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Nuclear Receptor 4A (NR4A) Family – Orphans No More

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Abstract

The orphan nuclear receptors NR4A1, NR4A2 and NR4A3 are immediate early genes induced by multiple stressors, and the NR4A1 receptors play an important role in maintaining cellular homeostasis and disease. There is increasing evidence for the role of these receptors in metabolic, cardiovascular and neurological functions and also in inflammation and inflammatory diseases and in immune functions and cancer. Despite the similarities of NR4A1, NR4A2 and NR4A3 and their interactions with common *cis*-genomic elements, they exhibit unique activities and cell-/tissue-specific functions. Although endogenous ligands for NR4A receptors have not been identified, there is increasing evidence that structurally-diverse synthetic molecules can directly interact with the ligand binding domain of NR4A1 and act as agonists or antagonists, and ligands for NR4A2 and NR4A3 have also been identified. Since NR4A receptors are key factors in multiple diseases, there are opportunities for the future development of NR4A ligands for clinical applications in treating multiple health problems including metabolic, neurologic and cardiovascular diseases, other inflammatory conditions, and cancer.

Keywords

NR4A; multiple functions; nuclear receptors; novel ligands

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INTRODUCTION

The orphan nuclear receptor (NR) family has been characterized as a collection of nuclear receptors which share many structural domain similarities with other NRs; however, their endogenous ligands are unknown [1]. These receptors include NR0B1 (adrenal hypoplasia congenita critical region on chromosome X gene), NR0B2 (small heterodimer partner), NR1D1/2 (Rev-Erbαβ), NR2C1 (testicular receptor 2), NR2C2 (testicular receptor 2), NR2E1 (tailless), NR2E3 (photoreceptor-specific NR [PNR]), NR2F1 chicken ovalbumin upstream promoter transcription factor 1 (COUP-TFI), NR2F2 (COUP-TFII), NR2F6 (verbA-related protein), NR4A1 (Nur77), NR4A2 (Nurr1), NR4A3 (Nor1), and NR6A1 (GCNF).

In contrast to the other NRs, the orphan NR0B1 (DAX-1) and NR0B2 (SHP) receptors do not express a DNA binding domain (DBD) and primarily function as nuclear cofactors that influence gene expression through protein-protein interactions [2–4]. The three NR4A receptors have significant structural similarities in their ligand binding domains (LBDs) and DNA BDs, whereas their N-terminal (A/B) domains containing activation function 1 (AF1) are highly divergent [5–8]. NR4A receptors were initially defined as nerve growth factor-induced-β (NGFI-β) receptors that bind as monomers to an NGFI-β response element (NBRE:AAAGGTCA) [8–12]. NR4A receptors also bind as a homo- or heterodimer to a Nur-responsive element (NuRE:TGATATTACCTCCAAATGCCA) which has been characterized from the pro-opiomelanocortin gene promoter [13, 14]. Both NR4A1 and NR4A2 can also bind as heterodimers with the retinoid X receptor (RXR) to a DR5 motif [15, 16]. These receptor-DNA interactions are characteristic of all NRs (except NR0B1 and NR0B2) and there is also evidence that NR4A1 regulates gene expression through interactions with the specificity protein 1 (Sp1) transcription factor bound to its cognate GC-rich motif [17–19]. NR4A1 acts as a cofactor (along with p300) of Sp1, and many other NRs bind Sp1 and are integral cofactors for expression of Sp1-regulated genes [19–28].

The initial discovery of NR4A receptors was linked to their rapid induction by multiple stimuli in various tissues/cells and organs and these responses play a role in coping with both exogenous and endogenous stressors and the tissue-specific expression and induction of NR4A receptors contributes to their specificity (reviewed in [29, 30]). For example, NR4A receptors are induced by nerve growth factors in neuronal cells and by apoptosis-inducing agents in cancer cell lines [31–37]. In contrast, extensive studies with NR4A1 demonstrate that this receptor is not only induced by diverse anti-apoptotic agents but is also highly expressed in solid tumors and exhibits pro-oncogenic activity. Over the past decade, several timely and informative reviews on NR4A receptors have been published [29, 30, 38–42] and therefore this paper will primarily focus on more recent advances in the field.

NR4A1 in Cellular Homeostasis and Diseases

Individual and combined knockouts of NR4A1, NR4A2 and NR4A3 in mice have been described and extensively investigated to demonstrate the function of these receptors in maintaining cellular homeostasis and their role in disease. Thus, contributions of NR4A1 in

metabolic disease, inflammation, atherosclerosis and other responses will be discussed in subsequent sub-sections of this review. One of the earliest functions identified for NR4A1 was its induction in T-cell hybridomas or thymocytes undergoing apoptosis [43, 44]. Surprisingly, T-cell receptor-mediated apoptosis in NR4A1 knockout mice was not defective and other apoptosis inducers were also functional in these mice [45]. NR4A1 also modulates adrenocortical function by regulation of CYP21 expression; however, in NR4A1 knockout mice the function of the hypothalamic pituitary axis was intact and it was concluded that other factors expressed in these mice compensated for loss of NR4A1 [46, 47]. However, the loss of NR4A1 in mice has dramatic effects on inflammatory, immune, metabolic and neurological functions and these will be discussed under the proceeding subsections. The direct effects of NR4A1 loss in mice were more pronounced in some double knockout mice containing the loss of another NR4A gene. For example, the loss of both NR4A1 and NR4A3 in mice led to the rapid development of lethal acute myeloid leukemia (AML) in mice indicating tumor suppressor-like activity for these receptors [48]. Using a similar approach, it was shown that a decreased dose of NR4A1 and NR4A3 (e.g. $NR4A1^{+/-}$/$NR4A3^{-/-}$ and $NR4A1^{-/-}$/$NR4A3^{+/-}$) resulted in a condition resembling a mixed myelodysplastic/myoproliferative neoplasm [49]. It was also recently reported that loss of NR4A1, NR4A2 and NR4A3 in T-cells blocked development of Treg-cells and resulted autoimmune diseases in multiple organs [50]. Thus, the future development of tissue-specific knockout of one or more NR4A receptors in mice will be important for understanding the underlying functions of these receptors.

NR4A1 and Metabolic Diseases

Pearen and Muscat have reviewed the roles of NR4A1 and other NR4A genes in metabolic diseases [30] and have summarized the diverse stimuli associated with metabolic function that induce expression of NR4A receptors and their role in glycogen metabolism in skeletal muscle has been reviewed [51]. Several recent reports have expanded on the role of NR4A1 in obesity and type2 diabetes and the potential for using NR4A1 ligands for treating this disease which has been increasing dramatically in western industrialized countries. NR4A1, NR4A2 and NR4A3 are highly upregulated in obese individuals and significantly decrease after fat loss [52]. NR4A1, NR4A2 and NR4A3 are rapidly induced by cAMP in mouse hepatocytes and by glucagon in mouse liver and overexpression of NR4A1 induced gluconeogenic gene expression [53]. In mice injected with an adenoviral-NR4A1 construct there was an increase in blood and hepatic glucose levels, whereas a dominant negative adenoviral-NR4A1-M1 construct decreased blood glucose levels and other parameters consistent with a diabetic-like condition [53]. In contrast the protective effects of NR4A1 knockdown in normal mice is not observed in $NR4A1^{-/-}$ mice maintained on a high fat diet since these animals exhibit increased insulin resistance and hepatic steatosis [54]. This study also demonstrated that loss of NR4A1 increases insulin-resistance suggesting that NR4A1 expression in muscle and other tissue may influence whole body glucose metabolism and metabolic disease [54]. In diabetic db/db mice expressing NR4A1, blood glucose levels were higher than in the db/db/$NR4A1^{-/-}$ mice, whereas levels were similar in normal mice and high fat diet plus streptozotocin (STZ) mice which represent a non-genetic model for obesity and T2DM [55]. The rationale for the differences in NR4A1 function in these mouse models requires further investigation.

Wu and coworkers have been investigating the identification and effects of NR4A1 ligands on metabolic disease and have identified cytosporone B (CsnB) and related analogs and ethyl [2,3,4-trimethoxy-6-(i-octanoyl)phenyl] acetate (TMPA) as compounds that bound the ligand binding domain (LBD) of NR4A1 (Fig. 1) [34, 55, 56]. CsnB was characterized as an NR4A1 agonist that increased blood glucose levels and induced hepatic gluconeogenesis in C57BL/6 mice [56]. TMPA also interacted with the LBD or NR4A1 but in contrast to CsnB, TMPA decreased blood glucose in db/db mice and had lower levels of insulin and the effects were not observed in db/db/NR4A1$^{-/-}$ mice [55]. Moreover, in the non-genetic high fat diet/STZ-treated mice TMPA also decreased blood glucose levels and the effects were not observed in these mice after loss of NR4A1. In addition, TMPA also inhibited hepatic gluconeogenesis in db/db mice as evidenced by increased phosphorylation of AMPKα and repression of glucose-6-phosphatase (G6pc) and phosphoenolpyruvate carboxykinase (Pepck) gene expression which was not observed in db/db mice crossed with NR4A1$^{-/-}$ mice. NR4A1-dependent activation of hepatic gluconeogenesis has been reported in several studies [53, 56, 57] and using TMPA as an NR4A1 antagonist, an interesting pathway has been uncovered (Figure 1B) [55]. High levels of gluconeogenesis are associated, in part, with constitutive inactivation of AMPKα due to the inactivation of liver kinase B1 (LKB1) which is sequestered by NR4A1 in the nucleus. Inactivation of NR4A1 in cells treated with TMPA results in nuclear export of free LKB1 which in turn activates (phosphorylates) AMPKα resulting in the inhibition of gluconeogenesis [55].

Thus, it is apparent that NR4A1 plays an important role in metabolic disease and T2DM and is a potential target for treatment of metabolic diseases and their complications.

NR4A1 and Cardiovascular Disease

Since cardiovascular disease is associated with chronic inflammation, it is not surprising that NR4A receptors play a role in this disease (reviewed in [58–60]). NR4A1 is expressed and functional in many of the cell subtypes that contribute to the damage of arterial vessel cell walls and this includes vascular smooth muscle cells, endothelial cells, invading macrophages and monocytes. De Vries and coworkers first detected NR4A1 as a gene induced in human smooth muscle cells treated with growth factors and cytokines [61] and also in atherosclerotic lesions in mouse models [62–65]. Perturbation of smooth muscle cells increases NR4A1 expression, and results of knockdown or overexpression experiments suggest that this receptor inhibits proliferation [63, 64]. Balloon-injury induced neointimal hyperplasia in rat carotid arteries was inhibited after treatment with the antioxidant α-lipoic acid which also induced formation of cytoplasmic NR4A1 [66]. The protective effects of α-lipoic acid were decreased after NR4A1 knockdown *in vivo*. In vascular smooth muscle cells in culture, NR4A1 was important for α-lipoic acid-induced apoptosis, suggesting that the cytosolic NR4A1 is critical for induction of apoptosis and this is consistent with studies in cancer cells and tumors [41]. Similar results were observed in neonatal heart cells cultured under conditions resembling a high fat diet where reactive oxygen species (ROS) induced apoptosis and this was accompanied by increased cytosolic NR4A1 expression and interactions with the mitochondria as observed in some cancer cell lines [41].

NR4A1 is also induced by multiple factors in endothelial cells and plays a role in endothelial cell proliferation and angiogenesis [67–69]. A recent study reported that both histamine and serotonin are pro-angiogenic factors in endothelial cells and *in vivo* and these effects are dependent on the histamine and serotonin receptors and NR4A1 but are independent of vascular endothelial growth factor (VEGF). These responses are also transitory since after an extended period (10 day), the angiogenesis inhibitor thrombospondin 1 was induced by serotonin and histamine and this response was NR4A1-independent [70]. The role of NR4A1 in inflammation and macrophages will be discussed separately; however, macrophages in areas of plaque formation express NR4A1 [70, 71]. Moreover, in both cell models and ApoE$^{-/-}$ mice maintained on a high fat/cholesterol diet, increased expression of NR4A1 or activation of the receptor by CsnB decreased macrophage derived foam cells and decreased atherosclerotic plaque formation [72]. This was also accompanied by decreased expression of inflammatory and adhesion genes and decreased hepatic lipid deposition and intestinal absorption of lipids, whereas the opposite effects were observed after NR4A1 knockdown. These results were consistent with transgenic animal studies showing that expression of NR4A1 results in inhibition of macrophage accumulation and matrix metalloproteinase levels in mouse models [62]. Complementary results [73] were also observed in ApoE$^{-/-}$/NR4A1$^{-/-}$ mice that exhibited increased atherosclerosis after 11 weeks on a western diet, and the loss of NR4A1 enhanced atherosclerosis, enhanced toll-like receptor signaling and pro-inflammatory macrophages. The importance of NR4A1 in inflammatory lymphocyte antigen bC (Ly-bC^{high}) and its function in healing after myocardial infarction has also recently been reported [74]. Ly-bC^{high} regulates a biphasic inflammatory and reparative response in the healing process and the loss of NR4A1 impairs healing and macrophages.

Thus, NR4A1 essentially plays a protective role in cardiovascular disease, and the protective effects of NR4A1 and Csn in the high fat/cholesterol mouse model [72] were dissimilar to those observed in db/db and non-genetic models of metabolic disease where NR4A1 promotes metabolic disease [55, 56]. It will be important to determine the role of human NR4A1 in these responses prior to clinical applications of NR4A1 ligands.

NR4A1 and Neurological Functions

NR4A2 (Nurr1) has been extensively investigated with respect to neuronal function since Nurr1$^{-/-}$ mice exhibit a well characterized selective loss of dopamine biosynthesis in the substantial Nigra/Ventral Tegmental area of the brain but not in hypothalamic neurons [75]. However, there is not only substantial evidence for expression of NR4A1 in various regions of the brain [76, 77] but also an increasing number of reports demonstrating the neuronal functions of this receptor [78]. cAMP response element binding protein (CREB) is an important nuclear transcription factor involved in neuroprotection, and results of cell culture and *in vivo* studies indicate that NR4A receptors mediate CREB-dependent neuroprotection [79]. Induced learning in mice by contextual fear conditioning increased expression of NR4A1, NR4A2 and NR4A3 in the hippocampus and similar results were observed for histone deacetylase inhibitor-induced enhanced memory [80]. A recent study delineated differences in the functions of NR4A1 and NR4A2 in the brain; NR4A2 was important for long term memory, object location and recognition, whereas NR4A1 was required only for

object location [81]. NR4A1 has also been linked to synaptic remodeling, response to L-DOPA, behavioral changes and dopaminergic loss after administering 1-methly-4-phenyl-1,2,3,6-tetrahychopyridine (MPTP) in mice [82–85]. MPTP-induced loss of dopaminergic neurons is more severe in NR4A1$^{-/-}$ mice compared to wild-type mice, and MPTP-dependent downregulation of NR4A1 is mediated by decreased expression of myocyte enhancer factor 2D (MEF2D) [85]. Patients chronically treated with antipsychotic drugs may develop tardive dyskinesia (TD) and in rodent models of this disease, there is an increase in NR4A1 expression [83, 86]. This has also been observed in non-human primates and it has been suggested that NR4A1 may be a target for intervention [86]. Another possible chemotherapeutic role for NR4A1 ligand may for treatment of strokes since NR4A1 is decreased in neural cells deprived of oxygen and glucose, and neural damage is rescued by NR4A1 overexpression [87]. Thus, the development of NR4A1-specific ligands for treatment of some neurological disorders represents both an opportunity and challenge for the future.

NR4A1 and Arthritis

Arthritis is another example of an inflammatory disease, and both NR4A1 and NR4A2 are induced in experimental models of inflammation [88–90]. For example, type II collagen-induced arthritis was significantly decreased in mice overexpressing NR4A1 compared to wild-type mice [88], suggesting another possible therapeutic target for an NR4A1 agonist such as Csn.

NR4A1 and Inflammation and Immune Responses

The rapid induction of NR4A receptors in response to diverse inflammatory agents and their rolls in T-cell receptor-mediated apoptosis has been reviewed [91] and noted in the Introduction to this article. Moreover, the roles of NR4A1 in metabolic, cardiovascular and neurological disease and arthritis are associated with inflammatory conditions. With the exception of metabolic disease models, most studies on inflammation and immune responses suggest that although NR4A1 is induced under inflammatory conditions, the receptor tends to be protective and is a potential target for NR4A1 agonists. Key recent papers include the observation that (i) NR4A receptors (all 3) play an important role in T-cell development [50], (ii) NR4A1 regulates subsets of genes important for differentiation of Treg cells [92], and (iii) NR4A1 is important for the anti-inflammatory effects of apoptotic cells in macrophages [93].

NR4A1 and Cancer

NR4A1 and its role in cancer have been recently reviewed [38–42] and only the important key concepts will be included in this article. Results of animal studies in which NR4A1 and NR4A3 have been knocked out and subsequent work on cell culture models indicate that NR4A1 is a tumor suppressor for the AML form of leukemia [48, 49]. In contrast, studies in other leukemia cell lines suggest a possible oncogenic role for NR4A1 [94] and research on the leukemia-type dependent differences in the function of NR4A1 is ongoing. The expression and function of NR4A1 in solid tumors is consistent in multiple tumor types. For example, NR4A1 is overexpressed in colon, pancreatic, breast (estrogen receptor positive and negative), and lung tumors, and in breast, colon and lung tumor patients high expression

of NR4A1 predicts decreased survival [17, 95–100]. The functional activity NR4A1 in cancer has been extensively investigated in cancer cell lines by either knockdown or overexpression, and results have shown that in lung, melanoma, lymphoma, pancreatic, colon, cervical, ovarian, and gastric cancer cell lines, NR4A1 regulates one or more of cancer cell proliferation, survival, cell cycle progression, migration, and invasion [17, 96, 98–107].

The mechanisms of action of NR4A1 are highly complex and involve both the nuclear and cytosolic receptors. Some of the earliest studies on NR4A1 in cancer cells demonstrated a novel pathway in which the caged retinoid compound CD437 and several analogs and diverse apoptosis-inducing agents induce apoptosis in cancer cell lines by inducing nuclear export of NR4A1 [108–120]. This nuclear export pathway has been linked to formation of a pro-apoptotic mitochondrial NR4A1-bcl2 complex, and this is also observed using peptide mimics and paclitaxel which simulates NR4A1 interactions with bcl2 [121, 122]. Cytosolic NR4A1 plays an important role in the mechanism of action of several pro-apoptotic antineoplastic agents including platinum-based drugs [123]. It was also observed that increased expression of chromodomain helicase/adenosine triphosphatase (ATPase) DNA-binding protein 1-like (CHD1L), which inhibits nuclear export of NR4A1, was associated with the increased survival of hepatocellular carcinoma cells [124].

The extranuclear activity of NR4A1 is a drug-induced response which invariably results in the induction of apoptosis; however, results of most knockdown or overexpression studies demonstrate a role for NR4A1 in cell proliferation, survival, migration and invasion. Presumably these responses are primarily due to NR4A1-regulated genes, and results in pancreatic cancer cells have identified genes that fit into each of these categories [101]. Mechanistic studies in colon cancer cells have identified several pathways that are consistent with the pro-oncogenic functions of nuclear NR4A1. Figure 2 summarizes some of these pathways observed in colon cancer cells. NR4A1 interacts with and inactivates p53 and, based on results of RNAi experiments, this results in activation of mTOR due to decreased expression of p53-regulated sestrin 2 and inactivation of AMPKα [96]. NR4A1 also regulates expression of survivin and other Sp-regulated genes containing GC-rich promoters [17, 18], and NR4A1 also regulates redox genes such as isocitrate dehydrogenase1 (IDH1) and thioredoxin domain containing 5 (TXNDC5) to maintain low levels of intracellular stress [18, 101]. NR4A1 also activates the pro-invasion gene MMP9 and suppresses E-cadherin [98] and also modulates β-catenin expression through multiple pathways [100, 125, 126]. These are examples of some NR4A1-regulated genes and pathways in colon cancer cells and other pathways including the cooperative role of NR4A1 in TGFβ-induced epithelial-mesenchymal-transition (EMT) in breast cancer cells [99], demonstrating the pro-oncogenic functions of the receptor. Thus, development of NR4A1 antagonists will be highly advantageous for cancer chemotherapy due to their potential for disabling multiple pro-oncogenic pathways (Fig. 2).

Wu and coworkers identified 3 structurally diverse compounds that bind NR4A1-LBD, namely CsnB and related compounds, TMPA, and 1-(3,4,5-trihydroxyphenyl)nonan-1-one (THPN) (Figure 1) [34, 55, 56, 127]. Results of modeling and receptor mutation studies show that these compounds interact with different amino acid side-chains within the LBD.

CsnB exhibits NR4A1 agonist activity in transactivation assays and inhibits cancer cell and tumor growth and these effects are associated with nuclear export of NR4A1 [65]. The antineoplastic activity of THPN is also due to nuclear export of NR4A1 [127]. There is some evidence that TMPA may act as a nuclear NR4A1 antagonist; however, the anticancer activates of this compound has not been characterized [55]. Studies in this laboratory have identified 1;1-bis(3′-indolyl)-1-(*p*-substituted phenyl)methane (C-DIM) compounds that modulate NR4A1-dependent transactivation [17, 18, 96, 97, 101, 128]. Early studies identified the *p*-methoxy-phenyl analogy (DIM-C-pPhOCH_3) as a potential NR4A1 agonist in pancreatic cancer cells using rodent derived NR4A1 constructs [128]; however, in subsequent studies using human NR4A1 we observed that this compound was only a weak agonist and most C-DIMs inhibited NR4A1-dependent transactivation [18, 39]. In collaboration with the Wu laboratory, we have now shown that many C-DIM analogs including the *p*-hydroxyl, trifluoromethyl, bromo, unsubstituted, cyano, chloro, iodo and carboxymethylphenyl analogs all directly bind the NR4A1-LBD [18]. Modeling studies for the highly active *p*-hydroxyphenyl compound (DIM-pPhOH) (Kd = 0.11 μM) show a unique interaction with the LBD of NR4A1 that differs from other ligands identified in the Wu laboratory. DIM-C-pPhOH and related compounds act directly on nuclear NR4A1 and exhibit NR4A1 antagonist activity, and results in cancer cell lines and tumors show that DIM-C-pPhOH is a highly effective anticancer agent [17, 18, 96, 97, 101]. As an NR4A1 antagonist, DIM-C-pPhOH inhibits the pro-oncogenic NR4A1-dependent pathways outlined in Figure 2, suggesting that C-DIM compounds and other NR4A1 antagonists represent an important new class of mechanism-based anticancer agents.

NR4A2 in Cellular Homeostasis and Disease

The nuclear receptor NR4A2 (Nurr1, HZF-3, RNR1, NOT, DHR38) is the second member of the NR4A family and possesses structural motifs and complex patterns of transcriptional activity similar to NR4A1 and NR4A3. The DNA-binding domain of NR4A2 is over 92% homologous to the same domain of NR4A1 (Nur77), conferring similarities both in sequence identity and function between these receptors [129]. Research over the past two decades has demonstrated activities of NR4A2 associated with energy metabolism, atherosclerosis and vascular function, T-cell receptor (TCR)-mediated apoptosis, inflammatory responses, regulation of the hypothalamic-pituitary axis (HPA) and reproductive processes [59]. Additionally, NR4A2 plays a significant role in development and homeostasis of the central nervous system and has been associated with functional working memory as well as neurological disorders such as Parkinson’s disease [78, 130]. Like NR4A1 and NR4A3, NR4A2 modulates target gene transcription by binding as a monomer, homodimer or heterodimer with RXR to *cis*-acting response elements such as the NGFI-B-responsive element (NBRE) located in gene promoter regions, which exhibit a canonical AAAGGTCA consensus sequence [131, 132]. Additionally, NR4A2-RXR heterodimers bind to a related sequence motif, DR5 (5′-AGGTCANNNAAAGGTCA-3′) in the presence of 9-*cis*-retinoic acid, whereas NR4A2 homodimers bind to another related sequence with the palindromic structure, 5′-TGACCTTTNNNNNAAAGGTCA-3′ [133]. The transcriptional activity of NR4A2 is not limited to transactivation but also includes transcriptional repression or “transrepression” by a mechanism involving recruitment of

nuclear co-repressor proteins that stabilize histone-DNA binding and suppress gene expression [134, 135]. Genome-wide transcriptomic and ChIP-on-ChIP studies have described a large number of target genes that are regulated by NR4A2 but the effects of NR4A2 activation on gene expression are highly dependent on cell type and on the nature of the signaling event studied. For example, NR4A2 activation can induce apoptosis in cancer cell lines [136, 137], but can also stimulate development and maturation of dopaminergic neurons [138–140], as well as block inflammatory responses in macrophage cells [130]. The loss of NR4A2 in mice results in the failure of dopamine neurons to differentiate [77] and like NR4A1, NR4A2$^{-/-}$ mice exhibit many other deficits as outlined below. To understand the regulatory effects of NR4A2, it will therefore be important to elucidate cell-specific signaling mechanisms and to identify distinct protein complexes that associate with either transcriptional activation or repression. Although the effects of NR4A2 are diverse, it has been proposed that NR4A transcription factors are the first-wave transcriptional response to environmental cues that cause diverse adaptive changes in cellular physiology [78].

NR4A2 and Metabolic Disease

Because of its effects in regulating genes important for metabolism, small molecular activators of NR4A2 are highly sought after as potential therapeutic agents for metabolic disease. The crystal structure of the human NR4A2 LBD indicates that it does not apparently contain a classical ligand binding cavity, as seen with other NR4A family members and with other steroid hormone receptors, due to the intrusion of side chains from several bulky hydrophobic residues in the region normally occupied by ligands [8]. These structural studies also suggested that the conformation of the NR4A2 LBD might confer a level of constitutive activity, due to the resemblance to the ligand-bound conformation of RXR. Later computer-based modeling of the NR4A2 LBD identified a hydrophobic region opposite the classical co-activator-binding site that is critical for transcriptional activity, as demonstrated by site-directed mutagenesis studies [141]. Mutations in this region reduced or abolished transcriptional activity of the NR4A2 LBD and indicated that proteasome-dependent degradation was important for NR4A2 protein turnover and modulation of the transcriptional effects of the receptor. Although the endogenous ligand for NR4A2 has yet to be identified, a number of compounds have been reported to activate or otherwise modulate the activity of the receptor. Among these, the anti-metabolite cancer drug 6-mercaptopurine, which is widely used for the treatment of acute childhood leukemia and chronic myelocytic leukemia, was shown to induce NR4A2 and NR4A3 through a motif in the N-terminal AF-1 domain of the receptor [142]. However, direct binding of 6-mercaptopurine to NR4A2 remains to be determined. It has also been reported that several benzimidazole compounds have high affinity for the NR4A2, as well as a series of isoxazolopyridinone compounds [30]. Another synthetic small molecule activator of NR4A2, 1,1-bis(3′-indolyl)-1-(*p*-chlorophenyl)methane (C-DIM12), caused ligand-dependent activation of NR4A2 and subsequent poly(ADP-ribose) polymerase (PARP) cleavage and apoptosis in bladder cancer cells that was abolished by RNAi knockdown of NR4A2 [136]. Recent modeling and receptor binding studies with this class of compounds have identified several C-DIM analogs that directly binding to NR4A1 in a groove along the co-activator interface of the LBD [18]. This domain is highly conserved between NR4A family members, suggesting

that selected C-DIM compounds with small, polar substituents on the phenyl ring could modulate NR4A2 transcriptional activity through direct binding to the receptor

The ability of NR4A2 to regulate the expression of multiple genes associated with metabolism and gluconeogenesis suggests that this factor may also be an important regulator of metabolic disease. The hepatic expression of NR4A2 is induced by cAMP in response to glucagon as well as a number of other compounds acting on the β-adrenoreceptor signaling axis, including fatty acids, glucose, insulin, cholesterol and thiazolidinediones [30]. NR4A2, as well as NR4A1 and NR4A3, are induced in rat liver after dietary restriction, underscoring the importance of the NR4A receptor subgroup with dietary inputs positively regulating metabolism [143]. Furthermore, dietary restriction in these studies also increased expression of Ucp-3, Ampkg3, Pgc-1a and Pgc-1b in muscle, which is consistent with increased activity of both NR4A1 and NR4A3 and which positively correlated with improved glucose utilization and insulin sensitivity. Other metabolically associated genes regulated by NR4A2 include the ATP-binding cassette subfamily G members 5 and 8 (AbcG5/8), Apolipoprotein B and E (ApoB/E), fatty acid synthase (Fas), fructose-1,6-bisphosphatase 1 and 2 (Fbp1/2), glucose transporter 4 (Glut4), uncoupling protein 2 and 3 (Ucp2/3), and the peroxisome proliferator-activated receptor-γ (Pgc1a), as extensively reviewed by Pearen and Muscat [30]. The NR4A subgroup, including NR4A2, is likely critically important for glucose utilization and for maintaining homeostasis in cholesterol and fatty acid metabolism. Small molecular ligands of NR4A2 could therefore be effective in treating aspects of metabolic disease and are being intensively studied for this purpose.

NR4A2 and Cardiovascular Disease

Atherosclerosis is characterized by hardening of arteries as a result of formation of plaques that compromises normal blood flow over time. Athersclerotic plaques contain fat, cholesterol and calcium deposits that stimulate a proliferative response in smooth muscle cells within the media of arteries, resulting in further constrictions to blood flow that may lead to myocardial infarction, stroke and death. Activation of endothelial cells at atherosclerotic plaques attracts circulating monocytes that represent an early event in the development of atherosclerotic lesions. NR4A receptors are moderately induced in atherosclerotic endothelial cells and macrophages and it has been proposed that amongst this receptor subfamily, NR4A3 promotes the development of atherosclerotic lesions, whereas NR4A1 and NR4A2 attenuate atherosclerosis [132]. NR4A2 also appears to have an anti-mitogenic effect in smooth muscle cells, which antagonizes the formation of atherosclerotic plaques [144]. The ability of NR4A2 to inhibit NFκB-dependent expression of inflammatory genes in macrophages may also contribute to the anti-atherogenic activity of this factor [59, 135]. This activity may be of particular importance, given that activated macrophages release cytokines and growth factors that aggravate local inflammation and activate underlying smooth muscle cells, leading to excessive uptake of lipids and the transition of macrophages into lipid-laden foam cells that remain resident in the atherosclerotic lesion [58]. Supporting the role for NR4A2 in protection against atherogenesis, it was discovered that NR4A2 is negatively regulated by miR-145 in smooth muscle cells and that mice lacking miR-145 are resistant to the development of atherosclerotic plaques, owing to their high expression of NR4A2 [145]. Additionally, studies in human macrophages using

lentiviral-mediated overexpression or knockdown of NR4A2 demonstrated that NR4A2 inhibited the uptake of oxidized LDL by macrophages and reduced the expression of pro-inflammatory cytokines and chemokines [146]. Collectively, these data support a role for NR4A2 in protection against cardiovascular disease.

NR4A2 and Neurological Function

NR4A2 has pleiotropic effects on gene expression in the brain which are highly dependent on cell type and on the specific extracellular signal or stressor encountered. Studies in human neural SK-N-AS cells were conducted in which a number of stable clonal lines were constructed with graded NR4A2 gene expression to approximate levels of NR4A2 seen in dopaminergic neurons present in human substantia nigra [147]. Transcriptomic data acquired from these NR4A2-expressing clonal lines revealed that the effects of NR4A2 on target genes varied considerably as a function of its concentration. Nearly one-fifth of NR4A2-responsive transcripts showed bidirectional changes with increasing NR4A2 expression where some genes were induced and others suppressed. Transcripts that were induced by increasing concentrations of NR4A2 included genes such as the neurodevelopment factors Crmp1, Kif1a and Tubb2a, whereas a number of transcripts were decreased at all higher concentrations of NR4A2, including the NFκB-related transcripts (Nfkb1, Nfkb1a), TNF-related transcripts (Tnf, Tnfip1, Tnf4sf4), and the peroxisome proliferator-activated receptor γ (Pgc1) [147]. These data strongly suggest that NR4A2 exerts concentration-dependent effects that dramatically influence transcriptional programs in neural cells.

NR4A2 is widely expressed throughout the brain and is present in telencephalic structures such as the cortex and hippocampus, although it is most well studied in context of its effects in dopaminergic neurons. As an immediate-early gene encoding a member of the steroid-thyroid hormone receptor family, NR4A2 is also rapidly induced following stress and injury in the CNS. In postnatal mice exposed to the glutmate receptor agonist, kainic acid, NR4A2 protein levels were rapidly induced in pyramidal neurons in the CA1 and CA3 layers of the hippocampus, as well as more transiently in the dentate gyrus, a region generally more resistant to neuronal in injury from kainic acid exposure [148]. NR4A2 is also involved with memory and learning, which may be mediated in part by MAP kinase signaling pathways that alter its phosphorylation state and its nuclear localization, described in studies where stimulation of ionotropic glutmate receptors (AMPA, NMDA) resulted in increased phosphorylation of NR4A2 and its export from the nucleus [78]. Interactions between NR4A2 and the cyclic-AMP response element binding protein (CREB) are important for memory and knockdown of NR4A in the hippocampus using antisense oligonucleotides impaired long-term memory and reversal learning in an appetitive spatial learning task [149].

In dopaminergic brain regions, particularly the substantia nigra (SN) and the ventral tegmental area (VTA), NR4A2 is important both for development and homestasis of dopamine producing neurons. This was initially demonstrated by studies using NR4A2 knockout mice, in which mice lacking NR4A2 failed to generate midbrain dopaminergic neurons, were hypoactive and died during the early postnatal period [77]. NR4A2 is also

required for maintenance and maturation of adult midbrain dopamine neurons [139], in part, through its regulation of dopamine synthesis and metabolism [150, 151]. Mice heterozygous for NR4A2 display an age-dependent decline in the number of dopaminergic neurons in the SN compared to wild-type mice and also exhibit a decrease in peak evoked DA release that is only partly compensated by increased expression of the dopamine transporter [152]. The age-related decline in neurological function in heterozygous NR4A2 knockout mice correlates with the effects of decreased NR4A2 expression in models of Parkinson's disease (PD), where reduced expression of NR4A2 increases the vulnerability of mesencephalic dopamine neurons to injury from 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [153]. In a study of 201 individuals affected with PD and 221 age-matched unaffected controls, two mutations were identified that associated with PD and mapped to the first exon of NR4A2 [154]. A subsequent study in 278 patients with PD, 166 healthy controls (HC), and 256 neurological disease controls revealed that lower expression of NR4A2 resulted in a significant increase in the risk for developing PD [155]. Selected point mutations in human NR4A2 are also implicated in PD by decreasing expression of tyrosine hydroxylase, the rate limiting enzyme in dopamine synthesis [156]. NR4A2 also regulates the expression of α-synuclein, the major protein constitutent of Lewy Body aggregates in PD, and decreased expression of NR4A2 transcriptionally increases α-synuclein expression [157]. The importance of NR4A2 in PD was also highlighted in recent studies using a synthetic small molecular activator of NR4A2, 1,1-bis(3′-indolyl)-1-(*p*-chlorophenyl)methane, which protected against MPTP-induced loss of dopaminergic neurons in a mouse model of PD and increased expression and nuclear localization of NR4A2 in the SN [158]. Collectively, these data indicate that NR4A2 is an important factor regulating multiple physiologic functions in the CNS but also suggest that this transcription factor in important in protection against oxidative and inflammatory stress relevant to neurodegenerative disorders including PD.

NR4A2 in Inflammatory and Immune Responses

NR4A2 appears to have both constitutive and inducible anti-inflammatory activity in monocyte/macrophage lineage immune cells, as well as in brain glial cells including both astrocytes and microglia. This anti-inflammatory activity appears to be directed towards the NFκB signaling pathway in response to inflammatory stimuli such as tumor necrosis factor α (TNFα) and bacterial lipopolysaccharide (LPS). Following exposure to TNFα or LPS, the p65 (RelA) subunit of NFκB rapidly translocates to the nucleus, where its histone deacetylase activity and subsequent phosphorylation by GSK3β facilitates opening of chromatin and removal of constitutively bound nuclear co-repressor complexes [135]. These studies also report that sumoylation of NR4A2 on K577 of the LBD is essential for this transrepressive activity. Earlier studies support this conclusion, because K577 in the NR4A2 LBD is part of a consensus SUMO-modification sequence and mutation of this lysine to arginine results in decreased transcriptional activity, suggesting that sumoylation of K577 is important for transcriptional modulation by NR4A2 [141]. In brain glial cells, the anti-inflammatory effects of NR4A2 are mediated by docking to NFκB-p65 on target inflammatory gene promoters, followed by recruitment of the CoREST co-repressor complex, resulting in clearance of NFκB-p65 and transcriptional repression [135]. Because inflammatory activation of glial cells is critical to the progressive loss of dopaminergic neurons in PD, these studies suggest that NR4A2 protects against neuronal loss in part by

limiting the production of neurotoxic mediators by microglia and astrocytes. NR4A2 also plays a pro-inflammatory role in synoviocytes associated with arthritis and a recent report that NR4A2 regulation of prolactin expression contributes to this response [159].

NR4A2 also influences maturation and differentiation of Th17 T-cells and may thereby have a role in both autoimmunity and in resolution of infections [160]. NR4A2, but not NR4A1 or NR4A3, is upregulated in rheumatoid arthritis, where its expression is induced by PGE2, IL-1β, and TNF-α [161], suggesting that this factor may have broad effects in limiting inflammatory responses through its function as a transrepressor of the NFκB pathway. Additionally, NR4A2 regulates expression of the Forkhead transcription factor Foxp3, which is important for differentiation of regulatory T cells (Treg cells) and is mediated through direct interaction of NR4A2 with Runx1 [30]. Induction of NR4A2, along with other soluble and cell-surface mediators with anti-inflammatory activities (e.g., IL-10, TGF-β, resolvins, ligands for TAM receptors), is important to attenuate responses to inducers or amplifiers of inflammation [130]. Based on these and other studies, NR4A2 appears to be broadly important for regulating both inflammation and resolution of inflammatory signaling in activated immune cells and glial cells.

NR4A2 and Cancer

A number of receptor knockdown or overexpression studies both *in vivo* and in cancer cell lines demonstrate that NR4A orphan receptors exhibit pro-oncogenic or tumor suppressor-like activity that is dependent on the type of tumor [38]. NR4A receptors have been shown to enhance cell proliferation, apoptosis, and differentiation in a tissue-specific context [29]. NR4A2 is upregulated in normal breast epithelium compared to breast cancer cells, suggesting an inverse correlation between breast cancer and the level of NR4A2 expression [162]. These studies also reported that short hairpin RNA (shRNA)-mediated silencing of NR4A2 gene expression in breast tumor xenografts in mice significantly reduced tumor growth [132]. Immunohistochemical analyses of human prostate cancer biopsies indicated that expression of NR4A2 was significantly higher than in normal controls, suggesting an inverse relationship between expression of NR4A2 and tumor growth [163]. Likewise, silencing of NR4A2 expression *in vitro* in prostate cancer cells reduced cell proliferation, invasion and migration, indicating that NR4A2 could a biomarker for the progression of breast and prostate cancer [132].

NR4A2 is more highly expressed in estrogen receptor-positive breast and bladder tumors compared with normal tissue, and higher levels of cytoplasmic NR4A2 were a prognostic factor for high tumor grade, decreased survival, and increased distant metastasis in a cohort of bladder cancer patients [38]. NR4A2 is also more highly expressed in prostate tumors compared to normal prostate and correlates with tumor classification and Gleason score as a negative prognostic factor [163]. Small molecules that stimulate the inhibitory effects of NR4A2 may have promise in treating cancer, demonstrated by the effects of the NR4A2-acting compound 1,1-bis(3′-indolyl)-1-(*p*-chlorophenyl)methane (C-DIM12 or DIM-C-pPhCl) which induced TRAIL protein expression and PARP cleavage in bladder cancer cells that was significantly decreased by inhibition of NR4A2 with RNAi [136]. Interestingly, overexpression of NR4A2 in colorectal cancer cells revealed that ectopic expression of

NR4A2 increased resistance to the chemotherapeutic agents 5-fluorouracil and oxaliplatin and attenuated the chemotherapeutic-induced apoptosis [164]. Using tissue microarray analysis, these studies also found that NR4A2 expression was increased in colorectal cancer specimens collected from 51 adenomatous colorectal cancers, 14 familial adenomatous polyposis colorectal cancers, 17 stage IV colorectal cancers with adjacent mucosa, and 682 stage I–III colorectal cancers. Increased expression of NR4A2 was related to protein kinase A activation and was correlated with chemoresistance [164]. These data demonstrate that NR4A2 expression predicts poor survival and drug resistance in various cancers, including breast, prostate, bladder and colon cancers.

NR4A3 in Cellular Homeostasis and Disease

The nuclear receptor NR4A3 (Nor1, TEX, MINOR, CHN) is the third member of the NR4A family and shares many of the same characteristics reported for NR4A1 and NR4A2. NR4A3-mediated transactivation and interactions with various *cis*-elements, except that unlike NR4A1 and NR4A2, NR4A3 does not form a heterodimer with RXR [15, 16]. Although there is some redundancy in the functions of the three NR4A receptors since these receptors are induced as early immediate genes by some of the same stressors [29, 30], each receptor also exhibits unique functions. One study reported that loss of NR4A3 in mice was embryo-lethal [165]; however, subsequent studies indicate that NR4A3$^{-/-}$ mice survive but exhibit deficits in the semicircular canals of the inner ear and in hippocampal development [166, 167]. This latter response can lead to several neuronal deficits and enhanced kainic acid-induced seizures. As indicated previously, double knockout NR4A1$^{-/-}$/NR4A3$^{-/-}$ mice rapidly develop acute AML-type leukemia and have been designated as tumor suppressors for this type of cancer [48, 49]. Moreover, studies on knockdown of NR4A3 and other NR4A receptors demonstrate a role for NR4As in immune homeostasis and regulation of T-cell development and aspects of metabolic disease [50, 52]. Muscat and coworkers have previously reviewed the physiological and pathophysiological roles of NR4A3 and other NR4A receptors [29, 30], and this article will highlight some of these functions and more recent studies.

NR4A3 and Metabolic Disease

Knockdown of NR4A3 in c2c12 skeletal muscle cells resulted in changes in gene expression consistent with a shift from oxidative to anaerobic gene expression [168], and subsequent studies in NR4A3-overexpressing mice demonstrated that NR4A3 increases type II muscle fibers and resistance to fatigue [169]. A role for NR4A3 in high vs. low running capacity in rodents was also reported [170]. NR4A3 induced cAMP in hepatocytes and in mouse liver in fasted mice [53] and levels were upregulated in obese patients [52]. The role of this receptor in mouse models of obesity and T2DM have not been extensively investigated.

NR4A3 and Cardiovascular Disease

NR4A3 is expressed in atherosclerotic lesions and is induced by diverse stressors in smooth muscle cells [69, 171–174], and knockdown experiments in smooth muscle cells suggest that this receptor plays a role in proliferation of these cells [174, 175]. NR4A3-induced proliferation has been linked to regulation of cyclins D1 and D2 [174, 175] and S-phase

kinase-associated protein 2 (Skp2) which results in decreased expression of the cyclin-dependent kinase inhibitor p27 [176]. A recent study showed that miR-638 is also a key regulator of smooth muscle cell proliferation by targeting NR4A3 which results in silencing of NR4A3-regulated genes involved in cell proliferation [177]. These results coupled with data from a mouse model overexpressing NR4A3 in smooth muscle cells demonstrate that in contrast to NR4A1, NR4A3 serves to enhance neointintima hyperplasia. A recent study showed that NR4A3 inhibited NFκB signaling in vascular smooth muscle cells demonstrating an anti-inflammatory function in this cell type [178]. NR4A receptors including NR4A3 are induced by VEGF in endothelial cells [69] and this proliferative response is inhibited after NR4A3 knockdown [171, 179]. A recent study reported that NR4A3 regulated vascular cell adhesion molecule-1 (VCAM-1) in endothelial cells through direct binding to an NBRE promoter element [180]. NR4A3 plays a role in monocyte adhesion and in an *in vivo* model for atherosclerosis using ApoE$^{-/-}$/NR4A3$^{-/-}$ mice, it was confirmed that NR4A3 regulates recruitment of monocytes to the vascular wall. NR4A3 facilitates macrophage recruitment, whereas the opposite response is observed for NR4A1 [72].

NR4A3 and Neurological Functions

NR4A genes have important neuronal functions [80–82] and studies with NR4A3$^{-/-}$ mice show specific hippocampal functions for this receptor. NR4A3 is a CREB-regulated gene and plays a role in memory enhancement by HDAC inhibitors [80]. More recent studies show differential expression of NR4A receptors [181, 182] and studies on dopamine neurons showed that in the ventral tegmental area, haloperidol rapidly induced NR4A3 and NR4A1 (but not NR4A2) and this was accompanied by induction of tyrosine hydroxylase and the dopamine transporter-mRNA. Functional studies also showed that NR4A3 expression in the Wistar-Kyoto rat contributed to depressive behavior [183]. Moreover, polymorphisms within the NR4A3 gene are correlated with nicotine addiction in patients with mental health disease [184], and it is possible that NR4A polymorphisms may also be associated with other receptor mediated health problems.

Inflammation and Immune Responses

NR4A3 like all NR4A receptors is induced by stressors and is upregulated by inflammatory conditions and also plays an integral role in T-cell receptor-induced apoptosis [29, 30, 49, 50]. Knockdown of NR4A1, NR4A2 and NR4A3 (combined) in mice resulted in death within 3 weeks and among the double knockout mice, only the NR4A1$^{-/-}$/NR4A3$^{-/-}$ mice died within 3–4 weeks [50]. Moreover, in this same study development of Treg cells was also decreased and it was concluded that “NR4A1 and NR4A3 were the main contributors to Treg cell homeostasis and the prevention of autoimmunity” [50].

NR4A3 in Cancer

The combined loss of NR4A3 and NR4A1 in mice results in acute AML-type leukemia [48, 49] and HDAC-inhibitor mediated apoptosis in AML cells is accompanied by induction of NR4A3 [185]. These results demonstrate a role for NR4A3 (in combination with NR4A1) as a tumor suppressor for AML; however, the function of this receptor in other leukemias has

not been determined. NR4A3 is downregulated in nasopharyngeal carcinomas due to promoter hypermethylation, and in cell lines overexpression of NR4A3 decreased cell proliferation and colony formation and this was consistent with tumor suppressor activity [186, 187]. NR4A3 is one of only a few NRs overexpressed in ER-positive and ER-negative breast tumors, and a recent report shows that NR4A3 expression is higher in triple negative vs. luminal tumors [95, 188]; however, the function of this receptor in breast cancer has not yet been determined. NR4A3 is also overexpressed in human hepatocellular carcinomas and induces hepatocyte proliferation, and the function of this gene in liver cancer cells has not been determined [189]. Genomic studies in horses have also linked NR4A3 to susceptibility to melanoma [190] but expression and functions in human melanomas have not been determined. Prostaglandin A2 is the only reported NR4A3 ligand [191], and future clinical applications for targeting NR4A3 will require additional insights on tumor-specific functions of this gene and development of new ligands.

Summary

NR4A1, NR4A2 and NR4A3 are orphan nuclear receptors and immediate early genes induced by multiple stressors. All three receptors bind the same genomic *cis*-elements; however, their distinct differences in activities are due, in part, to their more unique N- and C-terminal domains that differentially interact with various cofactors and ligands and their tissue-specific expression. Complete and tissue-specific knockout mouse models uniquely distinguish between the different roles for these receptors in metabolic, cardiovascular, neurological, immune and inflammatory functions, cancer and related diseases. Although endogenous ligands for NR4A receptors have not been identified, several recent studies have identified structurally diverse compounds that bind and activate or inactivate nuclear NR4A1 or induce nuclear export of NR4A1, and these compounds show some promise in the treatment of conditions such as metabolic diseases and cancer. For example, a recently published paper showed that the pro-fibrotic effects of transforming growth factor-β (TGF-β) signaling was inhibited by NR4A1 which recruits inhibitory chromatin-modifying complexes to the promoters of TGF-β-regulated genes [192]. The NR4A1 agonist cytosporone B inhibits experimentally-induced fibrosis in multiple tissues “demonstrating the first proof of concept for targeting NR4A1 in fibrotic diseases” [192]. Future clinical applications of NR4A ligands will require the synthesis and development of ligands specific for NR4A1, NR4A2 and NR4A3 and characterization of selective NR4A modulators that can be used for their tissue-specific agonist/antagonist activities.

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Highlights

- NR4A receptors play a critical role in maintaining cellular homeostasis.
- NR4A receptors also play critical roles in multiple diseases.
- Ligands for NR4A receptors have been characterized.
- NR4A ligands have applications for treating multiple diseases and cancer.

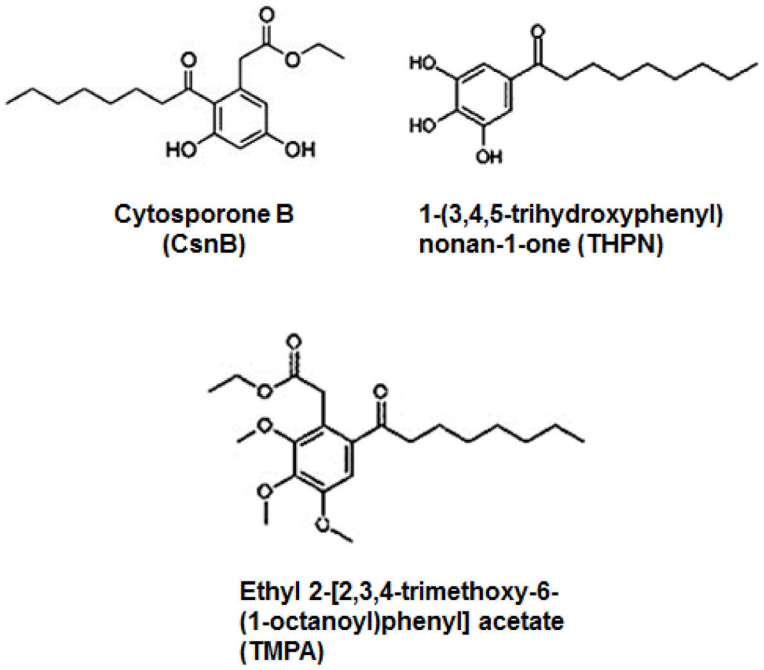

Figure 1.

Ligands that bind NR4A1. Studies in the Wu laboratory have identified a series of polyhydroxyaromatic compounds containing medium chain alkylketone groups that bind NR4A1 and act as agonists and antagonists (CsnB, THPN and TMPA) (34, 55, 56, 127).

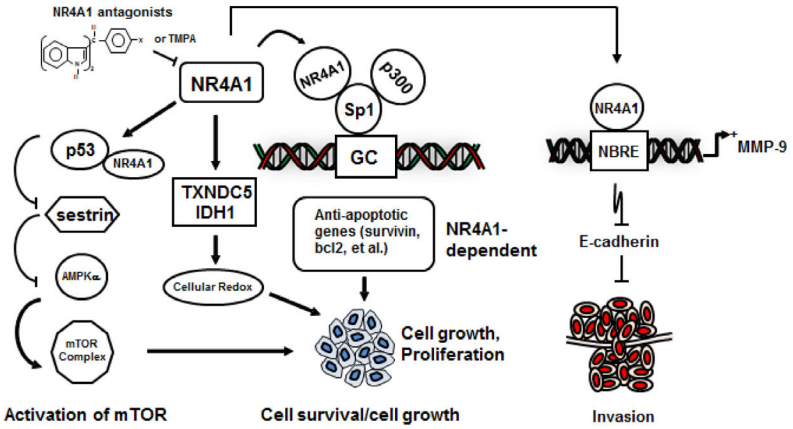

Figure 2.

NR4A1-regulated pathways in cancer cells that are inhibited by C-DIM/NR4A1 antagonists. Treatment of cancer cell lines with C-DIM/NR4A1 antagonists such as DIM-C-pPhOH or knockdown of NR4A1 by RNA interference results in inhibition of mTOR signaling by activation of p53, resulting in the induction of sestrin 2 and activation of AMPKα [96]. This is also accompanied by induction of ROS and ER stress through downregulation of TXNDC5 and IDH1 [18, 101] and also decreased expression of NR4A1/Sp1-regulated pro-survival/growth promoting genes [17]. Cancer cell invasion is also inhibited by antagonizing NR4A1 which results in decreased expression of MMP9 and E-cadherin [98].