February 2007

The functional significance of nuclear receptor acetylation.

Vladimir M. Popov
Thomas Jefferson University

Chenguang Wang
Thomas Jefferson University

L . Andrew Shirley
Thomas Jefferson University

Anne Rosenberg
Thomas Jefferson University

Shengwen Li
Thomas Jefferson University

See next page for additional authors

Authors
Vladimir M. Popov, Chenguang Wang, L . Andrew Shirley, Anne Rosenberg, Shengwen Li, Marja Nevalainen, Maofu Fu, and Richard G. Pestell

The functional significance of nuclear receptor acetylation

Vladimir M. Popov[a], Chenguang Wang[a], L. Andrew Shirley[c], Anne Rosenberg[c], Shengwen Li[a], Marja Nevalainen[a], Maofu Fu[a], Richard G. Pestell[a,b,*]

[a] Department of Cancer Biology, Kimmel Cancer Center, Thomas Jefferson University, 233 S. 10th Street, Philadelphia, PA 19107, United States [b] Department of Medical Oncology, Kimmel Cancer Center, Thomas Jefferson University, 233 S. 10th Street, Philadelphia, PA 19107, United States [c] Department of Surgery, Kimmel Cancer Center, Thomas Jefferson University, 233 S. 10th Street, Philadelphia, PA 19107, United States

Corresponding author at: Department of Cancer Biology, Thomas Jefferson University, 233 S. 10th Street, Philadelphia, PA 19107, United States. E-mail address: Dawn.Scardino@mail.jci.tju.edu (R.G. Pestell).

Abstract

The endocrine signaling governing nuclear receptor (NR) function has been known for several decades to play a crucial role in the onset and progression of several tumor types. Notably among these are the estrogen receptor (ER) in breast cancer and androgen receptor (AR) in prostate cancer. Other nuclear receptors may be involved in cancer progression including the peroxisome-proliferator activating receptor gamma (PPARγ), which has been implicated in breast, thyroid, and colon cancers. These NR are phylogenetically conserved modular transcriptional regulators, which like histones, undergo post-translational modification by acetylation, phosphorylation and ubiquitination. Importantly, the transcriptional activity of the receptors is governed by the coactivator p300, the activity of which is thought to be rate-limiting in the activity of these receptors. Histone acetyltransferases (HATs) and histone deacetylases (HDACs), modify histones by adding or removing an acetyl group from the ε amino group of lysines within an evolutionarily conserved lysine motif. Histone acetylation results in changes in chromatin structure in response to specific signals. These enzymes can also directly catalyze the NRs themselves, thus modifying signals at the receptor level. The post-translational modification of NR which is regulated by hormones, alters the NR function toward a growth promoting receptor. The deacetylation of NR is mediated by TSA-sensitive and NAD-dependent deacetylases. The regulation of NR by NAD-dependent enzymes provides a direct link between intracellular metabolism and hormone signaling.

Keywords: Acetylation, Breast cancer, Prostate cancer, SIRT1

1. Epigenetics

Histone modification promotes changes in chromatin structure that may effect genetic expression in a heritable manner without directly altering the genome. The term epigenetics was coined by Conrad Waddington in the 1940s. Originally, epigenetics referred to the study of the way in which genes and their products bring a phenotype into being [1]. The Waddington equation originally referred to an equation; that epigenesis + genetics = epigenetics. In this context, Waddington referred to a neoclassical embryology debate on epigenesis versus

preformationism. Current thinking has limited the notion of epigenetics to modifications in gene expression that do not involve changes in DNA nuclear sequences. The study of heritable changes in gene expression that occur without a change in DNA sequence have identified families of enzymes that modify DNA, histones and other proteins which indirectly impact gene expression. Other examples of epigenetic regulation in mammalian genomes such as X chromosome inactivation, imprinting, and aberrant methylation in neoplasia have been recently reviewed elsewhere [2,3]. The last decade has witnessed the cloning and functional characterization of many of the genes known to govern the post-translational modification of histones and DNA that govern epigenetic changes. Histone modification by acetylation, methylation, phosphorylation and sumoylation occurs in an integrated manner. Furthermore these enzymes have been shown to regulate the activity of non-histone proteins, including structural proteins, transcription factors and transport proteins. Histone acetylases which function to regulate gene expression independently of DNA sequence modulate the activity of diverse proteins including the nuclear receptors. The biological significance of histone acetylation and their function in nuclear receptor signaling will be reviewed in the context of human cancer and endocrine signaling.

1.1. *Histone acetylation and deacetylation*

The packaging of euchromatic DNA into nucleosomes involves an octamer of four core histones (H2A, H2B, H3 and H4). Posttranslational modification of these histones within the nucleosome profoundly influences the architecture of chromatin. Histone acetylation occurs in a steady state equilibrium determined by the relative activity of acetylases and deacetylases at a given lysine residue. Histone acetylation occurs at the ε amino group of evolutionarily conserved lysine residues. The histone acetyl transferases target specific lysine motifs and are broadly divided into the nuclear, or Type A, and the cytoplasmic, or Type B (recently reviewed in [3]).

Deacetylation is mediated by the classical or TSA-sensitive (Types I and II) HDACs and the Type III HDACs or Sirtuins. Class I HDACS [1–3,8] are related to the S*accharomyce*s *cerevisia*e transcriptional repressor RPD3 and Class II HDACs [4–7,9,20] share homology with HDA1. Class I HDACs are primarily nuclear and class II HDACs are dynamically shuttled between the nucleus and cytoplasm. Broadly speaking acetylation of histones is associated with increased gene expression and reduced compaction of local chromatin. HDACs are recruited to DNA in association with co-repressors, via proteins such as methylated CpG-binding proteins, methyl CpG-binding domain containing proteins and *via* DNA methyl transferases.

The catalytic domain of an HDAC is formed by a stretch of approximately 390 amino acids. Deacetylation involves a charge relay system and several essential cofactors to remove acetyl groups from the lysine residues. These essential cofactors include Zn^{+2}. HDACs form multiprotein complexes, many of which contain nuclear receptor binding proteins, mSin3A, N-CoR and SMRT [4].

The NAD-dependent HDACs are members of the conserved SIRT family. SIRT1 is a human homolog of the yeast HDAC *s*ilent *i*nformation *r*egulator 2 (Sir2). This sirtuin family is conserved from *archaebacteri*a to eukaryotes [5,6]. SIRT1 is one of seven human SIRT [6].

SIRT1 couples the removal of the acetyl group from its protein substrate with the cleavage of a high-energy bond in NAD, thus synthesizing the novel products 2′- and 3-*O*-acetyl-ADP-ribose [7]. These products are thought to function as secondary messengers. The SIRT1 enzyme is in turn inhibited by nicotinamade which, as a metabolic product serves to coordinate intracellular metabolism with the activity of SIRT1. In *C*. *elegan*s and in mice the Sirtuins regulate the Insulin/IGF1 signaling axis. In *Saccharomyces cerevisiae*, SIR2 extends the replicative lifespan [8].In *C*. *Elegan*s increased dosage of the worm *SIR2*-1 gene extends the lifespan of mother cells after caloric restriction [9,10]. In recent studies, deletion of the *SIRT*6 gene resulted in mice with lordokyphosis, defective base-excision repair, severe metabolic defects with reduced circulating IGF1 and serum glucose, and premature death [11]. The phenotype of these mice, interpreted as an acute degenerative aging like phenotype is considered evidence for a role of mammalian SIRT6 in aging [12].

SIRT1 is thought to deacetylate histones, DNA Polß, the coactivator p300, and several downstream transcription factors [11,13]. It has been suggested that the deacetylation of Polß by SIRT6 contributes to the defect in base-excision repair of *SIRT6*$^{-/-}$ Mice. The deacetylation of p300 has been linked to the ability of SIRT1 to regulate several transcription factors [13]. Given the importance of p300 in NR function, it is important to consider the role of SIRT1 in governing p300 function.

The coactivators p300 and its related ortholog CBP are transcriptional integrators regulating NR function. The relative abundance of p300 is considered rate-limiting in diverse signaling pathways involved in metabolism and cellular differentiation [14]. The coordination of these activities involves a scaffold function of the protein to tether transcription factors to the basal transcription apparatus, and both an intrinsic and associated histone acetyltransferase activity which modifies local chromatin to thereby alter access of transcription factors to their cognate DNA binding site. In addition p300 directly acetylates transcription factors and thereby modifies their function. The modular structure of p300 facilitates these diverse functions through distinct domains. The bromo domain regulates protein-protein interactions and facilitates association with chromatin. Three cysteine histidine rich domains (CH) serve as docking modules for transcription factors and the glutamine rich carboxyl terminus interacts with the NR coactivators, including the steroid receptor coactivators. Located between the amino acids 1004 and 1044 in p300 is a domain referred to as the CRD (cell cycle regulatory domain). This domain was so named based on evidence that that his domain was a target of p21^{CIP1} activity. Recent studies however identified the CRD1 domain as the key site of p300 sumoylation [15]. As several transcription factors, known to be repressed by SIRT1 were also known to be activated by p300, Bouras et al. examined the role of SIRT1 in regulating p300 activity [13]. SIRT1 was shown to repress p300 in an NAD-deacetylase dependent manner. SIRT1 repression of p300 involved the CRD1 domain. Two lysine residues were identified within the p300 CRD1 domain that were required for SIRT1 repression. p300 was shown to function as a substrate for SIRT1-mediated deacetylation *in vitro* and by proteomic analysis. The lysine residues shown to function as substrates within p300 for SIRT1-mediated deacetylation also functioned as acceptor sites for SUMO [13]. The SUMO specific protease SSP3 antagonized SIRT1-mediated repression of p300. Because p300 is a limiting coactivator for many transcription factors, it is likely that the deacetylation and repression of p300 by SIRT1 may integrate metabolic signals within the cell to in turn regulate the diverse metabolic process regulated by transcription factors and nuclear receptors.

Several transcription factors have been identified as targets of SIRT1. Lysine residues of p53 [16,17], MyoD, FOXO [18–20], and TAF168 [21] are all targets of hSIRT1. The effect of Sirt1 on cellular proliferation and growth appears to be cell type specific. Sir2-dependent deacetylation of p53 repressed its function, thus promoting cell growth [16,17,22]. In contrast, SIRT1 profoundly inhibits prostate cellular proliferation, and does so in a manner dependent upon the presence of the androgen receptor [23]. SIRT1 has been found to specifically interact with the AR and PPARγ . Indirect evidence also suggests the ERα may be regulated by endogenous SIRT activity.

2. Nuclear receptors

*2.1. Estroge*n *recepto*r

Estrogen and progesterone receptors (ER and PR) have now been studied in clinical breast cancer for more than 20 years [24]. Estrogen receptors are intracellular proteins that can bind with estrogens, phytoestrogens, or xenoestrogens. States of increased exposure to serum levels of estradiol [25], including early menarche, late menopause, and late or no pregnancies, which increase activation of the estrogen receptor in breast tissue, are risk factors for the development of breast cancer. This increased activation of the estrogen receptor leads to increased cell proliferation and is thought to lead to the progression of abnormal growth of breast tissue.

The estrogen receptor (ER) exists in two isoforms: ERα and ERß. Despite their considerable homology, these two receptor isoforms have important structural and functional differences that are important for tissue and promoter-specific regulation of gene expression, and likely contribute to hormonal sensitivity and resistance. The ER is a modular transcription factor with functional domains that are conserved between the receptor superfamily members. Posttranslational modification by phosphorylation, acetylation and ubiquitination modulate ERα activity and subcellular localization in response to hormonal and growth factor signals.

Currently, only ERα has an established clinical role as it predicts the likely response of a patient to hormone treatment, both in the adjuvant and metastatic breast cancer setting. Patients with breast cancers expressing ERα are approximately seven to eight times more likely to benefit from endocrine therapy than ERα -negative patients. For the initial three to five years after primary diagnosis, ERα-positive patients generally have a better outcome than ERα-negative patients, even if mutant for the BRAC1 gene [26]. Overall, however, the prognostic value of ERα is relatively weak and only of limited value in the clinically important subgroup of patients with lymph node-negative disease. Further work is required to establish if ERß has a clinical role in breast cancer. Additionally, the probability of objective response to the endocrine therapy increases with an increase in the quantity of estrogen receptor in the cancer [24].

Selective estrogen receptor modulators (SERMs) take advantage of the ER activity of breast cancers. Currently available agents include, Tamoxifen (Nolvadex), Raloxifene (Evista) and Toremifene (Fareston). For the past twenty years, Tamoxifen has been used to treat ER positive tumors in the adjuvant and metastatic setting. Results of the Study of Tamoxifen and Ralozifene (STAR) trial, will likely see the introduction of Raloxifene into this algorithm. Toremifene is not currently available in the United States. The data currently available suggest that receptor assays carried out on the primary tumor can be used for prediction of subsequent response to the endocrine therapy, even at a later time of recurrent disease. It is well accepted that ERα expression level in breast cancer patients not only correlates with higher response to hormone therapy but also a better prognosis. Positive receptor status is associated with favorable prognostic features including a lower rate of cell proliferation and histologic evidence of tumor differentiation. Finally, prognosis can be predicted from the presence or absence of estrogen receptor expression. While sequential assays of receptors in lesions from the same patients are likely to be in agreement, when changes occur they tend to be reductions in amount of receptor or loss of receptor during disease progression. A loss of ERα expression portends a poor prognosis as these cells can now grow independently of estrogen regulation and have gained resistant to endocrine inactivation therapy [27]. ERα expression status is an important biomarker that helps physicians individualize systemic therapy. However, a significant number of breast cancers lose expression of the ER□ gene due to gene silencing by a combination of DNA methylation and/or histone deacetylation.

Recently, several studies have focused on the posttranslational modification of ERα. It has been shown that ERα is directly acetylated by the coactivator p300 at the well-conserved lysine residues in the hinge/ligand domain [28]. The lysine motif was well conserved between nuclear receptors. In subsequent studies the lysine motif in other nuclear receptors has been shown to function as a target of histone acetylases as predicted in this original study. The histone acetyl transferase P/CAF bound to the ERα, and binding required the HAT domain [28]. However P/CAF failed to acetylate the ERα while readily acetylating histone. This finding illustrated the specificity of interaction between HATs and their NR substrates. A comparison of ERα and histone in careful dose-response curves demonstrated that the ERα peptide served as an excellent substrate for p300 HAT activity, not dissimilar to histones. Proteomic analysis, including Edman degradation assays, identified lysine 302 and 303 as the preferential sites for acetylation by p300, with an additional site at lysine 299.

When lysine residues 302 or 303 were mutated to arginine or glutamine, the ERα had increased estradiol-dependent activation, suggesting a role for ERα acetylation in ligand sensitivity [28] (Fig. 2A). Independent clinical studies, identified the lysine residue as frequently a site for mutation [29].In 34% of atypical breast hyperplasia samples, a Lys-to-Arg substitution was found at residue 303 (K303R) of the ERα [29]. Expression of this mutant led to a hypersensitivity to estradiol, showing maximal stimulation at physiologic levels. In the presence of the p160 coactivator and p300 the ERα was shown to be acetylated at lysines 266 and 268 [30]. In previous studies, an ERα fragment from aa 1-282 was not acetylated by p300 alone, suggesting an important independent role for SRC1 in acetylation of aa 266 and 268. The finding that the ERα is acetylated at multiple different sites by distinct complexes is not surprising and is consistent with analysis of other transcription factors such as p53 [31]. p300/CBP acetylates carboxyl-terminal lysine residues of p53 (lysines 372, 373 and 382). DNA damage induces acetylation of lysine 320 and lysine 373 with distinguishable kinetics and these two residues regulate distinct clusters of genes. The high affinity p53 binding site

promotes cell survival [31] and K373 regulates interactions with DNA binding sites of proapoptotic genes leading to cell death. Original studies had shown, using anti-acetyl lysine antibody-mediated immune precipitation, that the ERα is acetylated in human breast cancer cell lines [28]. It will be of interest to determine, using proteomic analysis, which additional residues of the ERα are acetylated *in vivo*.

Several previous studies have demonstrated the role of TSA-dependent HDACs in regulating ERα activity. Recent studies of NR have shown a role for NAD-dependent histone deacetylases in regulating AR and ERα function. The studies of AR in cultured cells were direct and used expression vectors encoding wild type or catalytically defective SIRT1 [23]. The SIRT1 activator, resveratrol, led to the inhibition of estrogen-dependent cell proliferation [32] providing indirect support for a role of SIRT in regulating ERα activity. *In vitro* experiments showed that the addition of recombinant SIRT1, along with nicotinamide adenine dinucleotide (NAD^+), with ERα, led to a strong deacetylation of the receptor [30]. However, the addition of nicotinamide, a known SIRT1 inhibitor, prevented the deacetylation. These lines of study imply that SIRT1, by deacetylating ERα, could become a novel therapeutic target for breast cancer, as SIRT1 activation could lead to modifications of estrogen signaling that would be beneficial to decreasing the proliferation of abnormal breast tissue.

2.2. Androgen receptor

2.2.1. Prostate cancer and androgen receptor

Prostate cancer is the most frequently diagnosed cancer in men in the United States and the second leading cause of male cancer deaths [33,34]. The androgen receptor's function is a critical determinant of human prostate cancer pathogenesis and progression. The growth of prostate cancer is androgen-sensitive and these growth signals initially go through the AR. Current androgen ablation therapy (AAT) remains a major therapeutic intervention in metastatic disease, resulting in 60–80% initial response rates. However, prostate cancer unresponsive to androgen ablation therapy subsequently emerges in the majority of patients resulting in short survival time. AR mutations are frequently associated with AAT resistant tumors. The AR is involved in both differentiation and proliferation as specific mutations in the AR can selectively affect either differentiation (AR insensitivity) or proliferation.

2.2.2. Androgen receptor hyperactivity, acetylation and deacetylation

Therapies directed at the AR initially work in a majority of patients with prostate cancer, but many develop resistance over time. Hyperactivity of AR expression or function is seen in >20–30% of prostate cancers. Activated growth factor signaling, loss of tumor suppressors, altered coactivator expression and increased AR expression may contribute to prostate tumorigenesis in different patients. Posttranslational modification of the AR contributes to enhanced activation of the AR. The AR is acetylated in response to physiological stimuli including DHT and bombesin [35,36] (Fig. 2B). Modification by acetylation enhances AR transactivation function at a subset of target promoters [37], in particular those cell cycle control genes that induce cellular proliferation.

The AR is acetylated at a conserved lysine motif. Edman degradation and sequence analysis identified the individual residues preferentially acetylated as lysine 630 and 632 with additional acetylation at lysine 633. Gain of function mutations of the acetylated lysine residues within the

AR, either glutamine or threonine substitutions, enhance DHT-dependent gene transcription at androgen-responsive gene promoters [35,38,39]. Conversely small polar substitutions (arginine or alanine mutations), reduced ligand-dependent transactivation. Gain-of-function mutants demonstrated enhanced cellular growth properties in prostate cancer cells, enhanced cellular proliferation and colony size in soft agar [38,39] (Fig. 1). Prostate cancer cells expressing acetylation mimic mutants were resistant to their androgen antagonist flutamide [38]. When implanted in nude mice human prostate cancer cells expressing either wild type AR or single residue point substitutions that function as acetylation mimic mutants demonstrated a dramatic enhancement of tumor growth *in vivo* [38]. Collectively these studies demonstrated for the first time that acetylation of a transcription factor, like phosphorylation, directly regulated cellular growth.

Analysis of the mechanisms by which AR regulated cellular growth showed reduced apoptosis in prostate cancer cells expressing AR acetylation mimic mutants [38]. It is known that AR mediated apoptosis involves a JNK-mediated pathway and that AR acetylation mimic mutants evade JNK-mediated apoptosis [38]. In addition AR acetylation mimic mutant expression was associated with enhanced cellular proliferation and Ki67 staining in cells. The AR acetylation site was shown to regulate transactivation but did not affect several other functions of the AR including transrepression, sumoylation or protein stability (Fig. 2). When expressed at physiological levels, the AR acetylation site did not affect protein abundance or subcellular distribution in prostate cancer cells. Superphysiological expression of the AR acetylation dead mutants in Hela cells appeared to increase the rate of nuclear translocation in response to androgen, increase the nuclear exclusion in the absence of ligand, and reduce the completion of nuclear translocation of the mutant associated with the formation of cellular aggregates [40]. It will be of interest to determine whether the acetylation site plays a role in disease states associated with misfolded proteins.

Investigation of the multiprotein complex recruited to the AR in a manner dependent upon the acetylation site, demonstrated that the lysine residues regulated recruitment and coprecipitation of p300. The charge of the lysine residues directly affected the biochemical association with p300 *in vitro*, and in cultured cells. Conversely the enhanced association with p300 correlated with reduced binding of an NCoR/HDAC/Smad3/Sin3A complex in cultured cells. These studies suggested the AR acetylation site regulates cellular growth through recruitment of HDAC/NCoR/Smad complexes to the promoters of a subset of cell-cycle regulatory genes, including cyclin D1 [23].

The AR acetylation and phosphorylation are functionally linked, with the AR acetylation site governing cAMP and AKT, but not ERK-induced AR activity [37]. In prostate cancer cell lines, the AR is phosphorylated and dephosphorylation inhibits AR activity. Activation of the cAMP pathway leads to a rapid dephosphorylation of the AR likely through induction of PKA inducible phosphatases. Activity of the AR is enhanced by induction of the MAPK pathway. Mutation of the lysine residues reduced ligand induced phosphorylation [37]. Conversely point mutation of phosphorylation sites in the AR reduced HDAC-mediated regulation of the AR. Together these studies demonstrated that the AR phosphorylation and acetylation events are linked.

Recent studies demonstrated that AR function is regulated by the NAD-dependent histone deacetylases (SIRT1) [42]. Sirt1 was shown to colocalize in nuclear cord like structures with the AR. Addition of the SIRT1 inhibitor nicotinamide induced androgen-regulated gene

transcription. SIRT1 was found to bind directly to the AR and the association was regulated by DHT. Sirt1 expression inhibited AR activity, requiring the catalytic activity of SIRT1. A point mutation of the core histidine residue of SIRT1, which abrogates its deacetylase activity, also reversed the repression of androgen receptor signaling [13,19]. SIRT1 was also found to inhibit the contact-independent growth stimulated by AR-expressing cancer cells. Sirt1 inhibited the activity of the AR in cultured cells and inhibited activating mutants of the AR identified in patients with resistance to current prostate cancer therapy. These AR mutants, which arise in the ligand binding domain, were readily repressed by Sirt1, however a single point substitution in the AR acetylation site evaded Sirt1-mediated repression. Growth suppression of prostate cancer cellular growth by Sirt1 was observed in cells expressing the AR, but not in cells that failed to express the AR. Sirt1 expression and function is regulated by dehydrotestosterone (DHT) and local metabolism suggesting SIRT1 may function as a physiological regulator of AR function.

Recent studies have shown alterations in histone acetylation occur in patients with prostate cancer [43], Histone modification patterns predicted tumor recurrence independently of tumor stage, preoperative PSA and capsule invasion [43]. A number of studies have shown metabolic changes occur with tumor progression in prostate cancer, with increased lactate level occurring with tumor grade progression [44,45]. During prostate cancer progression, metabolism shifts towards cytosolic glycolysis, which results in increased production of lactate. As SIRT1 activity is NAD-dependent, and because lactate decreases the NAD/NADH ratio, the dependence of cancer cell on glycolysis and thus lactate production [19,46] could also inhibit the function of SIRT1 *in vivo* and thereby enhance AR function. Since Sirt function is induced by lactate it will be of interest to determine whether the alteration in metabolism that occurs in prostate cancer contribute to the alterations in global chromatin structure and thereby deregulated gene expression that occurs in prostate cancer tumor progression.

2.2.3. SIRT1 as a novel therapy target

Although several distinct substrates for SIRT1 have been identified, (e.g., acetylated lysine residues of p53 and FOXO [6]), the dominant effect of Sirt1 in prostate cancer cell lines is to inhibit prostate cancer cellular proliferation in an AR-dependent manner [42]. Structural analysis of the AR/Sirt1 complex has been described. The minimized structure of the hSIRT1/AR peptide/NAD complex was modeled using the molecular display program Chimera from the University of San Francisco (Fig. 3). In this model the KLKK peptide (yellow) and the NAD molecule (cyan) is shown as “a ball and stick model”. The hydrophobic part of the lysine K630 side chain packs favorably against the aromatic ring in the side chain of F309 (Fig. 3). Determination of the molecular mechanism of the SIRT1/AR interaction and its physiologic effect on the function of the AR mutants that arise in patients with androgen ablation therapy resistant tumors may lead to the identification of a novel and effective new prostate cancer therapy. Collectively these studies of the AR have led to a working model in which the AR is bound to co-repressors in the absence of ligand, including HDAC and Sirt (Fig. 4). The derepression, of AR activity by addition of ligand induces AR activity in a manner that is dependent upon the AR acetylation site (Fig. 4).

2.3. Peroxisome proliferator-activating receptor γ

Peroxisome proliferator-activating receptor γ (PPARγ) mediates adipocyte differentiation, insulin sensitivity and inhibits cellular proliferation. PPARγ functions in either ligand-dependent or ligand-independent manner. Upon ligand binding, PPARγ interacts with several coactivators (CBP, p300, SRC-1, PRIP, Med220) for transcriptional activation and in the absence of ligand, complexes with PGC-1, or N-CoR, SMRT, or HDAC3. PPARγ ligands inhibit growth of several types of cancer cells, including breast, gastric adenocarcinoma, thyroid, lung, and colon cancer. PPARγ is expressed in both colon epithelial cells as well as colon cancer cells, and may function early to block of colon cancer progression [47] which is inactivated by increased expression of cyclin D1 [48,41,49]. The PPARγ ligand, troglitazone, induced apoptosis of thyroid papillary cancer cells [50] and the PPARγ ligand rosiglitazone partially reversed the mesenchymal-to-epithelial change in anaplastic thyroid cancer [51]. PPARγ decreased cell proliferation in both breast and non-small cell lung cancer [52]. In some studies in transgenic mice however PPARγ activation functions as a collaborative oncogene rather than a tumor suppressor [53]. Resolving the question of whether PPARγ is a tumor suppressor in the breast needs to be resolved, given the millions of patients taking PPARγ agonists for non-insulin dependent diabetes [53].

PPARγ regulates the expression of cyclin D1 through association of HATs and HDACs [48], SIRT1 regulates lipid metabolism through PPARγ [6,13,54].PPAR□ is repressed by SIRT1 and upon starvation in mice, PPARγ -regulated genes are repressed by SIRT1, causing fat mobilization in adipocytes. These studies imply that downregulation of PPARγ activity by pharmacological activation of SIRT1 might be beneficial for obesity. Given the importance of NR acetylation in their growth function, the mechanism by which Sirt1 regulates PPARγ function, either indirectly through co-activators, or directly, may be of interest in developing new compounds to modulate PPARγ growth function.

3. Conclusion

The androgen receptor is a well defined traditional target for the chemotherapy of prostate cancer. The deacetylation of the AR by SIRT1 may provide an alternative approach to inactivating the AR to benefit patients with prostate cancer. The functional activity of ERα is an important promoter of human breast cancer, and is regulated by acetylation. Discovering the exact role of ERα acetylation and the deacetylation caused by SIRT1 may also lead to new therapies for ERα positive cancer. Finally, PPARγ , known to be involved in several cancer types, associates with both HATs and HDACs. Modification of nuclear receptors by acetylation provides an important new target for therapeutic intervention in disease states driven by abnormal nuclear receptor function.

Competing interests statement

The authors declare that they have no competing financial interests.

Acknowledgements

This work was supported in part by R01CA70896, R01CA75503, R01CA86072, R01CA86071 (RGP) and a generous support grant from the Dr. Ralph and Marian C. Falk Medical Research Trust (R.G.P.). The Kimmel Cancer Center was supported by the NIH Cancer Center Core grant P30CA56036 (RGP). This project is funded in part under a grant with the Pennsylvania Department of Health. The Department specifically disclaims responsibility for any analysis, interpretations or conclusions.

References

[1] Waddington CH. Organisers and genes. Cambridge University Press; 1940.

[2] Neumeister P, Albanese C, Balent B, Greally J, Pestell R. Senescence and epigenetic dysregulation in cancer. Int J Biochem Cell Biol 2002;34:1475.

[3] Fu M, Wang C, Wang J, Zafonte BT, Lisanti MP, Pestell RG. Acetylation in hormone signaling and the cell cycle. Cytokine Growth Factor Rev 2002;13:259–76.

[4] de Ruijter AJ, van Gennip AH, Caron HN, Kemp S, van Kuilenburg AB. Histone deacetylases (HDACs): characterization of the classical HDAC family. Biochem J 2003;370:737–49.

[5] Frye RA. Phylogenetic classification of prokaryotic and eukaryotic Sir2-like proteins. Biochem Biophys Res Commun 2000;273:793–8.

[6] Yang T, Fu M, Pestell R, Sauve AA. SIRT1 and endocrine signaling. Trends Endocrinol Metab 2006;17:186–91.

[7] Sauve AA, Celic I, Avalos J, Deng H, Boeke JD, Schramm VL. Chemistry of gene silencing: the mechanism of NAD+-dependent deacetylation reactions. Biochemistry 2001;40:15456–63.

[8] Kaeberlein M, McVey M, Guarente L. The SIR2/3/4 complex and SIR2 alone promote longevity in *Saccharomyces cerevisiae* by two different mechanisms. Genes Dev 1999;13:2570–80.

[9] Tissenbaum HA, Guarente L. Increased dosage of a sir-2 gene extends lifespan in *Caenorhabditis elegans*. Nature 2001;410:227–30.

[10] Tissenbaum HA, Guarente L. Model organisms as a guide to mammalian aging. Dev Cell 2002;2:9–19.

[11] Mostoslavsky R, Chua KF, Lombard DB, Pang WW, Fischer MR, Gellon L, et al.

Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. Cell 2006;124:315–29.

[12] Rodgers JT, Puigserver P. Certainly can't live without this: SIRT6. Cell Metab 2006;3:77–8.

[13] Bouras T, Fu M, Sauve AA, Wang F, Quong AA, Perkins ND, et al. SIRT1 deacetylation and repression of P300 involves lysine residues 1020/1024 within the cell-cycle regulatory domain 1. J Biol Chem 2005;280:10264–76.

[14] Goodman RH, Smolik S. CBP/p300 in cell growth, transformation, and development. Genes Dev 2000;14:1553–77.

[15] Girdwood D, Bumpass D, Vaughan OA, Thain A, Anderson LA, Snowden AW, et al. p300 transcriptional repression is mediated by SUMO modification. Mol Cell 2003;11:1043–54.

[16] Luo J, Nikolaev AY, Imai S, Chen D, Su F, Shiloh A, et al. Negative control of p53 by Sir2alpha promotes cell survival under stress. Cell 2001;107:137–48.

[17] Vaziri H, Dessain SK, Ng Eaton E, Imai SI, Frye RA, Pandita TK, et al. A. hSIR2(SIRT1) functions as an NAD-dependent p53 deacetylase. Cell 2001;107:149–59.

[18] Brunet A, Sweeney LB, Sturgill JF, Chua KF, Greer PL, Lin Y, et al. Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. Science 2004;303:2011–5.

[19] Fulco M, Schiltz RL, Iezzi S, King MT, Zhao P, Kashiwaya Y, et al. Sir2 regulates skeletal muscle differentiation as a potential sensor of the redox state. Mol Cell 2003;12:51–62.

[20] Motta MC, Divecha N, Lemieux M, Kamel C, Chen D, Gu W, et al. Mammalian SIRT1 represses forkhead transcription factors. Cell 2004;116:551–63.

[21] Muth V, Nadaud S, Grummt I, Voit R. Acetylation of TAF_I68, a subunit of TIF-IB/SL1, activates RNA polymerase I transcription. EMBO J 2001;20:1353–62.

[22] Barlev NA, Liu L, Chehab NH, Mansfield K, Harris KG, Halazonetis TD, et al. Acetylation of p53 activates transcription through recruitment of coactivators/histone acetyltransferases. Mol Cell 2001;8:1243–54.

[23] Fu M, Sauve AA, Liu M, Jiao X, Zhang X, Powell M, et al. Hormonal control of androgen receptor function through SIRT1. Mol Biol Cell 2006;26:8122–35.

[24] Dickson RB, Pestell RG, Lippman ME. In: De Vita VT, Hellman S, Rosenberg SA, editors. Molecular biology of breast cancer. Cancer: principles and practice of oncology. 7th ed 2005. p. 1399–414.

[25] Pike MC, Spicer DV, Dahmoush L, Press MF. Estrogens, progestogens, normal breast cell proliferation, and breast cancer risk. Epidemiol Rev 1993;15:17–35.

[26] Rosen EM, Fan S, Pestell RG, Goldberg ID. BRCA1 gene in breast cancer. J Cell Physiol 2003;196:19–41.

[27] Giacinti L, Claudio PP, Lopez M, Giordano A. Epigenetic information and estrogen receptor alpha expression in breast cancer. Oncologist 2006;11:1–8.

[28] Wang C, Fu M, Angeletti RH, Siconolfi-Baez L, Reutens AT, Albanese C, et al. Direct acetylation of the estrogen receptor alpha hinge region by p300 regulates transactivation and hormone sensitivity. J Biol Chem 2001;276:18375–83.

[29] Fuqua SA, Wiltschke C, Zhang QX, Borg A, Castles CG, Friedrichs WE, et al. A hypersensitive estrogen receptor-alpha mutation in premalignant breast lesions. Cancer Res 2000;60:4026–9.

[30] Kim MY, Woo EM, Chong YTE, Homenko DR, Kraus WL. Acetylation of estrogen receptor a by p300 at lysines 266 and 268 enhances the deoxyribonucleic acid binding and transactivation activies of the receptor. Mol Endocrinol 2006;20:1479–93.

[31] Knights CD, Catania J, Giovanni SD, Muratoglu S, Perez R, Swartzbeck A, et al. Distinct p53 acetylation cassettes differentially influence gene-expression patterns and cell fate. J Cell Biol 2006;173:533–44.

[32] Le Corre L, Chalabi N, Delort L, Bignon YJ, Bernard-Gallon DJ. Resveratrol and breast cancer chemoprevention: molecular mechanisms. Mol Nutr Food Res 2005;49:462–71.

[33] Amanatullah DF, Reutens B, Zafonte B, Fu M, Mani S, Pestell R. Cell-cycle dysregulation in prostate cancer. Front Biosci 2000;1:372–90.

[34] Jemal A, Murray T, Samuels A, Ghafoor A, Ward E, Thun MJ. Cancer statistics, 2003. CA Cancer J Clin 2003;53:5–26.

[35] Fu M, Wang C, Reutens AT, Wang J, Angeletti RH, Siconolfi-Baez L, et al. p300 and p300/cAMP-response element-binding protein-associated factor acetylate the androgen receptor at sites governing hormone-dependent transactivation. J Biol Chem 2000;275:20853–60.

[36] Gong J, Zhu J, Goodman OB, Pestell RG, Schlegel PN, Nanus DM, et al. Activation of p300 histone acetyltransferase activity and acetylation of the androgen receptor by bombesin in prostate cancer cells. Oncogene 2006;25:2011–21.

[37] Fu M, Rao M, Wu K, Wang C, Zhang X, Hessien M, et al. The androgen receptor acetylation site regulates cAMP and AKT but not ERK-induced activity. J Biol Chem 2004;279:29436–49.

[38] Fu M, Wang C, Wang J, Sakamaki T, Zhang X, Yeung Y-G, et al. The androgen receptor acetylation governs transactivation and MEKK1-induced apoptosis without affecting in vitro sumoylation and transrepression function. Mol Cell Biol 2002;22:3373–88.

[39] Fu M, Wang C, Wang J, Sakamaki T, Di Vizio D, Zhang X, et al. Acetylation of the androgen receptor enhances coactivator binding and promotes prostate cancer cell growth. Mol Cell Biol 2003;23:8563–75.

[40] Thomas M, Dadgar N, Aphale A, Harrell JM, Kunkel R, Pratt WB, et al. Androgen receptor acetylation site mutations cause trafficking defects, misfolding, and aggregation similar to expanded glutamine tracts. J Biol Chem 2004;279:8389–95.

[41] Fu M, Wang C, Rao M, Wu X, Bouras T, Zhang X, et al. Cyclin D1 represses p300 transactivation through a CDK-independent mechanism. J Biol Chem 2005;28:29728–42.

[42] Fu M, Liu M, Sauve AA, Jiao X, Zhang X, Wu X, et al. Hormonal control of androgen receptor function through SIRT1. Mol Cell Biol 2006;26:8122–35.

[43] Seligson DB, Horvath S, Shi T, Yu H, Tse S, Grunstein M, et al. Global histone modification patterns predict risk of prostate cancer recurrence. Nature 2005;435:1262–6.

[44] Baron A, Migita T, Tang D, Loda M. Fatty acid synthase: a metabolic oncogene in prostate cancer? J Cell Biochem 2004;91:47–53.

[45] Rossi S, Graner E, Febbo P, Weinstein L, Bhattacharya N, Onody T, et al. Fatty acid synthase expression defines distinct molecular signatures in prostate cancer. Mol Cancer Res 2003;1:707–15.

[46] Altenberg B, Greulich KO. Genes of glycolysis are ubiquitously overexpressed in 24 cancer classes. Genomics 2004;84:1014–20.

[47] Girnun GD, Spiegelman BM. PPARgamma ligands: taking Ppart in chemoprevention. Gastroenterology 2003;124:564–7.

[48] Wang C, Pattabiraman N, Zhou JN, Fu M, Sakamaki T, Albanese C, et al. Cyclin D1 repression of peroxisome proliferator-activated receptor gamma expression and transactivation. Mol Cell Biol 2003;23:6159–73.

[49] Hulit J, Wang C, Li Z, Albanese C, Rao M, Di Vizio D, et al. Cyclin D1 genetic heterozygosity regulates colonic epithelial cell differentiation and tumor number in ApcMin mice. Mol Cell Biol 2004;24:7598–611.

[50] Ohta K, Endo T, Haraguchi K, Hershman JM, Onaya T. Ligands for peroxisome proliferator-activated receptor gamma inhibit growth and induce apoptosis of human papillary thyroid carcinoma cells. J Clin Endocrinol Metab 2001;86:2170–7.

[51] Aiello A, Pandini G, Frasca F, Conte E, Murabito A, Sacco A, et al. Peroxisomal proliferator-activated receptor-gamma agonists induce partial reversion of epithelial–mesenchymal transition in anaplastic thyroid cancer cells. Endocrinology 2006;147:4463–75.

[52] Keshamouni VG, Reddy RC, Arenberg DA, Joel B, Thannickal VJ, Kalemkerian GP, et al. Peroxisome proliferator-activated receptor-gamma activation inhibits tumor progression in

non-small-cell lung cancer. Oncogene 2004;23:100–8.

[53] Saez E, Rosenfeld J, Livolsi A, Olson P, Lombardo E, Nelson M, et al. PPAR gamma signaling exacerbates mammary gland tumor development. Genes Dev 2004;18:528–40.

[54] Picard F, Kurtev M, Chung N, Topark-Ngarm A, Senawong T, Machado De Oliveira R, et al. Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-gamma. Nature 2004;429:771–6.

[55] Leader JE, Wang C, Fu M, Pestell RG. Epigenetic regulation of nuclear steroid receptors. Biochem Pharmacol 2006;72:1589–96.

[56] Fan S, Wang J, Yuan R, Ma Y, Meng Q, Erdos MR, et al. BRCA1 inhibition of estrogen receptor signaling in transfected cells. Science 1999;284:1354–6.

[57] Wang C, Fan S, Li Z, Fu M, Rao M, Ma Y, et al. Cyclin D1 antagonizes BRCA1 repression of estrogen receptor alpha activity. Cancer Res 2005;65:6557–67.

[58] Abate-Shen C. Deregulated homeobox gene expression in cancer: cause or consequence? Nat Rev Cancer 2002;2:777–85.

FIGURES

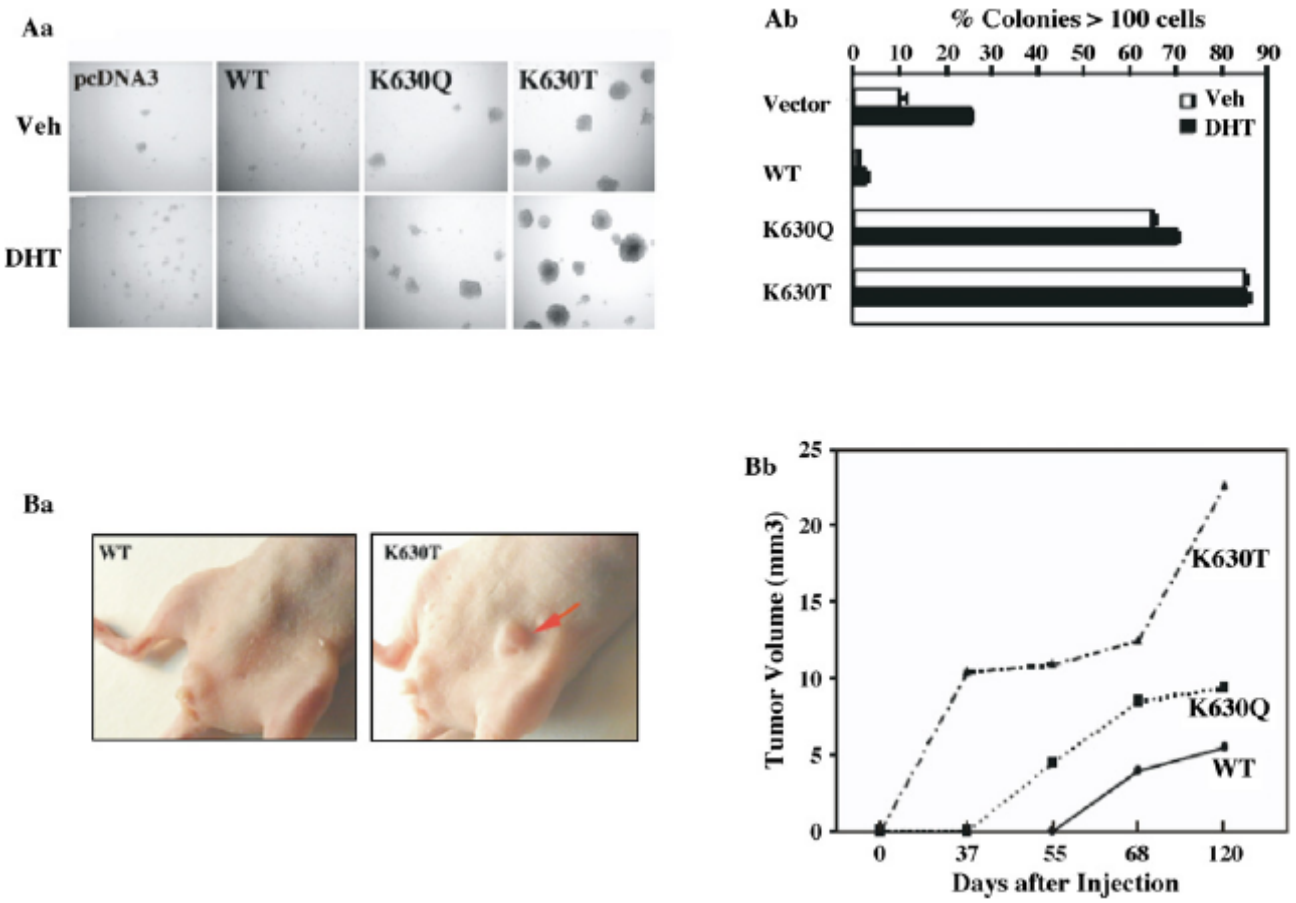

Fig. 1 – The AR acetylation mutants conveys contact-independent growth. (Aa and b) The human prostate cancer DU145 cells, stably expressing the ARwt or AR acetylation site mutants, were seeded in soft agar. The AR acetylation site mutants (ARK630Q and ARK630T) represent gain of function mutants that function as acetylation mimics. Phase contrast image of the colonies from a representative experiment is shown (100×). Colony numbers and size (percentage of colonies with more than 100 cells) determined on day 14. Compared with the ARwt, the AR acetylation site point mutant mimics dramatically enhance the size of the colonies. (Ba and b) Nude mice were implanted with 1×10^6 cells of stable lines expressing either the ARwt or AR acetylation site mutants. Mean volume of DU145 tumors grown in nude mice are shown at each time point (From Fu et al., 2003, with permission) [39]. Collectively these studies demonstrate that a single amino acid residue substitution of the AR acetylation site enhances the growth function of the human AR in human prostate cancer cell lines.

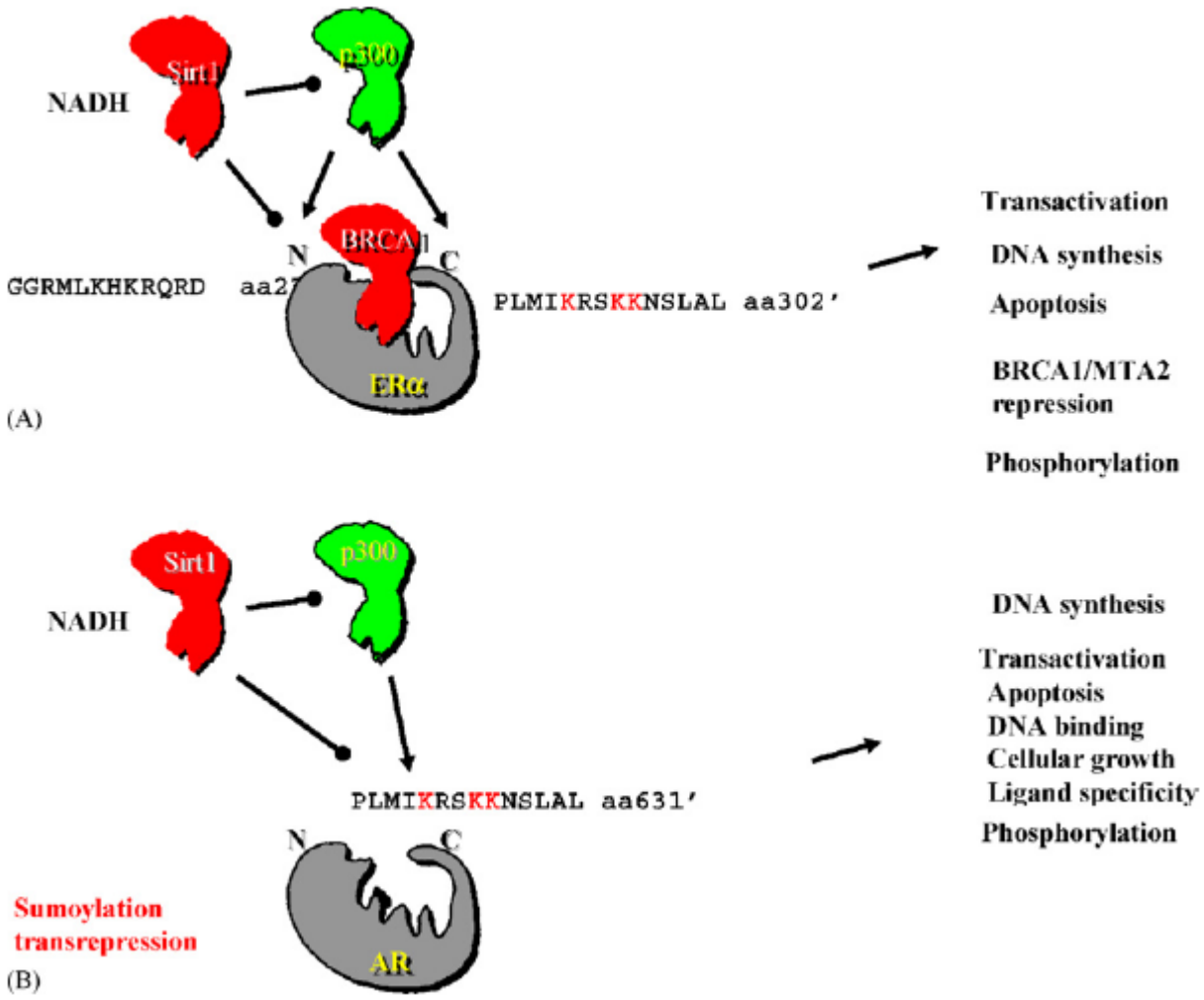

Fig. 2 – Acetylation of the ER and AR are regulated by both TSA and NAD sensitive histone deacetylases.

ERα and AR share conserved lysine residues that are acetylated by distinct HATs (reproduced with permission from [55]). The ERα is shown associated with the BRCA1 protein, which is known to repress activity of the ERα [56,57]. The two known acetylation sites of the ERα and their amino acid sequence is shown. p300 is a coactivator of the ERα and the AR. SIRT1 is known to deacetylate and thereby repress the activity of p300 [13]. The ERα may be a target of SIRT1 as chemical inhibitors of SIRT1 regulate ERα activity. The acetylation site of the ERα has been shown to govern several functions [29] (transactivation, DNA synthesis, apoptosis, BRCA1 repression). (B) The AR acetylation site is shown as a substrate for p300 acetylation and for deacetylation by SIRT1-Acetylation of the AR has been shown to regulate several key functions of the AR (DNA synthesis, transactivation, and cellular growth [35,37,39,58] but does not affect AR-mediated transrepression or sumoylation.

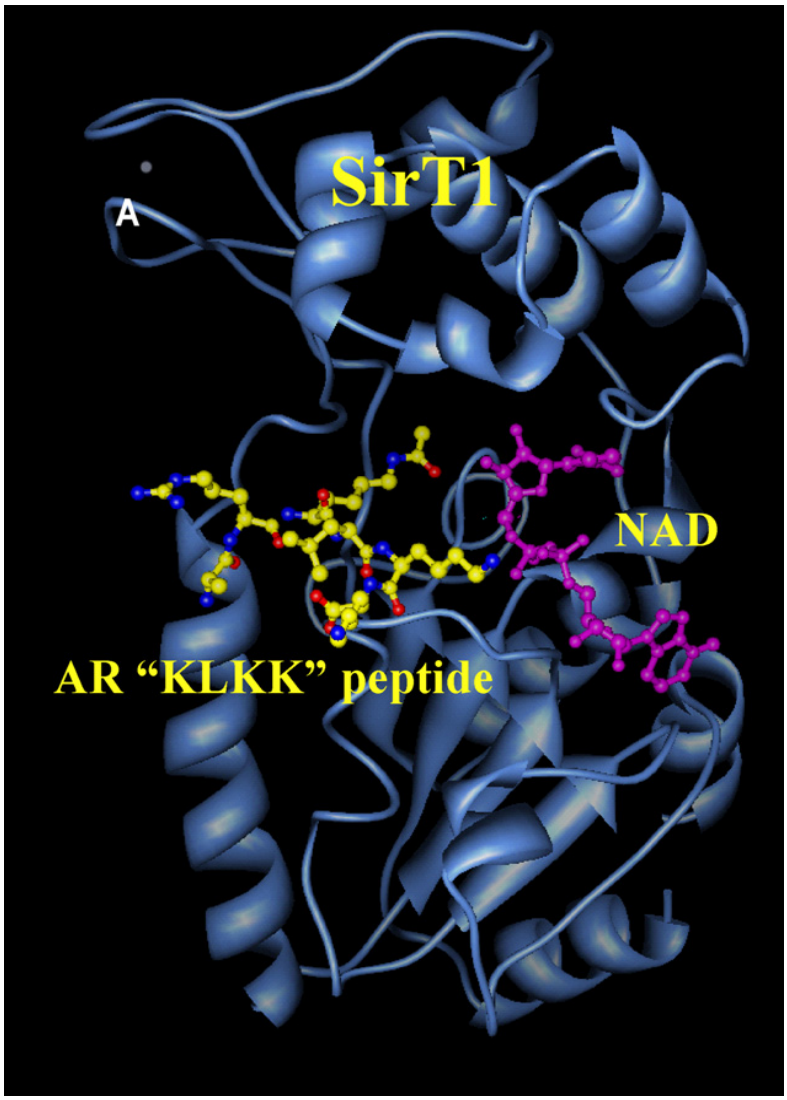

Figure 3 – Homology model of AR-"KLKK" peptide with NAD bound SIRT1.
The structure of the hSirT1 and AR "KLKK" peptide-NAD complex is shown as the ribbon model generated using the molecular display program CHIMERA. The "KKK" peptide (yellow) and the NAD molecule (cyan) are shown as the "ball-and-stick" model.

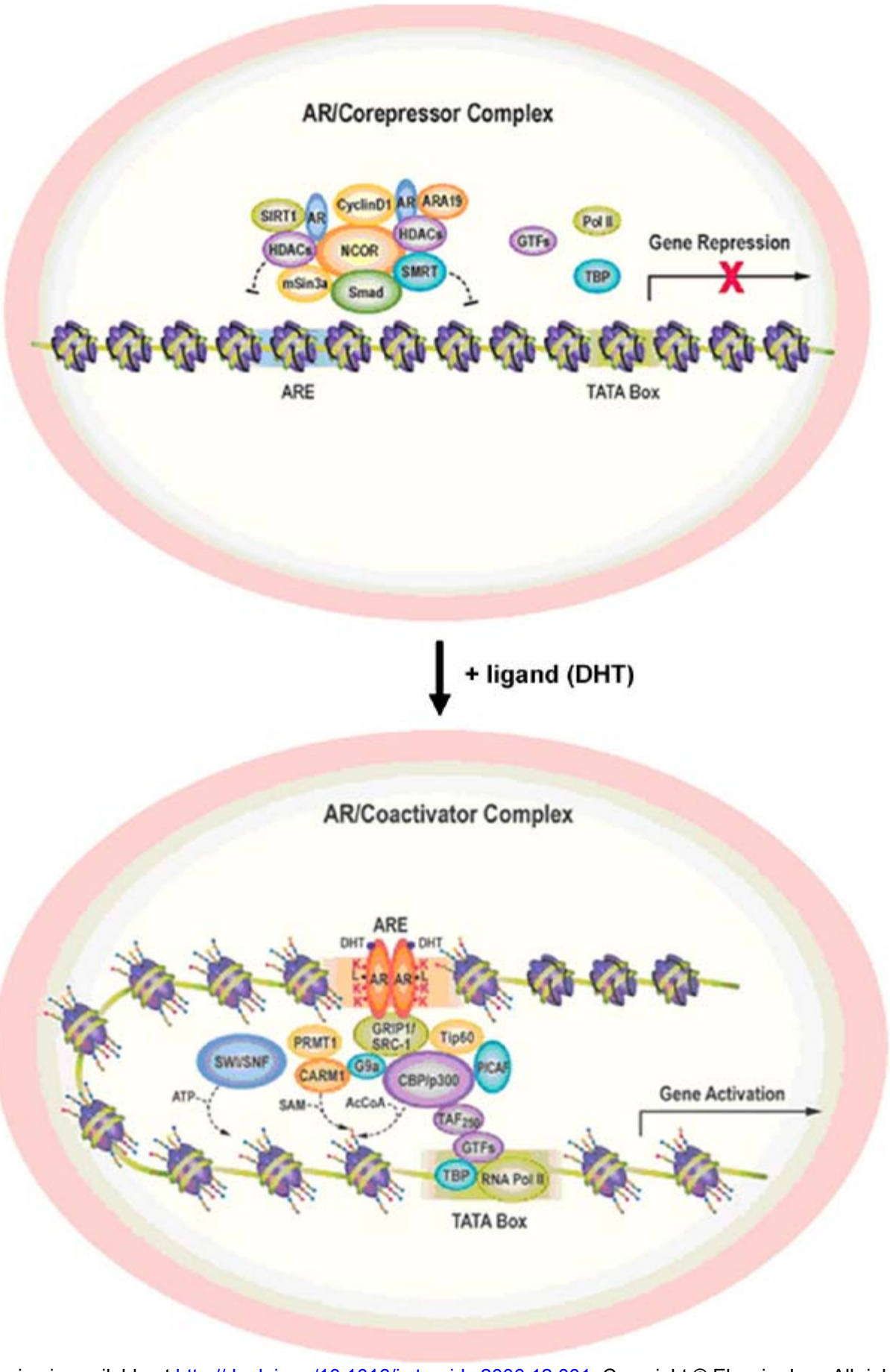

Fig. 4 – (A) Schematic representation of AR bound to corepressors in the absence of ligand in the context of local chromatin, dependent in part upon the AR lysine motif. SIRT1 inhibits AR activity. SIRT1 is shown to associate with the NCoR/HDAC repression complex. (B) Upon the addition of ligand (DHT), disengagement of the corepressor complex and recruitment of the coactivator complex engages gene expression, dependent in part upon the AR lysine motif.