114:1611-22. Epub 2014/03/15.

131. Hamers AA, Vos M, Rassam F, Marinkovic G, Kurakula K, van Gorp PJ, de Winther MP, Gijbels MJ, de Waard V, de Vries CJ. Bone marrow-specific deficiency of nuclear receptor Nur77 enhances atherosclerosis. Circ Res. 2012;110:428-38. Epub 2011/12/24.

• Key study reporting that Nur77 is crucial for maintaining the macrophage M2-specific phenotype. 132. Mahajan S, Saini A, Chandra V, Nanduri R, Kalra R, Bhagyaraj E, Khatri N, Gupta P. Nuclear Receptor Nr4a2 Promotes Alternative Polarization of Macrophages and Confers Protection in Sepsis. J Biol Chem. 2015;290:18304-14. Epub 2015/05/09.

133. Pei L, Castrillo A, Chen M, Hoffmann A, Tontonoz P. Induction of NR4A orphan nuclear receptor expression in macrophages in response to inflammatory stimuli. J Biol Chem. 2005;280:29256-62. Epub 2005/06/21.

• Key study demonstrating that the three NR4A are induced by inflammatory insults via NF-κB trans-activation of the NR4A promoters in macrophages.

134. Pei L, Castrillo A, Tontonoz P. Regulation of macrophage inflammatory gene expression by the orphan nuclear receptor Nur77. Mol Endocrinol. 2006;20:786-94. Epub 2005/12/13.

135. Holla VR, Mann JR, Shi Q, DuBois RN. Prostaglandin E2 regulates the nuclear receptor NR4A2 in colorectal cancer. J Biol Chem. 2006;281:2676-82. Epub 2005/11/19.

136. O'Kane M, Markham T, McEvoy AN, Fearon U, Veale DJ, FitzGerald O, Kirby B, Murphy EP. Increased expression of the orphan nuclear receptor NURR1 in psoriasis and modulation following TNF-alpha inhibition. J Invest Dermatol. 2008;128:300-10. Epub 2007/08/03.

137. Doi Y, Oki S, Ozawa T, Hohjoh H, Miyake S, Yamamura T. Orphan nuclear receptor NR4A2 expressed in T cells from multiple sclerosis mediates production of inflammatory cytokines. Proc Natl Acad Sci U S A. 2008;105:8381-6. Epub 2008/06/14.

138. Murphy EP, McEvoy A, Conneely OM, Bresnihan B, FitzGerald O. Involvement of the nuclear orphan receptor NURR1 in the regulation of corticotropin-releasing hormone expression and actions in human inflammatory arthritis. Arthritis Rheum. 2001;44:782-93. Epub 2001/04/24.

ACCEPTED MANUSCRIPT

139. Aherne CM, McMorrow J, Kane D, FitzGerald O, Mix KS, Murphy EP. Identification of NR4A2 as a transcriptional activator of IL-8 expression in human inflammatory arthritis. Mol Immunol. 2009;46:3345-57. Epub 2009/09/08.

140. Shao Q, Shen LH, Hu LH, Pu J, Qi MY, Li WQ, Tian FJ, Jing Q, He B. Nuclear receptor Nur77 suppresses inflammatory response dependent on COX-2 in macrophages induced by oxLDL. J Mol Cell Cardiol. 2010;49:304-11. Epub 2010/04/13.

141. Diatchenko L, Romanov S, Malinina I, Clarke J, Tchivilev I, Li X, Makarov SS. Identification of novel mediators of NF-kappaB through genome-wide survey of monocyte adherence-induced genes. J Leukoc Biol. 2005;78:1366-77. Epub 2005/10/06.

•• Key study identifying the NR4A as NF-□B potent inhibitors using systematic, genome-wide survey in monocyte adherent cells.

142. Li L, Liu Y, Chen HZ, Li FW, Wu JF, Zhang HK, He JP, Xing YZ, Chen Y, Wang WJ, Tian XY, Li AZ, Zhang Q, Huang PQ, Han J, Lin T, Wu Q. Impeding the interaction between Nur77 and p38 reduces LPS-induced inflammation. Nat Chem Biol. 2015;11:339-46. Epub 2015/03/31.

143. Li XM, Zhang S, He XS, Guo PD, Lu XX, Wang JR, Li JM, Wu H. Nur77-mediated TRAF6 signalling protects against LPS-induced sepsis in mice. J Inflamm (Lond). 2016;13:4. Epub 2016/02/04.

144. Ipseiz N, Uderhardt S, Scholtysek C, Steffen M, Schabbauer G, Bozec A, Schett G, Kronke G. The nuclear receptor Nr4a1 mediates anti-inflammatory effects of apoptotic cells. J Immunol. 2014;192:4852-8. Epub 2014/04/18.

145. Zhang X, Yan G, Diao Z, Sun H, Hu Y. NUR77 inhibits the expression of TIMP2 and increases the migration and invasion of HTR-8/SVneo cells induced by CYR61. Placenta. 2012;33:561-7. Epub 2012/05/04.

146. Gruber F, Hufnagl P, Hofer-Warbinek R, Schmid JA, Breuss JM, Huber-Beckmann R, Lucerna M, Papac N, Harant H, Lindley I, de Martin R, Binder BR. Direct binding of Nur77/NAK-1 to the plasminogen activator inhibitor 1 (PAI-1) promoter regulates TNF alpha -induced PAI-1 expression. Blood. 2003;101:3042-8. Epub 2002/12/31.

147. Rodriguez-Calvo R, Ferran B, Alonso J, Marti-Pamies I, Aguilo S, Calvayrac O, Rodriguez C, Martinez-Gonzalez J. NR4A receptors up-regulate the antiproteinase alpha-2 macroglobulin (A2M) and modulate MMP-2 and MMP-9 in vascular smooth muscle cells. Thromb Haemost. 2015;113:1323-34. Epub 2015/03/27.

ACCEPTED MANUSCRIPT

• A recent study demonstrating that NR4A inhibits matrix metaloproteinase activity, at least partially by inducing A2M at transcriptional level.

148. Zhao Y, Liu Y, Zheng D. Alpha 1-antichymotrypsin/SerpinA3 is a novel target of orphan nuclear receptor Nur77. FEBS J. 2008;275:1025-38. Epub 2008/02/06.

149. Crean D, Cummins EP, Bahar B, Mohan H, McMorrow JP, Murphy EP. Adenosine Modulates NR4A Orphan Nuclear Receptors To Attenuate Hyperinflammatory Responses in Monocytic Cells. J Immunol. 2015;195:1436-48. Epub 2015/07/08.

150. Raveney BJ, Oki S, Yamamura T. Nuclear receptor NR4A2 orchestrates Th17 cell-mediated autoimmune inflammation via IL-21 signalling. PLoS One. 2013;8:e56595. Epub 2013/02/26.

151. Mix KS, Attur MG, Al-Mussawir H, Abramson SB, Brinckerhoff CE, Murphy EP. Transcriptional repression of matrix metalloproteinase gene expression by the orphan nuclear receptor NURR1 in cartilage. J Biol Chem. 2007;282:9492-504. Epub 2007/02/07.

152. Mix KS, McMahon K, McMorrow JP, Walkenhorst DE, Smyth AM, Petrella BL, Gogarty M, Fearon U, Veale D, Attur MG, Abramson SB, Murphy EP. Orphan nuclear receptor NR4A2 induces synoviocyte proliferation, invasion, and matrix metalloproteinase 13 transcription. Arthritis Rheum. 2012;64:2126-36. Epub 2012/01/26.

153. Wu Y, Ghosh S, Nishi Y, Yanase T, Nawata H, Hu Y. The orphan nuclear receptors NURR1 and NGFI-B modulate aromatase gene expression in ovarian granulosa cells: a possible mechanism for repression of aromatase expression upon luteinizing hormone surge. Endocrinology. 2005;146:237-46. Epub 2004/10/16.

154. Lammi J, Huppunen J, Aarnisalo P. Regulation of the osteopontin gene by the orphan nuclear receptor NURR1 in osteoblasts. Mol Endocrinol. 2004;18:1546-57. Epub 2004/02/28.

155. Attur MG, Dave MN, Stuchin S, Kowalski AJ, Steiner G, Abramson SB, Denhardt DT, Amin AR. Osteopontin: an intrinsic inhibitor of inflammation in cartilage. Arthritis Rheum. 2001;44:578-84. Epub 2001/03/27.

156. McCoy JM, Walkenhorst DE, McCauley KS, Elaasar H, Everett JR, Mix KS. Orphan nuclear receptor NR4A2 induces transcription of the immunomodulatory peptide hormone prolactin. J Inflamm (Lond). 2015;12:13. Epub 2015/02/27.

ACCEPTED MANUSCRIPT

157. Chen P, Li J, Huo Y, Lu J, Wan L, Li B, Gan R, Guo C. Orphan nuclear receptor NR4A2 inhibits hepatic stellate cell proliferation through MAPK pathway in liver fibrosis. PeerJ. 2015;3:e1518. Epub 2015/12/30.

158. Nomiyama T, Zhao Y, Gizard F, Findeisen HM, Heywood EB, Jones KL, Conneely OM, Bruemmer D. Deficiency of the NR4A neuron-derived orphan receptor-1 attenuates neointima formation after vascular injury. Circulation. 2009;119:577-86. Epub 2009/01/21.

159. Adya R, Tan BK, Chen J, Randeva HS. Nuclear factor-kappaB induction by visfatin in human vascular endothelial cells: its role in MMP-2/9 production and activation. Diabetes Care. 2008;31:758-60. Epub 2008/01/11.

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FIGURE 1

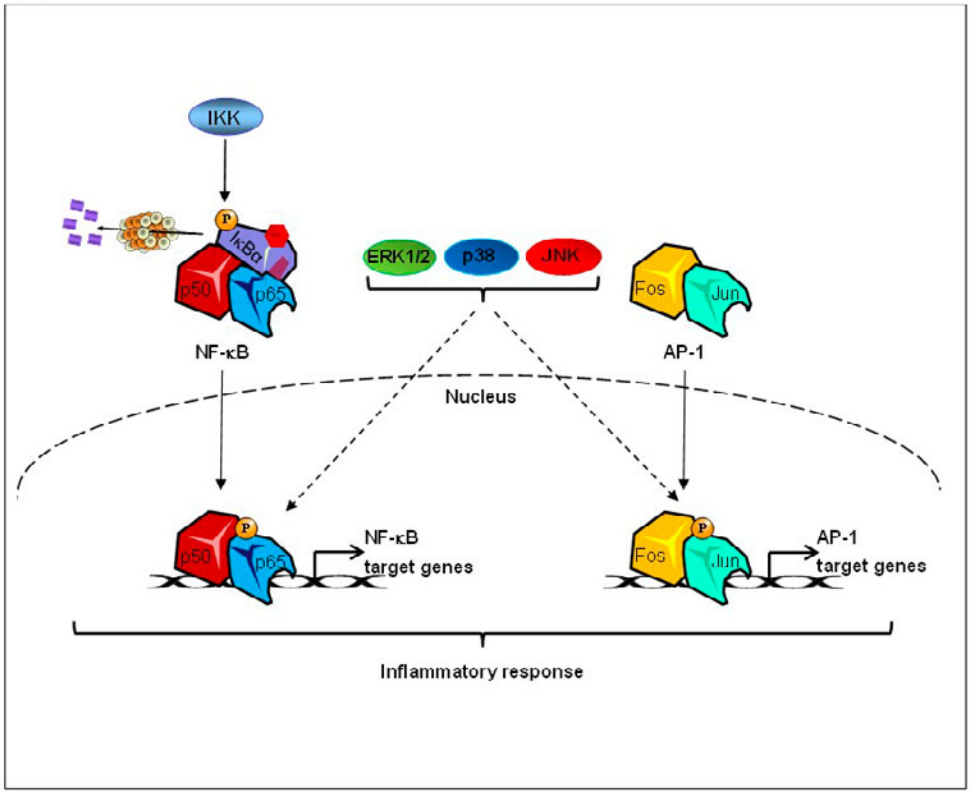

Figure 1. Schematic representation of the main cellular signaling pathways involved in the regulation of the inflammatory response. In the presence of pro-

inflammatory insults, the IKK complex phosphorylates IκBα, which is ubiquitinated and degraded by the proteasome. Additionally, the activation of MAPK such as ERK1/2, p38 and JNK activates both NF-κB and AP-1 transcription factors by phosphorylation, thereby leading their nuclear translocation to regulate the expression of their target genes. The coordinated actions of NF-κB and AP-1 induce the development of the inflammatory process by promoting the transcription of cytokines, chemokines and other pro-inflammatory genes. AP-1: activator protein-1; ERK1/2: extracellular signal-regulated kinases 1/2; IKK: IkB kinase; JNK: Jun N-terminal kinase; NF-κB: nuclear factor κB.

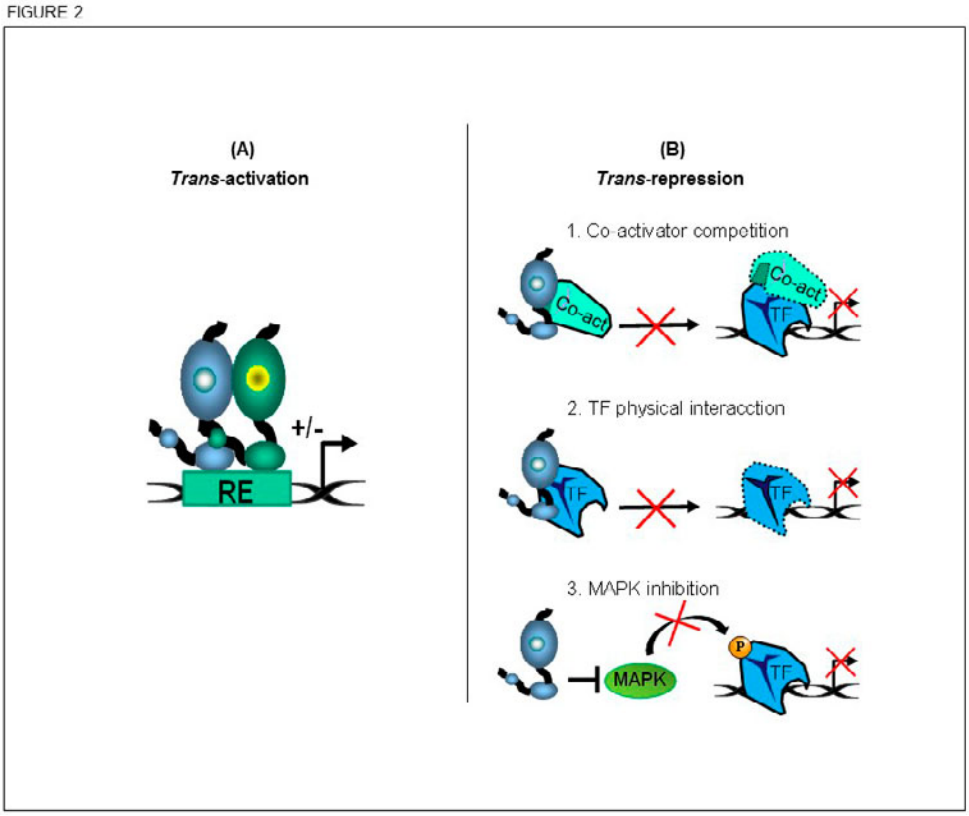

Figure 2. Main molecular mechanisms through which nuclear receptors drive their effects. NRs regulate gene expression through two different mechanisms. (A) In the transactivation mechanism, NRs act as typical transcription factors to induce or repress

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the transcription of their target genes by binding to DNA-specific sequences in their promoter region. (B) In the transrepression mechanism, NRs influence gene expression through mechanisms that are independent of their DNA-binding capacity, thereby inhibiting the activity of other transcription factors. There are several transrepression strategies, including co-activator competition, physical interaction with other transcription factors and the inhibition of MAPK pathways. MAPK: mitogen-activated protein kinase; RE: response element; TE: transcription factors.

FIGURE 3

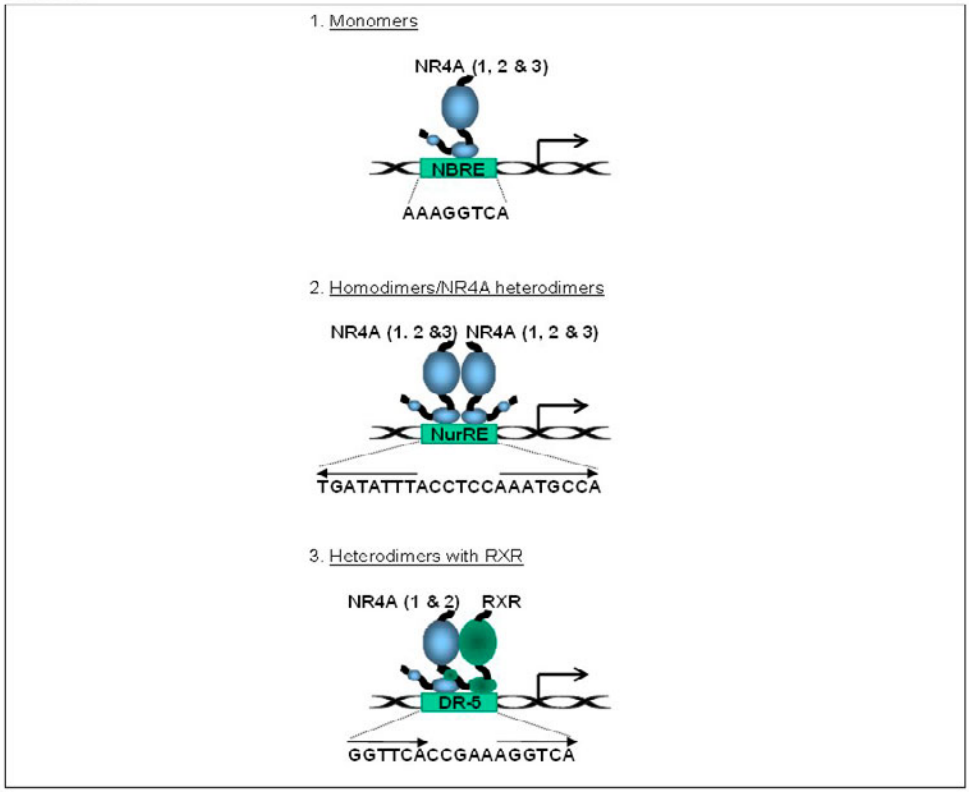

Figure 3. Specific binding sequences for the NR4A receptors. NR4A receptors act as transcription factors by regulating the transcription of their target genes through binding as monomers to NBRE sequences, as homodimers or NR4A-heterodimers to NurRE sequences, or heterodimers with RXR to DR-5 sequences. DR-5: direct repeat 5;

NBRE: NGFI-B-response element; NurRE: Nur-response element; RXR: retinoid X receptor.

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FIGURE 4

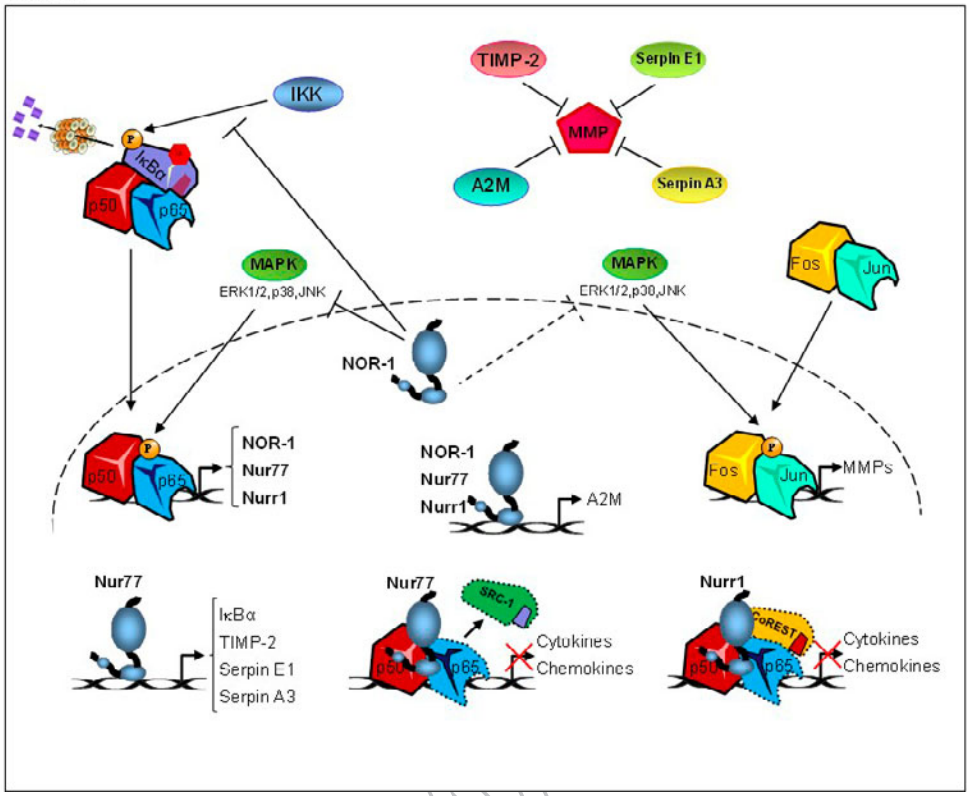

Figure 4. Molecular mechanisms of the NR4A receptors in the counter-regulation of the inflammatory response. The three NR4A receptors are rapidly induced by inflammatory insults via NF-κB transactivation of the NR4A promoters, and are all involved in the counter-regulation of inflammation in the later stages through several regulatory mechanisms. The three NR4A receptors are involved in MMP inhibition, thereby inducing the transcriptional regulation of the broad-spectrum antiprotease A2M. Additionally, Nur77 regulates the expression of other MMP inhibitors such as TIMP-2, Serpin E1 and Serpin A3, and the expression of the NF-κB inhibitor IκBα. Nur77 further binds to the p65 subunit of NF-κB, thus avoiding SRC-1 binding. Nurr1 also inhibits NF-κB activity via Nurr1-p65 docking and the recruitment of the CoREST corepressor complex. NOR-1 inhibits NF-κB activation by inducing IκBα phosphorylation/degradation and attenuating p65 phosphorylation. Moreover, NOR-1

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reduces MAPK pathway activation, and potentially reduces both NF-κB and AP-1 activation. Altogether, NR4A receptors are involved in a negative feedback mechanism responsible for maintaining the inflammatory balance. A2M: alpha-2 macroglobulin; AP-1: activator protein-1; MAPK: mitogen-activated protein kinase; MMPs: matrix metalloproteinases; NF-κB: nuclear factor κB; NOR-1: neuron-derived orphan receptor-1; Nurr1: Nurr-related factor 1; SRC-1: steroid receptor coactivator-1; TIMP-2: tissue inhibitor of metalloproteinases 2.

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