Nuclear receptors (NR) impact a myriad of physiological processes including homeostasis, reproduction, development, and metabolism. NRs are regulated by post-translational modifications (PTM) that markedly impact receptor function. Recent studies have identified NR PTMs that are involved in the onset and progression of human diseases, including cancer. The majority of evidence linking NR PTMs with disease has been demonstrated for phosphorylation, acetylation and sumoylation of androgen receptor (AR), estrogen receptor α (ERα), glucocorticoid receptor (GR) and peroxisome proliferator activated receptor γ (PPARγ). Phosphorylation of AR has been associated with hormone refractory prostate cancer and decreased disease-specific survival. AR acetylation and sumoylation increased growth of prostate cancer tumor models. AR phosphorylation reduced the toxicity of the expanded polyglutamine AR in Kennedy’s Disease as a consequence of reduced ligand binding. A comprehensive evaluation of ERα phosphorylation in breast cancer revealed several sites associated with better clinical outcome to tamoxifen therapy, whereas other phosphorylation sites were associated with poorer clinical outcome. ERα acetylation and sumoylation may also have predictive value for breast cancer. GR phosphorylation and acetylation impact GR responsiveness to glucocorticoids that are used as anti-inflammatory drugs. PPARγ phosphorylation can regulate the balance between growth and differentiation in adipose tissue that is linked to obesity and insulin resistance. Sumoylation of PPARγ is linked to repression of inflammatory genes important in patients with inflammatory diseases. NR PTMs provide an additional measure of NR function that can be used as both biomarkers of disease progression, and predictive markers for patient response to NR-directed treatments.

Introduction

Nuclear receptor (NR) function is regulated by post-translational modifications (PTM) including phosphorylation, acetylation, sumoylation, methylation, myristylation, nitration, ADP-ribosylation, and isoprenylation. These PTMs can be further divided into two categories: 1) reversible modifications that function by either addition or removal of functional chemical groups (i.e., phosphate, acetyl) on specific amino acid residues of target proteins [serine (S), tyrosine (Y), threonine (T), lysine (K)]; or 2) modifications involving addition of other proteins or polypeptides (e.g., sumoylation and ubiquitination).

Recently, many investigations have provided direct evidence for NR PTM in the pathophysiological progression of many diseases including cancers, diabetes, and obesity, among others. The majority of evidence linking NR PTMs with disease has been demonstrated for phosphorylation, sumoylation, ubiquitination and acetylation in the androgen receptor (AR), estrogen receptor (ER), glucocorticoid receptor (GR) and the peroxisome proliferator activated receptor γ (PPARγ). This report will be limited to a review of PTMs in ER, AR, GR and PPARγ and association with disease.

Androgen receptor

AR phosphorylation and prostate cancer

Advanced prostate cancer treatment has relied on hormone-deprivation therapy for the past 50 years. Response rates are initially high (70–80%); however, almost all patients relapse and develop hormone-refractory prostate cancer (HRPC), resulting in increased morbidity and death [McCall et al., 2008].

The majority of studies that demonstrate a relationship between AR phosphorylation and prostate cancer development have focused on the PI3K/Akt pathway (Figure 1). Studies in vitro demonstrate that the PI3K/Akt pathway is upregulated in HRPC and can result in phosphorylation of the AR. Akt is activated when phosphorylated at threonine 308 (T308), and subsequently serine 473 (S473), and these phosphorylations may play a similar role in the
Studies have shown that pAkt S473 is expressed in PIN (Prostatic Intraepithelial Neoplasia) and invasive prostate cancer with staining intensity positively correlated with PSA levels and Gleason grades [Altomare and Testa, 2005; Ghosh et al., 2003; Majumder and Sellers, 2005]. Increased phospho-Akt at S473 (pAkt S473) and phospho-AR S210 (pAR S210) was associated with decreased disease-specific survival [McCall et al., 2008]. In addition, phosphorylation of Akt and AR at S210 strongly correlated with HRPC [McCall et al., 2008] and HRPCs expressed significantly higher levels of pAR S210 compared to hormone-sensitive tumors [McCall et al., 2008].

Since upregulation of the PI3K/Akt pathway is associated with phosphorylation of AR during development of HRPC, Akt inhibitors are being developed as targeted therapeutics. Future clinical studies will determine whether activated Akt and/or phosphorylation of AR at S210 may be developed as predictive biomarkers for selecting patients who would respond to Akt inhibitors.

Studies in transgenic SBMA mouse models demonstrated that the reduction of serum androgen levels rescues the motor dysfunction and nuclear accumulation of mutant androgen receptors and improves survival of transgenic SBMA mice [Chevalier-Larsen et al., 2004; Katsuno et al., 2003a; Katsuno et al., 2003b]. Therefore, decreasing the effect of ligand on the AR could reduce disease progression. Furthermore, Palazzolo et al. demonstrated that in motor neuron-derived cells, phosphorylation of AR at the Akt consensus sites S215 and S792 reduced ligand-dependent nuclear translocation and toxicity of the expanded polyQ AR, as a consequence of reduced ligand binding [Palazzolo et al., 2007].

Leuprolrelin, a luteinizing hormone–releasing hormone agonist that reduces testosterone release from the testis and inhibits nuclear accumulation of mutant AR, rescued motor dysfunction in male transgenic mice carrying full-length mutant human AR with an expanded polyQ tract [Katsuno et al., 2003]. Phase II clinical trials of leuprolrelin suggested that androgen deprivation inhibited nuclear accumulation and/or stabilization of mutant AR in the motor neurons of the spinal cord and brainstem [Banno et al., 2009].

It is important to note that the phosphorylation sites in prostate cancer and SBMA are not different. AR may differ in length because of the polyQ and glycine repeats, but the most common size of AR is 919 amino acids. The Akt consensus phosphorylation sites of AR with 919 amino acid residues are S213 and S791 (Figure 1); these same sites were reported as S210 and S790 by McCall et al. [McCcall et al., 2008] and S215 and S792 by Palazzolo et al. [Palazzolo et al., 2007], although the sites are not different. The discrepancies in the phosphorylation sites mentioned in prostate cancer and SBMA might be due to the fact that AR differs in their length because of heterogeneity of the polyQ and glycine tracts.

**AR phosphorylation in spinal and bulbar muscular atrophy**

Spinal and bulbar muscular atrophy (SBMA; also known as Kennedy’s Disease) is a progressive neurodegenerative disease characterized by muscle weakness, muscle atrophy, and fasciculations. SBMA is caused by an abnormally high number of CAG trinucleotide repeats that encode for a stretch of uninterrupted polyglutamine amino acids in the AR known as a polyglutamine tract or polyQ tract [Mukherjee et al., 2009].

**AR sumoylation in prostate cancer and SBMA**

Sumoylation, the process of covalently attaching small ubiquitin-like modifiers to proteins, functions to regulate protein stability, protein–protein interactions, transcriptional activity, and subcellular localization [Hay, 2005; Seeler and Dejean, 2003]. The majority of identified SUMO substrates are active in transcription, RNA processing, DNA repair and chromatin remodeling (reviewed in [Karamouzis et al., 2008]). Androgen receptor was the first NR shown to be sumoylated [Poukka et al., 2000].

Sumoylation is mediated by activating, conjugating and ligating enzymes, and is reversed by a family of SUMO-specific proteases (SENPs); one member in particular, SENP1, was recently identified as a gene required for proliferation and survival of normal and prostate cancer cells [Schlabach et al., 2008]. Expression of SENP1 in prostate cancer cells increased the transcription activity of endogenous AR. Silencing of SENP1 attenuated the expression of several AR target genes and inhibited the androgen-stimulated growth of LNCaP cells (androgen-dependent prostate cancer cells).
Taken together, this suggests that SENP1 plays an important role in not only proliferation of prostate cancer cells, but in onset, as well [Bawa-Khalfe et al., 2007]. These data suggest the SUMO modification pathway as a potential target for prostate cancer therapy.

The two sumoylation sites of AR in human are K386 and K520 (Figure 2A). Cell culture studies have shown that sumoylation could reduce the formation of polyQ expanded AR aggregates without affecting the levels of the receptor, and the anti-aggregation effect by sumoylation was not dependent upon AR transcriptional activity [Mukherjee et al., 2009]. Chan et al. [Chan et al., 2002] reported that the Drosophila SBMA model with mutant polyQ AR exhibited a neurodegenerative phenotype.

**Figure 2.** Sumoylation and ubiquitination of the androgen receptor are involved in spinal and bulbar muscular atrophy. Spinal and bulbar muscular atrophy is caused by an abnormally high number of CAG trinucleotide repeats that encode for a stretch of uninterrupted polyglutamine amino acids in the AR known as a polyglutamine tract or polyQ tract. (A) Sumoylation of the androgen receptor has been shown to reduce aggregates of polyQ and occurs at lysine residues 386 and 520. (B) Overexpression of carboxyl terminus of Hsp70-interacting protein (CHIP), an ubiquitin E3 ligase, leads to ubiquitination of mutant AR, providing protection from SBMA.

Furthermore, expression of a catalytic-deficient mutant of the SUMO-1 activating enzyme Uba2 further exacerbated the polyQ AR-induced neurodegeneration [Chan et al., 2002; Dorval and Fraser, 2007]. In contrast, in other diseases associated with polyQ expanded regions in proteins such as the protein huntingtin in Huntington’s disease, it has been suggested that sumoylation intensifies the polyQ huntingtin-induced neurodegeneration in the Drosophila model [Steffan et al., 2004].

Taken together, sumoylation might have either beneficial or harmful effects, depending on the polyQ protein, and further studies using SBMA double transgenic mice with mutation at the sumoylation site of AR might provide more insight into the role of sumoylation in SBMA.

**AR ubiquitination and SBMA**

NRs are well known substrates for ubiquitination [Adachi et al., 2007], which is the covalent attachment of a peptide, ubiquitin, to lysine residues of a substrate protein. Similar to sumoylation, ubiquitination requires an activating enzyme (E1), a conjugating enzyme (E2), and an E3 ubiquitin ligase (Figure 2B). Although most NRs are ubiquitinated, discussion here will be limited to a review of ubiquitination of AR, ERα and PPARγ, since the ubiquitination of these NRs were clearly linked to human diseases.

Carboxyl terminus of Hsp70-interacting protein (CHIP) is an E2 ubiquitin ligase that interacts with heat shock proteins and chaperones to degrade toxic and misfolded proteins [McDonough and Patterson, 2003]. Double transgenic mice which overexpressed CHIP and mutant AR demonstrated that mutant AR protein was ubiquitinated and degraded by CHIP and the proteosomal system, which resulted in protection of mice from SBMA [Adachi et al., 2007]. Moreover, other neurodegenerative diseases that are caused by polyQ expansion of proteins ataxin-1 (type 1 spinocerebellar ataxia) and Huntingtin were also benefited by ubiquitination and proteosomal degradation [Al-Ramahi et al., 2006; Jana et al., 2005].

Overall, ubiquitination has been shown to play a vital role in protecting neuronal cells against the toxic properties of polyQ expanded proteins.

**AR acetylation and prostate cancer**

Acetylation of AR is in response to physiological stimuli including DHT (dihydrotestosterone) and bombesin enhanced AR transactivation function at a subset of target promoters [Fu et al., 2004]. In particular cell cycle control genes that induce cellular proliferation [Gong et al., 2006; Popov et al., 2007], which is the covalent attachment of a peptide, ubiquitin, to lysine residues of a substrate protein. Direct acetylation of AR at the highly conserved KKKK/RKXX lysine motif (lysine 630, 632 and 633), is mediated by coactivators p300, P/CAF and TIP60 (Tat-interactive protein) that possess intrinsic histone acetyltransferase (HAT) activity (Figure 3A) [Fu et al., 2003; Fu et al., 2000; Gaughan et al., 2002].

The proliferation of prostate cancer cell lines stably expressing the AR acetylation mimic mutants [AR lysine 630 to glutamine (AR K630Q/lysine 630 to threonine (AR K630T))] (Figure 3B) was increased in the presence of DHT compared to the wild-type AR. These acetylation-mimicking mutations in prostate cancer cells exhibited increased cell growth in vitro and showed increased tumor growth in vivo, and these tumors were resistant to the AR antagonist, flutamide [Fu et al., 2003].

Although these studies suggest that there is a link between AR acetylation and prostate cancer, specific experiments to test this have not yet been reported.
Antibodies to p-S118 and p-S167 on ER became available, the opportunity arose to test specific for phosphorylated amino acids within the ER survival (RFS) and overall survival (OS) in response, and to clinical outcomes such as release free samples to known biomarkers of prognosis and treatment investigating relationships of gene expression in tumor lines and xenograft models supported this hypothesis [Arpino et al., 2008]. Results from several cell signaling and the development of resistance to endocrine therapies [Arpino et al., 2008]. When it was found that ER generated at the plasma membrane by growth factor could be directly phosphorylated by several kinases [Lannigan, 2003]. When it was found that ER could be activated, similar to other steroid hormone receptors, can be phosphorylated at multiple sites within the protein [Murphy et al., 2011] (Figure 1).

In addition to estrogenic ligands, ER can be activated in a ligand-independent fashion, often by signals generated at the plasma membrane by growth factor receptors [Lannigan, 2003]. When it was found that ER could be directly phosphorylated by several kinases activated by membrane growth factor receptor signaling [Kato et al., 1995], the possibility arose of crosstalk between ERFs and growth factor receptor signaling, which could underlie ligand-independent activation of ER signaling and the development of resistance to endocrine therapies [Arpino et al., 2008]. Results from several cell line and xenograft models supported this hypothesis [Arpino et al., 2008].

One way to gain insights into the putative role of a gene in human breast cancer in vivo is frequently obtained by investigating relationships of gene expression in tumor samples to known biomarkers of prognosis and treatment response, and to clinical outcomes such as release free survival (RFS) and overall survival (OS) in retrospectively-collected patient cohorts. When antibodies specific for phosphorylated amino acids within the ER became available, the opportunity arose to test experimental hypotheses in human breast tumors in vivo.

Antibodies to p-S118 and p-S167 on ER have been commercially available since 2003. Our laboratory was the first to determine expression of p-S118 - ER in multiple breast cancer cases stored in the Manitoba Breast Tumor Bank (MBTB) [Skliris et al., 2009]. Subsequently, additional studies were published demonstrating the expression of one or both of these p-ERα sites by IHC in cohorts of breast tumor biopsy samples [Holm et al., 2009; Jiang et al., 2007; Murphy et al., 2004; Skliris et al., 2009].

More recently, detection of other phosphorylated sites on ERα has been published by our laboratory and others [Atrysiku et al., 2009; Britton et al., 2008; Williams et al., 2009]. In some of these studies, associations with other histopathological markers, clinical outcome and specific kinases were found [Jiang et al., 2007; Skliris et al., 2010]. Although sometimes contradictory results have been found, some common themes have emerged, especially with regard to p-S118 and p-S167, which have been the major focus to date.

Firstly, and in contrast to what was expected, detection of p-S118- and/or p-S167-ERα in breast tumors is generally associated with features of an intact estrogen responsive signaling pathway [Jiang et al., 2007; Murphy et al., 2004] and therefore more differentiated phenotypes. These data suggested that the detection of either p-S118- and/or p-S118-ERα may be surrogate markers for tumors that are more dependent on ERα signaling for growth, and are therefore more likely to respond to tamoxifen (Tam) therapy.

Indeed, a second emerging theme is that those patients whose tumors express p-S118- and/or p-S167-ERα more often have a better clinical outcome to Tam therapy in retrospective studies [Jiang et al., 2007; Murphy et al., 2004; Yamashita et al., 2005; Yamashita et al., 2008].

More recently, detection of p-S118-ERα in breast tumors was also associated with a better clinical outcome to aromatase inhibitors [Generali et al., 2009]. In our study, we found that measurement of progesterone receptor (PR), either by IHC or LBA (ligand binding assay) [Skliris et al., 2009; Weitsman et al., 2006], was still a stronger predictor of RFS and OS than the p-S118-ERα for patients on tamoxifen. Interestingly, however, we also found that addition of p-S118-ERα to PR further improved the prediction of response to endocrine therapy [Murphy et al., 2004]. Such data support combined use of biologically-relevant markers for the improved prediction of therapy response, at least with respect to endocrine therapy.

Although some studies find a weak positive association of ERBB2/HER2 expression and p-S118 [Jiang et al., 2007; Yamashita et al., 2008], we and others have found no significant differences in the expression of p-S118 and/or p-S167 in ER+ tumors that either overexpress or do not express ERBB2/HER2 [Jiang et al., 2007; Weitsman et al., 2006].

The data published so far therefore do not strongly support the hypotheses that ligand-independent activation of ERα by phosphorylation due to overexpression of

---

**Estrogen receptor α**

**Clinical relevance of phosphorylated forms of ERα in human breast cancer**

The estrogen receptor α (ERα) status of breast tumors has been used clinically since the late 1970s to identify those patients most likely to gain benefit from endocrine therapies. However, ERα status is an imperfect marker of endocrine therapy response and a current research priority is the search for more precise markers of treatment response and outcome. ERα, similar to other steroid hormone receptors, can be phosphorylated at multiple sites within the protein [Murphy et al., 2011] (Figure 1).

In addition to estrogenic ligands, ERα can be activated in a ligand-independent fashion, often by signals generated at the plasma membrane by growth factor receptors [Lannigan, 2003]. When it was found that ERα could be directly phosphorylated by several kinases activated by membrane growth factor receptor signaling [Kato et al., 1995], the possibility arose of crosstalk between ERFs and growth factor receptor signaling, which could underlie ligand-independent activation of ERα signaling and the development of resistance to endocrine therapies [Arpino et al., 2008]. Results from several cell line and xenograft models supported this hypothesis [Arpino et al., 2008].

One way to gain insights into the putative role of a gene in human breast cancer in vivo is frequently obtained by investigating relationships of gene expression in tumor samples to known biomarkers of prognosis and treatment response, and to clinical outcomes such as release free survival (RFS) and overall survival (OS) in retrospectively-collected patient cohorts. When antibodies specific for phosphorylated amino acids within the ERα became available, the opportunity arose to test experimental hypotheses in human breast tumors in vivo.

Antibodies to p-S118 and p-S167 on ERα have been commercially available since 2003. Our laboratory was the first to determine expression of p-S118 - ERα in multiple breast cancer cases stored in the Manitoba Breast Tumor Bank (MBTB) [Skliris et al., 2009]. Subsequently, additional studies were published demonstrating the expression of one or both of these p-ERα sites by IHC in cohorts of breast tumor biopsy samples [Holm et al., 2009; Jiang et al., 2007; Murphy et al., 2004; Skliris et al., 2009].

More recently, detection of other phosphorylated sites on ERα has been published by our laboratory and others [Atrysiku et al., 2009; Britton et al., 2008; Williams et al., 2009]. In some of these studies, associations with other histopathological markers, clinical outcome and specific kinases were found [Jiang et al., 2007; Skliris et al., 2010]. Although sometimes contradictory results have been found, some common themes have emerged, especially with regard to p-S118 and p-S167, which have been the major focus to date.

Firstly, and in contrast to what was expected, detection of p-S118- and/or p-S167-ERα in breast tumors is generally associated with features of an intact estrogen responsive signaling pathway [Jiang et al., 2007; Murphy et al., 2004] and therefore more differentiated phenotypes. These data suggested that the detection of either p-S118- and/or p-S118-ERα may be surrogate markers for tumors that are more dependent on ERα signaling for growth, and are therefore more likely to respond to tamoxifen (Tam) therapy.

Indeed, a second emerging theme is that those patients whose tumors express p-S118- and/or p-S167-ERα more often have a better clinical outcome to Tam therapy in retrospective studies [Jiang et al., 2007; Murphy et al., 2004; Yamashita et al., 2005; Yamashita et al., 2008].

More recently, detection of p-S118-ERα in breast tumors was also associated with a better clinical outcome to aromatase inhibitors [Generali et al., 2009]. In our study, we found that measurement of progesterone receptor (PR), either by IHC or LBA (ligand binding assay) [Skliris et al., 2009; Weitsman et al., 2006], was still a stronger predictor of RFS and OS than the p-S118-ERα for patients on tamoxifen. Interestingly, however, we also found that addition of p-S118-ERα to PR further improved the prediction of response to endocrine therapy [Murphy et al., 2004]. Such data support combined use of biologically-relevant markers for the improved prediction of therapy response, at least with respect to endocrine therapy.

Although some studies find a weak positive association of ERBB2/HER2 expression and p-S118 [Jiang et al., 2007; Yamashita et al., 2008], we and others have found no significant differences in the expression of p-S118 and/or p-S167 in ER+ tumors that either overexpress or do not express ERBB2/HER2 [Jiang et al., 2007; Weitsman et al., 2006].

The data published so far therefore do not strongly support the hypotheses that ligand-independent activation of ERα by phosphorylation due to overexpression of...
growth factor receptors such as HER2 is a mechanism of de novo tamoxifen and possibly aromatase inhibitor resistance in human breast cancer in vivo.

More recently, antibodies to other phosphorylation sites on ERα, i.e., p-S104/106, p-S282, p-S294, p-T311, p-S559, have been validated for IHC of formalin-fixed paraffin-embedded breast tumor tissues [Skiriris et al., 2009] and the results obtained support a third emerging theme that multiple phosphorylation forms of ERα can be detected in any one tumor sample [Jiang et al., 2007; Skiriris et al., 2009; Yamashita et al., 2008]. The relationship of these other phosphorylation sites to known biomarkers and response to endocrine therapies is currently unclear.

In contrast to the results for p-S118 and/or p-S167, detection of p-S305 in ERα in breast tumors from pre-menopausal women who had received tamoxifen for 2 years was associated with no benefit from tamoxifen [Holm et al., 2009]. Furthermore, experimental studies support a role for phosphorylation at S305 in tamoxifen resistance [Michalides et al., 2004], suggesting that not all types of phosphorylation of ERα predict a good outcome to endocrine therapies.

It is interesting that those phosphorylation sites that are so far associated with better clinical outcome to endocrine therapies are located in the N-terminus of the protein and those identified to be associated with poorer outcome (S305, T311, and S559 [Skiriris et al., 2010]), are located in a region at the border of the hinge region and the ligand binding domain.

Interestingly, S305 is adjacent to an acetylation site (K303) that has been implicated in modulating sensitivity to E2 [Fuqua et al., 2000] and regulating the efficiency of S305 as a substrate for PKA [Cui et al., 2004]. Inhibition of acetylation at this site by mutation of K303 to R resulted in reduced sensitivity to tamoxifen [Giordano et al., 2010] and aromatase inhibitors [Barone et al., 2010]. There is precedence for coupling regulation between phosphorylation and acetylation in adjacent areas of proteins and effects on protein function [Kramer et al., 2009].

ERα acetylation and breast cancer

Acetylation of ERα requires the interaction of ERα with coregulators, such as p300/CREB, that have intrinsic histone acetyltransferase (HAT) activity [Wang et al., 2001] and/or recruit HATs. ERα is selectively acetylated on lysine residues 299, 302 and 303 by p300 within the well-conserved hinge/ligand binding domain (Figure 4A) [Wang et al., 2001].

The mutation of lysine residues 302 or 303 to arginine (rendering these sites incapable of being acetylated) (Figure 4B) resulted in hypersensitivity to estradiol that led to increased estradiol-dependent activation of ERα, suggesting that ERα acetylation normally suppresses ligand sensitivity [Fuqua et al., 2000; Wang et al., 2001]. Additionally, independent clinical studies have identified a Lys-to-Arg substitution at nucleotide 908 (referred to as K303R) in 34% of atypical breast hyperplasia samples [Conway et al., 2005; Fuqua et al., 2000; Herynk et al., 2007]. A K303R mutation enhanced cellular proliferation in response to low concentrations of estradiol, suggesting the ERα K303R mutation provides a “gain of function” mutation in human breast cancer.

Furthermore, Conway et al. [Conway et al., 2005] reported similar mutations in 5.7% of screened breast tumors, with more frequent occurrences in high grade breast tumors and in mixed lobular/ductal tumors, compared with ductal carcinomas.

In another study, ~50% of breast cancer samples contained K303R mutations [Herynk et al., 2007], and in this cohort of patients, women over the age of 50 had more frequent mutations when compared to lower age groups (54.4% versus 37.5%). Moreover, a higher frequency of mutation was found in lymph node positive compared to negative tumors (70% versus 34.8%) [Herynk et al., 2007].

Figure 4. Acetylation of estrogen receptor α. (A) Acetylation of the estrogen receptor α requires the interaction of the receptor and of coregulators such as p300 that possess intrinsic histone acetyltransferase (HAT) activity. (B) Mutation of lysine residues 302/303 to arginine, rendering these sites incapable of being acetylated, resulted in hypersensitivity to estradiol in breast cancer cell lines.

ERα sumoylation and breast cancer

Sumoylation of ERα occurs through the modification of lysine residue(s) in the AF-1 domain [Choi et al., 2006]. Two ligand-dependent sumoylation sites, lysine 266 and 268 (K266 and K268), have been identified in the ERα hinge region that can result in increased ERα target gene expression in vitro (Figure 5A) [Sentis et al., 2005]. The hinge region of ERα is a specific target of SUMO-E3 ligases (catalyzes the covalent attachment of a SUMO), PIAS1 (Protein inhibitor of Activated STAT), and PIAS3 (Figure 5A) [Karamouzis et al., 2008]. An increase in expression of PIAS3 has been documented in breast cancer [Wang and Banerjee, 2004].

Future clinical studies are needed to determine whether ERα sumoylation in breast cancer has prognostic value or serves as a predictor for response to endocrine therapy.
Ubiquitination and ERα

The stability of ERα is affected differentially by agonists, antagonists, and selective estrogen receptor modulators [Reid et al., 2002; Wijayaratne and McDonnell, 2001]. Both ligand-stimulated, as well as unstimulated ERα, have been shown to be ubiquitinated and degraded by the proteasomal system (Figure 5B) [Tateishi et al., 2004].

Studies performed by the Gannon and O’Malley laboratories showed that the proteasomal degradation of transcription factors is a necessary step in the regulation of target gene transcription, possibly by enabling the sequential formation of protein complexes at the promoter region [Lonard et al., 2000; Reid et al., 2002]. Stable, unliganded ERα, which has a half-life of up to 5 days [Nirmala and Thampan, 1995], is ubiquitinated and degraded by carboxyl terminus of Hsp70-interacting protein (CHIP), an ubiquitin E3 ligase. Fulvestrant (ICI 182,780), a pure anti-estrogen, utilizes the ubiquitination mechanism to induce proteasome-dependent degradation of ERα.

Fulvestrant (ICI 182,780), a pure anti-estrogen that is currently used in adjuvant therapies for breast cancer, utilizes the ubiquitination mechanism to induce proteasome-dependent degradation of ERα [Lanvin et al., 2007]. Degradation of ERα induced by fulvestrant is independent of its transcriptional activity and new protein synthesis [Marsaud et al., 2003; Wijayaratne and McDonnell, 2001].

Glucocorticoid receptor

Anti-inflammatory effect of GR

Treatment with glucocorticoids is one of the most effective therapies for many chronic inflammatory diseases such as asthma, rheumatoid arthritis, and inflammatory bowel disease [Adcock and Ito, 2000]. Glucocorticoids bind to the cytosolic GR, which then translocates to the nucleus. GR can induce transcription of anti-inflammatory genes, such as secretory leukocyte proteinase inhibitor (SLPI) [Abbinante-Nissen et al., 1995] and mitogen-activated kinase phosphatase-1 (MKP-1) [Lasa et al., 2002], or inhibit transcription of pro-inflammatory genes such as interleukin-6 (IL-6) (Figure 6) [Ray et al., 1994]. GR also reduces inflammatory gene expression induced by NF-κB (Nuclear Factor-κB), AP-1 (Activator Protein-1) or via trans-repression through direct protein interaction with these proteins (reviewed in [De Bosscher et al., 2003]).

Figure 5. Estrogen receptor α sumoylation and ubiquitination. (A) Sumoylation of ERα occurs at lysine residues 266 and 268 (K266 and K268). (B) Unstimulated estrogen receptor is ubiquitinated and degraded by carboxyl terminus of Hsp70-interacting protein (CHIP), an ubiquitin E3 ligase. Fulvestrant (ICI 182,780), a pure anti-estrogen, utilizes the ubiquitination mechanism to induce proteasome-dependent degradation of ERα.

Figure 6. GR regulation of inflammatory genes. Upon ligand binding, the GR translocates to the nucleus, is acetylated by HATs, and binds to Glucocorticoid Response Elements (GRE) to induce transcription of anti-inflammatory genes. The liganded GR can also functionally repress NF-κB-mediated pro-inflammatory genes.

GR phosphorylation in inflammation and asthma in humans

As previously stated, glucocorticoids are used as primary treatment options for many inflammatory diseases; however, some patients are unresponsive to these treatments due to glucocorticoid resistance. Among the mechanisms for this resistance is decreased glucocorticoid signaling by changes in GR phosphorylation [Bloom, 2004; Li et al., 2004; Tsioura and Rothman, 2004]. GR is phosphorylated by cyclin-dependent kinases, MAPK and casein kinase II at 5 serine residues, S113, S141, S203, S211 and S226 [Ismaili and Garabedian, 2004]. p38 MAP kinase has been shown to be active in alveolar macrophages of asthmatic patients who showed poor response to glucocorticoids in comparison to patients with a normal response [Bhavsar et al., 2008]. The phosphorylation of GR by p38 MAPK subsequently affects GR function.
resulting in reduced responsiveness to glucocorticoid treatment. The serine residue of GR phosphorylated by p38 MAP kinase has yet to be elucidated; it is plausible that S211 or S226 could be phosphorylated [Irussen et al., 2002; Miller et al., 2005; Szatmary et al., 2004]. Use of p38 MAPK inhibitors increased the anti-inflammatory effects of glucocorticoids and reestablished the beneficial effects of glucocorticoids in glucocorticoid-resistant patients suffering from asthma [Irussen et al., 2002]. Studies have also shown that a large proportion of patients with glucocorticoid-resistant asthma showed reduced nuclear translocation of GR and DNA binding in PBMCs (Peripheral Blood Mononuclear Cells) after glucocorticoid exposure, and this could be due to changes in glucocorticoid receptor phosphorylation [Matthews et al., 2004; Szatmary et al., 2004].

Together, these studies suggest GR phosphorylation by p38 MAPK is one of the mechanisms leading to glucocorticoid resistance. Clinical studies are needed to determine if the clinical benefit of p38 MAPK inhibitors in asthmatic patients is related to changes in GR phosphorylation.

**GR acetylation increases glucocorticoid insensitivity**

Lysine 494 and 495 are the two ligand-dependent acetylation sites identified in GR [Ito et al., 2006]. GR binds to NF-κB and represses inflammatory gene transcription by NF-κB. Acetylated GR loses its affinity for NF-κB and therefore cannot repress inflammatory gene transcription induced by NF-κB (Figure 6). Histone deacetylase 2 (HDAC2) deacetylates GR, thereby enabling the association of GR with NF-κB and attenuation of pro-inflammatory gene transcription. Silencing HDAC2 by RNA interference resulted in reduced corticosteroid-sensitivity in primary alveolar macrophages and human lung epithelial cell lines.

Overexpression of HDAC2 in glucocorticoid-resistant alveolar macrophages from patients with COPD (chronic obstructive pulmonary disease) resulted in restoration of corticosteroid-sensitivity [Ito et al., 2006]. Taken together, acetylated GR could not inhibit NF-κB-dependent gene transcription, due to the reduced binding to NF-κB. It is plausible that hyperacetylation of GR, due to absence of HDAC2, may lead to glucocorticoid insensitivity.

**Peroxisome proliferator-activated receptors**

Peroxisome proliferator-activated receptors (PPAR) play an important role in the regulation of lipid homeostasis, energy metabolism, inflammatory responses and the induction of apoptosis [Issemann and Green, 1990; Rosen and Spiegelman, 2006]. The PPAR family consists of three subtypes, PPARα, PPARδ and PPARγ, encoded by separate genes, and among these subtypes, PPARγ has been studied extensively. The PPARs form heterodimers with the retinoid-X receptor (RXR), which then binds to response elements (PPRE) in the regulatory regions of target genes [Mangelsdorf and Evans, 1995].

**PPAR phosphorylation and insulin resistance and obesity**

Adipocyte differentiation is an important component of obesity and other metabolic diseases and this process is inhibited by mitogens and oncogenes. Several growth factors that inhibit fat cell differentiation cause MAPK-mediated phosphorylation of PPARγ and reduce its transcriptional activity. Growth factor-induced phosphorylation of S112 of PPARγ repressed its transcriptional and adipogenic functions [van Beekum et al., 2009], whereas expression of PPARγ with a non-phosphorylatable mutation at serine-112 (S112) yielded cells with increased sensitivity to ligand-induced adipogenesis and resistance to inhibition of differentiation by mitogens, as compared to cells expressing wild type PPARγ (Figure 1) [Hu et al., 1996; Iwata et al., 2001; Ristow et al., 1998]. Homozygous PPARγS112A knock-in mice did not develop insulin resistance and exhibited no change in body weight when fed a high fat diet [Rangwala et al., 2003]. These findings indicate that phosphorylation of PPARγ by growth factors is a major regulator of the balance between cell growth and differentiation in the adipose tissue.

A mutation of proline 115 to glutamine (P115Q) in PPARγ prevents phosphorylation at S112 in PPARγP115Q [van Beekum et al., 2009], would remove a canonical Ser-Pro phosphorylation motif at S112 and consequently prevent phosphorylation by Ser-Pro directed kinases. It is likely that the P115Q mutation and loss of S112 phosphorylation could lead to an increase in adipogenesis and possibly obesity and insulin resistance in humans. A heterozygous P115Q mutation was reported in four obese individuals in a cohort of 358 [van Beekum et al., 2009]. Three of these obese individuals (BMI 37.9-47.3) were diagnosed with type 2 diabetes. Ristow et al. reported the same mutation associated with obesity without type 2 diabetes [Ristow et al., 1998]. Blühner and Paschke [Bluher and Paschke, 2003] reported an individual with the P115Q mutation who exhibited high fasting insulin levels and profound insulin resistance. However, a German cohort of 85 [Hamer et al., 2002] and 67 [Evans et al., 2000] did not find an association of P115Q with obesity. Similarly, in a French cohort of 626, P115Q was not associated with obesity [Clement et al., 2000].

Additional studies on homozygous and heterozygous S112A and P115Q PPARγ mutations in different genetic backgrounds and under different dietary regimes will help to understand the role of PPARγ phosphorylation in obesity.

**Cdk5-mediated phosphorylation of PPARγ may be involved in the pathogenesis of insulin-resistance**

Cdk5 has been shown to phosphorylate PPARγ at Serine 273 (S273). Phosphorylation of S273 by Cdk5 did not impact the general transcriptional activity of PPARγ, but did alter the expression of specific genes such as adiponectin (insulin sensitizing hormone) [Choi et al.,...
Cdk5-mediated phosphorylation of PPARγ has been linked to obesity in mice induced by high-fat diet [Rangwala et al., 2003]. Anti-diabetic PPARγ ligands such as rosiglitazone and MRL24 directly inhibited S273 phosphorylation by Cdk5 without affecting S112 phosphorylation, resulting in restoration of a non-diabetic pattern of gene expression [Choi et al., 2010]. Moreover, inhibition of S273 phosphorylation by rosiglitazone in humans was associated with the anti-diabetic effects of the drug [Choi et al., 2010]. Further studies relating PPARγ phosphorylation to diabetes may offer the opportunity for development of improved next-generation anti-diabetic drugs.

**Sumoylation of PPARγ results in transrepression of inflammatory genes**

PPARγ has been demonstrated to have an anti-inflammatory effect and PPARγ ligands have been used in clinical trials for patients with inflammatory diseases [Giaginis et al., 2009; Spears et al., 2006]. The mechanism for the anti-inflammatory effect of PPARγ ligands may include sumoylation of the receptor (Figure 7). Expressed in macrophages, PPARγ functions to negatively regulate macrophage activation by repressing a subset of AP1 and NF-κB-dependent genes, ultimately repressing macrophage inflammatory genes [Jennewein et al., 2008; Pascual et al., 2005]. One mechanism regulating PPARγ inhibition of inflammatory genes is sumoylation of PPARγ at its consensus sumoylation sites, lysines 77 and 365 [Pascual et al., 2005]. The initial step in the sumoylation pathway involves ligand-dependent sumoylation of the PPARγ ligand-binding domain, that targets PPARγ for binding to nuclear receptor corepressor (NCOR)/histone deacetylase-3 (HDAC3) complexes at inflammatory gene promoters [Pascual et al., 2005]. This in turn, prevents recruitment of the ubiquitinylating/19S proteasome machinery that normally mediates the signal-dependent removal of corepressor complexes required for gene activation [Pascual et al., 2005]. As a result, NCOR complexes are not cleared from the promoter and target genes are maintained in a repressed state [Pascual et al., 2005]. Recent studies have demonstrated that PPARγ inhibits inflammatory gene expression in activated macrophages by the NCOR/sumoylation-dependent pathway [Ghisletti et al., 2007; Jennewein et al., 2008; Pascual et al., 2007].

**Ubiquitination and PPARγ**

The PPAR-γ protein has a short half-life of 2 hours [Christianson et al., 2008; Waite et al., 2001] and was found to be polyubiquitinated and degraded by the proteasome [Floyd and Stephens, 2002; Hauser et al., 2000]. Ubiquitination of PPAR-γ and degradation are strongly linked to ligand binding and activation, as TZDs (e.g., troglitazone, rosiglitazone) accelerated these processes [Floyd and Stephens, 2002; Hauser et al., 2000]. The exposure of adipocytes to the cytokine interferon-γ elicited by infiltrating interferon-γ-producing lymphocytes into adipose tissue [Kintscher et al., 2008], was also found to increase ubiquitination and degradation of PPAR-γ [Floyd and Stephens, 2002], indicating that ubiquitination can also be regulated by external stimuli. Studies by [Kubota et al., 1999; Miles et al., 2000] have shown that reduced PPARγ expression in mice (PPARγ−/−) is associated with resistance to weight gain along with protection from the insulin resistance that typically accompanies weight gain. In addition, genetic evidence indicates that decreased PPARγ activity may protect against insulin resistance in humans [Deeb et al., 1998]. Conversely, PPARγ is required for the formation of fat cells, and a lack of adipose cells is associated with insulin resistance and hyperglycemia [Willson et al., 2001]. All of these studies indicate that a careful balance between PPARγ expression and activity levels must be maintained to avoid development of diseases such as type II diabetes and obesity. The ubiquitin-proteasome pathway plays an important role in the regulation of PPARγ levels in adipocytes [Hauser et al., 2000].

**Interactions of PTM in human disease**

A complex network of signaling pathways does not rely only on phosphorylation or acetylation or any single PTM, rather they are likely controlled by the coordinated actions of phosphorylation, acetylation and a myriad of combinations of PTMs. One or more PTMs, or the same PTM at different residues, can occur on a protein. Multiple PTMs may occur simultaneously or sequentially that would be necessary for the distinct outcome of signaling pathways. Although the implications of PTMs such as phosphorylation and acetylation have been relatively well documented, interactions among these PTMs remain fertile ground for future investigations. Both positive and negative crosstalk occurs among NRs. In positive crosstalk, the first PTM serves as a signal for the addition or removal of another PTM, or for the recognition by a protein that induces a second modification. Phosphorylation-dependent ubiquitination and phosphorylation-dependent sumoylation are good examples. It is intriguing to note that phosphorylation of NRs has been linked to ubiquitination and protein turnover. A direct link between ERα phosphorylation and ubiquitination has been demonstrated by Alarid and colleagues [Valley et al., 2005]. Phosphorylation of ERα at S118 plays a role in estradiol-mediated ERα degradation by regulating recruitment of factors mediating ubiquitination [Valley et al., 2005]. Phosphorylation of ERα by P21 activated Kinase (PAK1) at ERα S305 influences the activation status of ER S118 and the
S305-associated ERα transactivation activity requires a functional S118. Transgenic mice expressing active PAK1 exhibited both activated ERα-305 and ERα-S118 phosphorylation (for a review of crosstalk of ER PTMs, see [Le Romancer et al., 2011]). Phosphorylation at AR S210 and S790 by Akt has been shown to promote AR ubiquitination and its subsequent proteosomal degradation [Lin et al., 2002]. Mutation at phosphorylation sites of GR (Serine 122, 150, 212, 220, 234, 315, 412, and threonine 159 to alanine) inhibited ligand-dependent GR degradation and consequently, GR-mediated transcriptional activity [Wallace and Cidlowski, 2001; Webster et al., 1997], which indicated yet another NR ubiquitination that is phosphorylation-dependent. A hypo-phosphorylated form of PPARγ that increased transcriptional activity was degraded faster compared to a phosphorylated form (S112), indicating that in this NR, phosphorylation inhibited ubiquitination and protein degradation [Floyd and Stephens, 2002]. Additionally, phosphorylation of PPARγ at S112 stimulated sumoylation at K107 and this was shown to reduce the ability of PPARγ to activate the adipogenic gene expression pathway.

In negative crosstalk, there is direct competition for modification of a single amino acid residue in a protein, or one modification masks the recognition site for the second PTM. In ERα, lysine residue K302 can be modified by ubiquitination, sumoylation, acetylation, or methylation. Since lysine is the target for ubiquitin enzymes, acetylation and methylation status might affect the protein turnover. ERα phosphorylation at S305 has been shown to inhibit acetylation of ERα at K303 [Cui et al., 2004]. A phosphomimicking mutation at S305 to E305 blocked K303 acetylation and produced an enhanced transcriptional response. However, all these interactions among different PTMs have yet to be analyzed in clinical studies.

It is important to note the caveats associated with evaluation of PTMs in nuclear receptors. PTMs are both dynamic and reversible processes that can be rapidly altered in response to changes in the cellular and environmental conditions that occur following tissue excision, including time to preserve or fix harvested tissue samples from patient biopsies or resection samples. It should be noted that the quality of the tissue and labile modifications, most notably phosphorylation, can be dramatically affected by pre-analytical variables (i.e., ischemic time, hypoxia, temperature or fixation time, exposure of patients to anesthetics and other drugs [Pinhel et al., 2010; Siddiqui and Rimm, 2010]). Alterations in phosphorylation and sumoylation status can occur as a result of ischemic and hypoxic conditions due to the change in the activities of endogenous phosphatases, kinases and sumo ligases and proteases during sample collection [Ahmed and Gardiner, 2011; Cimarosti et al., 2008]. Developing common tissue procurement guidelines to collect and store the clinical samples for the analysis of protein PTMs will avoid the alteration in the PTMs by other factors. All these caveats should be accommodated for when evaluating and interpreting results of PTMs in NRs from clinical samples.

Summary and future perspectives

This review has summarized basic and clinical research studies that link specific NR PTMs to a wide range of human diseases. In many cases, perturbation of a PTM can be associated with a change in receptor transcriptional function that underlies a pathological consequence in a tissue or organ system. Ongoing clinical studies will likely identify additional NR PTMs that are associated with disease progression and resistance to standard of care therapies that target NRs. Additionally, the development of more stringent in vivo models will also aid in better understanding of the mechanisms by which NR PTMs impact physiological processes and disease. To date, much of the in vivo research on NR PTMs has been conducted using transgenic mouse models. While these models have provided valuable insight into the functioning of PTMs, the creation of mouse models in which specific PTMs are altered in the endogenous NR will be crucial. Examination of the effects of PTMs on gene expression and chromatin interaction is another important avenue of research. As previously mentioned, the vast majority of NR PTMs ultimately affect transcriptional activity and determining the effect of PTMs on systemic gene expression will not only provide insight into the global effects of aberrant PTMs associated with disease, but can also indicate potential gene targets for the development of targeted therapeutics. Finally, research examining the role of PTMs of NR in disease must also evaluate the actual addition of PTMs and determining what role PTM turnover plays in disease pathophysiological progression. Current research has focused on PTMs that result in the addition of specific phosphate groups, ubiquitin tags, sumo proteins, etc. However, there appears to be a dearth of information regarding PTM turnover. As has been elegantly demonstrated for ERα phosphorylation in breast cancer, future studies will firmly establish NR PTMs as valuable surrogate measures of NR function, that may also be used as biomarkers for disease progression, as well as predictive markers for patient response to NR-directed therapies.

References

Abbinante-Nissen, J. M., Simpson, L. G. and Leikauf, G. D. (1995) Corticosteroids increase secretory leukocyte protease inhibitor transcript levels in airway epithelial cells *Am J Respir Cell Mol Biol* **28**, L601-6.

Adachi, H., Waza, M., Tokui, K., Katsuino, M., Minamiyama, M., Tanaka, F., Doyu, M. and Sobue, G. (2007) CHIP overexpression reduces mutant androgen receptor protein and ameliorates phenotypes of the spinal and bulbar muscular atrophy transgenic mouse model *J Neurosci* **27**, 5115-26.

Adcock, I. M. and Itó, K. (2000) Molecular mechanisms of corticosteroid actions *Monatsh Arch Chem D* **55**, 256-86.

Ahmed, M. M. and Gardiner, K. J. (2011) Preserving protein profiles in tissue samples: differing outcomes with and without heat stabilization *J Neurosci Methods* **196**, 99-106.

Al-Ramahi, I., Lam, Y. C., Chen, H. K., de Gouyon, B., Zhang, M., Perez, A. M., Branco, J., de Haro, M., Patterson, C., Zoghbi, H. Y. and Botas, J. (2006) CHIP protects from the neurotoxicity of expanded and wild-type...
Stearoyl-CoA desaturase 2 is required for peroxisome proliferator-activated receptor gamma expression and adipogenesis in cultured 3T3-L1 cells J Biol Chem 283, 2306-14.

Cimarosti, H., Lindberg, C., Bornfelt, S. F., Ronn, L. C. and Henley, J. M. (2008) Increased protein SUMOylation following focal cerebral ischemia Neuropharmacology 54, 280-9.

Clement, K., Herberg, S., Passinge, B., Galan, P., Varroud-Vial, M., Shulidier, A. R., Beamer, B. A., Charpentier, G., Guy-Grand, B., Foguel, P. and Vaisse, C. (2000) The Pro115Gln and Pro12Ala PPAR gamma mutations in obesity and type 2 diabetes Int J Obes Relat Metab Disord 24, 391-3.

Conway, K., Parrish, E., Edmiston, S. N., Tolbert, D., Tse, C. K., Geradts, J., Livasy, C. A., Singh, H., Newman, B. and Millikan, R. C. (2005) The estrogen receptor-alpha A908G (K303R) mutation occurs at a low frequency in invasive breast tumors: results from a population-based study Breast Cancer Res 7, R871-80.

Cui, Y., Zhang, M., Pestell, R., Curran, E. M., Welschon, W. V. and Fuqua, S. A. (2004) Phosphorylation of estrogen receptor alpha blocks its acetylation and regulates estrogen sensitivity Cancer Res 64, 9199-208.

De Bosscher, K., Vanden Berge, W. and Haegeerman, G. (2003) The interplay between the glucocticotoid receptor and nuclear factor-kappaB or activator protein-1: molecular mechanisms for gene repression Endocrin Rev 24, 488-522.

Deeb, S. S., Fajas, L., Nemoto, M., Phiajiama, M., Mykkanen, L., Kuisisto, J., Laakso, M., Fujimoto, W. and Auwero, J. (1998) A Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity Nat Genet 20, 288-7.

Dorval, V. and Fraser, P. E. (2007) SUMO on the road to neurodegeneration Biochim Biophys Acta 1773, 694-706.

Evans, D., Mann, W. A., de Heer, J., Michel, U., Wendt, D., Kortner, B., Wolf, A. and Beisiegel, U. (2000) Variation in the gene for human peroxisome proliferator activated receptor gamma (PPARgamma) does not play a major role in the development of morbid obesity Int J Obes Relat Metab Disord 24, 647-51.

Fan, M., Park, A. and Nephew, K. P. (2005) CHIP (carboxyl terminus of Hsc70-interacting protein) promotes basal and geldanamycin-induced degradation of estrogen receptor-alpha Mol Endocrinol 19, 2901-14.

Floyd, Z. E. and Stephens, J. M. (2002) Interferon-gamma-mediated activation and ubiquitin-proteasome-dependent degradation of PPARgamma in adipocytes J Biol Chem 277, 4062-8.

Fu, M., Rao, M., Wang, C., Sakamaki, T., Wang, J., Di Vizio, D., Zhang, X., Albanese, C., Balk, S., Chang, C., Fan, S., Rosen, E., Palivimo, J., Janne, O. A., Muratoglu, S., Avantaggiati, M. L. and Pestell, R. G. (2003) Acetylation of androgen receptor enhances coactivator binding and promotes prostate cancer cell growth Mol Cell Biol 23, 8563-75.

Fu, M., Wang, C., Reuten, A. T., Wang, J., Angeletti, R. H., Siconolfi-Baez, L., Ogrzyko, V., Avantaggiati, M. L. and Pestell, R. G. (2000) p300 and p300/cAMP response element-binding protein-associated factor acetylate the androgen receptor at sites governing hormone-dependent transactivation J Biol Chem 275, 20853-60.

Fuqua, S. A., Wiltschke, C., Zhang, Q. X., Borg, A., Castles, C. G., Friedrichs, W. E., Hopp, T., Hilsenbeck, S., Mohsini, S., O’Connell, P. and Allred, D. C. (2000) A hypersensitive estrogen receptor-alpha mutation in premalignant breast lesions Cancer Res 60, 4026-9.

Fu, M., Rao, M., Wu, K., Wang, C., Zhang, X., Hessien, M., Yeung, Y. G., Gioeli, D., Weber, M. J. and Pestell, R. G. (2004) The androgen receptor acetylation site regulates cAMP and AKT but not ERK-induced activity J Biol Chem 279, 29436-49.

Gaughan, L., Logan, I. R., Cook, S., Neale, D. E. and Robson, C. N. (2002) Tip60 and histone deacetylase 1 regulate androgen receptor activity through changes to the acetylation status of the receptor J Biol Chem 277, 25904-13.

www.nursa.org  NRS  | 2012  | Vol. 10 | DOI: 10.1621/nrs.10001  | Page 1 of 13

ataxon-1 and promotes their ubiquitination and degradation J Biol Chem 281, 26714-26.

Altomare, D. A. and Testa, J. R. (2005) Perturbations of the AKT signaling pathway in human cancer Oncogene 24, 7455-64.

Arpino, G., Wiechmann, L., Osborne, C. K. and Schiff, R. (2008) Crosstalk between the estrogen receptor and the HER tyrosine kinase receptor family: molecular mechanism and clinical implications for endocrine therapy resistance Endocr Rev 29, 217-33.

Atsriku, C., Britton, D. J., Held, J. M., Schilling, B., Scott, G. K., Gibson, B. W., Benz, C. C. and Baldwin, M. A. (2009) Systematic mapping of posttranslational modifications in human estrogen receptor-alpha with emphasis on novel phosphorylation sites Mol Cell Proteomics 8, 467-90.

Bannoh, N., Katsuno, M., Suzuki, K., Takeuchi, Y., Kawashima, M., Suga, N., Takamori, M., Ito, M., Nakamura, T., Matsuo, K., Yamada, S., Oki, Y., Adachi, H., Minamiyama, M., Waza, M., Atsuta, N., Watanabe, H., Fujimoto, Y., Nakashima, T., Tanaka, F., Doyu, M. and Sobue, G. (2009) Phase 2 trial of leuprolerein in patients with spinal and bulbar muscular atrophy Ann Neurol 65, 140-50.

Barone, I., Iacopetta, D., Covingtion, K. R., Cui, Y., Tsimelzon, A., Beyer, A., Ando, S. and Fuqua, S. A. (2010) Phosphorylation of the mutant K303R estrogen receptor alpha at serine 305 affects aromatase inhibitor sensitivity Oncogene 29, 2404-14.

Bawa-Khalfe, T., Cheng, J., Wang, Z. and Yeh, E. T. (2007) Induction of the SUMO-specific protease 1 transcription by the androgen receptor in prostate cancer cells J Biol Chem 282, 37341-9.

Bhavsar, P., Hiew, M., Khorasani, N., Torrego, A., Barnes, P. J., Adcock, I. and Chung, K. F. (2008) Relative corticosteroid insensitivity of alveolar macrophages in severe asthma compared with non-severe asthma Thorax 63, 784-90.

Bloom, J. W. (2004) Mitogen-activated protein kinase pathways: therapeutic targets in steroid resistance? J Allergy Clin Immunol 114, 1055-8.

Bluher, M. and Paschke, R. (2003) Analysis of the relationship between PPAR-gamma 2 gene variants and severe insulin resistance in obese patients with impaired glucose tolerance Exp Clin Endocrinol Diabetes 111, 85-90.

Britton, D. J., Scott, G. K., Schilling, B., Atsriku, C., Held, J. M., Gibson, B. W., Benz, C. C. and Baldwin, M. A. (2008) A novel serine phosphorylation site detected in the N-terminal domain of estrogen receptor isolated from human breast cancer cells J Am Soc Mass Spectrom 19, 729-40.

Chan, H. Y., Warrick, J. M., Andriola, I., Merry, D. and Bonini, N. M. (2002) Genetic modulation of polyglutamine toxicity by protein conjugation pathways in Drosophila Hum Mol Genet 11, 2895-904.

Cheng, J., Bawa, T., Lee, P., Gong, L. and Yeh, E. T. (2006) Role of desumoylation in the development of prostate cancer Neoplasia 8, 667-76.

Chevalier-Larsen, E. S., O’Brien, C. J., Wang, H., Jenkins, S. C., Holder, L., Lieberman, A. P. and Merry, D. E. (2004) Castration restores function and neurofilament alterations of aged symptomatic males in a transgenic mouse model of spinal and bulbar muscular atrophy J Neurosci 24, 4778-86.

Choi, J. H., Banks, A. S., Estall, J. L., Kajimura, S., Bostrom, P., Laznik, D., Ruas, J. L., Chalmers, M. J., Kamenecka, T. M., Bluher, M., Griffin, P. R. and Spiegelman, B. M. (2010) Anti-diabetic drugs inhibit obesity-linked phosphorylation of PPARgamma by Cdk5 Nature 466, 451-6.

Choi, S. J., Chung, S. S., y. E. J., Lee, H. W., Lee, M. H., Choi, H. S., Seol, J. H., Baek, S. H., Bang, O. S. and Chung, C. H. (2006) Negative modulation of RXRalpha transcriptional activity by small ubiquitin-related modifier (SUMO) modification and its reversal by SUMO-specific protease SUSP1 J Biol Chem 281, 30669-77.

Christianson, J. L., Nicoloro, S., Straubhaar, J. and Czeck, M. P. (2008) Stearoyl-CoA desaturase 2 is required for peroxisome proliferator-activated receptor gamma expression and adipogenesis in cultured 3T3-L1 cells J Biol Chem 283, 2306-14.
**Review**

Generali, D., Buffa, F. M., Berutti, A., Brizzi, M. P., Campo, L., Bonardi, S., Bersigia, A., Allevi, G., Miliani, M., Aggugini, S., Papotti, M., Dogliotti, L., Bottini, A., Harris, A. L. and Fox, S. B. (2009) Phosphorylated ERAlpha, HIF-1alpha, and MAPK signaling as predictors of primary endocrine treatment response and resistance in patients with breast cancer J Clin Oncol 27, 227-34.

Ghisletti, S., Huang, W., Ogawa, S., Pascual, G., Lin, M. E., Willison, T. M., Rosenfeld, M. G. and Glass, C. K. (2007) Parallel SUMOylation-dependent pathways mediate gene- and signal-specific transrepression by LRXs and PPARgamma Mol Cell 25, 57-70.

Ghosh, P. M., Malik, S., Bedolla, R. and Kreisberg, J. I. (2003) Akt in prostate cancer: possible role in androgen-independence Curr Drug Metab 4, 487-96.

Giaginis, C., Giagini, A. and Theocharis, S. (2009) Peroxisome proliferator-activated receptor-gamma (PPAR-gamma) ligands as potential therapeutic agents to treat arthritis Pharmacol Res 60, 160-9.

Giordano, C., Cui, Y., Barone, I., Ando, S., Mancini, M. A., Berno, V. and Fuqua, S. A. (2010) Growth factor-induced resistance to tamoxifen is associated with a mutation of estrogen receptor alpha and its phosphorylation at serine 305 Breast Cancer Res Treat 119, 71-85.

Gong, J., Zhu, J., Goodman, O. B., Jr., Pestel, R. G., Schlegel, P. N., Nanus, D. M. and Shen, R. (2006) Activation of p300 histone acetyltransferase activity and acetylation of the androgen receptor by bombesin in prostate cancer cells Oncogene 25, 2011-21.

Hamre, O. W., Forstner, D., Ottinger, I., Ristow, M., Bollheimer, L. C., Scholmerich, J. and Paltich, K. D. (2002) The Pro115Gln polymorphism within the PPAR gamma2 gene has no epidemiological impact on morbid obesity Exp Clin Endocrinol Diabetes 110, 230-4.

Hauser, S., Adelmant, G., Sarraf, P., Wright, H. M., Mueller, E. and Spiegelman, B. M. (2000) Degradation of the peroxisome proliferator-activated receptor gamma is linked to ligand-dependent activation J Biol Chem 275, 18527-33.

Hay, R. T. (2005) SUMO: a history of modification Mol Cell 18, 1-12.

Herynik, M. H., Parra, I., Cui, Y., Beyer, A., Wu, M. F., Hilsenbeck, S. G. and Fuqua, S. A. (2007) Association between the estrogen receptor alpha A908G mutation and outcomes in invasive breast cancer Clin Cancer Res 13, 3235-43.

Holm, C., Kok, M., Michalides, R., Fles, R., Koornstra, R. H., Wesseling, J., Hauptmann, M., Neeffjes, J., Peterse, J. L., Stal, O., Landberg, G. and Linn, S. C. (2009) Phosphorylation of the estrogen receptor alpha at serine 305 and prediction of tamoxifen resistance in breast cancer J Pathol 217, 372-9.

Hu, E., Kim, J. B., Sarraf, P. and Spiegelman, B. M. (1996) Inhibition of adipogenesis through MAP kinase-mediated phosphorylation of PPARgamma Science 274, 2100-3.

Iruen, E., Matthews, J. G., Takahashi, A., Barnes, P. J., Chung, K. F. and Adcock, I. M. (2002) p38 Mitogen-activated protein kinase-induced glucocorticoid receptor phosphorylation reduces its activity: role in steroid-insensitive asthma J Allergy Clin Immunol 109, 649-57.

Ismaili, N. and Garabedian, M. J. (2004) Modulation of glucocorticoid receptor function via phosphorylation Ann N Y Acad Sci 1024, 86-101.

Issmann, I. and Green, S. (1990) Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators Nature 347, 645-50.

Ito, K., Yamamura, S., Essilfie-Quaye, S., Cosio, B., Ito, M., Barnes, P. J. and Adcock, I. M. (2006) Histone deacetylase 2-mediated deacetylation of the glucocorticoid receptor enables NF-kappaB suppression J Exp Med 203, 7-13.

Iwata, M., Haruta, T., Usui, I., Takata, Y., Takano, A., Uno, T., Kawahara, J., Ueno, E., Sasaoka, T., Ishibashi, O. and Kobayashi, M. (2001) PgiIgllatzone ameliorates tumor necrosis factor-alpha-induced insulin resistance by a mechanism independent of adipogenic activity of peroxisome proliferator-activated receptor-gamma diabetes 50, 1083-92.

Jana, N. R., Dikshit, P., Goswami, A., Kotliarova, S., Murata, S., Tanaka, K. and Nukina, N. (2005) Co-chaperone CHIP associates with expanded polyglutamine protein and promotes its degradation by proteasomes J Biol Chem 280, 11635-40.

Jennewein, C., Kuhn, A. M., Schmidt, M. V., Meiliead-Julig, V., von Kretthen, A., Gonzalez, F. J. and Brune, B. (2008) Sumoylation of peroxisome proliferator-activated receptor gamma by apoptotic cells prevents lipopolysaccharide-induced NCoR removal from kappaB binding sites mediating transrepression of proinflammatory cytokines J Immunol 181, 5646-52.

Jiang, J., Sarwar, N., Peston, D., Kulminskaya, E., Shousha, S., Coombes, R. C. and Ali, S. (2007) Phosphorylation of estrogen receptor-alpha at Ser167 is indicative of longer disease-free and overall survival in breast cancer patients Clin Cancer Res 13, 5769-76.

Kailkkonen, S., Jaaskelainen, T., Karvonen, U., Rytinki, M. M., Makkonen, H., Gioeli, D., Paschali, B. M. and Palvimo, J. J. (2009) SUMO-specific protease (SENPI) reverses the hormone-augmented SUMOylation of androgen receptor and modulates gene responses in prostate cancer cells Mol Endocrinol 23, 292-307.

Karamouzis, M. V., Konstantinopoulos, P. A., Badia, F. A. and Papavassiliou, A. G. (2008) SUMO and estrogen receptors in breast cancer Breast Cancer Res Treat 107, 195-210.

Kato, S., Endoh, H., Masuhiro, Y., Kitamoto, T., Uchiyama, S., Sasaki, H., Masushige, S., Gotoh, Y., Nishida, E., Kawashima, H., Metzger, D. and Chambon, P. (1995) Activation of the estrogen receptor through phosphorylation by mitogen-activated protein kinase Science 270, 1491-4.

Katsuno, M., Adachi, H., Doyu, M., Minamiyama, M., Sang, C., Kobayashi, Y., Inukai, A. and Sobue, G. (2003a) Leuprolrelin rescues polyglutamine-dependent phenotypes in a transgenic mouse model of spinal and bulbar muscular atrophy Nat Med 9, 768-73.

Katsuno, M., Adachi, H., Inukai, A. and Sobue, G. (2003b) Transgenic mouse models of spinal and bulbar muscular atrophy (SBMA) Cytogenet Genome Res 100, 243-51.

Kintzcher, U., Hartge, M., Hess, K., Foryst-Ludwig, A., Clemenz, M., Wabitsch, M., Fischer-Posovszky, P., Barth, T. F., Dragon, D., Steurk, T., Hauner, H., Blüher, M., Unger, T., Wolf, A. M., Knipschild, U., Hombach, V. and Marx, N. (2008) T lymphocyte infiltration in visceral adipose tissue: a primary event in adipose tissue inflammation and the development of obesity-mediated insulin resistance Arterioscler Thromb Vasc Biol 28, 1304-10.

Kramer, O. H., Knauer, S. K., Greiner, G., Jandt, E., Reichardt, S., Guhrs, K. H., Stauber, R. H., Bohmer, F. D. and Heinzl, T. (2009) A phosphorylation-acetylation switch regulates STAT1 signaling Genes Dev 23, 223-35.

Kubota, N., Terauchi, Y., Miki, H., Tamemoto, H., Yamauchi, T., Komeda, K., Sato, S., Nakano, R., Ishii, C., Sugiyama, T., Eto, K., Tsumamoto, Y., Okuno, A., Murakami, K., Sekihara, H., Hasegawa, G., Naito, M., Toyoshima, Y., Tanaka, S., Ishita, K., Kitamura, T., Fujita, T., Ezaki, O., Azawa, S. and Kadowaki, T. (1999) PPAR gamma mediates high-fat diet-induced adipocyte hypothyrosis and insulin resistance Mol Cell 4, 597-609.

Lannigan, D. A. (2003) Estrogen receptor phosphorylation Steroids 68, 1-9.

Larvin, O., Bianco, S., Kersual, N., Chalbos, D. and Vanacker, J. M. (2007) Potentiation of IC1182,780 (Fulvestrant)-induced estrogen receptor-alpha degradation by the estrogen receptor-related receptor-alpha inverse agonist XCT790 J Biol Chem 282, 28328-34.

Lasa, M., Abraham, S. M., Boucheron, C., Saklatvala, J. and Clark, A. R. (2002) Dexamethasone causes sustained expression of mitogen-activated protein kinase (MAPK) phosphatase 1 and phosphatase-mediated inhibition of MAPK p38 Mol Cell Biol 22, 7802-11.

Le Romancer, M., Poulard, C., Cohen, P., Sentis, S., Renoir, J. M. and Corbo, L. (2011) Cracking the estrogen receptor's posttranslational code in breast tumors Endocr Rev 32, 597-622.
Liao, Y., Grobohlz, R., Abel, U., Trojan, L., Michel, M. S., Angel, P. and Mayer, D. (2003) Increase of Akt/PKB expression correlates with gleason pattern in human prostate cancer J Int Cancer 107, 567-90.

Lin, H. K., Yeh, S., Kang, H. Y. and Chang, C. (2001) Akt suppresses androgen-induced apoptosis by phosphorylating and inhibiting androgen receptor Proc Natl Acad Sci U S A 98, 7200-5.

Lin, H. K., Wang, L., Hu, Y. C., Altuwajir, S. and Chang, C. (2002) Phosphorylation-dependent ubiquitilation and degradation of androgen receptor by Akt require Mdm2 E3 ligase Embo J 21, 4037-48.

Lin, H. K., Hu, Y. C., Yang, L., Altuwajir, S., Chen, Y. T., Kang, H. Y. and Chang, C. (2003) Suppression versus induction of androgen receptor functions by the phosphatidylinositol 3-kinase/Akt pathway in prostate cancer LNCaP cells with different passage numbers J Biol Chem 278, 50902-7.

Li, L. B., Goleva, E., Hall, C. F., Ou, L. S. and Leung, D. Y. (2004) Superantigen-induced corticosteroid resistance of human T cells occurs through activation of the mitogen-activated protein kinase/extracellular signal-regulated kinase (MEK-ERK) pathway J Allergy Clin Immunol 114, 1059-69.

Lonard, D. M., Nawaz, Z., Smith, C. L. and O'Malley, B. W. (2000) The 26S proteasome is required for estrogen receptor-alpha and coactivator turnover and for efficient estrogen receptor-alpha transactivation Mol Cell 5, 939-48.

Majumder, P. K. and Sellers, W. R. (2005) Akt-regulated pathways in prostate cancer Oncogene 24, 7465-74.

Mangelsdorf, D. J. and Evans, R. M. (1995) The RXR heterodimers and orphan receptors Cell 83, 841-50.

Marsaud, V., Gougelet, A., Maillard, S. and Renoir, J. M. (2003) Various phosphorylation pathways, depending on agonist and antagonist binding to endogenous estrogen receptor alpha (ERalpha), differentially affect ERalpha transcriptional activity and transcellular activity in human breast cancer cells Mol Endocrinol 17, 2013-27.

Matthews, J. G., Ito, K., Barnes, P. J. and Adcock, I. M. (2004) Defective glucocorticoid receptor nuclear translocation and altered histone acetylation patterns in glucocorticoid-resistant patients J Allergy Clin Immunol 113, 1100-8.

McCullagh, S. J. (2008) Phosphorylation of the androgen receptor is associated with reduced survival in hormone-refractory prostate cancer patients Br J Cancer 98, 1094-101.

McDonough, H. and Patterson, C. (2003) CHIP: a link between the chaperone and proteasome systems Cell Stress Chaperones 8, 303-4.

Michalides, R., Grieksboor, A., Balkenende, A., Vervoord, D., Janssen, L., Jaink, K., Floore, A., Velds, A., van't Veer, L. and Neefjes, J. (2004) Tamoxifen resistance by a conformational arrest of the estrogen receptor alpha after PKA activation in breast cancer Cancer Cell 5, 597-605.

Miles, P. D., Barak, Y., He, W., Evans, R. M. and Olafsky, J. M. (2000) Improved insulin-sensitivity in mice heterozygous for PPAR-gamma deficiency J Clin Invest 102, 587-92.

Miller, A. L., Webb, M. S., Copik, A. J., Wang, Y., Johnson, B. H., Kumar, R. and Thompson, E. B. (2005) p38 Mitogen-activated protein kinase (MAPK) is a key mediator in glucocorticoid-induced apoptosis of lymphoid cells: correlation between p38 MAPK activation and site-specific phosphorylation of the human glucocorticoid receptor at serine 211 Mol Endocrinol 19, 1569-83.

Mukherjee, S., Thomas, M., Dadgar, N., Lieberman, A. P. and Inguez-Llui, J. A. (2009) Small ubiquitin-like modifier (SUMO) modification of the androgen receptor attenuates polyglutamine-mediated aggregation J Biol Chem 284, 21296-306.

Murphy, L. C., Seekallu, S. V. and Watson, P. H. (2011) Clinical significance of estrogen receptor phosphorylation Endocr Relat Cancer 18, R1-14.

Murphy, L. C., Niu, Y., Snell, L. and Watson, P. (2004) Phospho-serine-118 estrogen receptor-alpha expression is associated with better disease outcome in women treated with tamoxifen Clin Cancer Res 10, 5902-6.

Nirmala, P. B. and Thampan, R. V. (1995) Ubiquitination of the rat uterine estrogen receptor: dependence on estradiol Biochem Biophys Res Commun 213, 24-31.

Palazzolo, I., Burnett, B. G., Young, J. E., Brenne, P. L., La Spada, A. R., Fischbeck, K. H., Howell, B. W. and Pennuto, M. (2007) Akt blocks ligand binding and protects against expanded polyglutamine androgen receptor toxicity Hum Mol Genet 16, 1593-603.

Pascual, G., Pang, A. L., Ogawa, S., Gamliel, A., Li, A. C., Perissi, V., Rose, D. W., Willson, T. M., Rosenfeld, M. G. and Glass, C. K. (2005) A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-gamma Nature 437, 759-63.

Pascual, G., Sullivan, A. L., Ogawa, S., Gamliel, A., Perissi, V., Rosenfeld, M. G. and Glass, C. K. (2007) Anti-inflammatory and antidiabetic roles of PPARgamma Novartis Found Symp 286, 183-96; discussion 196-203.

Pinhel, I. F., Macneill, F. A., Hills, M. J., Salter, J., Dette, S., A’Hern, R., Nerurkar, A., Osin, P., Smith, I. E. and Dowsett, M. (2010) Extreme loss of immunoreactive p-Akt and p-ERk/2 during routine fixation of primary breast cancer Breast Cancer Res 12, R76.

Popov, V. M., Wang, C., Shirley, L. A., Rosenberg, A. L., Li, S., Nevalainen, M., Fu, M. and Pestell, R. G. (2007) The functional significance of nuclear receptor acetylation Steroids 72, 221-30.

Poukka, H., Karvonen, U., Janne, O. A. and Palvimo, J. J. (2000) Covalent modification of the androgen receptor by small ubiquitin-like modifier 1 (SUMO-1) Proc Natl Acad Sci U S A 97, 14145-50.

Rangwala, S. M., Rhodeas, B., Shapiro, J. S., Rich, A. S., Kim, J. K., Shulman, G. I., Kaestner, K. H. and Lazar, M. A. (2003) Genetic modulation of PPARgamma phosphorylation regulates insulin sensitivity Dev Cell 5, 657-63.

Ray, A., Prefontaine, K. E. and Ray, P. (1994) Down-modulation of interleukin-6 gene expression by 17 beta-estradiol in the absence of high affinity DNA binding by the estrogen receptor J Biol Chem 269, 12940-6.

Reid, G., Denger, S., Kos, M. and Gannon, F. (2002) Human estrogen receptor-alpha: regulation by synthesis, modification and degradation Cell Mol Life Sci 59, 821-31.

Ristow, M., Muller-Wieland, D., Pfeiffer, A., Krone, W. and Kahn, C. R. (1998) Obesity associated with a mutation in a genetic regulator of adipocyte differentiation N Engl J Med 339, 953-9.

Rosen, E. D. and Spiegelman, B. M. (2006) Adipocytes as regulators of energy balance and glucose homeostasis Nature 444, 847-53.

Schlabach, M. R., Luo, J., Solimini, N. L., Hu, G., Xu, Q., Li, M. Z., Zhao, Z., Smogorzewska, A., Sowa, M. E., Ang, X. L., Westbrook, T. F., Liang, A. C., Chang, K., Hackett, J. A., Harper, J. W., Hannon, G. J. and Elledge, S. J. (2008) Cancer proliferation gene discovery through functional genomics Science 319, 620-4.

Seeler, J. S. and Dejean, A. (2003) Nuclear and unclear functions of SUMO Nat Rev Mol Cell Biol 4, 690-9.

Sents, S., Le Romancer, M., Bianchin, C., Rostan, M. C. and Corbo, L. (2005) Sumoylation of the estrogen receptor alpha hinge region regulates its transcriptional activity Mol Endocrinol 19, 2671-84.

Siddiqui, S. and Rimm, D. L. (2010) Pre-analytic variables and phospho-specific antibodies: the Achilles heel of immunohistochemistry Breast Cancer Res 12, 113.

Skliris, G. P., Nugent, Z. J., Rowan, B. G., Penner, C. R., Watson, P. H. and Murphy, L. C. (2010) A phosphorylation code for oestrogen receptor-alpha predicts clinical outcome to endocrine therapy in breast cancer Endocr Relat Cancer 17, 589-97.
Skliris, G. P., Rowan, B. G., Al-Dhaheri, M., Williams, C., Troup, S., Begic, S., Parisien, M., Watson, P. H. and Murphy, L. C. (2009) Immunohistochemical validation of multiple phospho-specific epitopes for estrogen receptor alpha (ERalpha) in tissue microarrays of ERalpha positive human breast carcinomas Breast Cancer Res Treat 118, 443-53.

Spears, M., McSharry, C. and Thomson, N. C. (2006) Peroxisome proliferator-activated receptor-gamma agonists as potential anti-inflammatory agents in asthma and chronic obstructive pulmonary disease Clin Exp Allergy 36, 1494-504.

Steffan, J. S., Agrawal, N., Palios, J., Rockabrand, E., Trotman, L. C., Slepkov, N., Illes, K., Lukacsovich, T., Zhu, Y. Z., Cattaneo, E., Pandolfi, P. P., Thompson, L. M. and Marsh, J. L. (2004) SUMO modification of Huntington and Huntington’s disease pathology Science 304, 100-4.

Szatmary, Z., Garabedian, M. J. and Vilcek, J. (2004) Inhibition of glucocorticoid receptor-mediated transcriptional activation by p38 mitogen-activated protein (MAP) kinase J Biol Chem 279, 43708-15.

Tateishi, Y., Kawabe, Y., Chiba, T., Murata, S., Ichikawa, K., Murayama, A., Tanaka, K., Baba, T., Kato, S. and Yanagisawa, J. (2004) Ligand-dependent switching of ubiquitin-proteasome pathways for estrogen receptor Embo J 23, 4813-23.

Tsoura, D. C. and Rothman, P. B. (2004) Enhancement of MEK/ERK signaling promotes glucocorticoid resistance in CD4+ T cells J Clin Invest 113, 619-27.

Valley, C. C., Metivier, R., Solodin, N. M., Fowler, A. M., Mashek, M. T., Hill, L. and Alarid, E. T. (2005) Differential regulation of estrogen-inducible proteolysis and transcription by the estrogen receptor alpha N terminus Mol Cell Biol 25, 5417-28.

van Beekum, O., Fleskens, V. and Kalkhoven, E. (2009) Posttranslational modifications of PPAR-gamma: fine-tuning the metabolic master regulator Obesity (Silver Spring) 17, 213-9.

Waite, K. J., Floyd, Z. E., Arbour-Reily, P. and Stephens, J. M. (2001) Interferon-gamma-induced regulation of peroxisome proliferator-activated receptor gamma and STATs in adipocytes J Biol Chem 276, 7062-8.

Webster, J. C., Jewell, C. M., Bodwell, J. E., Munck, A., Sar, M. and Cidlowski, J. A. (1997) Mouse glucocorticoid receptor phosphorylation status influences multiple functions of the receptor protein J Biol Chem 272, 9287-93.

Wellsman, G. E., Li, L., Skliris, G. P., Davie, J. R., Ung, K., Niu, Y., Curtis-Snell, L., Tomes, L., Watson, P. H. and Murphy, L. C. (2006) Estrogen receptor-alpha phosphorylated at Ser118 is present at the promoters of estrogen-regulated genes and is not altered due to HER-2 overexpression Cancer Res 66, 10162-70.

Wijayaratne, A. L. and McDonnell, D. P. (2001) The human estrogen receptor-alpha is a ubiquitinated protein whose stability is affected differentially by agonists, antagonists, and selective estrogen receptor modulators J Biol Chem 276, 35684-92.

Williams, C. C., Basu, A., El-Gharbawy, A., Carrier, L. M., Smith, C. L. and Rowan, B. G. (2009) Identification of four novel phosphorylation sites in estrogen receptor alpha: impact on receptor-dependent gene expression and phosphorylation by protein kinase CK2 BMC Biochem 10, 36.

Willson, T. M., Lambert, M. H. and Kliwer, S. A. (2001) Peroxisome proliferator-activated receptor gamma and metabolic disease Annu Rev Biochem 70, 341-67.