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HISTONE ACETYLATION AND METHYLATION IN THE SIGNALING OF STEROID HORMONE RECEPTORS

K. TRTKOVÁ, J. BOUCHAL AND Z. KOLÁŘ

Laboratory of Molecular Pathology and Institute of Pathology, Faculty of Medicine, Palacky University, Hnevotinska 3, 77515 Olomouc, Czech Republic.
Fax: +420 585 632 966; E-mail trtkova@post.cz

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Abstract – Molecular changes associated with malignancy are extremely complex. Early epigenetic events occuring in the common tumor types such as breast or prostate cancer might determine the subsequent genetic changes leading to tumor development and progression. Covalent modifications of histones play a major role as determiners of epigenetic information and are important in the regulation of gene expression. Acetylation generally corelates with transcriptional activation, while methylation can signal either activation or repression. However, little is known about the interplay of different epigenetic events. Steroid hormones regulate many cellular processes through signal transduction pathways that result in a variety of posttranslational modifications. Such modifications can be triggered by steroid hormones in cooperation with coactivators (p160 family proteins, CBP, p300, p/CAF) and/or corepressors (N-Cor, SMRT, TZF). There is still much to learn about their regulation and the molecular and physiological consequences of these modifications.

Key words: Epigenetic modifications, steroid hormone receptors, cellular coregulators, prostate cancer

INTRODUCTION

Epigenetic gene silencing associated with transcriptional repression of tumor suppressor genes is an important mechanism for the loss of gene function in cancer. Epigenetically silenced genes do not have any genetic mutations, even though they are transcriptionally repressed in many different cancer-cell types. In fact, more genes might suffer loss of function through epigenetic modifications than through genetic defects (50). Covalent modifications of histone tails have a key role in regulating chromatin structure and controlling transcriptional activity. Histone modifications constitute a histone code serving as epigenetic markers for gene expression. Consequently, the "histone code hypothesis" (105) predicts that a pre-existing modification affects subsequent modifications on histone tails (124).

The nucleosome is the basic unit of chromatin. In the nucleosome, 146 bp of DNA wrap around an octamer of core histones, consisting of pairs of H3, H4, H2A and H2B (66). Histones, especially residues of the N-terminal regions of histones H3 and H4 and the N- and C-terminal regions of histones H2A, H2B and H1, are susceptible to a variety of posttranslational modifications, such as acetylation, phosphorylation, methylation. Histones may be methylated on either lysine (K) or arginine (R), and the most studied sites of lysine methylation lie in the N-terminal regions of H3 and H4 histone proteins.

Abbreviations: **ACTR**, activator of thyroid and retinoic acid receptors; **AD**, activation domain; **AF**, activation function; **AIB1**, amplified in breast cancer 1; **AIS**, androgen insensivity syndrome; **AR**, androgen receptor; **ARE**, androgen response element; **ATP**, adenosine triphosphate; **CARM1**, cofactor-associated arginine methyltransferase 1; **CBP**, CREB-binding protein; **CHD1**, chromodomain helicase DNA-binding protein 1; **DBD**, DNA-binding domain; **ER**, estrogen receptor; **GRIP1**, glucocorticoid receptor-interacting protein 1; **H**, histone; **HAT**, histone acetyltransferase; **HDAC**, histone deacetyltransferase; **HDACI**, histone deacetylase inhibitor; **HKMT**, histone lysine methyltransferase; **HMT**, histone methyltransferase; **HP1**, heterochromatin protein 1; **IL-6**, interleukin-6; **ING2**, inhibitor of growth 2; **LBD**, ligand-binding domain; **LSD1**, lysine specific demethylase 1; **NaB**, sodium butyrate; **N-Cor**, nuclear receptor corepressor; **NR**, nuclear receptor; **p/CAF**, p300/CBP-associated factor; **PcG**, Polycomb group; **PHD**, plant homeodomain; **PRMT**, protein arginine methyltransferase; **PSA**, prostate-specific antigen; **RAC3**, receptor-associated coactivator 3; **SMRT**, silencing mediator of retinoid and thyroid hormone receptor; **SRC-1**, steroid receptor coactivator-1; **TIF2**, transcriptional intermediary protein 2; **Tip 60**, Tat interactive protein, 60 kDa; **TRAM1**, thyroid receptor activator molecule 1; **TSA**, trichostatin; **TZF**, testicular zinc finger

Chromatin structure plays an integral role in the control of gene expression, and it is apparent that cancer is a disease that is driven by epigenetic changes (24, 50). Chromatin appears as an array of nucleosomes, but in the nucleus, the chromatin fibres that form chromosomes undergo several levels of folding, resulting in increasing degrees of condensation (39). Folding alters the accessibility of DNA to the proteins or protein complexes that regulate diverse chromatin functions such as DNA replication, gene expression and chromosome segregation (71, 124). Transcriptional silencing is correlated with a condensed chromatin structure called heterochromatin, whilst loosely packaged transcriptionally active chromatin is called euchromatin.

A major challenge in chromatin biology has centered on efforts to define the connection between specific methylation states and distinct biological outputs impacting on function (26). Methylation of lysine residues is important both in blocking acetylation and mediating protein-protein interactions that can activate or repress transcription (71). A methyl group is relatively small and its addition to lysine or arginine residues does not neutralize their charge, so it is unlikely that methylation alone can significantly affect chromatin structure (6). In contrast, acetylation of lysine residues in histones is known to antagonize the folding of chromatin *in vitro* (39). Unlike acetylation, which generally correlates with transcriptional activation (92), histone lysine methylation can signal either activation or repression, depending on the sites of methylation (124). In general, H3-K9 methylation is associated with heterochromatin formation (84, 97) and euchromatic gene repression (97). Genes within euchromatin have the potential to be active and are associated with methylated H3-K4 (94).

Histone modifications can be triggered by transcription coactivators and corepressors, or by DNA methylation, which triggers sequential binding by methyl-binding proteins (62, 75). Loss of DNA methylation at CpG dinucleotides was the first epigenetic abnormality to be identified in cancer cells (23). Most CpG sites in the human genome are methylated, except for clusters of CpG dinucleotides (CpG islands) that are normally free of methylation. In cancer cells, a switch from unmethylated to methylated CpG islands results in permanent loss of expression of genes that are crucial for recognition of tumor cells. However, the mechanism of DNA methylation associated gene silencing is not entirely known (111) and is under intensive study (32, 65, 72).

Steroid receptors belong to a large family of structurally related proteins that regulate transcription. Their transcriptional activities are modulated by cellular coregulatory proteins (coactivators and corepressor). Many of these coregulators exist as a part of large complexes that can be recruited by nuclear hormone receptors and function as chromatin remodeling factors. Androgen receptors are involved in the development and progression of prostate cancer, which is one of the most frequently diagnosed cancers in males. It is known, that the functional activity of the androgen receptor is enhanced by coactivators. Furthermore, inspite of the increasing number of androgen receptor coactivators that have been shown to be expressed in the prostate, their role in prostate cancer progression has not yet been clarified.

EFFECTS OF DNA METHYLATION

It is recognised that gene silencing correlates with DNA hypermethylation and the addition of a methyl group to cytosine base can influence transcription by preventing transcription factor binding and/or by forming silent chromatin structures (50). Most CpG sites (*i.e.*, CpG dinucleotide with p, indicating the phosphate backbone that connects the cytosine and guanine nucleotides) in the human genome are methylated, except for clusters of CpGs, termed CpG islands that are normally free of methylation. In cancer cells, a switch from unmethylated to methylated CpG islands results in a loss of gene expression. CpG island hypermethylation occurs at the 5'end in the regulatory regions of genes (*i.e.*, the promoter and the first exon) and it may result in silencing of the corresponding genes (8). Jarrard et al. (49) reported that aberrant methylation of the 5'end of CpG islands correlated with the loss of AR expression in prostate cancer cells. Aberrant methylation of the androgen receptor was not detected in human normal prostate or primary prostate cancer tissue. However, the loss of AR expression can occur in earlier stage, androgen-dependent prostate tumors. Both enhanced AR heterogeneity and a greater percentage of AR protein-negative cancer cells correlated with a poorer response to hormone ablation treatment and survival (87). Hence, AR methylation may

represent a phenotype associated with the development of androgen-independence in a subset of prostate cancer that does not express the AR (54). This finding is of therapeutic interest because Jarrard et al. (49) demonstrated that *AR* gene methylation is reversible when prostate cancer cells are exposed to demethylating agents 5-azacytidine and 5'aza-2-deoxycytidine.

Hypermethylation of promoter CpG islands is frequently observed in breast cancer (119, 121). Yoshida et al. (123) found that methylation of the promoter regions of *estrogen receptor α* (*ERα*) gene directly suppressed transcription of this gene. Transcription of the *ERα* gene occurs from two different promoters (35), with the distal promoter B located 2 kb upstream of the proximal promoter A (123). It follows that the biological actions of estrogens are mediated by binding to two specific estrogen receptors (ERs), Erα or Erβ. Estrogens are known to bind Erβ with affinity similar to ERα, and both ER subtypes regulate gene expression in several ways; mainly via the classical pathway through direct DNA binding via estrogen response elements (EREs) and/or via the nonclassical pathway through protein-protein interaction with other transcription factors (73). Currently, only ERα has an established clinical role as it predicts the likely response of a patient to hormone treatment. A loss of ERα expression portends a poor prognosis as these cells can now grow independently of estrogen regulation and have gained resistance to endocrine inactivation therapy. However, a significant number of breast cancers lose expression of the *ERα* gene due to gene silencing by a combination of DNA methylation and/or histone deacetylation (85). Widschwendter et al. (118) showed that *ESR1* gene, encoding ERα, proved to be the best predictor of progesterone receptor status, whereas methylation of *PGR* gene, encoding progesterone receptor (PGR), was the best predictor of estrogen receptor status. It appears from this that *PGR* methylation indicates the absence of the estrogen receptor. However, the loss of progesterone receptor in ER-positive breast cancer may be a distinctive molecular event associated with activation of transcriptional factors such as epidermal growth factor receptors: EGFR (or ErbB-1) and ErbB-2 (or Her-2/neu) (33). There is probably some kind of cooperation between EGFR and ErbB-2 that triggers a kinase pathway resulting in the final outcome to the nuclear stimulation nuclear factor-κB that activates the transcription of tumor-linked genes (91, 104). The *Her-2/neu* gene is overexpressed in 20% - 30% of invasive breast carcinomas and is associated with increased metastatic potential (76). Her-2/neu positive-cancers show a lower level of PGR in the tumor (56), and a higher prevalency of DNA methylation for *PGR*, *HSD17B4* (coding for type 4 17-β-hydroxysteroid dehydrogenase) and *CDH13* (coding for H-cadherin) in Her-2/neu-positive cancers was confirmed in a set of primary breast cancers (25). Moreover, it is known that breast cancers in patients with *breast cancer susceptibility gene 1* (*BRCA1*) gene germ line mutations are more often negative for *Her-2/neu* (58). In concordance with this finding, Fiegl et al. (25) demonstrated sufficient levels of *BRCA1* methylation only in Her-2/neu-negative tumors.

Both DNA hypomethylation and DNA hypermethylation may promote cancer development. Widschwendter et al. (119) demonstrated inverse correlation between satellite DNA hypomethylation and hypermethylation of *RNR1* (coding for rRNA) and *CDH13*. Genomic hypomethylation can result in activation of tumor-promoting genes and chromosomal instability (44). On the other hand, promoter CpG island hypermethylation induces the transcriptional silencing of tumor suppressor genes and contributes to oncogenesis as well. One of the hallmarks of cancer is abnormal methylation patterns (7) with malignancies generally governed by widespread DNA hypomethylation of tumor-promoting genes along with site-specific DNA hypermethylation of tumor suppressor genes (7, 21). Whereas hypermethylation of tumor suppressor genes has great attention, very little attention has been paid to hypomethylation of tumor-promoting genes attracts considerable, which is often observed in late-stage cancers (99). The hypomethylation often involves satellite 2 (Sat2) DNA in the juxtacentromeric (centromere-adjacent) region of chromosome 1 and 16 (22). Importantly, extensive hypomethylation of Sat2 DNA in chromosome 1 has been a highly significant marker of poor prognosis and more informative than ovarian cancer grade or stage (119).

METHYLATION IS A REVERSIBLE PROCESS

Enzymes that mediate methylation have been shown to be important to changes of specific

chromatin states and to be responsible for transcription regulation (6). Histone methylation is catalyzed by histone methyltransferases (HMTs). HMTs can be grouped into two divergent families: histone lysine methyltransferases (HKMTs) and protein arginine methyltransferases (PRMTs).

The histone lysine methylation plays a role in regulating the state of chromatin compaction, and thus the establishment and maintenance of heterochromatin and euchromatin. In general, methylation at H3-K4, H3-K36 and H3-K79 is correlated with euchromatin and transcriptional activation, whereas methylation at H3-K9 and H3-K27, and at H4-K20 is associated with heterochromatin and transcriptional repression (60, 101). Lysine can be mono-, di- and tri-methylated. Tri-methylated H3-K4 is preferentially associated with the transcription start sites of active genes, whereas di-metylated H3-K4 is a mark for both active and inactive gene expression (94). The degree of methylation of histone lysine residues may vary according to the context in which it occurs or the specific enzyme that makes the modification (60). Lysine methylation has been viewed as a permanent histone mark until recently when the first histone lysine demethylase, LSD1 (lysine specific demethylase 1), was discovered. LSD1 specifically demethylates histone H3-K4 (98), and in complex with androgen receptors, demethylates the repressing mono- and di-methylated H3-K9 (77). Thus, depending on the specific interacting partners, LSD1 action results in either gene silencing or activation. The LSD1 demethylase is not part of a big family and does not have many obvious homologues. This is rather surprising as there are many methylated lysines in histones and LSD1 seems to be very specific for H3-K4 (6). Nevertheless, the androgen receptor as a specific transcription factor appears to alter the specificity of LSD1 from H3-K4 to H3-K9, and thereby converts the demethylase from a repressor to an activator of transcription (77).

The methylation of arginine residues has only been linked to active transcription because this modification is only found on chromatin when genes are actively transcribed (60). PRMTs can be divided on the basis of whether they catalyze symmetric or asymmetric di-methylation. Type I PRMT enzymes form mono-methylarginine and asymmetric di-methylarginine products. Type II PRMT enzymes catalyze the formation of mono-methylarginine and symmetric di-methylarginine (74). Seven mammalian PRMT genes have been identified: PRMT1, PRMT2, PRMT3, CARM1/PRMT4, JBP1/PRMT5, PRMT6 and PRMT7. PRMT5 is the only example of a type II enzyme, whereas the other PRMTs (except PRMT7) are all type I enzymes. PRMT7 makes only mono-methylarginine and contains two methyltransferase domains in a single polypeptide chain (79) and thus may represent a third class of PRMTs (60). Despite the high degree of conservation within the methyltransferase domain, there is relatively little overlap in the protein substrate specifities of the seven PRMTs (102). Deletion of the N-terminal region of PRMT3, which contains a zinc-finger motif, impairs its enzymatic activity and implies its role in protein substrate recognition (27). Cofactor-associated arginine methyltransferase 1 (CARM1) has been identified as an interacting protein for the p160 coactivator (11). Both the methyltransferase activity and the association with p160 coactivators are essential for CARM1 coactivator function with hormone-activated nuclear receptors (12, 61, 108). CARM1 methylates histone H3 *in vitro* (11) at H3-R2, H3-R17 and H3-R26 (95), while PRMT1 methylates histone H4 at H4-R3 both *in vitro* and *in vivo* (106, 115). PRMT1 also interacts with the p160 coactivators to enhance transcriptional activation by both hormone-dependent and orphan nuclear receptors (55, 115). CARM1 and PRMT1 have been found to cooperate synergistically. CARM1 (arginine methylation of histone H3) cooperates with PRMT1 (arginine methylation of histone H4) (55), and CARM1 cooperates with CBP, p300 and p/CAF (acetylation of histones H3 and H4) (61). It is possible that some of the histone modifications may occur prior to the others (60). Therefore, PRMT1-mediated methylation of H4-R3 facilitates subsequent acetylation of histone H4 by p300 (115). Similarly, CARM1 methylation at H3-R17 is not increased if transfected CBP is unable to acetylate histone H3 (16).

EFFECTORS MEDIATE READING OF THE HISTONE CODE

Histone modifications may affect transcription by recognition of sites for the recruitment of effectors. While histone acetyltransferases (HATs), histone deacetylases (HDACs) and histone methyltransferases (HMTs) serve to write the histone code, other mechanisms must exist for its reading (89).

Despite a wide variety of known histone marks, only a few protein domains have been identified that are able to recognize or read histone tail modifications. These include the bromodomain, which binds acetylated lysine residues of histone H4 (48), and the chromodomain, which targets methylated lysine residues of histone H3 (5). Furthermore, Wysocka et al. (120) described a plant homeodomain (PHD) finger as a highly specialized methyl-lysine-binding domain. Pena et al. (83) found that the PHD finger of tumor suppressor ING2 (inhibitor of growth 2) binds tri-methylated H3-K4 (*i.e.* lysine may be mono-, di- or trimethylated) and is one of a new family of effectors that target this epigenetic mark (96). Tri-methylated H3-K4 marks the transcription start sites of virtually all active genes (94), but the molecular effectors involved in specific recognition of tri-methylated H3-K4 remain poorly understood. The ING2 PHD finger seems to be more specific for tri-methylated H3-K4 than chromodomains (83), which show little preference for tri-methylated over di-methylated histone peptides (47).

Repressive proteins, such as heterochromatin protein 1 (HP1) or the Polycomb group (PcG) proteins, contain a chromodomain. The PcG proteins were first identified in *Drosophilla melanogaster*, but homologs have been identified in all higher organisms (82). Mutations in PcG proteins have been linked to cancers of the immune system and prostate (100); however, the mechanisms by which PcG proteins repress transcription are unknown. Methylation on H3-K9 provides binding sites for the chromodomain of HP1 (5), and on the other hand, the chromodomain helicase DNA-binding protein 1 (CHD1) uses its chromodomain to bind the activating methylated H3-K4 (86). Therefore, the function of the methyl group is a reflection of the type of protein it has evolved to recruit - either an activator or a repressor of transcription (6).

CREB-binding protein (CBP) and p300 are coactivators that function by interacting with transcription factors and/or modifying chromatin structure through their histone acetyltransferase activity. Comparison of the amino acid sequences of CBP and p300 from different species has revealed the presence of regions that are practically identical, including the bromodomain, three cysteine (C)-histidine (H)-rich regions (CH1, CH2 and CH3) and the HAT domain (3). The HAT domains are preceeded by the bromodomains (48), and based on the sequence homology, part of the CH2 region has been classified as a PHD type zinc finger (1). The PHD zinc finger has been found as an integral part of the HAT domain of CBP (52), but is dispensable for p300 HAT activity (10). Bromo- and chromo-domains often occur together with PHD zinc fingers (1), and the occurrence of PHD zinc fingers in proteins involved in chromatin function suggests that nucleosome binding may be a general function for PHD zinc fingers (89). In the p300 protein, the PHD zinc finger is situated next to the bromodomain. It has been reported that p300 can interact with chromatin *in vitro* and this interaction requires the bromodomain (69). Ragvin et al. (89) have showed that both the bromodomain and the PHD zinc finger contact the nucleosome while simultaneously interacting with each other. It is reasonable to assume that the bromodomain requires acetylated histone tails for binding, while the PHD zinc finger recognises another part of the nucleosome.

COACTIVATOR COMPLEX ASSEMBLY

Activation of transcription involves two general mechanisms: alteration of the chromatin structure around the promoter by ATP-dependent nucleosome remodeling and the recruitment of the RNA polymerase II transcription preinitiation complex (37, 110). Transcriptional activation is accomplished with the help of complexes of coactivator proteins that bind to the activation domains of nuclear receptors (110). Stallcup et al. (103) hypothesizes that the nuclear receptors lie upstream and RNA polymerase II downstream of these domains.

The nuclear receptors (NRs) are a family of transcriptional activators that play important roles in the regulation of cell growth and diferentiation by providing a link between signalling molecules and the transcriptional response (68, 112). These receptors are grouped into a large superfamily which includes receptors for steroid hormones (*e.g.* estrogens, glucocorticoids, progesterone, mineralocorticoids, androgens, vitamin D, *etc.*), retinoic acids, thyroid hormones, fatty acids, leukotrienes and prostaglandins (59). In addition, a large number of NRs have also been identified by homology, but have no identified natural ligand, and are referred to as nuclear orphan receptors.

Coactivators associate with NRs in a ligand-dependent manner are essential for ligand-

induced NR activation (31). Among the most well characterized of the NR coactivators is a family of three related 160 kDa protein complexes called the p160 coactivators (1. steroid receptor coactivator-1 (SRC-1), 2. glucocorticoid receptor-interacting protein 1 (GRIP1)/transcriptional intermediary protein 2 (TIF2), and 3. activator of thyroid and retinoic acid receptors (ACTR) amplified in breast cancer 1 (AIB1)/ receptor-associated coactivator 3 (RAC3)/thyroid receptor activator molecule 1 (TRAM1). As depicted in Fig. 1, the p160 coactivators (GRIP1/TIF2) bind directly to hormone activated NR and recruit secondary coactivators, including histone acetyltransferases such as CBP, p300 and p300/CBP-associated factor (p/CAF), as well as CARM1, PRMT1 and other proteins in the transcription complex (42, 55). Two separate domains of p160 coactivators can bind to the activation functions of the NRs. In the central region of their polypeptide chains, the p160 coactivators contain three NR box LXXLL motifs (L - Leu; X - any amino acid) that interact directly with the AF-2 domains of NRs (20, 109). The C-terminal region of the p160 coactivators can also interact with the AF-1 domain of some of the nuclear receptors (67, 81, 117).

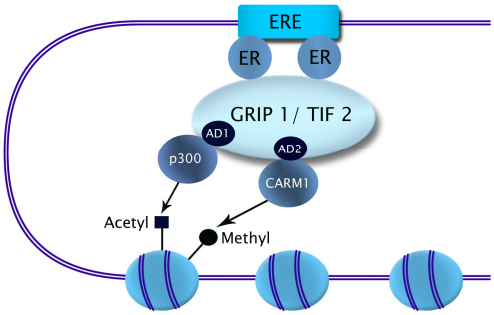

Figure 1. Hypothetical model for transcriptional activation by estrogen receptor (ER). ER is modified by ERE (Estrogen response elements) and by three coactivators, GRIP1, p300 and CARM1 (modified from Chen et al., 2000).

Upon ligand-binding, androgen receptor (AR) bind DNA elements through a highly conserved DNA-binding domain (DBD) and mediate transcription through transactivating domains, referred to as activation function-1 (AF-1) and 2 (AF-2), localized respectively in the N-terminal region and in the ligand-binding domain (LBD) within the C-terminal region (Fig. 2) (68, 112). The AR is thought to be quite unique among the NRs, because its AF2 activity is not detected in a variety of mammalian cell lines (9, 80) despite its homology to other nuclear receptors. A majority of recurrent prostate cancers express high levels of AR and two nuclear receptor coactivators, SRC1 and TIF2 (36). However, He et al. (40) demonstrated that weak interactions between the LBD of the AR and the SRC1 and TIF2 coactivators correspond with weak AF-2 activity. Consequently, the AF-2 surface in the LBD can more likely function as a strong interaction site for the N-terminal region that is required for AR activity *in vivo* (40). Some patients with androgen insensitivity syndrome (AIS) lack a coactivator interacting with the AF-1 region of the AR (2). The overall transcriptional activation by the NRs is mediated by the combined effects of AF-1 and AF-2 activating domains; the relative importance of which depends on the specificity of the NR, cell type and target gene promoter (12). The contribution of activation domains to transcriptional activation lies in the catalysis of an enzymatic reaction (*e.g.* acetylation or methylation) or in promotion of a protein-protein interaction (103).

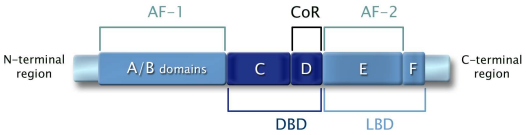

Figure 2. Structural organization and functional domains (A/B, C, D, E, F) of nuclear receptors (NRs). The N-terminal A/B region harbors transcriptional activation function (AF-1). The highly conserved domain C harbors the DNA-binding domain (DBD), in which several sequence elements (termed P-, D-, T- and A-boxes) have been characterized that contribute to response element specificity, a dimerization and interacts with DNA backbone and residues flanking the DNA core recognition sequence. The D region appears to correspond to a „hinge“ between the C and E domains and contains corepressor-binding site (CoR). This domain is less conserved than the surrounding regions C and E. The hallmark of NR is its ligand-binding domain (LBD) in the E region. The LBD harbors the ligand-dependent activation function AF-2, a major dimerization interface and often a repression function. Some receptors possess at the C-terminal region of the LBD a region F, which displays little evolutionary conservation. The F region might play a role in coactivator recruitment to the E domain and in determining the specificity of the ligand-binding coactivator interface (modified from Freedman, 1999; Laudet and Gronemeyer, 2002).

After being recruited to the promoter by NRs, the p160 coactivator transmits an activating signal via two AD1 and AD2 C-terminal activation domains. AD1 located approximately between amino acids 1040 and 1120 in the GRIP1/TIF2 coactivator, binds CBP and p300 (12). However, CBP and p300 can bind directly to estrogen receptors (ER) and other NRs (53).

Deletion of AD1 has been shown to eliminate CBP and p300 binding and to cause a substantional reduction in the coactivator activity of p160 coactivators (67, 114). Chen et al. (12) established conditions under which wild type GRIP1 and p300 acted synergistically as coactivators for ERs and they found little if any enhancement of NR function by p300 alone. The synergy between p300 and CARM1 indicates that they activate or recruit different downstream targets. CARM1 bound to AD2 in the C-terminal region (between amino acids 1122 – 1462) of GRIP1 (67) and enhanced transcriptional activation by NRs when it was coexpresed with a p160 coactivator (11). Deletion of the AD1 region from GRIP1 eliminated the ability of p300 to enhance ER function but had no effect on the ability of CARM1 to cooperate with GRIP1 in enhancing ER function (12). The action of AD2 is independent of AD1 and requires neither CBP nor p300 (67). Moreover, GRIP and other p160 coactivators bind directly to NRs and act as primary coactivators, whereas p300 and CARM1 are recruited indirectly to the NR and thus act as secondary coactivators (11, 55). PRMT1 is also a coactivator for NRs and functions as a secondary NR coactivator in a manner similar to CARM1 (55).

The strong coactivator synergy among CARM1, p300 and GRIP1 in NR-mediated transcription requires the methyltransferase activity of CARM1 (61). Both CARM1 and PRMT1 can methylate histones *in vitro* (11, 64) and their coactivator potential is dependent upon an intact metyltransferase domain (11, 55, 61, 115). Histone methylation might be one mechanism of CARM1 coactivator function (11) but on the other hand, histone methylation can cooperate with acetylation by coactivators to remodel nucleosomes (105).

ACETYLATION AND DEACETYLATION AS A MECHANISM OF REGULATION OF COACTIVATORS AND COREPRESSORS

The histone acetyltransferases (HATs) and histone deacetylases (HDACs) regulate acetylation and deacetylation of the conserved lysine residues present in the amino terminal tails of all four core histones. In general transcriptionally active chromatin (euchromatin) is associated with hyperacetylated histones, whereas heterochromatin is associated with hypoacetylated histones (31, 105). The discovery that transcriptional coactivators have histone acetylation activity, while repressors possess histone deacetylation activity, strongly links histone acetylation to transcriptional activation, and deacetylation to repression.

Regulation of gene expression by NRs involves interactions with HAT and HDAC complexes. Transcriptional activity of androgen and other steroid hormone receptors is mediated through a large number of interacting proteins which function as coactivators and corepressors (4, 15). In contrast to NR coactivators, NR corepressors regulate the transcriptional activity of nuclear receptors by interacting with unliganded NRs (Fig. 3). The androgen receptor (AR) is a classical NR that binds androgen ligand. Coactivators of the AR, proteins with acetyltransferase activity, interact with one or more domains of the AR, thus leading to remodelling of the chromatin structure through the acetylation of histones (15). Deletional mutagenesis of the AR indicates that whereas the AF-2 domain in the C-terminal region is dispensable, the AF-1 domain in the N-terminal region is required for augmentation of AR action by the CBP coactivator (57). Comuzzi et al. (14) demonstrated inhibition of CBP at both mRNA and protein levels, in the LNCaP cell line, by either androgen or interleukin-6 (IL-6). Progression from androgen-dependence to androgen-independence is a critical step in prostate cancer development. IL-6 has been found to regulate growth of prostate cancer and to transactivate AR-dependent gene expression in the absence of androgens (43). Similarly, SRC-1 is involved in ligand-independent activation of the AR by IL-6 (113). The coactivator p300 has been shown to interact with the AR during its androgen-dependent activation. Debes et al., (17) demonstrated that, after silencing p300, there was no induction of AR activity by IL-6. These findings indicate that IL-6 may be involved in the androgen-independent progression of prostate cancer, but the pathway by which IL-6 induces AR gene expresion remains undefined. Nuclear localization of CBP observed by Comuzzi et al. (14) is concordant with the observation that Tat interactive protein (60 kDa - Tip60), an AR coactivator (38), was recruited in the absence of androgen to the promoter of the PSA (*prostate-specific antigen*) gene in the androgen-independent LNCaP cell line. Increased expression of coactivators in prostate cancer, enhancing expression of AR target genes in late

tumor stages, may be a novel mechanism relevant to prostate cancer progression.

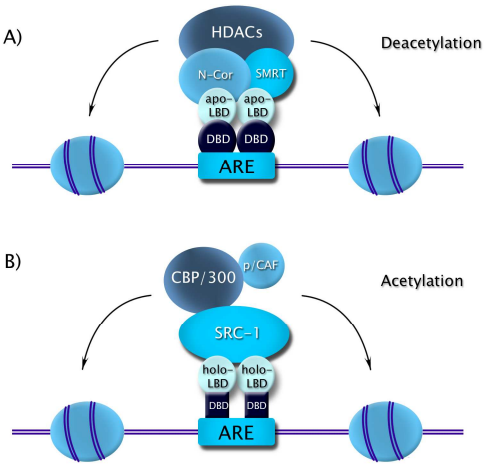

Figure 3. AR corepressors and coactivators. A) In ligand absence, the AR forms complexes with corepressors including SMRT, N-Cor, HDACs and exerts histone deacetylase (HDAC) activity. Unliganded receptors are referred to as apo-receptor forms. Corepressor binding occurs on the surface of the non-liganded LBD and is similar to coactivator binding which is mediated by CoRNR box. B) The liganded receptor corresponds to the holo-receptor form. Ligand-binding dissociates this complex and recruits coactivator complex such as p300, p/CAF and SRC1 that displays histone acetyltransferase (HAT) activity. Finaly, activation function (AF-1) linked to DBD can activate transcription (modified from Laudet and Gronemeyer, 2002).

The overexpression of some AR coactivators is associated with prostate cancer progression (4, 13, 18). Besides CBP and Tip60, high levels of SRC-1 and TIF2 in prostate cancer, during tumor relapse, may also contribute to hyper-responsiveness of the androgen-signalling pathway (14). The upregulation of SRC-1 and TIF2 was documented in patients with recurrent carcinoma of the prostate (36), and the expression of RAC3 in prostate carcinoma specimens correlates with tumor grade and stage, as well as with disease-specific survival (34). CBP and p300 have a tumor suppressor-like activity, which is naturally altered in a variety of human cancers. Debes et al. (18) showed that p300 is involved in prostate cancer proliferation and is a predictor of aggressive features of prostate cancer. Furthermore, transfection of p300 into prostate cancer cells grown in culture induces quantifiable nuclear alterations and these alterations correlate with more aggressive prostate cancer (19).

The fact that expression of AR coactivators in prostate cancer tissue correlates with AR expression, therefore suggests that acetylation of the AR may contribute to the development of androgen-independent prostate cancer (31). In living cells, acetylation of the AR is induced by ligand-binding and is also facilitated by histone deacetylase inhibitors (HDACIs) (30). HDACIs are potential therapeutic agents that inhibit tumor cell growth and survival. In some cases, growth arrest is induced at low doses, and apoptosis is induced at higher doses; in other cases growth arrest precedes apoptosis (93, 116). Suberoylanilide hydroxamic acid (vorinostat) inhibited growth of androgen-dependent LNCaP prostate cancer cell line at low micromolar concentrations and induced caspase-dependent apoptosis associated with chromatin condensation (70). Frønsdal and Saatcioglu (28) assessed the ability of three different HDACIs, trichostatin A (TSA), sodium butyrate (NaB) and FR901228 (depsipeptide) to induce cell death in androgen-dependent LNCaP cells or androgen-independent DU145 and PC-3 prostate cancer cell lines. They found that all three HDACIs mediated cell death in LNCaP and DU145 cells, but not in PC-3 cells, and that the extent of death varied with different HDAC inhibitors. Furthermore, trichostatin A induced cell death in androgen-dependent cell lines (CWR22R, LNCaP) to higher extent compared with androgen-independent cell lines (DU145, PC3), but TSA-induced AR suppression and cell death were caspase -independent (90). This implies, there is no definitive evidence that distinct HDACs have a defined role in cancer. On the other hand, HDACIs would constitute a useful experimental tool to address the issue of assigning distinct biological functions to individual HDACs. Given the known function of histone acetylation in transcription, it seems logical to postulate that inhibition of HDACs alone is unlikely to lead to generalized increase in the transcription of all known genes. In fact, acetylation works together with other posttranslational modifications, and blocking deacetylation might have different outcomes depending on the previous chromatin state (78).

The DNA-binding domain of the AR consists of two zinc finger clusters, the first of which is involved in specific recognition of androgen-responsive elements (AREs) in target genes (41). The AR is acetylated at a lysine-rich motif KXKK (K – Lys; X - any amino acid) which is conserved between species (29). The AR acetylation sites are located in the hinge region of the C-terminal region in close proximity to the second zinc finger of the DNA-binding domain

of AR and might serve as a site for coactivator or corepressor binding (30). The corepressor of ARs, testicular zinc finger protein (TZF) (46) has a zinc-finger motif in its C-terminal region (45). TZF interacts with the AF-1 sequence of ARs and transient over-expression of TZF in LNCaP cells results in decreased AR activity in a ligand-dependent fashion. Moreover, nuclear receptor corepressor (N-Cor) additively decreases the transcriptional activity of AR with TZF (46). N-Cor and its homolog silencing mediator of retinoid and thyroid hormone receptor (SMRT) are well-characterized corepressors interacting with nuclear receptors in the absence of ligand or in the presence of antagonists, and confering the transcriptional repression (41, 51, 88). A central portion of TZF (amino acids 512 – 663) does not contain CoRNR motif, which has been found in the corepressors N-Cor and SMRT (107). It is suggested, that TZF and N-Cor might act through different pathways. Corepressor complexes containing N-Cor and SMRT are considered to recruit HDAC and then repress transcription (51). The HDAC inhibitor TSA prevented the transcriptional repression by TZF, suggesting that recruitment of HDAC2 into the AR/TZF complex is the important mechanism of repression (107). However, competition for agonist-bound AR between corepressors and coactivators is a possible mechanism of repression (63). Increased levels of TZF dissociated TIF2 coactivator from ARs and *vice versa*. Thus the AR-mediated transactivation depends on the TZF/TIF2 ratio (107). Furthermore, inhibition of N-Cor and SMRT enhanced the recruitment of the coactivators SRC-1 and p300 by agonist-bound AR and led to increase hyperacetylation of histone H3 and H4, suggesting that the corepressors compete with coactivators for binding to agonist bound AR (122).

CONCLUSION

At present, a substantial number of methylated or acetylated proteins and enzymes that make histone modifications have been identified. Covalent modifications of histones play a major role as carriers of epigenetic information. Histone acetylation together with methylation can modulate the activity of many genes by modifying both core histones and non-histone substrates. The androgen and estrogen receptor, members of the nuclear receptor superfamily, are acetylated by histone acetyltransferase at a motif that is conserved between species and other nuclerar receptors. Residues within the acetylated motifs of these steroid hormone receptors are mutated in prostate cancer as well as in breast cancer tissue. Acetylation of the receptors regulates cellular coregulatory proteins (coactivators and/or corepressors) and can enhanced growth properties correlating with altered promoter specifity tor target genes. The fact that expression of coactivators of androgen receptor in prostate cancer tissue correlates with expression of androgen receptor, therefore suggests that inhibition of the acetylation process and coactivator-binding of androgen receptor may be a promising approach for the treatment of prostate cancer. Consequently, the early epigenetic changes occuring in many tumors may determine the subsequent genetic changes that lead to tumor development and progression. By understanding and identifying the mechanisms controling a basic epigenetic mechanism, we may be able to influence the process of carcinogenesis and the development of new treatment strategies.

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