Molecular Pathways: The Role of NR4A Orphan Nuclear Receptors in Cancer

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Abstract

Nuclear receptors are of integral importance in carcinogenesis. Manipulation of classic ligand-activated nuclear receptors, such as estrogen receptor blockade in breast cancer, is an important established cancer therapy. Orphan nuclear receptors, such as nuclear family 4 subgroup A (NR4A) receptors, have no known natural ligand(s). These elusive receptors are increasingly recognized as molecular switches in cell survival and a molecular link between inflammation and cancer. NR4A receptors act as transcription factors, altering expression of downstream genes in apoptosis (Fas-ligand, TRAIL), proliferation, DNA repair, metabolism, cell migration, inflammation (interleukin-8), and angiogenesis (VEGF). NR4A receptors are modulated by multiple cell-signaling pathways, including protein kinase A/CREB, NF-κB, phosphoinositide 3-kinase/AKT, c-jun-NH2-kinase, Wnt, and mitogen-activated protein kinase pathways. NR4A receptor effects are context and tissue specific, influenced by their levels of expression, posttranslational modification, and interaction with other transcription factors (RXR, PPAR-γ). The subcellular location of NR4A “nuclear receptors” is also important functionally; novel roles have been described in the cytoplasm where NR4A proteins act both indirectly and directly on the mitochondria to promote apoptosis via Bcl-2. NR4A receptors are implicated in a wide variety of malignancies, including breast, lung, colon, bladder, and prostate cancer; glioblastoma multiforme; sarcoma; and acute and/or chronic myeloid leukemia. NR4A receptors modulate response to conventional chemotherapy and represent an exciting frontier for chemotherapeutic intervention, as novel agents targeting NR4A receptors have now been developed. This review provides a concise clinical overview of current knowledge of NR4A signaling in cancer and the potential for therapeutic manipulation. Clin Cancer Res; 18(12); 3223–8. ©2012 AACR.

Background

Targeting ligand-activated steroid nuclear receptors is an important established cancer therapy. Orphan nuclear receptors are similar to steroid nuclear receptors as they act as transcription factors to modulate downstream gene expression. However, orphan nuclear receptors have no known natural ligand(s). These intriguing receptors comprise more than half the total number of nuclear receptors. Nuclear family 4 subgroup A (NR4A) orphan nuclear receptors NR4A1 (Nur77, testicular receptor 3, nerve growth factor 1β), NR4A2 (Nur-related factor 1), and NR4A3 (neuron-derived orphan receptor 1, mitogen inducible orphan nuclear receptor) are thought to be incapable of classic ligand binding due to bulky ligand-binding domains, unlike “adopted” receptors, for example, PPAR-γ (NR1C3), in which putative ligands have since been discovered. Diverse and paradoxical transcriptional and direct roles have been described for these receptors; physiologic functions of NR4A receptors are context and tissue specific. NR4A receptors have emerged as important molecular switches in processes associated with carcinogenesis, including apoptosis, DNA repair, proliferation, migration, inflammation, metabolism, and angiogenesis (Fig. 1). Unlike classic steroid hormone receptors, which need to be activated by a ligand, these proteins are constitutively active (1). Emerging techniques using cell-specific knockdown of NR4A receptors in vivo have recently accelerated discovery of NR4A receptor functions (2). The actions of NR4A receptors depend on NR4A receptor subcellular localization, levels of NR4A expression, transcriptional modulation by coactivators and/or corepressors, posttranslational modification, and interaction with other nuclear receptors (3, 4).

NR4A Receptors and Cancer

Apoptosis

Inhibition of apoptosis. NR4A receptors can promote cell growth and survival, activating transcription of downstream antipoptotic and proproliferative genes. Physiologically, NR4A2 is essential for dopaminergic neuronal survival in the central nervous system, and reduced NR4A2 is
implicated in Parkinson disease (5). In vitro, NR4A2 inhibits p53-mediated induction of downstream proapoptotic genes like BAX, a proapoptotic member of the Bcl-2 family (6). NR4A2 also inhibits apoptosis via convergence with Wnt and mitogen-activated protein kinase (MAPK) pathways (7, 8). Kitagawa and colleagues have reported that β-catenin binds NR4A2, releasing NR4A2 from the corepressor protein Lef-1 in 293F cells, allowing transcription of downstream Wnt and NR4A2 targets. Physiologically, these interactions are required for normal neuronal development and the survival of dopaminergic neurons (7). NR4A receptors and β-catenin modulate each other’s transcriptional activity in a cell-specific manner (7, 9). In colon cancer cell lines, a bile acid carcinogen (deoxycholic acid) has been shown to stimulate β-catenin-dependent increased expression of NR4A1 (10). Conversely, NR4A1 has been shown to promote degradation of cytoplasmic β-catenin in a transcription-independent mechanism, whereas in murine models, NR4A1 has been shown to reduce tumor cell proliferation by transcriptional inhibition of Wnt signaling (11, 12).

**Proapoptotic roles for the NR4A receptors.** Proapoptotic roles have also been described for the NR4A receptors. Nuclear export of NR4A1 is important functionally, as cytosolic NR4A1 has a nongenomic proapoptotic role in cancer cell lines in vitro. NR4A1 induces apoptosis by direct interaction with Bcl-2 in the mitochondria, exposing proapoptotic BH3, or indirectly by stimulating other cytosolic proapoptotic proteins, such as BAX binding to the mitochondria to initiate the apoptotic cascade (Fig. 1A; refs. 13–16). Bcl-2 modulation by NR4A1 is also important physiologically in the negative selection of T cells (13). Nuclear NR4A receptors can also have proapoptotic effects by inducing proapoptotic and antiproliferative genes (1, 17).

**DNA repair**

A novel function for NR4A receptors in DNA double-strand break (DSB) repair has recently been identified (18).
NR4A receptors translocate to sites of double-strand DNA damage in a mechanism dependent on PARP-1 and are phosphorylated by DNA protein kinases. Interestingly, the DNA repair action of NR4A receptors is not dependent on their transcriptional activity, but rather due to a direct interaction at the DNA repair site, the precise mechanisms of which remain incompletely understood (18). In melanoma, repression of NR4A4 receptors impairs UV-induced DNA damage repair via the melanocortin-1 receptor (19). These studies suggest a pro–cell-survival role for NR4As by regulating DNA repair. Conversely, a role for NR4A1 in inhibition of DNA repair has been described in hepatocellular carcinoma cell lines (20).

Inflammation
NR4A receptors are an important molecular link between inflammation and cancer. Proinflammatory effects of NR4A receptors are seen in the tumor-like growth of pannus in rheumatoid arthritis, in which inflammatory synovial hyperplasia becomes an invasive front of destructive tissue causing cartilage damage (3). Conversely, anti-inflammatory effects have been shown in transformed normal vascular macrophages, endothelial cells in atherosclerosis, and in the central nervous system (21, 22). Proinflammatory prostaglandins are strongly implicated in cancer; prostaglandin E2 (PGE2) induces NR4A2 in colon cancer, which leads to a myriad of downstream procancer effects. Meanwhile, COX-2 inhibitors repress NR4A2 expression and NR4A-regulated genes, including osteopontin (23). This repression may represent a mechanism for the reduced colon cancer risk observed in population studies of nonsteroidal anti-inflammatory drugs (NSAID). NR4A receptors are downstream targets of the cAMP-responsive element binding protein (CREB); binding sites for CREB have been identified in the promoter region of all 3 NR4A receptors. PGE2 induction of NR4A receptors involves CREB and NF-κB signaling, as PGE2 induces phosphorylation of CREB; this phosphorylated CREB can then bind to NR4A promoters and enhance gene transcription (23).

Metabolism and angiogenesis
NR4A receptors are involved in fatty acid oxidation and hepatic glucose metabolism (24). In normal skeletal muscle physiology, NR4A3 has been shown to promote fatty acid oxidative pathways and is induced by β-adrenergic signaling via protein kinase A, MAPK, and CREB-dependent pathways (25, 26). In colon cancer, NR4A2 with peroxisome proliferator-activated receptor γ coactivator 1α (PGC1α) induces expression of fatty acid oxidation enzymes, allowing cells to switch to alternative oxidative pathways, promoting cell survival (27).

Tumor growth also depends on formation of new blood vessels (angiogenesis) to facilitate delivery of oxygen and nutrients to the tumor. NR4A receptors are downstream targets of VEGF, promoting proliferation of endothelial cells in vitro and in vivo (25, 28). Transcription of NR4A receptors is increased by VEGF, hypoxia, and CREB activation in vascular endothelial cells (25). In vivo, the transcriptional activity of NR4A1 and NR4A2 is involved in VEGF-mediated angiogenesis (28, 29).

NR4A receptors and chromosomal translocations
Chromosomal translocation of NR4A receptors can lead to oncogenic conversion. For example, translocations involving NR4A3 in extraskeletal myxoid chondrosarcoma, most commonly between Ewing sarcoma region-1 (EWS) and NR4A3 (t(9, 22)(q22; q12), result in a fusion protein, EWS-NOR1, which activates NR4A3 target genes, such as PPAR-γ, and leads to oncogenesis (30).

Clinical–Translational Advances
NR4A receptors: identified roles in human cancer
Altered NR4A receptor expression has been identified in many solid tumors, with a plethora of in vitro roles described (Table 1). Meanwhile, reduced expression of NR4A1 and NR4A3 receptors is associated with hematologic malignancies, including acute myeloid leukemia and chronic myelodysplastic and/or myeloproliferative disease (31).

Novel drug targets. Drug development targeting NR4A receptor signaling is challenging due to the lack of a natural ligand, their bulky ligand-binding domains, the diversity of their cell- and context-specific functions, and redundancy between members of the NR4A family. Despite these challenges, several strategies have now been developed to enable therapeutic targeting of NR4A receptor signaling (1). These strategies include targeting their expression, nuclear export, and interaction with coactivators and/or repressors.

The proapoptotic nongenomic action of NR4A1 in the cytoplasm has therapeutic potential and has been manipulated in 2 ways. The first way is by drugs inducing nuclear export of NR4A1. Several drugs already in clinical use are now recognized to induce nuclear export of NR4A1, including 5-fluorouracil (5-FU) and certain NSAIDs (15). Novel drugs targeting nuclear export of NR4A1 include n-butylephedrine (BP) and cytosporone B. BP and its derivatives (PCH4) have shown therapeutic potential in glioblastoma multiforme, in vitro and in vivo, and in oral squamous cell carcinoma in vitro (32). PCH4 induces apoptosis in oral squamous cell carcinoma and glioblastoma multiforme cell lines in a mechanism dependent on PCH4-mediated increased NR4A1 expression and cytoplasmic translocation (32, 33). Cytosporone B, a compound isolated from fungi, is a ligand for NR4A1 and induces apoptosis by transactivation of NR4A1 target genes and by inducing expression and mitochondrial localization of NR4A1 in vitro and in vivo (34).

Second, a novel nanopeptide (nu-BCP9) has been derived from NR4A1, which mimics the action of NR4A1 on mitochondrial Bcl-2 to promote apoptosis (14). As the functions of the NR4A receptors are further elucidated, further peptide mimetics may be developed to emulate or block specific actions of the NR4A receptors, at both a nongenomic and transcriptional level.

Another class of drugs that can promote cell death via NR4A receptors is the diindoylmethane derivatives (CDIM).
CDIMs are unusual because they can cause apoptosis via induction or inhibition of NR4A1 transcriptional activity, in addition to NR4A1-independent induction of apoptosis via endoplasmic reticular (ER) stress. The derivative DIM-C-pPhOCH3 [1, 1-bis (3'-indoyl)-1-(p-anisyl)] methane is an "activator" of NR4A1-mediated transcription of proapoptotic genes, for example, cystathionase, p21, and ATF3, leading to apoptosis in pancreatic cancer cell lines in vitro. Intriguingly, DIM-C-pPhOH [1, 1-bis (3'-indoyl)-1-p-hydroxyphenylmethane], an "inhibitor" of NR4A1 nuclear transactivation, also causes apoptosis in pancreatic cancer cell lines, but by reducing transcription of NR4A1-dependent antiapoptotic and proproliferative genes [1, 17, 35, 36]. This modulation of the opposing effects of NR4A1 on cell survival by CDIMs has potential for selective receptor modulation in pancreatic cancer. Interestingly, in bladder cancer, a CDIM (DIM-C-pPhCl) can induce apoptosis and inhibit growth via activation of NR4A2 (1).

### Table 1. Summary of NR4A receptors in tumors, showing altered expression of NR4A receptors in tumor tissue and functional effects of NR4A receptors shown in vitro

<table>
<thead>
<tr>
<th>Cancer (reference)</th>
<th>NR4A expression in tumors</th>
<th>Function in vitro</th>
<th>Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon (15, 23, 27, 39)</td>
<td>NR4A1+ NR4A2+</td>
<td>Apoptosis A1+, A2 – proliferation A1+, A2+, metabolism A2+, angiogenesis A1+</td>
<td>CDIMs, 5-FU, butyrate</td>
</tr>
<tr>
<td>Breast (40–43) Melanoma (19, 44, 45)</td>
<td>NR4A1+ NR4A3+</td>
<td>Apoptosis A1+ A3+ migration A1–</td>
<td>NSAIDs/COX-2 inhibitors</td>
</tr>
<tr>
<td>Bladder (4, 46) Liver (20, 47)</td>
<td>NR4A1+ NR4A2+</td>
<td>Apoptosis A1+, A2±, migration A2+</td>
<td>CDIMs</td>
</tr>
<tr>
<td>Thyroid (48) Lung (1, 49–51)</td>
<td>NR4A1+, NR4A2– (mets)</td>
<td>Apoptosis A1+, DNA repair A1</td>
<td>BP</td>
</tr>
<tr>
<td>Pancreatic (1) Prostate (1) Gastric (52)</td>
<td>NR4A1+ NR4A2–</td>
<td>Apoptosis A1± proliferation A1±, Apoptosis A1±, A2–</td>
<td>CDIMs</td>
</tr>
<tr>
<td>Extraskeletal myxoid chondrosarcoma (30) Cervical (45)</td>
<td>T(9;22)q22; q12 NR4A3/EWS1</td>
<td>Apoptosis A3–, proliferation A3+</td>
<td>BP/PCH4</td>
</tr>
<tr>
<td>Ovarian (1, 53) Oral squamous cell carcinoma (33, 37)</td>
<td>NR4A2+</td>
<td>Apoptosis A1+, Apoptosis A1+, A2–</td>
<td>Vitamin K2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>BP/PCH4, 5-FU (resistance)</td>
</tr>
</tbody>
</table>

**NOTE:** Drugs targeting NR4A receptors are listed. +, increased; –, decreased.

**Abbreviations:** ACTH, adrenocorticotropic hormone; CDIM, diindoylmethane derivative; HCC, hepatocellular carcinoma; NSCLT, non-small cell lung cancer therapy; SCLT, small cell lung cancer therapy.

**Chemotherapy resistance.** NR4A receptors contribute to resistance to chemotherapy; understanding the mechanisms of this resistance may enable therapeutic targeting. Induction of NR4A2 by PGE2 in a cAMP/protein kinase A–dependent manner promotes resistance to 5-FU in squamous cell carcinoma (37). This finding has potential relevance for colorectal cancer, as PGE2-mediated induction of NR4A2 also occurs in colon cancer cell lines, whereas 5-FU is a commonly used chemotherapeutic agent in colorectal cancer. Similarly, Riggins and colleagues have suggested roles for NR4A receptors in mediating resistance to doxorubicin in breast cancer (6). The DNA repair effect of NR4A receptors may contribute to resistance to radiotherapy or chemotherapy (e.g., bleomycin; refs. 18, 20). Manipulation of NR4A/DSB binding may be a strategy to increase tumor sensitivity to chemoradiotherapy.

Other nuclear receptors, like retinoic acid receptor β (RARβ), can influence the expression and function of NR4A
receptors and are targetable. For example, by combining a histone deacetylase inhibitor with fenretinide (a synthetic retinoid), the expression, interaction, and nuclear export of NR4A1 and RARβ are increased, leading to apoptosis in hepatocellular cell lines in vitro, which are otherwise relatively insensitive to fenretinide (38).

Conclusions
In summary, the study of NR4A receptors represents an exciting new chapter in our understanding of the molecular changes that occur during carcinogenesis. NR4A receptors have diverse cellular effects (15) and function as molecular sensors, which, depending on their cellular microenvironment, may promote or inhibit cell death. Targeting expression levels, activity, and nuclear export of NR4A receptors, in a tissue-dependent manner, to manipulate their role in cell survival, has potential in the development of novel anticancer strategies.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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