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Focus On

Estrogen receptor acetylation and phosphorylation in hormone responses

C. Wang, M. Fu, R. G. Pestell

Lombardi Comprehensive Cancer Center, Department of Oncology, Georgetown University, Washington, DC, USA.

Abstract Histone acetylation is thought to facilitate binding of transcription factors (TFs) to specific target DNA sequences by destabilizing nucleosomes bound to the promoter region of a target gene. In addition, non-histone proteins including a subset of TFs and co-activators are acetylated by p300/CBP and P/CAF. The regulation of estrogen signaling by direct estrogen receptor alpha (ERα) post-translational modification reveals a novel role for histone acetyltransferase in hormone signaling. ERα is acetylated and phosphorylated and phosphorylation occurs at multiple sites in response to kinase signaling. The finding that mutations with the ERα hinge domain lysine residues enhance hormone sensitivity suggests these residues may be involved in ligand-dependent transcriptional repression or transcriptional attenuation. Phosphorylation and acetylation of the ER regulates hormone signaling and is being assessed for a role in resistance to anti-estrogen therapy of ERα-positive patients.

Keywords: Acetylation; Breast cancer; Estrogen receptor; Metastasis

In the USA, breast cancer accounts for approximately 30% of all cancers diagnosed and remains the second leading cause of cancer death in women. The importance of estrogen receptor alpha (ERα) activity in breast cancer onset is evidenced by the efficacy of ERα antagonists for breast cancer prevention in high-risk individuals [1,2]. The Stathmin staging classification (TNM) includes ERα status [3], as the abundance of ERα determined by immunoreactivity provides independent evidence for high survival rate and lower relapse [4,5].

Estrogens regulate cellular proliferation and differentiation of the normal breast. The two related nuclear receptors, ERα and ERβ, function as transcription factors (TFs) to regulate downstream target genes, co-ordinating cell-cycle progression within the mammary epithelial cell and contribute to intracrine and paracrine signaling. ERs are members of the nuclear steroid hormone receptor family, which function as ligand-dependent transcriptional regulators. The subcellular location of the ERα also contributes to distinct signaling pathways. Thus, in addition to the well-known nuclear function, membrane-associated ERα also functions through membrane tyrosine kinase receptor signaling pathways [6]. Additional types of functional ERs associated with plasma membranes, such as GPR30, are thought to regulate non-genomic signaling through second messenger Ca^{2+}, nitric oxide and protein–lipid kinases [7]. The ERα encodes distinct functional domains (termed A–F) conserved with other members of the classical receptor subclass. The conserved DNA-binding domain consists of 68 amino acids with two zinc finger structures. The activation function (AF) domains, AF-1 and AF-2, of the ERα contribute synergistically to transcriptional induction. AF-1 function is induced by oncoproteins, growth factors, the co-activator p300, p68 RNA helicase A and several other signaling pathways [8–10]. The

Correspondence to: Richard G. Pestell, Lombardi Comprehensive Cancer Center, Department of Oncology, Georgetown University, Washington, DC 20057, USA.

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ligand-binding domain consists of α helices projecting away from the hormone-binding pocket in the absence of ligand. In the presence of ligand, the ERα and its carboxyl terminal helix 12 undergo conformational change, creating new structural surfaces that alter binding of co-repressors and recruit co-activators, promoting transcriptional activity.

The function of ERα is regulated by post-translational modification and functional interaction *in trans* with an array of distinct proteins. Post translational modification of the nuclear receptors co-ordinate distinct functional activities. ERα is phosphorylated by distinct kinases, including extracellular-regulated kinases (ERK) 1/2, p38 MAP kinase (MAPK), cyclin A/cdk2, cdk7, c-Src, pp90-RSK1, AKT, IKKα and protein kinase A. ERα forms multiprotein complexes with co-activator and co-repressor proteins. These complexes encode multiple distinct enzyme activities, including histone acetyl transferase (HAT), histone methyl transferases and chromatin remodeling proteins. The nuclear receptor co-activators of the p160 family (SRC1, SRC2, SRC3) interact with the nuclear receptor in a ligand-regulated manner through the AF-2 domain. α-Helical motifs within the p160 proteins are required for interaction with nuclear receptors. ERα co-activators include P/CAF, p68 RNA helicase, p300/CBP, BRG1, the SRC family, TIP60 and others (reviewed in [11]). It has been hypothesized, as growth factor stimulation regulates many of these mitogenic signaling kinases, that phosphorylation of the ERα may contribute to proliferative signaling and/or clinical resistance to anti-estrogen therapies, such as tamoxifen.

The co-elution of ERα with the HATs prompted biochemical studies which identified the ERα as a *bona fide* substrate for p300 [12]. Acetylation of histones involves the transfer of an acetyl group from acetyl co-enzyme A (acetyl CoA) to the ε-amino group of lysine side chains. This reaction is conducted by a growing family of HATs, including p300/CBP and SRCs, which have distinct substrate specificities [13]. Acetylation is now known to regulate the function of many proteins, including histones, structural proteins, cell-cycle control protein, TFs and the HAT enzymes themselves [11,14]. Histone acetylation leads to local changes in electrostatic interactions, changes α-helical content of histone tails and creates recognition sites to recruit other TFs and co-regulatory proteins. The sequential recruitment of other proteins forms the basis of local signaling modules within the chromatin of the histone tails, referred to as the 'histone code hypothesis' [15]. A growing list of non-histone substrates for HATs, known as Factor Acetyl Transferase Substrates (FATS), underscores the broad importance of this modification in biology (Fig. 1).

Recent studies have demonstrated the ERα is acetylated in breast cancer cells [12]. The ERα acetylation site was identified through MALDI-TOF

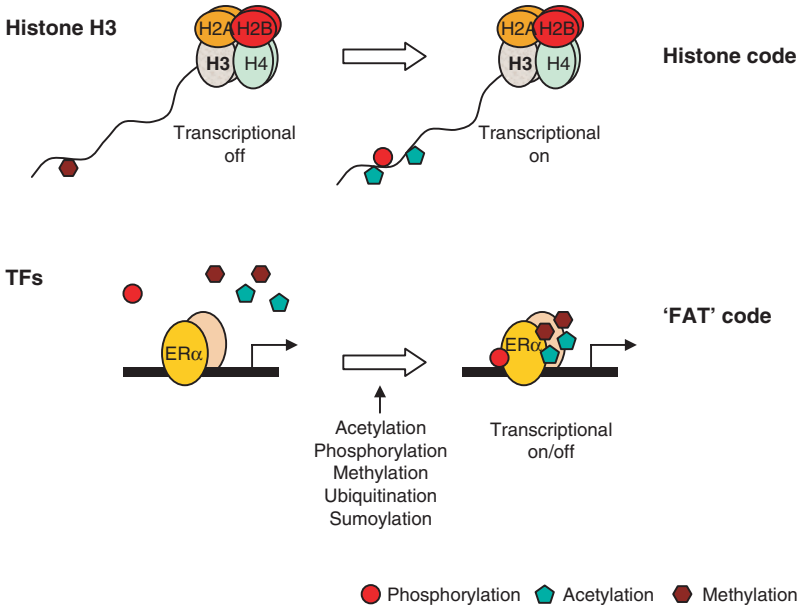

Figure 1.
A schematic representation of a nucleosome core particle, consisting of a histone octamer wrapped in DNA. The histone tail is shown extending to the left of core histones. The core histone tails contain motifs that can be post-translationally modified by phosphorylation, acetylation and methylation. Below TFs are shown bound to DNA. Post-translational modification of TFs by acetylation in turn integrates intramolecular signaling by phosphorylation, methylation and other post-translational modifications. The intramolecular signal between phosphorylation, acetylation and other post-translational modifications are referred to as the FAT code.

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mass spectrometry and sequencing by Edman degradation. The acetylated residues (lysines 299, 302, 303) are located within the ERα hinge region, predicted to reside in proximity to the hydrophobic ligand-binding pocket. Although the ERα binds several HATs, including P/CAF, ERα is preferentially acetylated by p300, and lysines 302 and 303 were preferentially acetylated, suggesting an important selectivity in substrate recognition [12]. Mutational analysis of the ERα lysine residues demonstrated a role in basal-level and ligand-regulated activity. Substitution of the lysine residues enhanced activation of the ERα at low concentrations of estradiol [12,16].

Post-translational modification by acetylation and phosphorylation are integrated in several physiological circumstances. Histone acetylation and phosphorylation occurs contemporaneously upon induction of immediate early-gene induction. Histone H3 is both acetylated and phosphorylated [17]. Mutation of the androgen receptor (AR) acetylation site reduced cAMP and AKT but not MAPK signaling [18,19]. The AR acetylation site affected AR phosphorylation. Mutation of each of the six AR phosphorylation sites demonstrated a single phosphorylation site, Ser94, regulated the AR response to histone deacetylation. Together, these studies suggested the AR lysine residues may co-ordinate the function of a subset of kinase modules signaling to the AR. To assess the possibility that phosphorylation and acetylation site events were interdependent in regulating ERα activity, growth factor regulation of ERα activity was examined. AF-1 functions, including growth factor-induced, ligand-independent ERα activity, mediated through MAPK, and activation by p68 RNA helicase A, were not affected by ERα acetylation [12]. However, mutation of the PKA phosphorylation site at residue 305, to mimic constitutive phosphorylation, blocked *in vivo* acetylation of the ERα at K303, suggesting these two post-translational modifications are coupled within the ERα receptor [20]. Resistance to the anti-estrogen tamoxifen involves PKA signals to ERα S305 [21]. cAMP-dependent phosphorylation site mutants of this residue enhanced hormone sensitivity of the ERα and inhibited ERα acetylation [20]. Together, these studies implicate the ERα lysine motif in an intramolecular signaling response in tamoxifen.

The ERα-acetylated lysine motif is conserved with many other nuclear receptors [12]. Subsequent studies have confirmed this motif is a site of acetylation in other receptors, which regulates nuclear receptor function in a profound manner [13,18,19,22]. The AR is acetylated by several HATs including P/CAF, p300 and TIP60 [18,23,24]. Functional analysis has provided evidence for TF acetylation in growth control. Generation of AR acetylation mimic mutants revealed a role for TF acetylation in growth control [18,23,24]. Since acetylation of lysine residues can neutralize their positive charge and increase their hydrophobicity, AR mutants encoding lysine residues substituted with polar uncharged or polar residues were generated as acetylation mimic mutants. Comparison was made with mutants encoding charged residue to create 'dead' mutants. AR acetylation mimic mutants enhanced binding to co-activator proteins (p300) and reduced binding to co-repressors (HDAC/NCoR/Smad3). The acetylation mimic AR mutants conveyed enhanced prostate cancer cellular growth in soft agar and in nude mice. The acetylation mimic mutants were resistant to the effects of the androgen antagonist flutamide [18,24]. The ERα acetylation site also appears to govern co-regulator recruitment and cellular growth. MCF7 cells expressing ERα K303R show enhanced proliferation in response to ligand and increased binding to SRC2 [16].

Based on studies examining acetylation of histone and the AR, several predictions can be made for ERα acetylation. AR acetylation mimic mutants are defective in repression by and recruitment of HDAC/NCoR-containing complexes. Several ERα co-repressors contain histone deacetylases. The metastasis-associated protein family (MTA) members, MTA1 and MTA2 for example, are found within nucleosome remodeling and histone deacetylation (NuRD) complexes, and BRCA1 is co-associated with HDACs [25] (Fig. 1). BRCA1 and MTA1 inhibit ERα signals. It would be predicted that the ERα acetylation site may convey resistance to BRCA1 or MTA repression. If confirmed, such findings would provide a key link between breast tumor suppression and estrogen hormone signaling. Acetylation of nuclear receptors occurs through a conserved motif. In addition, functional properties of nuclear receptors, including tumor growth *in vivo*, are regulated by acetylation. Therefore, receptor acetylation is being assessed as a new target for cancer therapies [26].

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