Review

Transcriptional Crosstalk between Nuclear Receptors and Cytokine Signal Transduction Pathways in Immunity

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The nuclear receptor superfamily and the transcriptional factors associated with cytokines are inherently different families of signaling molecules and activate gene transcription by binding to their respective responsive element. However, it has become increasingly clear from our works and others that nuclear receptors are important regulators of cytokine production and function through complex and varied interactions between these distinct transcriptional factors. This review provides a general overview of the mechanism of action of nuclear receptors and their transcriptional crosstalk with transcriptional factors associated with cytokine transduction pathways. One of the most important mechanistic aspects is protein to protein interaction through a direct or co-regulator-mediated indirect manner. Such crosstalk is crucially involved in physiological and therapeutic roles of nuclear receptors and their ligands in immunity, inflammation and cytokine-related tumors. *Cellular & Molecular Immunology*. 2004;1(6):416-424.

Key Words: nuclear receptor, cytokine, signaling transduction, crosstalk, transcriptional factor, immunity

Introduction

Nuclear receptors encompass a large family of ligand-inducible transcription factors, including the receptors for sex steroids (progestins, estrogens and androgens), adrenal steroids (glucocorticoids and mineralocorticoids), vitamin D, thyroid hormones, retinoids (9-*cis* and all-*trans*), and lipid metabolites, and many homologous receptor proteins (orphan receptors) with unknown ligands (1-5). Nuclear receptors are the ligand-activated transcriptional factors that are essential in embryonic development, maintenance of differentiated cellular phenotypes, metabolism and cell death (4-6). Dysfunction of nuclear receptor signaling leads to proliferative, reproductive and metabolic diseases such as cancer, infertility, obesity and diabetes (6-12). Notably, there is a wealth of evidence that nuclear receptors are important regulators of cytokine production and actions.

The cytokines are a large family of secreted molecules consisting of more than 50 secreted factors that regulate processes ranging from body growth, lactation and adiposity to haematopoiesis (13-15). They can act in autocrine manner, affecting the behavior of the cell that releases the cytokine, in a paracrine manner, affecting the behavior of adjacent cells, and some cytokines are stable enough to act in an endocrine manner, affecting the behavior of distant cells, although this depends on their ability to enter the circulation and on their half-life in the blood. Cytokines are especially important for regulating inflammatory and immune responses, and have crucial functions in controlling both the innate and adaptive arms of the immune response. Not only do cytokines govern the development and homeostasis of lymphocytes, but they also direct the differentiation of helper T cells and promote the generation of memory cells. The transcriptional regulation of secretion and gene expression of cytokines is complex and involves general transcriptional factors as well as nuclear receptors.

Cytokines induce a variety of biological responses by binding to specific cell surface receptors and activating cytoplasmic signal transduction pathways, such as the Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway, which transmits information received from extracellular polypeptide signals, through transmembrane receptors, directly to target gene promoters

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Abbreviations: AP-1, Activation protein 1; AR, androgen receptor; CBP, CREB-binding protein; C/EBP, CCAT/enhancer-binding protein; CREB, cAMP response element-binding protein; DBD, DNA binding domain; ER, estrogen receptor; GR, glucocorticoid receptor; GRE, GR response element; Jaks, Janus kinases; LBD, ligand binding domain; MAPK, mitogen-activated protein kinase; MM, multiple myeloma; NCoR, nuclear receptor co-repressor; NFAT, nuclear factor of activated T cells; NF-κB, nuclear factor-kappa B; Oct, octamer transcription factors; PIAS, protein inhibitor of activated STAT; PPAR, peroxisome proliferator-activated receptor; PPRE, PPAR response element; RAR, retinoc acid receptor; RXR, retinoid-X receptor; STAT, signal transducer and activator of transcription; SMRT, silencing mediator of retinoid and thyroid receptors; SRC, steroid receptor co-activator.

in the nucleus, providing a mechanism for transcriptional regulation without second messengers (15-17). JAKs bind specifically to intracellular domains of cytokine receptor signaling chains and catalyze ligand-induced phosphorylation of themselves and of intracellular tyrosine residues on the receptor, creating STAT docking sites. Phosphorylation of STATs on activating tyrosine residues leads to STAT homo- and heterodimerization. STAT dimers are rapidly transported from the cytoplasm to the nucleus and are competent for DNA binding. Once the activated STAT dimer recognizes a target promoter, a primary transcription complex forms and the transcription rate from this promoter is dramatically increased. The ability to induce transcription of target genes is an intrinsic property of the STAT dimers, reflecting the ability of STAT transcriptional activation domains to recruit nuclear co-activators that mediate chromatin modifications and communication with the core promoters (16-24). Several lines of evidence indicated that cytokine-mediated signal transduction pathways especially STATs are target sites for nuclear receptor modulation of immune responses.

Apart from the classical mode of nuclear receptor action which involves binding as a dimer to regulatory sequences in target gene promoters and subsequent activation of transcription, it has become increasingly clear from our works (25-29) and others (30-38) that numerous members of the steroid and non-steroid nuclear receptor families may be communicating and interacting with other transcriptional factors to regulate cytokine production or cytokine-mediated signal transduction and cell responses. In this review, we recapitulate molecular mechanisms of such molecular crosstalks and enumerate their physiological and pathological consequences in immune responses, inflammation and cytokine-responsive tumors.

Mechanisms of crosstalk between nuclear receptors and transcriptional factors

The structure of nuclear receptors (39-42) is comprised of: an amino-terminal activation function, AF-1 (A/B domain), which can activate transcription in a ligand-independent fashion; the DNA-binding domain (DBD) (C); a hinge region (D); and a carboxy-terminal ligand-binding domain (LBD) (E). The DBD allows them to bind to and activate target genes, thus defining them as transcription factors. The LBD also contains a second activation function (AF-2) that maps to a surface-exposed hydrophobic pocket, proving a docking site for co-regulatory proteins, and modulates their activities, making them hormone-dependent transcription factors (Figure 1). Although they have common structural features, divergence of the steroid and thyroid/retinoid/vitamin D3 receptor subclasses is supported by differences in their functional characteristics, as well as by their discrepant recognition of *cis*-acting hormone response elements. Upon ligand binding, nuclear receptors form homo- or heterodimers, which bind to specific response elements in DNA to modulate gene expression. Most nuclear receptors stimulate transcription by recruiting co-activators and components of the basic transcription complex to target genes, while some recruit co-repressors and suppress gene expression (43, 44).

The crosstalk between nuclear receptor-mediated and other signal transduction pathways can occur in at least three ways (12). The first is based on the interference between the transcriptional activities of certain nuclear receptors and other transcription factors. The glucocorticoid receptor (GR) and the activating protein 1 (AP-1) transcription factor interact on target gene promoters which contain only a binding site for either one of the two transcription factors. Mutational analysis in transfected cells has mapped the domains required for crosstalk to the zinc finger DNA-binding domain (DBD) of the nuclear receptor and the bZip domain of AP-1. Most frequently negative interference of both factors with each other's activity has been observed, for example, when AP-1 is composed of c-Fos and c-Jun; however, synergism is also possible under cell-specific conditions and when AP-1 is a homodimer of c-Jun (33-36). Many members of the nuclear receptor family such as peroxisome proliferator-activated receptor γ (PPARγ), sex steroids receptors, and vitamin D receptor have been shown to participate in such crosstalk. Moreover, the transcription factor families of NF-κB/Rel as well as STAT, Oct, NFAT and C/EBP are engaged in crosstalk with steroid receptors (26-30). Recently, the p53 tumor suppressor, which functions as a sequence-dependent transcription factor to activate or repress diverse sets of genes under different stress conditions, ultimately leading to growth arrest, apoptosis, or senescence, has been demonstrated to interact with GR (45-47), androgen receptor (AR) (48, 49) and estrogen receptor (ER) (50). Crosstalk between retinoic acid receptor and STAT3 has also been reported in acute promyelocytic leukemia (51, 52).

The second type of crosstalk arises from the fact that nuclear receptors themselves can be the target of other signaling pathways that modify the receptor posttranscriptionally and alter its function. A prominent example is phosphorylation, but several other types of modification, such as ubiquitylation and acetylation, have been demonstrated. Phosphorylation can modify all major domains of nuclear receptors, the N-terminal AF-1, the ligand- and DNA-binding domains. Phosphorylation of nuclear receptors

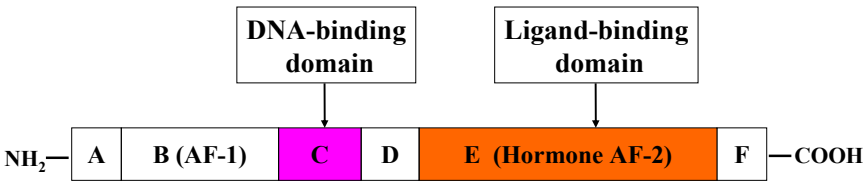

Figure 1. Structural and functional organization of nuclear receptors.

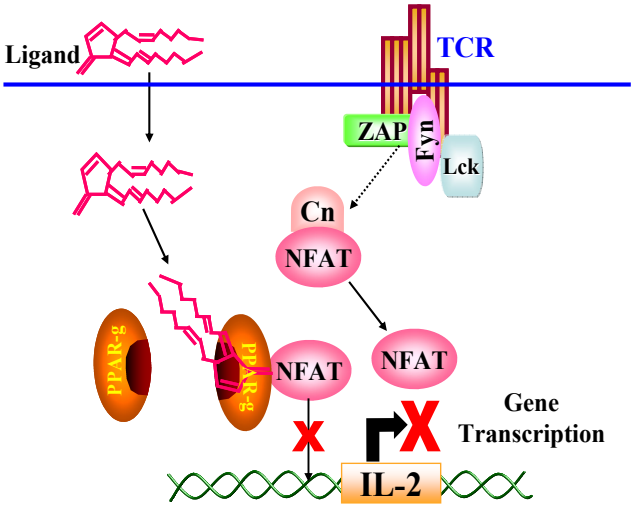

Figure 2. Regulation of IL-2 gene expression in T lymphocytes by direct interaction between NFAT and PPARγ. NFAT binds to the promoter region of the IL-2 gene and is needed to activate its transcription. The nuclear receptor PPARγ can be activated by its ligands. However, complex formation between NFAT and activated PPARγ leads to decreased DNA binding and transactivation of NFAT, in turn inhibiting gene expression of IL-2.

by kinases that are associated with general transcription factors, or that are activated in response to a variety of signals (MAPK, Akt, PKA, PKC), often facilitates the recruitment of co-activators, or of components of the transcription machinery, and therefore cooperates with the ligand to enhance transcription activation (53-55).

The third type of nuclear receptor crosstalk, which has only recently been recognized, is the so-called 'non-genomic' action of several nuclear receptors. However, some non-genomic actions of nuclear receptor ligands are apparently mediated through membrane receptors that are not part of the nuclear receptor superfamily (12, 56-61).

Multiplicity of crosstalk between nuclear receptors and transcriptional factors based on protein-protein interaction

Transcriptional crosstalk depends on the interaction of proteins whereas interaction with DNA in many cases appears secondary since only a recognition motif in the target gene for one of the factors is sufficient. Several types of interaction are conceivable, such as direct interaction, indirect interaction through "bridging proteins", and competition for limiting amounts of cofactors. Such interactions could result in tethering of the non-DNA-bound partner to the target gene through interaction with the DNA-bound partner or sequestration of both factors into an inactive, possibly non-DNA-binding complex. Interestingly, in the case of interactions between PPARγ and STAT3, two structurally distinct PPARγ ligands suppress IL-6 activated-STAT3 through the divergent types of crosstalk including direct or a co-repressor SMRT-mediated association (25, 26). Thus, the multiplicity of crosstalk between nuclear receptors and transcriptional factors is an important factor that contributes to both signal diversification and specification.

Direct interaction between nuclear receptors and transcriptional factors

Transcriptional regulation by nuclear receptors involves a direct protein-to-protein interaction with another transcription factor, such as Jun, NF-κB, or NFAT, which completely prevents these transcription factors from binding to their own response elements and therefore prevents their transcriptional activation. A well-established example is PPARγ co-association with the T-cell specific transcription factor NFAT in regulation of IL-2 gene expression (Figure 2) (28). The PPARγ is a prototypical member of the nuclear receptor superfamily and integrates the control of energy, lipid and glucose homeostasis (62-65). The transcription factor NFAT plays an essential role in gene expression of IL-2 by T lymphocytes and is also involved in the proliferation of peripheral T lymphocytes. Therefore, we evaluated transcriptional activity and DNA binding of NFAT to determine whether NFAT might be a target for negative regulation of T-cell activation by PPARγ ligands. Utilizing the EMSA, we have found that PPARγ ligands significantly inhibited the specific binding of NFAT probe corresponding to the human IL-2 promoter the transcriptional activation of the reporter construct directed by the NFAT distal site of the IL-2 promoter was abrogated by 15-d-PGJ2 or ciglitazone in the presence of PPARγ over-expression. We further tested for complex formation between PPARγ and NFAT in a co-immunoprecipitation experiment. The NFAT can be co-precipitated with PPARγ in T cells induced by PMA/PHA and 15-d-PGJ2 or ciglitazone. Furthermore, the addition of anti-PPARγ antibody induced high affinity binding of extracts to the NFAT probes as determined by EMSA, demonstrating that removal of PPARγ with this antiserum increases the target specificity of NFAT. The data indicates that a direct physical protein-protein interaction occurs between nuclear receptor PPARγ and transcription factors NFAT, in turn inhibiting transcription of IL-2 in T lymphocytes.

It is known that GR physically interacts with certain transcription factors. The interaction between the GR and AP-1 prevents their binding to either the AP-1 site or the GRE site and antagonizes the transactivational capacities of both regulators (33-35). However, the interaction between GR and STAT5 enhances STAT5-mediated transactivation but diminishes GR function at the GRE site (66). AF-1, but not the DNA-binding domain and AF-2, of GR is required for the interaction. Furthermore, GR association results in enhanced nuclear translocation, prolonged tyrosine phosphorylation and increased DNA binding capacity of STAT5 (67). The collective findings reveal that GR acts as a co-activator of STAT5 transcriptional function. In addition, IL-6-activated STAT3 interacts with ligand-bound GR to augment glucocorticoid signaling without association with a STAT DNA binding motif. STAT3 also may act as a potent co-activator of glucocorticoid receptor (68).

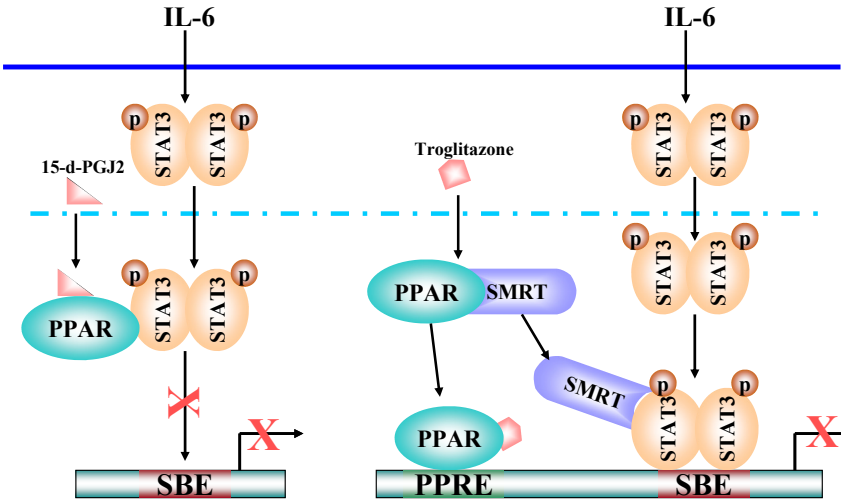

Figure 3. PPARγ Regulation of IL-6/Stat3 signaling mediated by direct or SMRT-mediated indirect interaction. Upon IL-6 binding, the IL-6R/gp130 dimer induces phosphorylation of JAK1 and JAK3, which in turn phosphorylate Stat3. The phosphorylated Stat3 dimerizes and translocates to the nucleus, where they bind to the Stat3 binding element (SBE) in the responsive gene to initiate transcription. Two structurally distinct PPARγ agonists suppress IL-6-activated STAT3 through diverse molecular mechanisms. 15-d-PGJ2 enhances direct physical protein-protein interaction between PPARγ and IL-6-activated STAT3. Troglitazone induces redistribution of co-repressor SMRT from PPARγ to activated STAT3, in turn transcriptionally inactivating STAT3.

Crosstalk mediated by co-activator or co-repressor of nuclear receptors

Most nuclear receptors stimulate transcription by recruiting co-activators and components of the basic transcription complex to target genes, while some recruit co-repressors and suppress gene expression (69-78). Co-activators, such as the steroid receptor co-activator (SRC) family, PGC-1, PGC-2, P300/CREB-binding protein (CBP), L7/SPA, steroid receptor RNA activator (SRA), E6-associated protein/RPF1, TIP60 (Tat-interacting protein), interact with nuclear receptors in a ligand-dependent manner and often contain the enzymatic activities necessary for an alteration in chromatin structure from a quiescent state to one allowing active gene transcription. Co-repressors, such as nuclear receptor co-repressor (NCoR), silencing mediator of retinoid and thyroid receptors (SMRT), BRCA1, ubiquitin-activating enzyme 3 (Uba3) and repressor of tamoxifen transcriptional activity (RTA), have the opposite effect on chromatin structure, making it inaccessible to the binding of transcription factors or resistant to their actions. These proteins are often associated with histone deacetylase (HDAC) activity, though other mechanisms for gene silencing clearly exist (Hermanson et al., 2002; Gao X and Nawaz Z, 2002). Both types of coregulators are required for efficient modulation of target gene transcription by nuclear receptors. It would be interesting to examine whether nuclear receptor co-activators or co-repressors are included in the crosstalk between nuclear receptor-mediated and other signal transduction pathways.

PGC-2 is a PPARγ AF-1-specific co-activator. It has been reported that NF-κB function is inhibited by PPARγ ligands, including troglitazone, which act as anti-inflammatory agents (29, 79, 80). It therefore seems that complexes formed by ligand-bound PPARγ, NF-κB and unknown factors lead to mutual inactivation of both classes of transcription regulatory factor, probably by suppressing their functions. Suzawa M et al. (81) demonstrated that although NF-κB is reported to associate with many other nuclear receptors, NF-κB seems to be recruited preferentially to PPARγ, at least in bone marrow stem cells, by its interaction with PGC-2 because the physical interaction of PGC-2 with PPARγ is highly selective among nuclear receptors.

The co-repressor SMRT has also to be demonstrated to mediate association between PPARγ and STAT3 (Figure 3) in multiple myeloma (MM) cells (25, 26). PPARγ can form weak interactions with the co-repressor NCoR/SMRT complex. PPARγ cannot bind to DNA while it is associated with the co-repressor complex. After ligand binding, PPARγ disassociates from the co-repressor complex, and then binds to DNA through a peroxisome proliferator response element. We first clarified that treatment of MM cells with troglitazone decreased association of SMRT with PPARγ, which results in redistribution of co-repressor SMRT from PPARγ to activated-STAT3. Furthermore this interaction between SMRT and IL-6-activated STAT3 can be attenuated by a PPARγ antagonist GW9662, confirming the specificity of the exchange of co-repressor SMRT induced by the liganded PPARγ. Recruitment of SMRT, which is associated with HDAC, by STAT3 leads to transcriptionally inactivating STAT3 and consequently down-regulating IL-6 mediated MM cell growth and gene expression. These observations support that co-activators or co-repressors function not only for regulation of the ligand-dependent DNA binding and transcriptional activities of nuclear receptors themselves, but also act as a bridge protein to modulate nuclear receptors crosstalk with other transcription factors.

Crosstalk mediated by co-regulatory molecules for transcription factors

Like co-activator or co-repressor for nuclear receptors, physiological protein inhibitors of STAT signaling comprise endogenous proteins that directly or indirectly down-regulate STAT activation (82-85). This family of inhibitors includes the cytokine-inducible SH2-containing (CIS) proteins (same as suppressor of cytokine signaling (SOCS), JAK binding protein (JAB), and STAT-induced STAT inhibitor (SSI), as well as the protein inhibitors of activated STATs (PIAS). Among these co-regulators of

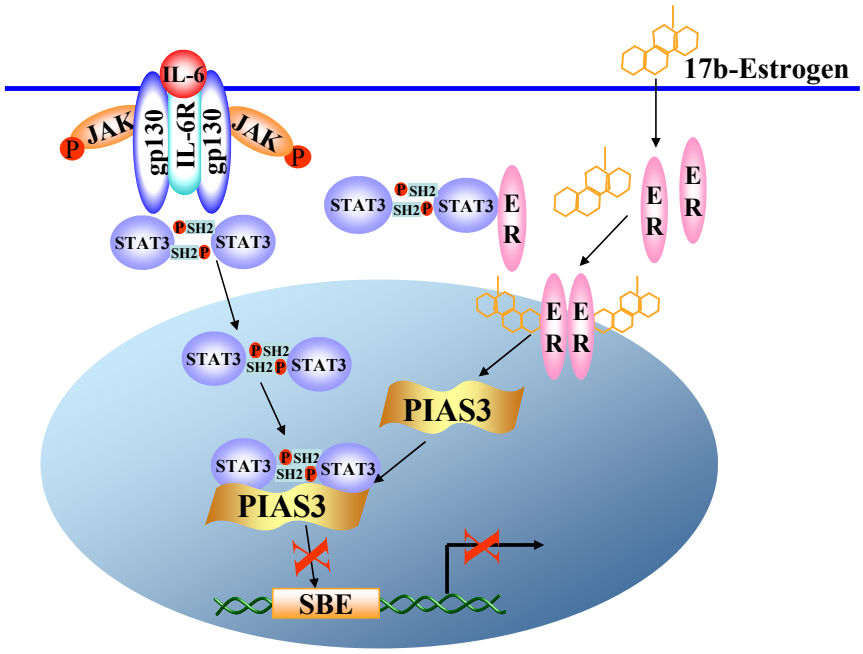

Figure 4. Estrogen receptor regulation of IL-6/STAT3 signaling mediated by PIAS3 as an interacting partner. In multiple myeloma cells, activated ER does not associate directly with STAT3. Estrogen inhibits the IL-6/Stat3 signaling by inducing PIAS3 expression, which suppresses STAT3 binding to the promoter regions of target genes and transactivation.

STATs, the CIS family regulates STAT signaling at the level of cytokine receptors or JAKs, whereas the PIAS family directly interacts with activated STATs, interfering with their DNA-binding activity, and inhibiting gene transcription (86, 87).

A critical role of PIAS3 in estrogen receptor interaction with IL-6-induced STAT3 has been demonstrated in multiple myeloma cells (27) (Figure 4). Estrogens mediate these activities through binding to a specific nuclear receptor protein, the estrogen receptor, which functions as a signal transducer and transcriptional factor to modulate expression of target genes (88). Yamamoto et al. (89) reported that activated ER directly associates with and acts as a transcriptional co-factor for STAT3 induced by IL-6 in breast cancer cells. The PIAS represents another group of proteins that normally serve to decrease DNA activation by blocking of STAT DNA-binding activity (90). The NH2-terminal region of the PIAS proteins contains a conserved motif, LXXLL, which is also present in a number of other nuclear receptor co-regulators (91). The PIAS3-STAT interaction seems to be dependent on cytokine stimulation, a finding that is consistent with the ligand-dependent interaction of other LXXLL motif-containg nuclear receptor co-regulators. Over-expression of PIAS1 and PIAS3, specific nuclear inhibitors of STAT1 and STAT3, respectively, suppress gene transcription mediated by these STATs. PIAS proteins have been identified that bind tyrosine-phosphorylated, dimeric STATs and prevent them from binding DNA, perhaps by potentiating tyrosine dephosphorylation (92). Down-regulation of PIAS3 protein, as is seen in anaplastic large cell lymphoma, may be in part responsible for maintaining high levels of activated STAT3 (93). Our experimental studies also suggested that members of the PIAS family may also function in estrogen signaling. In MM cells, IL-6-induced activation of STAT3 is blocked by pretreatment of myeloma cells with estrogen. It has been shown that myeloma cells upregulate PIAS3 synthesis upon estrogen receptor stimulation and that PIAS3 binds to and blocks STAT3 DNA-binding activity, suggesting a possible mechanism of STAT3 inhibition requiring PIAS3 as a co-regulator modulating the crosstalk between ER and STAT3. In contrast, we did not observe a significant change of PIAS3 in PPARγ ligands-treated MM cells stimulated by IL-6, indicating that PPARγ, unlike ER, inhibits IL-6/STAT3 signaling pathway independent of PIAS3.

Biological consequences of interactions between nuclear receptors and transcriptional factors

There is emerging evidence that nuclear receptors interact with transcriptional factors to modulate cytokine production and action in many physiological and clinical conditions. Nuclear receptor regulation may occur at the levels of gene expression of cytokines themselves and their receptors, or cytokine-mediated signaling transduction pathways. A number of specific protein-protein interactions are potential targets not only for physiological regulation of immune responses, but also for pharmaceutical interventions in inflammation, autoimmune diseases and haematological malignancies.

Immune responses and inflammation

Cytokines are small proteins that are released by various cells in the body, usually in response to an activating stimulus to regulate local and systemic immune and inflammatory responses (94). Many of the cytokines act as inflammatory mediators. The cytokines secreted by macrophages in response to pathogens include IL-1β, IL-6, IL-12, TNFα and chemokine IL-8. The genes of the above inflammation mediators are substantially regulated by the AP-1, C/EBP and particularly the NF-κB transcriptional factors. Thus repression of these transcription factors by nuclear receptors should repress at least in part the

expression of the inflammatory mediators. Suppression of proinflammatory cytokine secretion by glucocorticoids and estrogen contributes to the immunosuppressive and anti-inflammatory effects of these steroid hormones. On the other hand, retinoid acid induces IFN-α synthesis to potentate its antiproliferative and antiviral activities. The studies conducted at the whole animal level indicated that the interactions between the AP-1 and GC signaling pathways are much more extensive (94-96). AP-1-related signaling *via* the Jun N-terminal kinases can lead to increased levels of circulating GC, which eventually down-modulate AP-1 activity *via* transcriptional interference. This negative feedback loop is likely to be of great importance for maintenance of homeostasis and regulation of stress responses, including acute and chronic inflammation. In addition, the anti-inflammatory effects of PPARγ activation have been demonstrated with human and murine monocytes/macrophages and cell lines. Treatment with 15-d-PGJ2 or thiazolidinediones has been found to inhibit the secretion of many of these mediators including IL-6, TNFα and IL-1β.

Modulation of the cytokines expressed by T lymphocytes can inhibit autoimmune disease. We have demonstrated that a novel role of PPARγ, which when activated by the different ligands, potently blocks T cell activation through PPARγ association with transcription factors NFAT and NF-κB (28, 29). PPARγ ligands 15-d-PGJ2 or ciglitazone significantly inhibited PHA-mediated T-cell proliferation and IL-2 production in a dose-dependent manner. By contrast, the PPARα-specific ligand Wy14643 did not inhibit PHA-mediated T-cell proliferation.

Nuclear receptors also exert diverse effects on the expression of cytokine receptors. Glucocorticoids enhance the expression of IL-2Rα, IL-4Rα, IL-6Rα, IL-7Rα, gp130, IFNγR, and GM-CSFRα, but suppress that of GHR, IL-3R, IL-11R and OSMR. Upregulation of IL-2Rα and IL-7Rα may account for augmentation of the interleukin-induced T cell proliferation by glucocorticoids. Reduction of GHR and IL-3R expression may contribute to the inhibitory effects of glucocorticoid on GH-stimulated insulin-like growth factors 1 (IGF-1) production and IL-3-dependent mast cell proliferation, respectively. Studies of the mechanism involved have shown that dexamethasone activates the promoter of the IL-2Rα gene, suggesting a direct effect on transcription. Similar to glucocorticoids, sex steroids (estrogen, progesterone and androgen) exert complex effects on cytokine receptor expression. Regulation of cytokine receptor expression has also been demonstrated for vitamin D. An active form of vitamin D, 1α, 25-dihydroxyvitamin D3, induces the IL-10R expression, which may explain in part augmentation of the IL-10 anti-inflammatory action by the vitamin (97).

Haematological malignancies

Multiple myeloma, the second most common lymphoma, is a malignant B-cell tumor that is distributed at several sites within the bone-marrow compartment. At present, it accounts for ~12,000 deaths per year in the United States alone ~2% of all cancer deaths and nearly 20% of deaths caused by haematological malignancies. Most patients with multiple myeloma cannot be cured with currently available therapies (98-100). Our experiment results indicated that nuclear receptors ER or PPARγ interaction with transcription factors STAT3 or c/EBPβ through down-regulation of IL-6 secretion and IL-6/STAT3-mediated cell cycle or apoptosis gene expression may have significant therapeutic implications in multiple myeloma.

Interleukin-6 is the key growth and survival factor of multiple myeloma cells. IL-6 is particularly involved in the origin of all benign and malignant plasma cell expansions. Although some multiple myeloma cells secrete IL-6 and grow in an autocrine fashion, IL-6 is primarily produced in bone marrow stromal cells (BMSCs) and mediates paracrine MM cell growth. Its secretion is upregulated in BMSCs by either adhesion of MM cells or cytokines (TNFα, VEGF, IL-1β). We have found that a direct physical protein-protein interaction occurs between nuclear receptor PPARγ and c/EBPβ, which in partially mediated PPARγ agonists 15-d-PGJ2 and troglitazone significantly suppressing IL-6 transcription and secretion of BMSCs induced by MM cell adhesion.

On the other hand, IL-6-mediated activation of STAT3 is essential for the survival and growth of myeloma tumor cells. Catlett-Falcone et al. (101) reported that STAT3 is constitutively activated in bone marrow mononuclear cells from patients with multiple myeloma and in the IL-6-dependent human myeloma cell line U266. Moreover, U266 cells are inherently resistant to Fas-mediated apoptosis by upregulating antiapoptotic protein Bcl-xL. Blocking IL-6 receptor signaling from Janus kinases to the STAT3 protein inhibits Bcl-xL expression and induces apoptosis. Activated STAT3 translocates into the nucleus to activate target genes. STAT3 is required for the regulation of cell cycle regulatory genes such as *c-myc* and *cyclin D1*, as well as antiapoptotic genes *bcl-2*, *mcl-1*, and *bcl-xl* (102-106). As discussion in previous section, PPARγ indeed interacted with phosphorylated STAT3, which could be enhanced by 15-d-PGJ2 and decreased by troglitazone. The 15-d-PGJ2 is a naturally occurring ligand with low affinity of PPARγ, whereas a class of antidiabetic drugs known as thiazolidinediones are high affinity synthetic ligands of PPARγ. Since the ligand-binding pocket is not static, each PPARγ ligand has the potential to induce a different conformation of the receptor. Moreover, treatment of MM cells with troglitazone decreased association of SMRT with PPARγ, which results in redistribution of co-repressor SMRT from PPARγ to activated-STAT3. Consequently, 15-d-PGJ2 or troglitazone markedly decreased STAT3 binding to target genes *c-myc* and *mcl-1* promoter and inhibited mRNA expression of *c-myc* and *mcl-1*. Therefore, transcriptional blockade of STAT3 and its target downstream genes by crosstalk between PPARγ and STAT3 is a key step for activated PPARγ inhibition of IL-6-mediated cell signaling in human multiple myeloma cell growth.

Conclusions and perspectives

Cytokines induce a variety of biological responses by binding to specific cell surface receptors and activating

cytoplasmic signal transduction pathways, which are involved in not only immune/inflammatory responses, but also in malignancy pathological conditions. The areas of transcriptional control have received increasing attention. Nuclear receptors are the ligand-activated transcriptional factors that are essential in embryonic development, maintenance of differentiated cellular phenotypes, metabolism and cell death (107). Nuclear receptors functionally interact with general transcriptional factors to regulate gene expression of cytokines and their receptors, and transcriptional control of cell cycle or apoptosis genes mediated by cytokine signal circuits. The molecular basis of this interaction has remained elusive, despite the proposal of several distinct mechanisms. One of the most important mechanistic aspects is protein-protein interaction through a direct or co-regulator-mediated indirect manner (12, 26, 30, 36, 38). More genetically engineered screening systems to differentiate between these various signaling options, both in cells *in vitro* and in model mutant mice (108) need to be generated. Better understanding of the mechanisms on interaction between these two distinct families of transcriptional factors activated by different signaling pathways potentially leads to new drug design and/or therapeutic strategies for treatment of cytokine-related diseases range from inflammation to cancer.

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