Epigenetics meets estrogen receptor: regulation of estrogen receptor by direct lysine methylation

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Abstract

The nuclear hormone receptor estrogen receptor α (ERα) promotes cellular growth through ligand-dependent activation of specific target genes, a process which is targeted in the treatment of ERα-expressing breast cancers. ERα activity is regulated at the protein level by post-translational modifications including phosphorylation and acetylation. A study now shows that ERα can also be directly methylated at lysine 302 (K302) by SET7, a histone methyltransferase that is known to monomethylate H3K4 and is associated with transcriptional activation. It was shown that K302 methylation stabilizes ERα protein and is suggested to increase sensitivity of ERα to estrogens, enhancing transcription of estrogen response elements. Furthermore, SET7 methylation of K302 is enhanced by a breast cancer-associated mutation at K303 (K303R) in vitro. These findings provide an additional mechanism of SET7 mediated transcriptional activation, as well as potential insight into the complex regulation of ERα stability and ligand sensitivity.

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Estrogens such as 17β-estradiol (E₂) are synthesized predominantly in ovaries or via aromatization in peripheral tissues and play a pivotal role in the development of breast, ovarian, and endometrial cancers (Yager & Davidson 2006). The role of ER, particularly ERα, and its ligands in breast carcinogenesis has been recognized for some time (Yager & Davidson 2006). Indeed, about 70% of newly diagnosed invasive breast cancers express ERx protein and this expression identifies cancers that are potentially amenable to endocrine approaches designed to deplete ligand or receptor, or impede ligand–receptor interactions. Although other important factors affecting endocrine therapies have come to light in recent years (e.g. HER-2 expression and progesterone receptor status), molecular studies have indicated that ERα-related pathways are a major determinant of endocrine responsiveness (Rastelli & Crispino 2008).

Much work has focused on mechanisms that regulate ERx expression in human breast cancer cells. It has been hypothesized that epigenetic mechanisms including DNA methylation and/or histone modifications might contribute to silencing of ERx expression (Ottaviano et al. 1994, Leader et al. 2006). Indeed, several studies have demonstrated that the ERx CpG island is hypermethylated in ERx-negative human breast cancer cells and treatment of these cells with inhibitors of DNA methyltransferases such as 5-azacitidine or decitabine elicits reexpression of functional ERx protein. Furthermore, covalent histone modifications, such as the methylation or acetylation of lysine residues, can also contribute to the transcriptional silencing or activation of ERx (Sharma et al. 2006, Macaluso et al. 2007). Site-specific modifications in the N-terminal tail region of core and linker histones specify a histone code of epigenetic regulation that dictates a specific active or inactive chromatin state (Strahl & Allis 2000). Methylation of lysine residues can occur on histones H3 and H4, thereby contributing to a chromatin conformation compatible with either active or repressed gene transcription, depending on the residue modified and the degree of methylation (Sims et al. 2003). In particular, monomethylation at histone H3 lysine 4 (H3K4Me1) is associated with transcriptionally competent euchromatin (Noma et al. 2001), but the mechanism of H3K4Me1-associated transcriptional activation is poorly understood.

Several histone H3K4-specific methyltransferases have been identified including human SET7 (also known as SET9;
Wang et al. (2001b). The suppressor of variegation, enhancer of zeste and trithorax (SET) domain is an evolutionarily conserved sequence motif originally described in Drosophila; it is present in chromosomal proteins that function in epigenetic control of gene expression (Jenuwein et al. 1998). Early studies revealed that SET7 activates transcription by preventing chromatin condensation (Wang et al. 2001b, Nishioka et al. 2002). Importantly, SET7 can methylate specific lysines on non-histone proteins including transcriptional factors and regulators like p53 and TAF10. SET7 methylation stabilizes and inhibits nuclear export of p53, leading to the transcriptional activation of the p53 target genes (Chuikov et al. 2004). Likewise, SET7 methylation has a stimulatory effect on TAF10 mediated transcription by increasing TAF10 affinity for interaction with RNA polymerase II (Kouskouti et al. 2004). These studies suggest that the role of SET7 methylation in transcriptional activation reaches beyond the modification of histones.

Another such non-histone target appears to be ERα. Evidence has been emerging that lysine residues in the ERα hinge region can be targets for multiple types of post-translational modifications (PTMs) including acetylation, phosphorylation, and ubiquitination (Fig. 1). These modifications may affect hormone sensitivity through alteration of ERα stability or regulation of estrogen-dependent gene transcription. Histone acetyltransferases such as p300 and histone deacetylase inhibitors have been shown to induce acetylation at several lysines within the hinge region. Coactivator-dependent acetylation of K266 and K268 by p300 increases ERα transcriptional activity by enhancing DNA binding (Kim et al. 2006), whereas acetylation of K303 may attenuate hormone sensitivity through dissociation of transcriptional coactivators from the promoter (Wang et al. 2001a). Studies have found that hyper-phosphorylation of serine at 305 (S305) enhances estrogen-induced transcriptional activity and acetylation of lysine at 303 inhibits S305 phosphorylation (Cui et al. 2004). Other covalent modifications such as ubiquitination at K302 (Berry et al. 2008) and sumoylation at K266 and K268 (Sentis et al. 2005) have also been shown to affect estrogen receptor stability and activity. Together, these data suggest that the complex cellular responses to E2 are significantly affected by PTMs. However, it was not clear how ERα methylation might play a role in estrogen signaling until Subramanian and colleagues recently presented the first evidence that ERα is regulated by the SET7 lysine methyltransferase in human breast cancer cells (Subramanian et al. 2008). In fact, this is only the second account in the literature of regulation of a nuclear receptor by direct methylation, shortly following the discovery that retinoic acid receptor α activity is regulated by lysine trimethylation (Huq et al. 2007).

Using an in vitro methylation assay of peptide substrates (ERα, androgen receptor (AR), glucocorticoid receptor (GR) and mutant ERα), and recombinant purified HMTases, they found that the methylation of lysine in the ERα hinge region is specifically targeted by SET7. Through mass spectrometric analysis, they identified K302 as the only site of methylation.

Figure 1 Post-translational modifications of ERα. The subdomains of ERα are outlined with some of the major previously identified PTMs annotated with numbered shapes (defined in legend). (A) The major functional domains of ERα are shown. The poorly conserved AF-1 domain lies at the N-terminus, followed by the DNA binding domain (DBD). The C-terminal region of ERα houses the ligand-binding domain (LBD) of hormone receptors which contains the well-conserved AF-2 domain. (B) The DNA and ligand binding regions are connected by the hinge region, expanded to show the various PTM target residues previously identified. (C) Comparison of the SET7 binding motif for the hinge region of ERα, TAF10, p53, and histone h3 is shown. The recognition consensus sequence is shaded in grey with the target lysine residues aligned and in bold. The PTMs highlighted above were reported in the following publications: 1, (Ali et al. 1993), 2, (Cui et al. 2004), 3, (Kim et al. 2006), 4, (Sentis et al. 2005), 5, (Berry et al. 2008), 6, (Subramanian et al. 2008).
by SET7 within the ERα hinge region in vitro. To confirm these findings in vivo, the authors raised a specific antibody against an ERα peptide that is monomethylated at K302; its specificity and sensitivity for this methylated residue were convincingly demonstrated by dot-blot analysis. They then transiently transfected ERα-negative HeLa cells with expression constructs for SET7 and ERα, and cell lysates were immunoprecipitated for ERα, followed by western blot analysis. Their findings demonstrate that ERα is methylated at K302 by SET7 in cell culture and that this methylation can be decreased by mutation of K303 to arginine (K303R). This latter observation is of particular interest because an acquired missense mutation in the ERα gene resulting in K303R substitution has been reported in primary breast ductal hyperplasia and cancers from patients in the southern USA (Fuqua et al. 2000, Conway et al. 2005) but not in Japan or the UK (Tokunaga et al. 2004, Davies et al. 2005). These studies lead to the conclusion that SET7 serves as a specific HMTase to monomethylate K302 and SET7-mediated methylation of K302 may be altered by the mutation of the neighboring K303. The structural basis of these findings was elaborated with high-resolution X-ray data from co-crystals of full length SET7 protein with an ERα peptide.

The role of SET7-mediated methylation in the regulation of ERα function was carefully addressed by the authors. They stably transfected retroviral vectors that contained SET7 shRNA into the ERα-positive MCF-7 breast cancer cell line to generate shSET7 knockdown cells. Results show that knockdown of SET7 concomitantly decreases the steady-state levels of ERα protein but not ERα mRNA, suggesting that methylation of K302 by SET7 modulates ERα protein stability. Pulse chase experiments confirmed that ERα half-life is decreased by about twofold in shSET7 cells compared with control cells. Consistent with these observations, estrogen response elements (ERE)-coupled luciferase reporter assays showed that estrogen-driven transcription is dramatically inhibited in shSET7 knockdown clones. Further estrogen-induced transactivation of two classic endogenous ERα target genes, PS2 and PgR, was blunted in shSET7 cells, suggesting that SET7 is required for estrogen-induced transcriptional response. One potential mechanism of SET7 mediated enhancement of ERE transcription is through alteration of protein binding complexes in the ERα target gene promoters. Therefore, the authors used quantitative chromatin immunoprecipitation (ChIP) assays to determine that SET7 regulates ERα target genes by altering ERα binding to target gene regulatory sequences. Furthermore, they established that ERα and NCoA2 but not SET7 act as coactivators at the PS2 gene promoter in multiple cell lines. However, this does not preclude the possibility that SET7 could act on ER co-factors in addition to ERα, a matter that will not be resolved until the non-histone targets of SET7 are extensively studied.

This study by Subramanian and colleagues has substantially enhanced our knowledge of the role of PTMs of ERα hinge region in the estrogen-dependent gene transcription pathway. Also, the stabilization of ERα by SET7 provides additional complexity to a new-found dimension of the epigenetic code, whereby specific PTMs of transcription

**Figure 2** SET7 methylates multiple target proteins to activate transcription. H3K4 methylation by SET7 facilitates gene transcription by preventing chromatin condensation, while SET7 methylation of transcription factors such as p53, TAF10, and ERα enhances their ability to serve as transcriptional activators.
factors could make a significant contribution to the transcriptional activity commonly attributed to a specific histone modification (Fig. 2). These findings raise important questions, some relevant to the field of epigenomics as well as molecular endocrinology. First, to what extent is the SET7 induced upregulation of ERE transcription responsible for the transcriptional activation-associated with concordant H3K4 monomethylation in ERα-expressing tissues? Also, what of the reverse? Does SET7 activity play a role in ERα-dependent proliferation of breast cancer, or resistance to endocrine therapies? The answers to these questions could determine the potential of SET7 as a target for breast, or other endocrine-related cancers. Also, as noted earlier, extension of these studies to other cell lines is extremely important to show, whether the findings are general and to understand what other cell-type factors might play a role. Indeed, other studies have shown that, in addition to nuclear gene transcription, ERα carries out various cellular functions at the plasma membrane and the mitochondria in a cell-type-specific manner (Zhang et al. 2002, Pedram et al. 2006). It is quite plausible that the cellular localization and function of ERα is regulated by specific PTMs as well.

This study is one of several suggesting that PTMs of ERα may play a role in hormone response. Methylation of K302 is of particular interest in view of reports that the functional consequences may be of substantial impact and may be modulated by the adjacent K303R variant that has been reported in human breast cancers, especially those with more aggressive clinical features and poorer prognosis. Thus, these findings may ultimately lead to the development of new anticancer therapies that specifically manipulate SET7 and alter estrogen-signaling pathways.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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