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Therapeutic implications of the nuclear factor-kappaB/nuclear receptor cross-talk

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1. ABSTRACT

More and more evidence reveals that the transcription factor NF-kappaB plays a critical role in tumor development and progression and that it may constitute the missing link between inflammation and cancer. It turned out that many of the well known cancer drugs exert their anti-tumoral effect at least in part through modulating the activity of NF-kappaB. The potential of nuclear receptors to modulate the activity of this widespread transcription factor has repeatedly been reported and illustrates their enormous therapeutic potential. However, the efficacy of these liganded receptors is overshadowed by the occurrence of unwanted effects owing to their broad range of actions. Accordingly, researchers pursue the ambition to improve the specificity of nuclear receptor modulators. In this review we have explored the molecular mechanisms by which nuclear receptors interfere with NF-kappaB signalling and quoted the therapeutic implications of their cross-coupling. Strategies that are explored at the moment and that may hold great potential for the future are extensively reviewed.

2. NUCLEAR RECEPTORS

Nuclear receptors (NRs) are transcription factors (TFs) with essential and non-redundant roles in fundamental biological processes, including growth, development, homeostasis and cell death. The fact that these receptors are evolutionary related is reflected by their structural similarities. Indeed, most nuclear receptor family members contain three functional domains, i.e. an N-terminal transactivation domain, a DNA-binding domain (DBD) and a C-terminal ligand-binding domain (LBD) (1). Sequencing of the human genome has led to the identification of 48 NRs that can be divided in two main groups. Firstly, the orphan receptors, which can be further divided into two subgroups, being 1) true orphans, for which ligands are unknown or may not exist, or 2) adopted orphans, for which candidate ligands have only recently been identified (e.g. peroxisome proliferator-activated receptors, PPAR). Secondly, the liganded receptors, which contain 24 members, including the glucocorticoid receptor (GR), the estrogen receptor (ER), the mineralocorticoid receptor (MR), the progesterone receptor (PR) and the androgen receptor (AR) (1).

GR is expressed in almost all tissues of the human body. However, the levels of GR protein, of which different splice- and translation variants occur, are tissue- and cell cycle-specifically regulated (2-6). Cortisol or hydrocortisone is the major glucocorticoid (GC) in the human body. Due to its prominent role in a variety of biological actions, including carbohydrate, protein and fat metabolism, as well as its modulatory role in the central nervous system, hematopoietic, renal and immune systems, its expression by the adrenal cortex is tightly controlled via a negative feedback mechanism on the hypothalamo-pituitary-adrenal axis (7). Although cortisol can also bind the MR, this binding is inhibited in mineralocorticoid-target tissues due to the presence of 11beta-hydroxysteroid dehydrogenase 2, which converts cortisol into the inactive metabolite cortisone (8), thereby making GR its primary target in those tissues. Besides GRs, ERs, as well as PPARs, are known to regulate the inflammatory response. The effects of estrogens span further than the immune system. This is exemplified by their influence not only on reproduction, which is widely recognized, but also on skeletal, cardiovascular and central nervous systems (9-11). These hormones act through activation of two receptors, namely ERalpha and ERbeta (12), which can form homo- or heterodimers. PPARs, on the other hand, form a family of three nuclear receptors (PPARalpha, PPARbeta/delta, PPARgamma). They respond to various fatty acids (13-15) and are differentially distributed to distinct tissues (16). To be able to exert their effects on specific metabolic processes, PPARs form a heterodimer with the retinoid X receptor (RXR). Furthermore, PPARs function as regulators of cell proliferation and apoptosis, two cellular mechanisms of which deregulation can have detrimental outcomes and can result in diseases, such as cancer (15).

In the absence of ligands, GR, MR, PR and ER are kept inactive in the cytoplasm of the cell by chaperone proteins, such as heat shock proteins (hsp) and immunophilins (17). However, recently it became clear that this is a simplified model and that, in fact, a continuous shuttling of the receptors between the two cellular compartments occurs (18, 19). Only after binding of ligands to their corresponding receptor the dynamic equilibrium is disturbed, thereby resulting in a net nuclear shift. Indeed, due to their small, lipophilic nature, ligands such as steroid hormones (e.g. GCs, estrogens, progesterone, mineralocorticoids, androgens), fatty acids and prostaglandins diffuse freely through the cell membrane. Once inside the cell, these hormonal and metabolic substances bind to their corresponding receptor and induce a conformational change. A nuclear localization signal is exposed, thereby allowing the receptor to translocate to the nucleus and to influence transcription. More specifically, this conformational change allows nuclear receptors to recruit coactivator complexes, via their activation function 2 (AF-2) domain, which consists of a short conserved helical sequence within the C-terminus of the LBD (20). These coactivator complexes are composed of chromatin-modifying proteins, such as factors with ATP-dependent chromatin remodelling (e.g. BRG-1, BRM) or histone arginine methyltransferase activities (21), molecular scaffolds that assemble cofactor complexes (e.g. PPARγ coactivator-1 (PGC-1)), as well as members of the p160 family (e.g. steroid receptor coactivator 1 (SRC-1), transcription intermediary factor 2 (TIF2/GRIP-1) and p300/cAMP-responsive element-binding protein (CBP)). Coactivator molecules such as CBP, p300 and SRC-1 modulate the activity of the transcription apparatus through their histone acetyltransferase (HAT) activity (22, 23). Core histones are posttranslationally modified, thereby changing their electrical charge and pushing the DNA in a more relaxed chromatin structure. It is believed that the inverse process, deacetylation of histones by HDACs (histone deacetylases) results in a more condensed chromatin structure, thereby reducing the access of TFs to their binding sites and repressing transcription of target genes (24). It has been suggested that at the end of this initial chromatin-modifying step, the p160 family members are acetylated, thereby loosing their ability to interact with the receptor, or alternatively, that these coactivators are degraded by the proteasome (25, 26). Once the first cofactor complex is disassembled, nuclear receptors interact with members of the Mediator complex (TRAP/DRIP/ARC), which directly contact the basal transcriptional machinery (27). A bridge is formed with the RNA polymerase II holoenzyme that can subsequently be recruited onto the promoter. However, it has been shown that different ligands (28-30) or different NRs (31, 32) can exhibit a preference when it comes to the recruitment of different coactivators. In addition, the spatial and temporal modes in which the process of cofactor recruitment occurs can vary for different NRs and for different promoters. For example, Métivier and colleagues (33) suggested that at the ER-responsive pS2 promoter, the coactivators p300 and SRC-1 were first recruited to induce histone acetylation, followed by the recruitment of ERalpha and the Mediator complex. On the other hand, recruitment of AR and cofactors onto the PSA regulatory regions (34) support a combinatorial model. A similar model was already available for the cathepsin D promoter onto which p160 proteins, ERalpha and DRIP/TRAP are recruited in a combinatorial mode (35). Furthermore, when ligand is added for longer time periods, a cyclic and dynamic recruitment of coactivator complexes to the ER-responsive promoter can be observed (33). In contrast, association of the AR-coactivator complex on PSA regulatory regions gradually increases, with a maximum occupancy at 16 hr, followed by a gradual decline (34). Taking into account that specific cell types (36) or diverse promoters can display alternative requirements of different coactivators that can be recruited sequentially, combinatorially or in parallel, it seems logic that in this way a code for obtaining tissue and gene specificity is created. Moreover, the possibility of a rapid NR/DNA interaction turnover (37-39), together with the fact that coactivators and NRs themselves are targets for modification by different signal transduction pathways (reviewed in (40-42)) creates a frame in which it is possible for a cell to very quickly respond to changing environmental factors.

It has been proposed that the segregation of nuclear receptors in different subcellular compartments acts as an important regulatory checkpoint. It is hypothesized, e.g. for ER, that this mechanism of cellular segregation

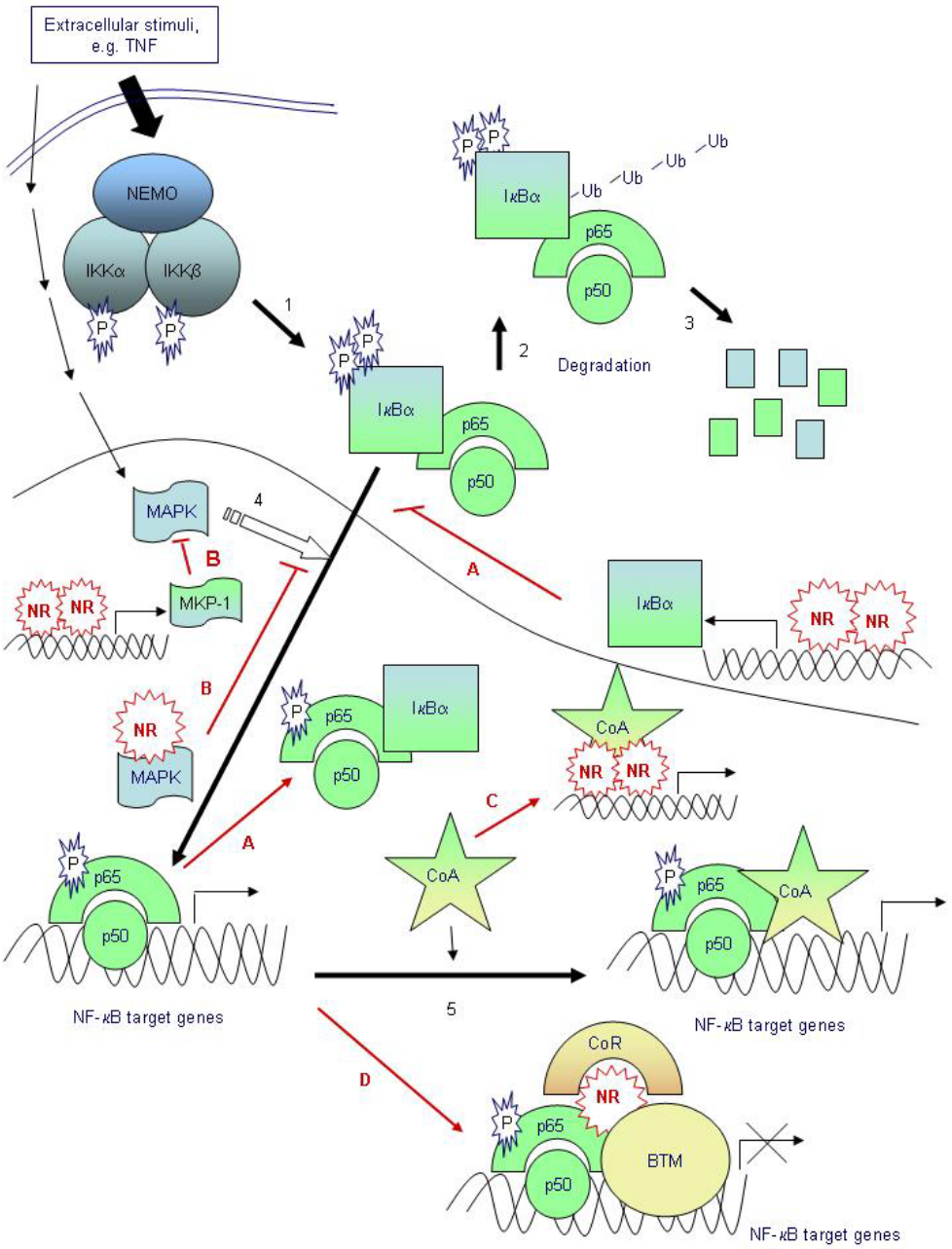

Figure 1. Interference of nuclear receptors (NRs) with the canonical pathway of NF-kappaB activation. Upon triggering with stimuli, such as TNFalpha, IkappaBalpha is phosphorylated by the activated IKK complex (1), and subsequently ubiquitinated (2) and degraded (3). NF-kappaB (p65/p50) enters the nucleus and after being post-translationally modified by kinase pathways, such as the TNF-activated MAPK pathway (4), it induces gene transcription (5). Different models have been hypothesized to describe the cross-coupling between NF-kappaB and NRs. Note that some of these mechanisms may be very cell type-dependent and this complication is discussed in more detail in the text: (A) NRs may inhibit NF-kappaB release through up-regulation of IkappaBalpha, thereby inhibiting NF-kappaB/DNA binding, (B) NRs may inhibit MAPK signalling via upregulation of the MAP kinase phosphatase MKP-1 or via direct interactions, thereby preventing post-translational modifications of NF-kappaB, (C) NRs may compete with NF-kappaB for co-activators, and (D) NRs inhibit the formation of a functionally active transcription complex and important non-exclusive mechanistic aspects hereof may include: a direct physical interaction between NRs and NF-kappaB, interference with the basal transcription machinery (BTM) and a cofactor exchange, namely the removal of coactivators (CoA) and the recruitment of corepressors (CoR). Non-genomic effects are at this moment not depicted in the figure due to the lack of a conclusive model.

may allow the receptor to exert both genomic (nuclear) and non-genomic (cytoplasmic) activities (43, 44). Recently, Carrigan and co-workers (45) have defined a nuclear retention signal in the hinge region of GR. Their data suggest that active nuclear retention of GR acts as a strong inducer of GR transcriptional activity (45). However, for other NRs, for instance PPARs, which can be found constitutively in the nucleus, even in the absence of ligand, a different mechanism must occur. In the unliganded state, DNA-bound PPAR is kept inactive through interaction with a nuclear corepressor complex, containing the nuclear corepressor (NCoR), silencing mediator of retinoic-acid and thyroid hormone receptors (SMRT) and HDACs, which keep the chromatin in a condensed state. In analogy with the previous model, ligand induction results in a corepressor/coactivator switch (46), a process called "de-repression" (47). The repressor complex is dissociated, through ubiquitination and degradation of NCoR, and substituted by a coactivator complex with HAT activity, thereby allowing transcriptional activation (47).

In general, multiple modes of action have been revealed and several common mechanisms exist. Ligand-activated NRs can homo- or heterodimerize and can activate the transcription of responsive genes via direct DNA binding to responsive elements in the promoter (transactivation). In contrast, liganded receptors can also regulate the activities of other major signalling pathways (transrepression) (48, 49), such as those driven by nuclear factor-kappa B (NF-kappaB), activator protein 1 (AP-1) (50-52), cAMP-responsive element-binding protein (CREB), signal transducers and activators of transcription (STATs) (53) or interferon regulatory factor 3 (IRF3) (54). It is mainly the latter characteristic of the NRs, in particular their interference with the functionality of NF-kappaB, that explains their success as drug targets, and this topic will be discussed further in this review.

3. NUCLEAR FACTOR-KAPPA B

The mammalian NF-kappaB family consists of a heterogeneous and commonly expressed group of TFs. The family contains 5 members that can be divided in two subgroups. The first class is composed of members that comprise a C-terminal transcription activation domain (RelA (p65), RelB and c-Rel), whereas the second class (NF-kappaB1 (p50/p105), NF-kappaB2 (p52/p100)) does not. Each member contains a Rel homology domain (RHD) that mediates DNA binding, dimerization, as well as interaction with members of the "inhibitor of NF-kappaB" (IkappaB) family, which keep NF-kappaB dimers in the cytoplasm of the cell. NF-kappaB can be activated by a wide array of extracellular stimuli, including cytokines, viruses, oxidative stress, phorbol esters, lipopolysaccharide and B- and T-lymphocyte activation (55-57). After activation of cell surface receptors, distinct signal transduction pathways may be activated, but most of these stimuli seem to converge on the level of IkappaB (56, 57). Whether it is via activation of the IkappaB kinase complex (IKK) (canonical pathway) or via the activation of the p38-activated serine/threonine kinase casein kinase II (CKII) (after exposure to UV-light; atypical pathway) (58, 59), IκB is phosphorylated and subsequently degraded (60-62). Indeed, although both kinases phosphorylate IkappaBalpha on different residues, i.e. IKK phosphorylates IkappaBalpha on N-terminal sites, while CKII targets a cluster of C-terminal sites, both pathways finally converge on the level of IkappaBalpha (59). The liberated NF-kappaB, which is predominantly composed of a p65 and p50 heterodimer, enters the nucleus and regulates the transcription of a diverse subset of genes encoding, amongst others, cytokines, chemokines, matrix metalloproteinases (MMPs), cell adhesion molecules, inducible NO-synthase (iNOS) and cyclooxygenase 2 (COX-2) (63-67). Alternatively, a select group of stimuli, e.g. B-cell activating factor (BAFF), lymphotoxin beta (LT-beta), CD40 ligand and viruses, such as human T-cell leukaemia (HTLV) and Epstein-Barr (EBV) activate NF-kappaB2 via an alternative, non-canonical pathway, i.e. in an IkappaB-independent manner, but via activation of NF-kappaB-inducing kinase (NIK) and IKKalpha, p100 is processed into p52 (58). Finally, recent studies highlighted the existence of another NF-kappaB-activating pathway that is signalling inside-out, i.e. from the nucleus to the cytoplasm. Indeed, although it was previously thought that DNA-damaging agents signalled via the same pathway, referred to as the atypical pathway, it became clear that these stimuli could not be grouped and in fact activate different patways. Recently gained insights are extensively reviewed in (58, 68). In brief, in contrast to UV-light that signals to NF-kappaB in an IKK-independent manner (as described above), other DNA-damaging agents, including genotoxic stress (i.e. the formation of double stranded breaks), oxidative stress, heat or electric shocks, do signal via a member of the IKK complex, namely via the NF-kappaB essential modulator (NEMO or IKKgamma). It is believed that, upon DNA damage, p53-inducible death domain-containing protein (PIDD) and receptor-interacting protein 1 (RIP-1) translocate to the nucleus were they sumoylate NEMO (69). In parallel, the ataxia telangiectasia mutated (ATM) kinase is activated, which recognizes and phosphorylates the sumoylated NEMO. Subsequently, NEMO gets mono-ubiquitinated (70, 71) a modification that serves as a nuclear export signal instead of being a tag for degradation by the proteasome. Finally, NEMO returns to the cytoplasm, where it triggers the activation of the IKK complex (72).

Different studies agree that NF-kappaB is highly activated at sites of inflammation (73-75), thereby further supporting the role of NF-kappaB as an important regulator of inflammation. However, it is only recently that NF-kappaB was pinpointed as being the missing link between inflammation and cancer (76). Indeed, several pro-inflammatory cytokines and chemokines (e.g. TNF, IL-6, IL-1, CXCL8) are under the transcriptional control of the IKKbeta-dependent NF-kappaB activation pathway and are associated with tumor development and progression (77-80). Furthermore, the transcription of proteins that are crucial for tumor cells to proliferate, to invade and to metastasize, such as anti-apoptotic proteins, growth factors (e.g. vascular endothelial growth factor (VEGF)) and MMPs, is NF-kappaB-regulated (77, 78, 80-83). As an example, the NF-kappaB-regulated pleiotropic cytokine IL-

6 is believed to be a crucial player, standing at the cross-roads between inflammation and cancer. This is due to its ability to act as a paracrine/autocrine growth factor of many tumor cells and is further supported by its implication in tumor progression, angiogenesis, invasion and motility (84). Furthermore, IL-6 can epigenetically modulate gene expression via reduction of the miRNA, miR-370, that controls the expression of the oncogene MAP3K8 (85). The recently gained insights that NF-kappaB is essential for promoting inflammation-associated cancer, paves the way for the use of anti-inflammatory agents also into anti-cancer protocols. As such, agents that target the NF-kappaB activation pathway, such as proteasome inhibitors and upstream kinase inhibitors, display anti-cancer properties in clinical or preclinical studies (86). Alternatively, a variety of studies have shown a mutually antagonistic cross-talk between activated NRs and NF-kappaB (51). The mutual repressive activities between NF-kappaB and PR were recently confirmed and this antagonism is shown to be important for the downregulation of cytokine expression in human leukocyte cells (87, 88). More information is available concerning the cross-coupling of GR, ER, AR and PPAR with NF-kappaB. The importance of ER/NF-kappaB cross-talk has been elucidated using ER-positive and ER-negative breast cancer cells. Whereas active DNA-bound NF-kappaB was absent in the ER-positive cells, a constitutively activated transcription factor could be found in ER-negative cells (89). In the same line, in AR-negative prostate cancer cells NF-kappaB was also found to be constitutively active (90). In addition, research has revealed that GR overexpression in the epidermis of transgenic mice dramatically inhibited skin carcinogenesis. The mechanistic basis of the tumor-suppressor effect of GR lies in the interference of GR with NF-kappaB (91). A similar study was performed in prostate cancer cells and suggested that GR inhibits multiple signalling pathways and TFs involved in proliferation and transformation, including NF-kappaB, thereby explaining the tumor-suppressive role of GR in the prostate (92).

4. CROSS-TALK MECHANSIMS

4.1. Cytoplasmic Models

4.1.1. Upregulation of inhibitors

One convenient way of how NRs could counteract the activity of NF-kappaB would be through upregulation of the inhibitor protein IkappaBalpha. Concomitantly, more than a decade ago Scheinman (93, 94) and Auphan (95) proposed that, after administration of GC, p65 is sequestered in an inactive cytoplasmic form, thereby reducing the NF-kappaB/DNA binding. These observations found follow-ups in other NR research fields, such as for ER, AR, PPAR (extensively reviewed in (50)) and retinoid-related orphan receptor alpha I (96). For instance, IkappaBalpha levels were found to be higher in ER-positive compared to ER-negative breast cancer cell lines (97). In addition, in rats suffering a transient cerebral ischemia, an increase in phosphorylated IkappaBalpha is coupled to activation of NF-kappaB (98). However, more and more evidence is arising showing that IkappaBalpha upregulation is not the main mechanism by which NRs exert their anti-inflammatory effects. First, the question still remains how GC could up-regulate IkappaBalpha when no classical glucocorticoid response element (GRE) can be found in the promoter region. However, an alternative mechanism, which does not require DNA-binding, may occur. Such a mechanism has been described before for PPARalpha, showing that this NR requires DRIP205, recruited onto the Sp1 sites flanking the kappaB site, to regulate IkappaBalpha expression. These data indicate that even in the absence of its functional response elements, PPARalpha may positively regulate gene expression (99). It seems not unreasonable that a similar mechanism may occur for other NRs. Nevertheless, it has been shown that even in the absence of new protein synthesis GR was able to efficiently repress NF-kappaB activity (100). Furthermore, using *in vivo* footprinting and Chromatin Immunoprecipitation (ChIP), it was revealed that NF-kappaB remains bound to the ICAM and IL-8 promoter respectively, even under conditions of gene repression by GCs (101, 102). Also the initial finding that estradiol (E_2) inhibits the expression of NF-kappaB-driven genes by interfering with the binding of NF-kappaB to the DNA (103), could not be generalized. In fact, recent ChIP data showed that E_2 treatment did not impair the binding of the p50 or p65 subunit of NF-kappaB to the TNFalpha promoter (104).

The seemingly contradictory results obtained by different research groups may reflect the tissue specificity of this mechanism, whereby different characteristics prone to the cell (e.g. a cell-specific subset of cofactors, differential GC effects on alternative pathways in different cells) eventually define the importance of IkappaBalpha upregulation (50).

4.1.2. Interference with other signal transduction pathways

Although the picture of NF-kappaB regulation seemed clarified for years and deemed relatively simple, it is only a few years ago that research has unravelled a more complex and sophisticated image of NF-kappaB activation. Post-translational modifications of the different members of the NF-kappaB family seem to be responsible for fine-tuning NF-kappaB activity (reviewed in (58)). This additional level of regulation provides a point for cross-talk with other signalling pathways, which, for some examples, may be under the tight control of NRs. Indeed, previous studies reported on the importance of ERK and p38, as well as the subsequent phosphorylation of p65 at Ser276 by the downstream nuclear kinase MSK-1, for obtaining full-blown NF-kappaB activation (105, 106). Since GC are able to upregulate MKP-1, a dual specificity phosphatase (DUSP) which in turn de-phosphorylates and thus inactivates ERK and p38, this might constitute an alternative mechanism by which GC exert their anti-inflammatory role (107-109). In addition, it is thought that p38 MAPK can post-transcriptionally regulate the expression of a variety of pro-inflammatory genes. Indeed, although steroids may influence the levels and activity of tristetraprolin (TTP), a factor that regulates mRNA stability and thus the expression of certain inflammatory genes (109-111), steroids can alternatively alter the stability of pro-inflammatory mRNAs via inhibition of p38. Through activation of its substrate MAPKAPK-2, p38 can stabilize

various mRNAs, such as IL-6, IL-8, COX-2 and TNF-alpha, and this process seems to depend upon the presence of an A/U-rich repeat (ARE) (112-114). The role of MKP-1 induction after GC treatment has been reported in synovial fibroblasts (115) and its significance was further elaborated by Abraham and colleagues, who show that in DUSP knock-out mice the *in vivo* anti-inflammatory effects of DEX on zymosan-induced inflammation is impaired (116). However, taking into account that the inhibition of novel protein synthesis does not hamper repression of IL-6 production by GC (100), reasons that GC-induced MKP-1 upregulation is not the only mechanism explaining the transrepressive effects of GC or, alternatively, that the importance of this mechanism is again cell type- and/or gene-dependent. This is in agreement with the observation that the dependency of DEX-mediated MKP-1 upregulation for the concomitant repression of inflammatory mediators can in fact vary for the different markers. Indeed, it is shown for IL-1alpha, COX-2 and IL-12 that the MKP-1 dependency decreases from highly dependent to not at all dependent (116), reviewed in (117). Alternatively to MKP-1 upregulation, GR might regulate the function of the MAPK family members through direct protein-protein interactions. Such a mechanism has already been described by Bruna and co-workers who have characterized a hormone-regulated JNK docking site in the GR ligand-binding domain. From their results these authors conclude that by binding to JNK, GR inhibits the interaction of JNK with its upstream kinase MKK-7, thereby inhibiting the activation of this MAPK. As a consequence inactive JNK accumulates on the AP-1-bound response elements of the *c-jun* gene (118). A direct interaction mechanism has also been described for ER-mediated activation of the Src/p21/ERK pathway, this via interaction of estrogen-activated ER with c-Src (119). However, such a direct interaction has, to our knowledge, not yet been described between p38 and ERK on the one hand and GR on the other hand.

Vice versa, the activity of NRs can be modulated by other pathways, such as the cAMP-dependent protein kinase (PKA) pathway. Not only has it been shown that GR is an effective substrate for phosphorylation mediated by the catalytical subunit of PKA (PKAc) (120), in addition, it has been reported that PKAc/retinoid acid receptor cross-talk occurs through direct phosphorylation of the receptor (121, 122), and furthermore, that PKAc regulates dimerization of human ER-alpha (123). A more recent study however, reported on the importance of PKAc in the cross-talk between GR and NF-kappaB (124). From their data, Doucas and co-workers conclude that while PKAc potentiates GR-dependent transcription, it attenuates the cross-repression between NF-kappaB and GR. Moreover, they suggest that both TFs may already interact in the cytoplasm of the cell (124). A similar observation was made by Widén *et al.*, who showed by means of immunoaffinity chromatography or immunoprecipitation and western blotting that the p65/p50/IkappaBalpha complex already interacts with GR in the cytoplasm, even in the absence of a hormonal ligand or a pro-inflammatory signal (125). However, just as for the IkappaBalpha and MKP-1 mechanisms, data exist claiming that a restricted cytoplasmic event is unlikely to be the main mechanism explaining the transrepression activity of GR. Indeed, by means of an exlusively nuclear set-up using a Gal4 (DBD)-p65 fusion protein, which could constitutively activate a Gal4-dependent luciferase construct, it was shown that the repressive effects of DEX were not influenced by the lack of interferences by upstream events (100). Although this experiment does not rule out the occurrence of an interaction between NF-kappaB and GR in the cytoplasm of the cell, unarguably it does indicate that this cytoplasmic encounter may not entirely explain the cross-coupling between both TFs. Further evidence supporting nuclear models will be discussed below.

4.2. Nuclear models

4.2.1. From physical interaction to the basal transcription machinery

In analogy with the transactivation function of GR, it was proposed almost 20 years ago that transcriptional repression by GCs occurs via interaction of GR with a negative GC response element (nGRE) in the promoter regions of repressed genes, analogous to the prototype described in the proximal region of the pro-opiomelanocotin (POMC) promoter (126, 127). However, in 1997, together with the characterization of a Nur response element (NurRE) which binds Nur77 (NGFI-B) dimers but not GR molecules, a novel POMC-promoter target for GC-mediated repression was identified (128). Later on it turned out that at the level of the NurRE, NGFI-B and GR interact via their DBDs; in the absence of GR DNA binding and GR homo-dimerization (129). Furthermore, it was shown that this is not an exclusive mechanism for NGFI-B, but instead, that all members of the orphan nuclear receptor Nur77 subfamily are able to interact with GR (129). At the prostate-specific antigen promoter an alternative mechanism has been described for AR. In this setting, the cross-modulatory activity of AR with p65 results from the binding of both factors to a common cis-DNA element (130).

Actually, the above-described direct physiological interaction between Nur77 and GR, closely resembles a model that is proposed for transrepression between GR and NF-kappaB (129). Indeed, first evidence concerning a direct physical interaction between the latter 2 TFs came from co-immunoprecipitation studies (131). By means of point mutants it became clear that GR interacts with the RHD and C-terminal transactivation domain of RelA via its DBD (100, 132). More specifically, the GR zinc-binding region (ZBR), which includes the DNA-binding and dimerization functions of the receptor, is sufficient to associate with the RelA subunit of NF-kappaB *in vivo* (102). However, in analogy with the model described for Nur77, also here DNA binding of the receptor *per se* was not necessary (131, 133). Some evidence is gathered that a similar mechanism also exists in the field of ER. One group has reported the ability of ERalpha to stably associate with DNA-bound NF-kappaB in gel shift experiments under conditions that NF-kappaB-driven gene expression is inhibited (134). Another group has elucidated that the NF-kappaB repressive activity of ER is fully dependent on the complete ER DBD. Again a mutation

approach pointed out that nucleotide residues within or overlapping the DBD of ER are essential to maintain ER's ability to repress the expression of the NF-κB-driven IL-6 gene (135). In a recent manuscript it was clarified that the RHD of p65 is necessary for the interaction of p65 with ER *in vitro* and *in vivo* (136). The occurrence of protein-protein interactions between PPARalpha and NF-kappaB was first brought to the attention by Delerive and co-workers in 1999 (137). In addition, it has been suggested that a weak interaction, as observed for AR and RelA, may explain their reciprocal negative cross-talk (138).

These pieces of evidence were used to further elucidate the role of this protein-protein interaction and it was questioned whether there would be a role for the basal transcription machinery (BTM) in the transrepression activity of NRs. Indeed, it was found by Meyer and colleagues that the repression of the osteocalcin gene is the result of a GRE overlapping with the TATA-box, thereby preventing the assembly of a functional basal transcription machinery (139). The significance of the TATA-box determining responsiveness towards GR-mediated transrepression was confirmed using NF-kappaB-driven recombinant constructs (140). Conclusive evidence for an interfering role of GR in the assembly of a functional BTM was given by Nissen & Yamamoto (102). Since TNF-alpha stimulated the formation of a pre-initiation complex (PIC) at the IL-8 and ICAM promoters, as well as the phosphorylation of the largest subunit of RNA polymerase II (pol II) at serines 2 and 5 situated in its carboxy-terminal domain (CTD), these authors reasoned that GR might interfere with these essential modifications. Indeed, although GR did not interfere with PIC assembly under repressive conditions, it did interfere with the phosphorylation of Ser 2 of the pol II CTD. In a follow-up paper the group of Yamamoto described that promoter-specific gene regulation by the glucocorticoid receptor resulted from the formation of diverse regulatory complexes at the different promoters (141). These results strongly suggest that GR might interfere with the recruitment of different cofactors to efficiently repress gene transcription (102).

4.2.2. Getting specificity: a role for cofactors?

Upon realizing that NRs counteract the repression of only a subset of NF-kappaB-driven genes we are left with the question of how signal-, promoter- and cell-specific modulation of the inflammatory response is obtained by these receptors (142). TFs themselves cannot drive inflammatory responses, but instead need the help of an overlapping set of coactivator proteins, thereby raising the possibility that these cofactors may carry the secret of context-dependent regulation. Since at least some of these coactivator proteins are present in cells at functionally limiting concentrations (143), it has been proposed that NRs compete with NF-kappaB for coactivators, such as CBP/p300. This cofactor competition model found support by data gathered in the AR field and the GR field (144, 145). However, a major draw-back of this model is its incapability to explain the gene specificity of transrepression, since a plethora of TFs that are homed in a cell converge at the level of CBP/p300 for their transcriptional activity (51, 140). Experimental evidence minimizing the importance of cofactor competition came from the observation that GC repression of p65-mediated gene expression is not relieved by overexpression of the coactivator molecules CBP/p300 and SRC-1. In addition, a nuclear GAL4-p65 point mutant, defective in CBP recruitment, could still be functionally repressed by GR (140).

Instead of competing for coactivators, it has been proposed that NRs exert their repressive effects on gene expression through the recruitment of HDACs. A role for these corepressors was elaborated using the HDAC inhibitor trichostatin A (146, 147). Subsequently, by means of a GR antagonist, namely RU486, Ito and co-workers were able to pin-point the importance of HDAC-2. By showing that the recruitment of HDAC-2 to the NF-kappaB complex was impaired after RU486 induction, these authors concluded that HDAC-2 recruitment is an essential step in the transrepression activity of GR (148). More recently, the importance of HDAC-2 was further highlighted through the use of RNA interference. Loss of HDAC-2 inhibited the association between GR and NF-kappaB. This can be understood by taking into account that GR becomes acetylated after ligand binding and by accepting that HDAC-2-mediated GR deacetylation is necessary to enable binding of GR to NF-kappaB (149). In the same study it was shown that overexpression of HDAC-2 in GC-insensitive alveolar macrophages from patients with COPD is able to restore GC sensitivity. A similar role for HDAC-2 was described in the cross-repression between NGFI-B and GR. The group of Drouin has reported on the importance of Brg1, the ATPase subunit of the Swi/Snf complex, for *in vivo* stabilization of the interactions between GR and NGFI-B on the one hand and between GR and HDAC-2 on the other hand. Whereas Brg1 resides constitutively on the POMC promoter, ligand induction is necessary for the recruitment of GR and HDAC-2, thereby resulting in histone H4 deacetylation and inhibition of pol II clearance from the promoter. In addition, there is convincing evidence showing that the lack of nuclear expression of both Brg1 and HDAC-2 gives rise to the occurrence of GC resistance (150). The significance of corepressors in NR-mediated transrepression mechanisms was further supported by studies of PPARgamma in macrophages. Genes that are subject to transrepression by PPARgamma have promoters that interact in their basal state with corepressor complexes containing NCoR-HDAC-3-TBL. In the absence of PPARgamma ligand, LPS signalling results in the clearance of this repressor complex from the promoter, thereby enabling NF-kappaB and its coactivator complex to bind. However, when PPARgamma is activated by ligand, a conformational change is elicited that enables SUMOylation of its ligand-binding domain, thereby stabilizing the interaction between the receptor and the NCoR/HDAC complex. As a consequence, LPS signalling fails to relieve the repressive effects on transcription (151).

Since different promoters may have a preference for different coactivators and corepressors, the above findings may in part explain the gene specificity of this

mechanism. In transactivation mechanisms, the mediator subunits MED14 and MED1 have been implicated in transcriptional regulation and seem to be used by the GR in a gene-specific manner (152). Evidence accumulates that different NR target genes may require different activation functions of the receptor (153), thereby creating the possibility of interaction with different coactivator proteins (such as the Mediator complex). Secondly, also for transrepression mechanisms, the promoter-specific recruitment of cofactors has been described. In a first example it was shown that regulatory complexes formed at the IL-8 and IkappaB promoters were distinguished by differential recruitment of the Ser2 CTD kinase, P-TEFb. This might be an explanation for the differential expression of two genes of which the proximal kappaB-elements are distinguished by a single base pair difference only (141). In addition, Ogawa and colleagues (154) describe a mechanism in which GR is able to repress the activation of functionally related NF-kappaB-driven genes by disturbing p65/interferon regulatory factor (IRF) complexes. NF-kappaB-driven genes, which are not negatively regulated by GR, seem to use other proteins as coactivators, e.g. Bcl-3. To make the story even more complex, it has been described that some cofactors can exert both transactivation and transrepression effects in a single cell type, albeit not at the same moment and in a response element-specific manner. The cofactor GRIP, for instance, displays different activity domains that make a coactivator/corepressor switch possible (155). A similar coactivator/corepressor switch is described for ER. A recent study shows that unliganded ERalpha behaves as a TNFalpha-induced coactivator that becomes a corepressor in the presence of E_2 by recruiting GRIP-1 (104).

The observation that GR-mediated repression of NF-kappaB-driven genes can only be observed after toll-like receptor (TLR)-4 and TLR-9 triggering, but on the contrary fails after TLR-3 triggering (154), brings us to a point of signal specificity. What will be the final outcome of transrepression in a cell where different pathways interact and influence each other and how are these messages combined into a combinatorial code? Indeed, it is shown for GR and NF-kappaB that both TFs only reside transiently on their DNA-binding sites. It would thus be interesting to explore what the turn-over is of both factors in repressive conditions, as a rapid turn-over would make continuous sampling of the cell environment possible (51). Moreover, NRs cannot be seen as functionally completely separated molecules, but in fact they can modulate the expression of distinct, as well as of an overlapping set of target genes. An example is the synergistic negative effect of GR and PPARgamma on iNOS expression, which probably resides in a simultaneous targeting of NF-kappaB by GR and of NCoR complexes by PPARgamma. The phenomenon that NRs block NF-kappaB can thus not be considered as a general mechanism, since only a subset of NF-kappaB-driven genes are influenced, but should be considered a more subtle encounter in which cell-, signal- and promoter-specific mechanisms play an essential role for defining the final response. Taken together, all these arguments favour a cofactor exchange model instead of a cofactor competition model (142, 143).

4.3. Alternative mechanisms

Since the genomic effects of NRs depend on transrepression of inflammatory gene expression and/or upregulation of anti-inflammatory genes, this mechanism takes a few hours to days. However, more and more evidence accumulates, stating that NRs can have an effect on cellular responses, which happen in a time frame from seconds to an hour (156, 157). Which receptor/pathway is responsible for mediating these non-genomic effects is yet to be elucidated. For GCs and estrogens for instance, their interaction with a specific membrane receptor, namely a G-protein-coupled receptor, has been described (158-160). Secondly, an alternative mechanism has been reported to explain the GC-mediated immunosuppressive effects on T-cell activation. As reviewed by Löwenberg and colleagues, it has been proposed that GR physically interacts with the T-cell receptor (TCR) complex and that this interaction gets disturbed after ligand activation of GR, leading to impaired T-cell signalling (157). Thirdly, it has been postulated that for some steroid ligands a plasma membrane-bound NR exists (159, 161-163). Alternatively, Buttgereit and colleagues showed that GC at high concentrations intercalate into the plasma membrane of immune cells, thereby interfering with calcium and sodium cycling across the membranes (164, 165). Although the mechanistic details of these rapid actions are still lacking, it seems that steroids can induce an increase in several second messengers such as inositol triphosphate, cAMP, Ca^{2+}. Furthermore, it has been proposed that phytoestrogenic isoflavones can selectively, in an ER-independent manner, block nuclear transactivation of NF-kappaB via successive attenuation of MEK/ERK and MSK-1 activity, hereby preventing the phosphorylation of p65 and histone H3 (166). By showing that steroids can rapidly interfere with the action of kinase pathways it becomes clear that genomic and non-genomic effects cannot completely be seen as two diverse mechanisms. Indeed, evidence is arising that membrane-initiated actions of NRs may provide an additional mechanism for the regulation of gene transcription. Firstly, research has unravelled that the PKA pathway induces the degradation of GRIP-1 through the ubiquitin-proteasome pathway, thereby providing a novel regulatory mechanism by which hormones down-regulate a cofactor (167). Secondly, a recent report showed that E_2 induces cell proliferation via a non-genomic pathway, through activation of ERK1/2 and subsequent upregulation of cyclin D1 (via mER and G protein) (168). In contrast, ER might cooperate with NF-kappaB for the expression of COX-2 via a non-genomic effect (169). This in turn results in the production of PGI2 and may thus explain the protective effects of estrogens on the cardiovascular system (52, 170). It would thus be very interesting to further define which mechanism (genomic versus non-genomic) and/or receptor type contributes most to the immunosuppressive actions and which mechanism lies at the basis of currently observed unwanted systemic effects. Furthermore, it is of the utmost importance to define which factors are crucial to determine whether TFs, such as ER and NF-kappaB, will cooperate or will antagonize each other's functions (52). Indeed, a synergistic cooperation between ER and NF-kappaB, whereby E_2 mediates an increase in activated

ERK, subsequently leading to NF-kappaB activation and expression of anti-oxidant enzymes, such as Mn-superoxide dismutase and gluthathion peroxidase seems highly promoter-specific (171).

5. THERAPEUTIC IMPLICATIONS

5.1. GR ligands

The broad-range anti-inflammatory effects of GCs are reflected by the many mechanisms by which their corresponding receptor can interfere with pro-inflammatory pathways. As such, GR ligands are able to inhibit the expression of a battery of pro-inflammatory mediators, thereby explaining their therapeutical relevance. Indeed, in contrast to e.g. antibody therapy, which targets only one cytokine or chemokine and is used in treatment regimens of inflammatory diseases such as rheumatoid arthritis and Crohn's disease, GCs display a much broader target range. A very attractive model has been described by Smolen and colleagues to explain the relevance of targeting more than one pro-inflammatory mediator in rheumatoid arthritis (172). These authors use the picture of an inflammatory house of cards to explain that once an inflammation process is initiated and a cascade of pro-inflammatory gene expression is started, targeting the initial cytokine, that triggered the process, will not be sufficient anymore for an optimal therapeutic response. On the contrary, to bring the inflammatory house of cards to full collapse and to avoid remission, multiple mediators should be targeted (172). However, this multiplicity of GC treatment is not only its strength, but at the same time it is responsible for the main weakness of cortisone treatment, as it originates from the essential role GCs play in the regulation of metabolic and stress responses. The frequent administration of GCs leads to a systemic increase of hormone levels and this in turn can lead to a dysregulation of sugar and lipid metabolism, as well as fluid and salt retention, resulting in the occurrence of unwanted effects, including diabetes, glaucoma, fat redistribution, hypertension, but also osteoporosis, muscle wasting, insomnia and psychiatric disorders (173). Keeping the unwanted effects of GC in mind, it has to be stated that, if cortisone would be discovered today, it would probably never get approved by the regulatory authorities. However, despite the scientific progress made in the last years concerning the molecular mechanisms of GR function and despite the continuous efforts of the pharmaceutical industry, no other drug has been able to kick GC-based therapy from its pedestal when it comes to therapeutic benefits. Consequently, it is still the most commonly used drug that does improve the quality of live of patients with chronic inflammatory and autoimmune diseases, as well as cancer. Different directions are however explored to optimize treatment protocols based on GR ligands, in the hope to find drugs that display the same anti-inflammatory potential as classical steroid hormones, but lacking the unwanted secondary effects (173). Firstly, although MR-containing tissues are protected from cortisol effects by an enzymatic activity converting cortisol to the weaker cortisone, synthetic GR ligands, such as DEX, are not sensitive to 11β-hydroxysteroid dehydrogenase 2 and thus retain a full capacity to bind to MR in certain tissues, e.g. the kidneys. As such, DEX imposes its effects on the kidney via both MR and GR and this mechanism is believed to form the basis of hypertension (173). Improvements thus came with the knowledge that local administration (e.g. in aerosols or creams for topical usage) instead of systemic usage would already limit the occurrence of some of the side-effects. Secondly, pro-drugs might be developed, which only release their active substances at sites of inflammation (174). Alternatively, GCs can be marketed as soft-drugs, which are rapidly metabolized and inactivated after exerting their pharmacologic effects (175). Nevertheless, localized therapy can only be used in certain conditions and therefore there is still an urgent need for drugs with a better benefit-risk profile (176, 177).

The observation that different (ant)agonists of GR can induce only a subset of the functions elicited by the natural ligands raised the intriguing possibility that it might be possible to find or create ligands, able to separate the beneficial effects from the side-effects (48, 178). Indeed, recent developments in the GR field have allowed the identification of so-called dissociated GR ligands. The first dissociated GC described was able to inhibit AP-1-dependent transcription, but failed to promote GRE-driven transcription and was called RU24858 (179). However, subsequent work showed that the transactivation, as well as the transrepression effects of this compound are strongly cell type-dependent (180, 181) and these combined data may explain the poor separation of wanted and unwanted effects *in vivo* (117, 182). A second promising selective GR agonist (SEGRA), was described by Schäcke *et al.* in 2004 and constitutes the non-steroidal compound ZK216348 (183). In a murine croton oil-induced ear inflammation model, this compound was able to suppress inflammation to the same extent as prednisolone does. In contrast, this compound showed a markedly superior side-effect profile regarding blood glucose levels, spleen involution, skin atrophy and osteoporosis (183, 184). However, the main draw-back of this compound arises from the fact that it is not solely modulating the activity of GR, but in addition exerts effects at the PR and MR level (183).

Recently, a plant-derived compound, referred to as CompoundA (CpdA), was discovered to selectively interfere with NF-kappaB-driven pro-inflammatory gene expression in a solely GR-dependent manner, yet fails to transactivate GRE-driven genes. Furthermore, CpdA is able to inhibit inflammation in a model of acute paw swelling without increasing blood glucose concentrations (185). To elaborate if CpdA would also be able to inhibit inflammation in a therapeutic protocol under chronic inflammatory conditions, the collagen-induced arthritis model was used to show that the therapeutic potential of CpdA is indeed present under conditions lacking the unwanted diabetogenic effects (186). It was additionally shown that these observations arise from the fact that CpdA actively induces the formation of a GR monomer (186). These data are in line with the observations made by Reichardt and co-workers, who found that knock-in mice which express a GR that is unable to dimerize and to bind DNA on classical GRE-elements were still able to suppress inflammation. In these GR^{dim} mice, induction of

gluconeogenic enzymes in the liver could not be observed (187, 188). Conclusively, it is postulated that the anti-inflammatory action of GR can be separated from its unwanted effects if a ligand is found or synthesised that is able to specifically induce the GR monomer. However, recently it has been shown that GR can transactivate some genes even in the absence of dimerization (153). Since CpdA can induce a differential conformation of the receptor as compared to DEX (185), a large scale gene expression array will allow to compare both GR-modulating compounds and extend this knowledge to different cell types and *in vivo* models.

Indeed, after a more detailed research it appeared that many of the compounds originally categorized as being dissociated, turned out to rather be gene-selective. An example is the characterization of AL-438, a compound that represses and activates only a subset of genes normally regulated by classical steroids. These characteristics do not affect the anti-inflammatory potential of the compound *in vivo*, but correlate with a decrease of the negative effects on bone metabolism, chondrocyte proliferation and glucose levels (30, 184, 189). The causal molecular mechanism resides in a differential cofactor recruitment in response to ligand. While prednisolone-induced GR interacts with both cofactors, i.e. PGC-1, a cofactor critical for upregulating glucose levels after steroid induction, and GRIP-1, induction of GR by AL-438 reduces the interaction of GR with PGC-1, while maintaining the interaction with GRIP-1 (30).

The complexity of the whole story is brought further to the attention by the realization that only subtle differences in ligand structure can have profound effects on the outcome of gene-expression (190). The knowledge that endogenous promoters behave as very sensitive detectors of only subtle differences pops the question if it would be feasable to design cell- or tissue-specific ligands (51, 117, 190). Furthermore, it is not inconceivable that in order to reach efficient treatment regimens in all patients, it might be necessary to adapt therapies to patient-specific needs. Indeed, our preliminary data using fibroblast-like synoviocytes (FLS) isolated from the inflamed tissues of patients with RA, show that different patients show a promoter- and ligand-dependent differential response to the GR modulators DEX and CpdA (191).

5.2. ER ligands

The role of estrogens in cancer is dual and complex. While the transcriptional activation of ER target genes, which promote cell proliferation, drives cancer progression (192-194), the ability of ER to interfere with the NF-kappaB activity is believed to be responsible for the protective anti-inflammatory and anti-tumoral effects (84). The search for safer drugs has resulted in the identification of so-called selective estrogen receptor modulators (SERM), that exert tissue-selective activities (i.e. they exert ER agonist effects in one tissue, while ER antagonist effects in another) and may display a preference for one of the two ER isoforms (195, 196). Due to its ER antagonistic effect in breast tissue, tamoxifen is a generally applied SERM, used for the treatment of ER-expressing breast cancer cells. Although it was previously assumed that tamoxifen displays a safer side-effect profile, this compound seems to display agonistic effects in the uterus, thereby elevating the risk of developing endometrial cancer (197). However, as small changes in ligand structure can lead to differential cofactor recruitment, thus resulting in a ligand-dependent cell type- and promoter-specific response, there are various trials ongoing, in order to develop promising non-steroidal ER modulators, which display an improved activity, as well as a higher tissue specificity (extensively reviewed by Barker (196) and Harnish (198)). For instance, the tissue-selective ER down-regulator GW5638 still exerts some of the agonist actions of tamoxifen, yet behaves as an antagonist in breast tissue and displays no effects in the uterus (194, 199, 200).

With the exception of raloxifene, which is currently used in osteoporosis therapy but under reviewing for its utility in treatment regimens for breast cancer and which is able to inhibit NF-kappaB activity in myeloma cells via the removal of p65 from its binding sites in an ERalpha-dependent manner, most other currently used SERMs do not interfere with NF-kappaB activity (201, 202). However, the relation of excessive NF-kappaB signalling with aberrant cell death pathways and continuous cell proliferation has repeatedly been reported, and higher levels of c-Rel, p65 and p50 have been reported in over 90% of breast cancers (89, 97, 203, 204). Furthermore, it has recently been reported that ERalpha acts as an important regulator to control epithelial to mesenchymal transition (EMT) by controlling de *de novo* synthesis of RelB, which in turn controls the expression of Bcl-2. This mechanism may explain the more invasive character of ERalpha-negative breast cancer cells and explains the need for ligands that can block NF-kappaB activity (204). Therefore, the recently characterized WAY-169916, which selectively antagonizes NF-kappaB activity, without stimulating uterine proliferation or ER-mediated gene expression, may hold great promise (205).

Another trail that is explored is the possibility of targeting one specific ER isoform. Indeed, while both ERalpha and ERbeta can contribute to the transrepressive effects on inflammatory genes, it is ERalpha that promotes proliferation of breast cancer cells, while ERbeta behaves as a tumor suppressor (104). Furthermore, ERbeta is believed to be more potent in inhibiting NF-kappaB than ERalpha (206). As a consequence, ERbeta-selective estrogens, including phytoestrogens, such as the herbal extract MF101, may constitute a safer alternative to estrogens (84, 207). In addition, as already mentioned above, soy isoflavones, e.g. genestein, daidzein and biochanin, inhibit the NF-kappaB pathway in an ER-independent manner, making them attractive candidates for the treatment of ER-negative breast cancers (166). However, the *in vivo* efficacy of these natural compounds in cancer treatment has still to be determined.

Conclusively, the high degree of tissue-specific responses following the ligand-dependent conformational change of ER has made the search for new targets extra difficult. As with GR, cofactors play a crucial role in

mediating these differential effects. Currently, peptides that selectively target ER/cofactor interactions are under intensive investigation and might hold promise for the development of a more direct mechanism to modulate ER activity (reviewed in (208)). The potential of these peptidomimetics are not restricted to the ER field, but in addition found their way into PPAR research (209).

5.3. PPAR ligands

As for GR and ER, PPARs exert their anti-inflammatory effects mainly by interfering with activities of other TFs, including nuclear factor of activated T-cells (NFAT), signal transducer and activator of transcription (STAT)-3, CCAAT/enhancer-binding protein beta (C/EBPbeta), AP-1 and NF-kappaB. As such, fibrates can inhibit in a PPARalpha-dependent manner the production of IL-6, a key player in inflammation and tumorigenesis, via the blockage of AP-1 and NF-kappaB (137). However, the anti-inflammatory effects of PPARgamma-ligands have more extensively been reported. For instance, Arnold and co-workers showed that the respiratory syncytial virus (RSV)-induced DNA binding of NF-kappaB could be inhibited by PPARgamma ligands, thus correlating with a decrease in IL-6, IL-8 and ICAM-1 mRNA expression (210, 211). A decrease in LPS-induced IL-8, as well as COX-2 mRNA expression was also observed in colon cells after concomitant treatment with PPARgamma ligands and may involve a delayed IkappaBalpha degradation (212). Alternatively, Rosiglitazone (ROSI) may attenuate acute colitis through modulation of the NF-kappaB and p38 MAPK pathways, subsequently leading to decreased COX-2 levels (213). Furthermore, although different causal molecular mechanisms are described, there is unanimity about the expression of iNOS being under the transcriptional control of PPARgamma (151, 214).

Despite the clear potential of PPAR ligands to interfere with the activity of pro-inflammatory and pro-tumorigenic TFs, e.g. NF-kappaB, *in vitro* (215) and despite the promising results obtained in *in vivo* models of inflammation (216-220) and in patients suffering ulcerative colitis (221), the role of their corresponding receptors in cancer remains controversial and totally conflicting results have been reported by different research groups (extensively reviewed in (15)). The complexity of PPAR signalling is underscored by the observation that PPAR ligands may exert their effects via NR-independent mechanisms (222, 223). It would thus be of high interest to further explore to what level exactly the different mechanisms of PPAR ligand action mediate either beneficial or detrimental effects of PPAR function. Indeed, as for the previously described receptors, signalling to transcription results from integrating cellular pathways via differential cofactor recruitment and post-translational modifications of cofactor, nuclear receptor and other TFs that may bind adjacent promoter sites (15). For instance, interactions between hormone receptors, e.g. GR, on the one hand and PPARs on the other hand allows the cell to integrate local and systemic responses (142). This interaction may hold great therapeutic potential as, according to Nie and colleagues, a synergistic inhibition of chemokine expression occurs upon combined treatment with PPARgamma agonists and GC (224). In analogy, the simultaneous activation of PPARalpha and GR dose-dependently enhances the repression of NF-kappaB-driven genes (225). These results suggest that a combination therapy with PPARalpha/gamma ligands may allow lowering the doses of synthetic GC in treatment regimens without affecting their anti-inflammatory potential.

6. GENERAL CONCLUSIONS AND PERSPECTIVES

More and more evidence reveals that many of the well known cancer drugs exert their anti-tumoral effect at least in part through modulating the activity of NF-kappaB, thereby stressing the importance of NF-kappaB in tumor development and progression (reviewed in (226)). However, we should not forget the central role NF-kappaB plays (when regulated normally) in maintaining immune homeostasis. Complete blockage of NF-kappaB might thus have detrimental effects by undermining the important function of the immune system, not only when it comes to pathogen infection, but also in the light of immune surveillance to prevent tumor development (227). Therefore, NR ligands that down-modulate the activity of this widespread TF, instead of completely blocking it, are of enormous value due to their therapeutic potential. However, the efficacy of NR ligands is overshadowed by the occurrence of unwanted effects owing to their broad range of actions. Accordingly, the main goal of NR research remains the improvement of specificity, and molecular research is advancing to pursue this ambition. In this review we have quoted several strategies explored at the moment. Firstly, we reviewed interesting data obtained with different NR modulators, showing that different NR ligands could manipulate gene expression in different signal-, cell- and gene-specific ways on account of differential cofactor recruitment. The importance of these cofactors is further highlighted by their role in alternative splicing and elucidates that these crucial proteins not only influence the abundance, but also the nature of their products (228, 229). Since splicing deregulation is associated with different pathologies, including cancer, it might thus be interesting to identify which splice variants are associated with cancer and are regulated by hormones (229). The story is even more complex as it becomes clear that cross-talk between different cellular pathways results in diverse patterns of post-translational modifications (PTMs) of cofactors as well as TFs, referred to as the "protein code", hereby modifying their activity (41). As discussed above, nuclear receptors can modulate cell signalling pathways via genomic and non-genomic pathways and it is therefore crucial to further elaborate to what extent both mechanisms influence NR signalling. Furthermore, drugs that target the pathways responsible for these PTMs can drastically interfere with NR function and might thus be of importance for usage in combination protocols. Next to the combination of agents that act on different NR pathways, as discussed for GR and PPAR ligands, therapeutic benefit may also come from combination with ligands that act on other key signalling pathways, e.g. the MAPK, Akt, PKA, PKC pathways. As an example, it has recently been shown that PKA-induced phosphorylation of ERalpha induces resistance to

tamoxifen in breast cancer cells (230). In analogy, it is described that phosphorylation of GR after excessive p38 MAPK activation interferes with the affinity of GR for corticosteroids (231). Drug cocktails including kinase inhibitors might thus reduce steroid resistance, allowing to lower the concentrations of NR ligands used.

New fields that are still at the beginning of exploration when it comes to NR signalling include epigenetics and micro-RNAs (miRNAs). Indeed, specificity of gene regulation can also be obtained at the chromatin level and is reflected by the cross-talk of different individual modifications of the histone tails (histon code) and the DNA itself (in general epigenetics). Increasing evidence reveals the importance of epigenetics in NF-kappaB and NR signalling and it might thus hold a great challenge to reveal novel epigenetic targets that determine specificity (232). Lastly, miRNAs, which constitute a subset of non-coding RNAs important in controlling the stability of mRNAs, are reported to be aberrantly expressed in cancer tissues (233-235). Since these miRNAs may constitute an extra gene regulatory mechanism it will be exciting to learn how these molecules might interfere with NF-kappaB/NR-signalling.

To conclude, blocking NF-kappaB seems a two-sided sword. While some reports claim an anti-apoptotic role of NF-kappaB, other reports declare that NF-kappaB exhibits a pro-apoptotic role after DNA-damage. It is thus of extreme importance to unravel under which conditions NF-kappaB behaves as a stimulus for apoptosis (68). Additionally, it may be interesting to explore if NR ligands could cooperate with the pro-apoptotic capacity of NF-kappaB, which may add great value to cancer therapies.

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Abbreviations: NR: nuclear receptor, TF: transcription factor, GR: glucocorticoid receptor, ER: estrogen receptor, AR: androgen receptor, PR: progesterone receptor, MR: mineralocorticoid receptor, PPAR: peroxisome proliferator-activated receptor, GC: glucocorticoid, RXR: retinoid X receptor, AF: activation function, LBD: ligand binding domain, DBD: DNA binding domain, PGC: PPARgamma coactivator, SRC: steroid receptor coactivator, TIF: transcription intermediary factor, CBP: cAMP responsive element binding protein, HAT: histone acetyltransferase, HDAC: histone deacetylase, SMRT: silencing mediator of retinoic acid and thyroid hormone receptors, NF-kappaB: nuclear factor-kappaB, AP-1: activator protein 1, STAT: signal transducer and activator of transcription, IRF: interferon regulatory factor, IkappaB: inhibitor of NF-kappaB, IKK: IkappaB kinase complex, MMP: matrix metalloproteinase, iNOS: inducible NO synthase, COX: cyclooxygenase, IL: interleukin, TNF: tumor necrosis factor, GRE: glucocorticoid response element, E_2: estradiol, MKP: MAP kinase phosphatase, ERK: extracellular regulated kinase, JNK: c-Jun N-terminal kinase, PKA: cAMP-dependent protein kinase, PKAc: the catalytical subunit of PKA, BTM: basal transcription machinery, pol II: RNA polymerase II, CTD: carboxy-terminal domain

Key Words: NF-kappaB, nuclear receptors, glucocorticoid receptor, estrogen receptor, peroxisome proliferator-activated receptor, cancer, inflammation, therapeutic implications

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