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Acetylation of nuclear receptors in cellular growth and apoptosis

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Abstract

Post-translational modification of chromatin histones governs a key mechanism of transcriptional regulation. Histone acetylation, together with methylation, phosphorylation, ubiquitylation, sumoylation, glycosylation, and ADP ribosylation, modulate the activity of many genes by modifying both core histones and non-histone transcription factors. Epigenetic protein modification plays an important role in multiple cellular processes including DNA repair, protein stability, nuclear translocation, protein–protein interactions, and in regulation of cellular proliferation, differentiation and apoptosis.

Histone acetyltransferases modify histones, coactivators, nuclear transport proteins, structural proteins, cell cycle components and transcription factors including p53 and nuclear receptors. The estrogen, PPARγ and androgen receptor are members of the nuclear receptor (NR) superfamily. The androgen receptor (AR) and estrogen receptor α (ERα) are directly acetylated by histone acetyltransferases at a motif that is conserved between species and other NR. Point mutations at the lysine residue within the acetylation motif of the AR and ERα have been identified in prostate cancer as well as in breast cancer tissue. Acetylation of the NR governs ligand sensitivity and hormone antagonist responses. The AR is acetylated by p300, P/CAF and TIP60 and acetylation of the AR regulates co-regulator recruitment and growth properties of the receptors in cultured cells and in vivo. AR acetylation mimic mutants convey reduced apoptosis and enhanced growth properties correlating with altered promoter specificity for cell-cycle target genes. Cell-cycle control proteins, including cyclins, in turn alter the access of transcription factors and nuclear receptors to the promoters of target genes.

Keywords: Nuclear receptor; Histone acetyltransferases; Histone deacetylases; Coactivators; Corepressors; Post-translational modification; Cellular growth; Apoptosis

1. Introduction

Activation of target genes by hormones requires chromatin remodeling and histone modifications. Whereas DNA sequences within chromatin serve as genetic code for gene expression, post-translational modifications of core histones comprise an epigenetic ''histone code'' that modulates the local chromatin structure and determines the accessibility of transcriptional co-regulators to the underlying DNA. A diverse array of covalent modifications of the amino acid residues in the histone tails including acetylation, phosphorylation, methylation and ubiquitylation have been reported. Distinct histone modifications, which act sequentially or in combination, dictate dynamic transitions between transcriptionally active or transcriptionally silent chromatin states [1–5]. Post-translational modification of histone proteins as well as non-histone proteins including nuclear receptors integrates signaling pathways mediating diverse biological processes. This review will focus on the biological significance of nuclear receptors modification by acetylation.

2. Histone acetyltransferases and deacetylases

In eukaryotes, DNA is packaged by histones into nucleosomes which are composed of 147 base pairs of DNA and core histone proteins H2A, H2B, H3 and H4. Alterations in the localize chromatin structure has an important impact on genetic transcriptional responses. Chromatin remodeling

Abbreviations: NR, nuclear receptor; AR, androgen receptor; ERα, estrogen receptor α; PPARγ, peroxisome proliferator-activated receptor γ; HATs, histone acetyltransferases; HDACs, histone deacetylases; SRC-1, steroid receptor coactivator-1; N-CoR, nuclear receptor corepressor; SMRT, silencing mediator of retinoid and thyroid hormone receptor; DHT, dihydrotestosterone; TSA, trichostatin A

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complexes and enzymes involved in post-translational modifications of the histone components of chromatin play important roles in transcriptional regulation.

The histone acetyltransferases (HAT) and histone deacetylases (HDAC) regulate acetylation and deacetylation of the conserved lysine residues present in the amino terminal tails of all four core histones. It is believed that acetylation of the lysine residues neutralize the basic charge of the histone tails and therefore reduces their affinity for the negatively charged chromosomal DNA. Furthermore, covalent modification of histones constitute a ''histone code'' serving as an epigenetic marker for gene expression which in turn provides recognition sites for factors involved in either the activation or repression of gene expression [6]. In general, transcriptionally active euchromatin domains tend to be relatively hyperacetylated whereas inactive heterochromatin domains are hypoacetylated [7].

Each of the HAT enzymes belongs to one of two categories: type A, located in the nucleus, or type B, located in the cytoplasm, although recent evidence indicates that some HAT proteins may function in multiple complexes or locations and thus not precisely fit these historical classifications. B-type HATs have a housekeeping role in the cell, acetylating newly synthesized free histones in the cytoplasm for transport into the nucleus, where they are deacetylated and incorporated into chromatin [7]. The A-type HATs, on the other hand, acetylate nucleosomal histone within chromatin in the nucleus, and are thereby linked to transcriptional regulation. The A-type HATs are divided further into five families, including the Gcn5-related acetyltransferases (GNATs), the MYST (for MOZ, Ybf2/Sas3, Sas2 and Tip60)-related HATs, p300/CBP HATs; the general transcription factor HATs (TAFII250), and the nuclear hormone-related HATs, SRC1 and ACTR (SRC3) [6].

The HDACs play a major role in keeping the balance between the acetylated and deacetylated states of chromatin. There are two protein families with HDAC activity: the classical HDAC family and the SIR2 family of NAD^+-dependent HDACs. The classical HDAC family is again divided into two groups: class I HDACs (HDACs 1, 2, 3, and 8) which are similar to the yeast (*Saccharomyces cerevisiae*) transcriptional regulator RPD3. They localize to the nucleus and are expressed in most cell types. Class II HDACs (HDACs 4, 5, 6, 7, 9, and 10) are homologous to the yeast HDA1 protein and are located in both the nucleus and cytoplasm. The expression pattern of class II HDACs is more restricted than that of class I HDACs. HDAC5, HDAC7 and HDAC9 are expressed in the heart, whereas HDAC4, HDAC8 and HDAC9 seem to be expressed more in tumor tissues than in normal tissues [8,9]. The newly identified HDAC11 is more closely related to the class I HDACs HDAC3 and HDAC8. HDAC11 contains a catalytic domain at the N-terminus with HDAC activity but it is found not to reside in any of the known HDAC complexes (Sin3, N-CoR, SMRT), indicating it may have distinct physiological roles from those of the known HDACs [8,10].

3. Acetylation of non-histone targets

Accumulating evidences suggest that substrates of HATs and HDACs are not limited to histones. A subset of transcription factors such as p53 [11,12], GATA-1 [13], GATA-2 [14], GATA-3 [15], EKLF [16], HMG box architectural factor UBF [Pelletier, 2000, no. 245], AML1 [17], and hormone nuclear receptors, such as AR [18–21], ER α [22] are regulated by acetylation. Through modification of histone and nonhistone substrates, histone acetyltransferase complexes are involved in diverse processes such as transcription regulation, protein degradation, gene silencing, DNA repair and cell-cycle progression.

4. Nuclear receptor superfamily

The NR superfamily encodes structurally related proteins including receptors for steroid and thyroid hormones, retinoic acid, vitamins, and other proteins for which no ligands have been found (orphan receptors) [23]. Nuclear hormone receptors function as ligand-activated transcription factors. The functional domains of the NR are conserved within the superfamily members and include the activation function region (AF), DNA binding domain (DBD), hinge region and ligand-binding domain (LBD). The DBD directs the receptors to bind specific DNA sequences as monomers, homodimers, or heterodimers. The LBD responds to binding of the cognate hormone [24]. The N-terminal domain and LBD interact with other transcriptional cofactors (coactivators and corepressors), resulting in ligand-regulated and ligand-independent effects on gene transcription [25–27]. Nuclear receptors are one of the most abundant classes of transcriptional regulators and regulate diverse functions, including homeostasis, reproduction, development and metabolism [28].

5. Nuclear receptor co-regulators

Co-regulators (coactivators and corepressors) convey both intrinsic enzymatic activities and recruit enzymes to molecular interactions to modulate gene expression in response to hormonal signals [29]. Coactivators associate with NR in a ligand-dependent manner and are essential for ligand-induced NR activation (see Fig. 1). A large number of coactivators/adaptors of NR family have been identified during recent years, including steroid receptor coactivator-1 (SRC-1), amplified in breast cancer 1/activator of the thyroid and RA receptor/steroid receptor coactivator-2

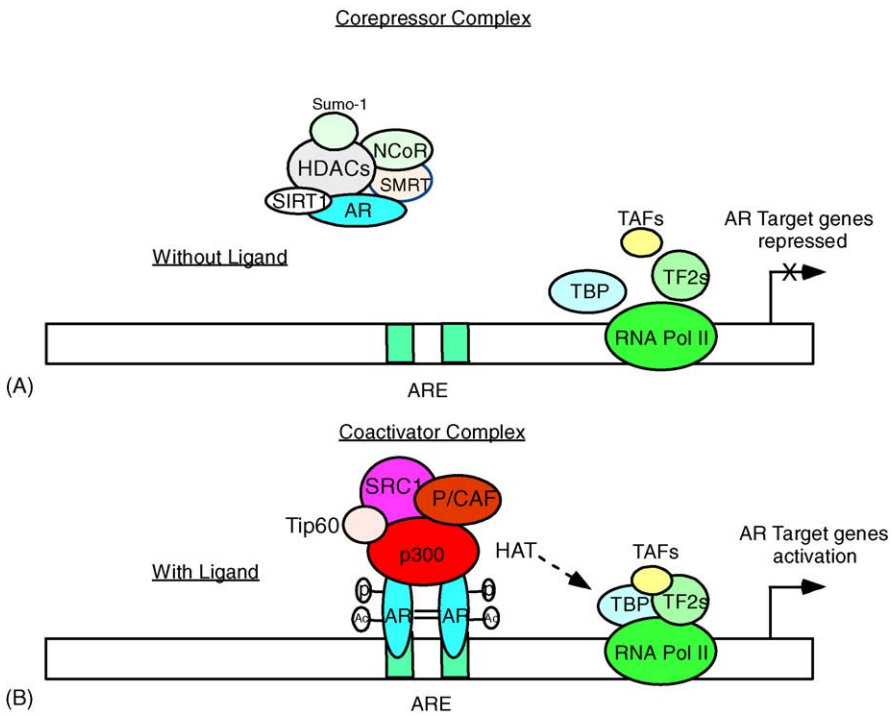

Fig. 1. AR corepressors and coactivators. (A) In absence of ligand, the AR forms complexes with corepressors including SMRT, N-CoR, HDACs, Sumo-1 and SIRT1. (B) In presence of ligand (DHT), the AR undergoes nuclear translocation, dimerization, and forms complexes with coactivators such as Tip60, p300, P/CAF, SRC1 and binds to androgen-responsive elements of the AR target genes. Ac: acetylation; P: phosphorylation.

(AIB1/ACTR/SRC-3), glucocorticoid receptor interacting protein 1/transcriptional intermediary factor 2/steroid receptor coactivator-3 (GRIP-1/TIF-2/SRC-2), p300/CBP (CREB-binding protein) and p/CAF (p300/CBP-associated factor) (reviewed in Refs. [24,30,31]). The coactivator proteins augment NR activity through several functions. They recruit other cofactors and serve as a molecular bridge between the NR and the basal transcription apparatus. The cointegrator proteins p300/CBP augment NR activity, in part related to their intrinsic histone acetyltransferase (HAT) activity. Histone acetylation contributes to nucleosome destabilization, facilitating transcription factor binding to specific target DNA sequences in the promoter region of hormone-responsive target genes. The LXXLL motif/nuclear receptor box, contained within a number of coactivator molecules, mediates the interaction with nuclear hormone receptors. Tat-interactive protein 60 kDa (Tip60) contains a single nuclear receptor box at its extreme C terminus. Unlike members of the p160 coactivator family that interact predominantly with the N terminus of the AR in an LXXLL motif-independent manner, the LXXLL motif of Tip60 is required and is sufficient for AR interaction [32]. Furthermore, Tip60 directly acetylates the AR, which is a requisite for Tip60-mediated transcription [20].

In contrast to NR coactivators, NR corepressors regulate the transcriptional activity of nuclear receptors by interacting with unliganded NRs. These proteins such as nuclear receptor corepressor (N-CoR) and its homolog silencing mediator of retinoid and thyroid hormone receptor (SMRT) contain repression domains and function as corepressors by recruiting a complex containing Sin3, HDACs and several additional proteins. Other corepressors of NR include thyroid hormone receptor uncoupling protein (TRUP) [33], BRCA1 [34], and NuRD [35] (reviewed in Refs. [24,30,31]). N-CoR and SMRT are structurally quite similar. The N-terminal repression domain of N-CoR/SMRT interacts with HDAC complexes and a transducin-like molecule that interacts with histones. Interaction surfaces within the N-CoR/SMRT bind NR with the prediction that a single corepressor interacts with a single NR in a DNA-bound dimer [36]. An alternative mechanism exists for transcriptional repression of ERα through recruitment of HDAC complexes to the ERα by BRCA1 [34].

6. Acetylation modification of nuclear receptors

Regulation of nuclear receptor gene expression involves dynamic and coordinated interactions with HATs and HDAC complexes, which are components of the NR coactivator or corepressor complexes. Nuclear receptor members, such as the AR and ERα, are direct substrates of histone acetyltransferase in vitro and in vivo [18–22]. The candidate acetylation motif KXKK/RXKK of the nuclear receptor members such as TR, RAR, PPAR, LXR, FXR, VDR, GR, PR, HNF4, and SF1 are phylogenetically conserved among different species including vertebrates, arthropods, and nematodes [22]. Such conservation suggests that acetylation might be a general

mechanism regulating hormone signaling thereby contributing to diverse functions including regulation of development, and homeostasis.

6.1. Acetylation of the AR

6.1.1. Acetylation of the AR determines its ligand-dependent activity and governs ligand sensitivity and specificity

The AR plays a key role in prostate cancer cellular proliferation by dihydrotestosterone (DHT) and the induction of secondary sexual characteristics. The AR can be modified by acetylation in vitro by p/CAF, p300, and Tip60 [18,20]. Immunoprecipitation-blotting has demonstrated that the AR is acetylated in vivo [21]. Both CBP and p300 augment ligand induced AR activity [18,37]. Trichostatin A (TSA), a specific HDAC inhibitor, enhanced activity of the androgen-responsive MMTV-LUC reporter in a dose-dependent manner [18]. A more dramatic effect of TSA was seen when cells were transfected with an androgen-responsive reporter and the AR and treated with the AR ligand, DHT. This finding suggested that the activity of endogenous deacetylases might constrain the full activation potential of androgen-responsive genes [18]. The AR acetylation sites are located in the hinge region of the AR, and is located in proximity to the second zinc finger of DBD. The AR acetylation motif is well conserved in different species. Mutations of the AR lysine residues 630, 632, and 633 abrogated p300 and reduced DHT-induced activity, suggesting that acetylation is an essential step in ligand-dependent AR activation [18]. Furthermore, the ligand-induced transcriptional activity of acetylation mimic mutant AR_{K630Q} and a somatic mutation identified in prostate cancer patients at the AR acetylation site, AR_{K630T}, is greater than that of wild-type AR. These AR acetylating site mimics also showed enhanced responsiveness at lower DHT concentrations than the wild-type AR. In addition, the DHT antagonist flutamide antagonized DHT-induced wild-type AR activity; the AR acetylation mimics however were relatively resistant [21].

The acetylation motif KLKK of the AR in the hinge region is in close proximity to the second zinc finger of the DNA binding domain and the bipartite nuclear localization sequence (NLS) of the receptor [38]. Early studies therefore examined the relative nuclear and cytoplasmic distribution of the AR and the AR acetylation mutants. These studies showed similar relative abundance in nuclear and cytoplasmic components by western blotting [21]. Subsequent studies suggest that the lysine residue in the AR acetylation motif may play a role in subcellular distribution dependent on cell type, interdependent upon the size of the AR glutamine tract, and may be dependent upon the amount of receptor expressed in cells. In stable prostate cancer cell lines expressing modest amounts of mutant receptor, the nuclear and cytoplasmic distribution of the AR were unaffected by mutation of the motif [21,39]. In the context of an expanded glutamine tract (Q24) AR, the AR acetylation site mutant (K632/633A) demonstrated a greater proportion of cytoplasmic distribution in the absence of ligand and delayed ligand-dependent nuclear translocation. Interestingly, in transient expression studies of HeLa cells using Fu-GENE6, cytoplasmic aggregates of $AR_{K632/633A}$ were identified [40], although the biological significance of these aggregates remains to be explored. An expanded glutamine repeats of the AR induces similar aggregates [40]. In DU145 and HEK293 cells, the AR_{K630A} and $AR_{K632/633A}$ mutants maintain nuclear transrepression function of NFκB, SP1 and AP-1 activities, demonstrate enhanced binding to nuclear HDAC1 and N-CoR with reduced binding to p300. Perhaps of greater importance is the finding that despite similar level of nuclear protein, the AR_{K630A} mutant displayed reduced recruitment to an endogenous androgen responsive element (ARE), in the context of its local chromatin structure [39]. Although recruitment of the AR_{K630A} mutant to DHT was partially compromised, a more substantial deficit in TSA-dependent recruitment to the ARE was observed [39]. Together these studies suggest the AR acetylation site may regulate recruitment of AR to specific endogenous AREs in the context of local chromatin.

6.1.2. Acetylation of the AR determines its affinity with its co-regulators

Acetylation-deficient AR mutants AR_{K630A} and $AR_{K632A/K633A}$ are selectively defective in DHT-induced transactivation of androgen-responsive reporter genes and coactivation by SRC1, Ubc9, TIP60, and p300. Acetylation-deficient AR mutants AR_{K630A} and $AR_{K632A/K633A}$ showed 10-fold increased binding of the N-CoR corepressor compared with the AR wild-type in the presence of ligand, whereas the acetylation mimic mutants, AR_{K630Q} and AR_{K630T}, a somatic mutation identified from prostate cancer patient [41], showed enhanced p300 binding and reduced N-CoR/HDAC/Smad3 corepressor binding. These findings suggest that the AR acetylation motif might serve as a docking site for the coactivators or corepressors binding, and acetylation of the lysine residue in the AR hinge region is a key ''switch'' for the recruitment of co-regulators to the basal transcriptional machinery of AR target genes [19,21].

6.1.3. Acetylation of the AR regulates prostate cancer cell growth

Several lines of experimental data strongly suggest that the AR acetylation site is involved in the regulation of prostate cellular growth and apoptosis. The cellular proliferation rate of prostate cancer cell lines stably expressing the AR acetylation mimic mutants (AR_{K630Q} and AR_{K630T}) was increased in the presence of DHT compared to the AR wild-type. In colony formation assays, the size and number of soft agar colonies of AR_{K630Q} and AR_{K630T} stable cell lines were substantially increased compared with those of

the wild-type AR cell line. A major growth advantage was observed in the absence of ligand, suggesting that a basal-level function of the AR acetylation mimic mutants may contribute to contact independent growth. In addition, apoptosis measured by terminal deoxynucleotidyl transferase-mediated dUTP-digoxigenin nick end labeling (TUNEL) staining was reduced three- to four-fold in tumors from nude mice harboring the AR acetylation mimic mutants compared with the wild-type AR expressing clones [21]. Both promoter activity and protein levels of cyclin D1 and cyclin E were increased in the AR acetylation mutant cell lines. Collectively, these studies indicate that the enhanced growth advantage may be due to increased activation of the cell-cycle control genes.

6.1.4. AR coactivators in prostate cancer

The transcriptional activity of the AR and other steroid hormone receptors is mediated through a large and growing number of interacting proteins which function as coactivators or corepressors [42]. p300 is an AR coactivator that contains intrinsic HAT activity. As discussed above, p300, together with p/CAF and TIP60, are direct AR acetylase in vitro. Recently, it has been demonstrated that p300 plays an important role in prostate cancer (PCa) cell proliferation as well as PCa progression [43]. p300 is overexpressed in PCa tissues and higher levels of p300 expression correlated with a higher Gleason score, larger tumor volumes and extraprostatic extension of PCa, suggesting that p300 might be involved in some of the genotypic and phenotypic cellular changes associated with PCa progression [43].

In addition to p300, recent studies have found increased expression of SRC-1 and SRC-2/TIF2 in tissues from androgen-independent prostate cancer. A majority of recurrent prostate cancers overexpress TIF2 and SRC1 that are coincident with the onset of recurrent prostate cancer growth. SRC1 and TIF2 were more intense in the nuclei of recurrent prostate cancer than in androgen-dependent prostate cancer or benign prostate hyperplasia, correlating with high AR expression in recurrent prostate cancer [44]. Prostate cancer cell lines also express AR coactivators, although the pattern of coactivator expression varies between different cell lines. The AR coactivator SRC1 was expressed ubiquitously at almost equal amounts in DU145, PC3, LNCaP, and LN-TR2, whereas ARA55, ARA54, TIF2, and RAC3 displayed cell line specific expression [45].

The fact that AR is a direct target of Tip60, p300, and P/CAF in vitro, that acetylation mimic mutants of the AR enhance prostate cancer cell growth, and that expression of AR coactivators in prostate cancer tissues correlates with AR expression, prostate cancer progression and recurrence suggest that acetylation of the AR may contribute to the development of androgen-independent prostate cancer. Inhibition of the AR acetylation process and AR-coactivator binding may be a promising approach for the treatment of prostate cancer.

6.2. Acetylation of the ERα

The estrogen receptor is involved in regulating important functions in development and reproduction and in human diseases including breast cancer, cardiovascular disease, osteoporosis, and Alzheimer's disease. The ERα contains two activation domains, AF-1 and AF-2. The AF-1 function is constitutive and is induced by p300/CBP, p68 RNA helicase A and by mitogen-activated protein kinases (MAPKs) [46–49]. The AF-2 domain contributes to ligand-induced ERα activity through further recruitment of coactivator proteins including the p160 family (SRC-1, TIF2/GRIP1, AIB1/ACTR), and coactivators with HAT activity (CBP, p300 and P/CAF) [48–50].

6.2.1. Acetylation of the ERα determines its hormone sensitivity

The ERα is also acetylated both in vitro and in vivo [22]. p300 selectively and directly acetylates the ERα at lysine residues within the ERα hinge/ligand binding domain. Compared with the wild-type ERα, glutamine or arginine substitutions at the ERα acetylation site enhanced E2-dependent activity of the ERα, suggesting that direct ERα acetylation normally suppresses ligand sensitivity. However, the ERα acetylation mutants are not altered in their responsiveness to MAPK activation, indicating that the mechanisms governing ligand-induced ERα activity through the ERα acetylation site are distinct from those governed by ACTR which involves MAPK. [22].

6.2.2. Acetylation site mutation of the ERα in breast cancer

Breast cancer is hypothesized to evolve from normal ductal epithelium through typical hyperplasia, atypical hyperplasia, carcinoma in situ, invasive carcinoma to metastatic carcinoma. Specific molecular alterations are correlated with the multi-step development of breast cancer. A somatic mutation at the ERα acetylation motif has been reported to occur at high frequency in Caucasians with breast hyperplasia [51]. Studies from atypical breast hyperplasia demonstrated a Lys-to-Arg substitution at residue 303 (K303R) in 34% of the patients examined. The ER_{K303R} mutation is frequently present in both premalignant lesions of the breast and may also occur in the adjacent normal-appearing breast epithelium [51]. Expression of the ER_{K303R} mutation confers a hypersensitivity to estrogen with maximal stimulation in response to physiological levels (10^{-12} M to 10^{-11} M) of hormone. Growth curve studies showed that MCF-7 clones expressing the ER_{K303R} mutation proliferate at low concentration of hormone (10^{-12} M) with nearly the same highly proliferative response seen at the highest concentration of estradiol used (10^{-9} M). Thus, the ER_{K303R} mutation is a gain-of-function mutation that

could have a significant biological role in early breast cancer development [51].

6.2.3. ERα coactivators in breast cancer

The transcriptional activity of ERα is regulated by coactivators, corepressors, and chromatin remodeling complexes. In the breast cancer tissues and breast cancer cell lines, expression of ERα is well correlated with expression of TIF2, AIB1, P/CAF, and N-CoR. The expression levels of ERα, TIF2, and CBP are significantly higher in intraductal carcinomas than those in normal breast tissue [52]. Low N-CoR expression is associated with shorter relapse-free survival in tamoxifen-resistant tumors [53]. AIB1 (SRC-3) is frequently amplified in breast tumors and correlates with ERα and progesterone receptor positivity as well as with tumor size [54]. These findings suggest a positive correlation between ERα and cofactors, and up-regulation of ERα and cofactors may play an important role in the development of breast cancer. This notion is further supported by the chromatin immunoprecipitation (ChIP) studies showing that ERα and a number of coactivators rapidly associate with estrogen-responsive promoters following estrogen treatment. Recruitment of the p160 class of coactivators to estrogen-responsive genes is sufficient for the growth stimulatory actions of estrogen in breast cancer [55].

Cyclin D1, which is frequently amplified in breast cancer, activates the ERα through direct binding with ERα in a CDK-independent manner [56]. By acting as a bridging factor between ERα and SRCs, cyclin D1 recruits SRC-family coactivators to ERα in the absence of ligand [57]. Furthermore, cyclin D1 also regulates ERα function by antagonizing BRCA1 repression of ERα (Wang and Pestell, unpublished data). Thus cyclin D1 may serve as an ERα coactivator, providing an indirect mechanism to regulate breast cancer cellular growth.

Cyclin D1 plays distinct roles in regulation of ERα versus AR. Cyclin D1 is considered as a coactivator of ERα, whereas cyclin D1 functions as a corepressor to inhibit ligand-dependent AR activation [58–60]. Cyclin D1 physically associates with the AR to inhibit AR activity independent of its CDK4-binding [58,59]. Intriguingly, both cyclin D1 and the AR bound to similar domain of P/CAF, and cyclin D1 displaced binding of the AR to P/CAF in vitro [59]. Taken together, cyclin D1 may repress ligand-dependent AR activity by directly competing for P/CAF binding or by recruitment of HDAC corepressor complex to the AR.

6.3. Histone acetylation and thyroid hormone receptor function

Thyroid hormone receptors (TRs) are members of the NR superfamily which regulate development, differentiation, homeostasis, and tumorigenesis. TRs function as heterodimers with the 9-*cis*-retinoic acid X receptors (RXR). Unliganded heterodimers of TR and RXR bind to thyroid hormone response elements (TREs) and repress transcription, whereas ligand-bound TRs are transcriptional activators [61]. The repression function of unliganded TR/RXR is complexed with HDAC and reduces the local histone acetylation. T3 treatment disrupts the normal nucleosomal array structure and reverses this repression, suggesting that chromatin remodeling, including histone acetylation and chromatin disruption, are important for T3 regulation in vivo [62–64]. Further evidence demonstrated that transcriptional activation by TRβ involves chromatin remodeling, histone acetylation, and synergistic stimulation by p300 and SRCs [61]. Binding of liganded TRβ to chromatin induces promoter-proximal chromatin remodeling and histone acetylation, and histone acetylation is correlated with increased TRβ-dependent transcription.

6.4. Co-regulators, histone acetyltransferase and PPAR γ function

Peroxisome proliferator-activated receptor γ (PPARγ) is a member of the nuclear receptor superfamily that mediates adipocyte differentiation, exerts anti-inflammatory effects in monocyte/macrophages, modulates insulin sensitivity, and inhibits cellular proliferation [65–68]. The regulation of gene transcription by ligand-bound PPARγ involves DNA binding and recruitment of coactivator proteins including p300, the SRC-1 class of coactivators and DRIP205 [69–71]. The hormone nuclear receptor coactivators such as SRC1 and p300 contain LXXLL motifs that interact with the AF-2 helix of the receptor molecule. p300 contacts the AF-2 region of PPARγ in a ligand-dependent manner, and contacts AF-1 in a ligand-independent manner [72]. PPARγ, like other nuclear receptors, also interacts with corepressor proteins such as N-CoR and SMRT [73].

PPARγ ligands inhibit cellular growth of different cancer types such as colon cancer, breast cancer, gastric adenocarcinoma, prostate cancer through its anti-proliferative and pro-apoptosis properties [74–81]. Upon ligand binding, PPARγ selectively inhibited expression of the cyclin D1 gene [68]. Cyclin D1 repression by PPARγ involved competition for limiting abundance of p300, directed through a c-Fos binding site of the cyclin D1 promoter [68]. Furthermore, expression of cyclin D1 inhibited ligand-induced PPARγ transactivation, expression, and promoter activity through a pRB- and cdk-independent mechanism. The cyclin D1 HLH (helix-loop-helix) region is required for repression of the PPARγ function [82]. The molecular mechanism of the cyclin D1 HLH region-mediated-repression of the PPARγ ligand dependent activation remained to be explored. Preliminary data suggested that cyclin D1 might antagonize p300-mediated PPARγ function by recruitment of HDAC to the promoter of PPARγ target genes (Fu and Pestell, unpublished data).

7. Sumoylation of the nuclear receptors

Sumoylation is an enzymatic process involving the attachment of a small protein moiety, SUMO, to substrate proteins. Although biochemically analogous to ubiquitylation, conjugation of SUMO does not typically lead to degradation of the substrate [83,84]. A subset of cellular proteins, including histone H4, MEK1, CCAAT/enhancer-binding proteins (CEBP), topoisomerase I, Tcf-4, Smad4, p53, MDM2, pancreatic duodenal homeobox-1 (Pdx1), the transcription corepressor CtBP are sumoylated [83,85–96]. Nuclear receptors including AR, glucocorticoid receptor (GR), PPARγ and progesterone receptor (PR) are also subjected to sumoylation modification [97–103]. Sumoylation of GR is not dependent on the presence of the ligand. SUMO-1 overexpression induces dramatic GR degradation. On the other hand, SUMO-1 stimulates the transactivation capacity of GRs in the context of multiple GR molecules [99]. PR undergoes sumoylation at lysine 388 located in its N-terminal domain and overexpression of SUMO-1 markedly enhances PR-mediated gene transcription. However, sumoylation of the receptor itself is not responsible for enhanced transcription. SRC1 is sumoylated through two major sites of conjugation at Lys-732 and Lys-774 and sumoylation increases PR/SRC-1 interaction prolonging SRC-1 retention in the nucleus [97]. The SUMO-1-conjugating enzyme Ubc9 interacts with the AR and the AR is covalently modified by SUMO-1 in the N-terminal domain. The sumoylation core motif found in AR is present in the N-terminal domains of GR, MR, and PR, suggesting that sumoylation, like acetylation modification, may be a general mechanism for regulation of steroid receptor function [102].

The protein inhibitor of activated STAT (PIAS) family proteins PIAS1 and PIASxα function as SUMO-E3 ligases for substrate including AR. PIAS1 and PIASxα but not PIAS3 or PIASxβ enhanced sumoylation of AR in cells. PIAS1 and PIASxα-bound Ubc9, the E2 enzyme for SUMO-1, in a RING finger-like domain-dependent manner. Repression of AR-dependent transcription by PIAS1 and PIASxα is dependent on the ectopic expression of SUMO-1, indicating that the sumoylation of AR is crucial for the AR repression [100]. PIASy-mediated AR repression however seems to be independent of sumoylation [98].

A PIAS-like protein, hZimp10, is an AR coactivator which physically associates with the N-terminal AR transactivation domain. The C-terminal proline-rich region of hZimp10 contains a strong intrinsic transactivation domain and expression of hZimp10 in human prostate cancer cells augmented the transcriptional activity of AR. hZimp10 co-localized with AR and SUMO-1 at replication foci and the augmentation of AR activity by hZimp10 is dependent on AR sumoylation [101].

Recent studies suggested that phosphorylation-dependent AR ubiquitylation and degradation may require the Mdm2 E3 ligase activity. Both AKT and Mdm2 can form a complex with AR and promote phosphorylation-dependent AR ubiquitylation, resulting in AR degradation by the proteasome. The E3 ligase activity of Mdm2 and phosphorylation of Mdm2 by AKT are essential for Mdm2 to affect AR ubiquitylation and degradation [104].

8. Conclusions

Post-translational medications of the NRs and interaction of NR with coactivators and corepressors play important roles in modulating NR functions. A subset of evolutionarily related NR family members contain a potential acetylation motif implicating acetylation is involved in regulating multiple distinct hormone signals. The NR coactivators SRC1, AIB1, and p300 are overexpressed in human cancer and residues within the acetylated motif of NR are mutated in cancer. Lysine acetylation mimic mutant AR and ERα receptors promote contact-independent growth. Development of coactivator-specific small molecules or HAT-specific inhibitors may be useful approaches to inhibit the transcriptional activity of NRs by blocking receptor–coactivator interaction [105–108].

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