The first decade of estrogen receptor cistromics in breast cancer

Koen D Flach and Wilbert Zwart

Division of Molecular Pathology, The Netherlands Cancer Institute, Amsterdam, The Netherlands

Correspondence
should be addressed
to W Zwart

Email
w.zwart@nki.nl

Abstract

The advent of genome-wide transcription factor profiling has revolutionized the field of breast cancer research. Estrogen receptor α (ER α), the major drug target in hormone receptor-positive breast cancer, has been known as a key transcriptional regulator in tumor progression for over 30 years. Even though this function of ER α is heavily exploited and widely accepted as an Achilles heel for hormonal breast cancer, only since the last decade we have been able to understand how this transcription factor is functioning on a genome-wide scale. Initial ChIP-on-chip (chromatin immunoprecipitation coupled with tiling array) analyses have taught us that ER α is an enhancer-associated factor binding to many thousands of sites throughout the human genome and revealed the identity of a number of directly interacting transcription factors that are essential for ER α action. More recently, with the development of massive parallel sequencing technologies and refinements thereof in sample processing, a genome-wide interrogation of ER α has become feasible and affordable with unprecedented data quality and richness. These studies have revealed numerous additional biological insights into ER α behavior in cell lines and especially in clinical specimens. Therefore, what have we actually learned during this first decade of cistromics in breast cancer and where may future developments in the field take us?

Key Words

- ▶ breast cancer
- ▶ cistromics
- ▶ estrogen receptor
- ▶ ChIP-seq

Journal of Endocrinology
(2016) **229**, R43–R56

Introduction

Breast cancer is the most prevalent form of cancer in women, with approximately 1.7 million annual new diagnoses (Ferlay *et al.* 2015). Despite the improvement of breast cancer treatment, still over half a million women die of this disease every year (Ferlay *et al.* 2015). Approximately 70% of breast tumors are estrogen receptor α (ER α) positive, and tumor cell proliferation is thought to be dependent on the activity of this hormone-mediated transcription factor (Hayashi *et al.* 2003, Dahlman-Wright *et al.* 2006).

The first evidence for a link between estrogens (produced in the ovaries) and breast cancer was reported by George Thomas Beatson in 1896 with a case report

describing a premenopausal breast cancer patient with metastatic disease (Beatson 1896). Although not aware of the exact mechanisms of hormonal action in human physiology, Beatson was familiar with a procedure performed in cattle in which lactation after giving birth can be extended by removal of the ovaries. Inspired by this phenomenon, Beatson performed a bilateral oophorectomy on his patient, which initially resulted in a complete remission of the disease (Beatson 1896, Thomson 1902). The protein responsible for this clinical benefit was found almost 80 years later, with the seminal discovery of the estrogen receptor (ER) in 1973 by Elwood Jensen (Jensen & DeSombre 1973). After first being cloned

in 1985 (Walter *et al.* 1985), in 1986 a complementary DNA clone of the translated mRNA of the ER from MCF-7 human breast cancer cells was sequenced and upon expression gave rise to a functional protein (Greene *et al.* 1986).

Today, ER α is recognized as the major drug target in hormonal breast cancer. In the adjuvant treatment of ER α -positive disease, receptor inhibition is achieved by either a direct blockage of ER α activation through competitive inhibition of estradiol association using tamoxifen (Katzenellenbogen *et al.* 1985, Jordan & Murphy 1990, Arpino *et al.* 2009) or by preventing estrogen synthesis using aromatase inhibitors (Fabian 2007). Despite the extensive use of these treatment modalities in adjuvant therapy, a significant number of patients still develop a recurrence (Early Breast Cancer Trialists' Collaborative *et al.* 2011). Although cross-resistance between the different endocrine therapy options can occur, patients that relapse on one type of endocrine therapy can still benefit from a different treatment modality (Vergote *et al.* 2006, Wang *et al.* 2009, Yoo *et al.* 2011), suggesting that multiple resistance mechanisms can exist that may be treatment selective. In order to directly administer the right drug to the right patient, it is vital to increase our knowledge about ER α functioning as well as its selective responses to prolonged exposure to hormonal agents.

Even though ER α inhibitors have been used in the clinic since the early 1980s, the direct mode of ER α 's genomic action on a genome-wide scale has remained elusive for many years. With the initial development of ChIP-on-chip (chromatin immunoprecipitation coupled with tiling array) technologies, this situation changed dramatically with the interrogation of ER α action for the first time on a human chromosome-wide scale (Carroll *et al.* 2005). With the development of massive parallel high-throughput sequencing techniques, a full genome coverage of ER α became possible (and importantly affordable) through ChIP sequencing (ChIP-seq) (Welboren *et al.* 2009). Now, 10 years after the first unbiased and systemic assessment of ER α -binding sites in human cell lines, we will discuss what we have learned from the cistromics of ER α and where future developments might take us.

ER complex formation and its mode of action

ER α is activated through the association of its natural ligand estradiol with the receptors' ligand-binding domain, which enables dissociation from chaperone protein Hsp90

(Catelli *et al.* 1985, Pratt & Toft 1997, Devin-Leclerc *et al.* 1998) and facilitates ER α -chromatin interactions (Kumar & Chambon 1988). Initial ChIP-on-chip experiments have shown ER α to mainly bind enhancer regions (Carroll *et al.* 2005). Computational DNA sequence motif analyses of ER α -binding sites resulted in the identification of a number of upstream transcription factors that facilitate the binding of ER α to the chromatin, including pioneer factor FOXA1 (Carroll *et al.* 2005, Hurtado *et al.* 2011) and putative pioneer factors PBX1 (Magnani *et al.* 2011) and AP-2 γ (Tan *et al.* 2011) (Fig. 1). Pioneer factors can associate with compacted chromatin and trigger enhancer competency by decondensing the chromatin, facilitating the binding of additional chromatin-binding factors (Zaret & Carroll 2011, Jozwik & Carroll 2012). Additionally, ER α -cooperating transcription factor GATA3 is capable of mediating enhancer accessibility at ER α regulatory regions and has properties similar to FOXA1 (Kong *et al.* 2011, Theodorou *et al.* 2013). Besides binding directly to the DNA, ER α can also associate with the chromatin via other transcription factors, a mechanism also known as tethering, including RUNX1 (Stender *et al.* 2010) and AP-1 (Umayahara *et al.* 1994, Kushner *et al.* 2000, Cheung *et al.* 2005).

After activation, ER α undergoes a conformational change (Paige *et al.* 1999), forming a coactivator-binding pocket at the receptors' carboxy-terminus (Shiau *et al.* 1998). This interaction surface subsequently leads to the recruitment of the members of the p160 coactivator family: SRC1 (NCOA1; Onate *et al.* 1995), SRC2 (NCOA2, TIF2, GRIP1; Voegel *et al.* 1996, Hong *et al.* 1997), and SRC3 (NCOA3, p/CIP, AIB1, ACTR; Anzick *et al.* 1997, Chen *et al.* 1997, Torchia *et al.* 1997, Suen *et al.* 1998). The binding of these SRCs to the coactivator-binding pocket of activated ER α has been described to occur both in a competitive manner (exclusive recruitment of one type of SRC) (Shiau *et al.* 1998, Margeat *et al.* 2001, Carraz *et al.* 2009) and in a joint manner, possibly through heterodimerization (Zhang *et al.* 2004). Reports on the exact stoichiometry within the p160/ER α complex are conflicting, describing a single p160 to associate with an ER α dimer (Margeat *et al.* 2001) or two SRCs per active ER α complex (Zhang *et al.* 2004, Yi *et al.* 2015), although both situations might occur side to side (Zhang *et al.* 2004). Recently, it has been shown, for SRC3, that these ER α interactions occur in a monomeric fashion, where two ligand-bound ER α monomers individually recruit one SRC3 protein, after which an ER α dimer (binding two SRC3 molecular) associates with a single p300 protein (Yi *et al.* 2015). The p160 composition of the ER α transcriptional

Figure 1

The ERα transcriptional complex pathway. When activated by its natural ligand estradiol or by direct phosphorylations, ERα binds to enhancers made accessible by pioneer factors (e.g. FOXA1). A transcriptional complex, including p300, CBP, SRC1-2-3, and other coactivators, is assembled and enhancer RNAs are transcribed. After cohesin-stabilized chromatin looping to associated gene promoters, RNA polymerase II is recruited and an active transcriptional complex is formed, capable of transcribing associated genes.

complex influences its genomic binding preferences on a genome-wide scale, consequently resulting in an altered transcriptional repertoire (Zwart *et al.* 2011) and altered phenotypic behavior (Fig. 2).

After ERα binding, p160 proteins can subsequently recruit other essential proteins for transcriptional regulation, including p300 and CBP (McKenna *et al.* 1999), which can modify chromatin accessibility through their acetyltransferase activity (Ogryzko *et al.* 1996). In order to further modify the chromatin toward a transcription favorable landscape, histone modifiers CARM1 (Chen *et al.* 1999a, Stallcup *et al.* 2000) and JMJD2B (Kawazu *et al.* 2011, Shi *et al.* 2011) and members of the SWI/SNF chromatin remodeling complex, including BAF57, are recruited (Belandia *et al.* 2002).

With the recent discovery of estradiol-induced enhancer RNAs (eRNAs) at a set of ~1200 ERα-bound enhancer elements (Hah *et al.* 2013, Li *et al.* 2013), an additional layer of ERα biology was revealed. This eRNA production is not just limited to ERα-bound enhancers but is, for example, also apparent for the androgen

receptor (AR; Wang *et al.* 2011a) and p53 (Melo *et al.* 2013). DNase I sensitivity assays demonstrated that eRNAs are capable of regulating genomic access of the transcriptional complex to regulatory regions (Mousavi *et al.* 2013). eRNAs found at ERα-binding sites strongly correlated with the enrichment of a number of genomic features associated with enhancers and enhancer looping to target gene promoters (Hah *et al.* 2013). The physiological relevance of eRNAs in ERα biology was further stipulated by the observation that knockdown of a subset of eRNAs (e.g. GREB1 enhancer) reduced the transcription of coding gene transcripts, as well as reducing promoter–enhancer interactions as shown by chromosome conformation capture (3C; Li *et al.* 2013), although conflicting 3C results have also been described (Hah *et al.* 2013). Hah and coworkers found that inhibition of eRNA production by flavopiridol, a CDK9 inhibitor blocking transcriptional elongation, did not affect other indicators of enhancer activity or estradiol-dependent promoter–enhancer looping (Hah *et al.* 2013), leaving the exact role of eRNAs somewhat elusive. These

Figure 2

ER α transcriptional complex composition, genomic profile, and transcriptional output. Illustration of ER α -induced transcription, where the genomic binding profile of ER α 's transcriptional complex leads to induced transcription and an expression profile on the basis of which a classification profile can be made (A). These genomic, transcriptional, and classification profiles can be altered by posttranslational modifications of cofactors (B), phosphorylations on ER α itself (C), and the composition of the transcriptional complex (D).

eRNA-associated promoter-enhancer interactions, also known as chromosomal looping structures, have been described to promote ER α -regulated gene transcription

and seem to be stabilized by Cohesin (Fullwood *et al.* 2009, Schmidt *et al.* 2010, Li *et al.* 2013). Although these observations hint toward an important role for eRNAs

in ER α -regulated transcription, only a subset of eRNAs has yet been investigated thoroughly, with conflicting roles in chromosomal looping, leaving the exact physiological roles for them currently elusive.

After ER α has recruited its cofactors, an active transcriptional complex can be formed by RNA polymerase II recruitment and transcription of responsive genes can be initiated (Glass *et al.* 1997) (Fig. 1). When treated with tamoxifen, the ligand-binding domain of ER α adopts an alternative conformation, impairing the docking of p160 proteins to ER α , preventing the correct assembly of the transcriptional complex (Shiau *et al.* 1998).

The genome-wide kinetics with which the ER α complex assembles on the chromatin is not yet fully understood. By using ChIP at three ER α -responsive gene promoters, Shang and coworkers have reported that ER α and a number of its coactivators associate on these estrogen responsive promoters in a cyclic fashion and that these cycles of ER α -complex assembly are followed by transcription (Shang *et al.* 2000). This cyclic recruitment of ER α and its coregulators could be confirmed by others, who reported cofactor recruitment to be preceded by histone deacetylases and nucleosome-remodeling complexes at the TFF1 promoter (Metivier *et al.* 2003). These data imply that transcriptional activation of ER α -responsive genes may require both activating and repressive epigenetic processes. Although both papers state that ER α -induced transcriptional activation occurs in a cyclic fashion, both papers only investigated the dynamic nature of ER α on a couple of sites and a comprehensive overview of ER α dynamics on a genome-wide scale is currently lacking. Furthermore, whether this cyclic ER α complex assembly occurs only on promoters, as studied in both papers, or whether it is also apparent at ER α -bound enhancers remains unclear.

ER α cistromics in breast cancer cell lines

Initially, most reports on ER α chromatin interactions, its dynamics and recruitment of coregulators were centred on single binding site-based analyses, often limited to the TFF1 promoter. With the technological development of tiling arrays, ER α genomic interactions could reliably be assessed on a chromosome-wide scale (Carroll *et al.* 2005). As technology progressed, this approach was quickly succeeded by massive parallel sequencing technologies, enabling the interrogation of ER α sites on a genome-wide scale in a cost-effective manner (Welboren *et al.* 2009). These initial reports resulted in a huge paradigm shift, completely changing the way we think about ER α

genomics. These studies illustrated that even though most pioneering studies on ER α genomics exclusively interrogated promoters, this genomic behavior of ER α clearly represents an exception. In fact, only a small proportion of about 5% of ER α -binding sites was found at gene promoters; a characteristic feature that has been validated by others (Carroll *et al.* 2005, Zwart *et al.* 2011) and is also apparent for other nuclear receptors, including AR (Yu *et al.* 2010) and glucocorticoid receptor (GR; Reddy *et al.* 2009). Approximately 95% of all ER α -binding sites are found at distal *cis*-regulatory elements (hence designated as 'cistromics' (Lupien *et al.* 2008)) that were later recognized as enhancer regions. These regions are putative regulatory elements and might not all be functional. Recently, a CRISPR-Cas9 screen has been used to functionally assess ER α enhancer elements and their effect on cell proliferation (Korkmaz *et al.* 2016). Out of the 99 ER α -binding sites that were targeted, the deletion of only three of them affected cell proliferation, further illustrating that only a subset of ER α -binding sites at cistromics might actually be functionally involved in cell proliferation processes.

The discovery of enhancer preference for ER α -binding repositioned the classical promoter-centered ER α studies considerably on the level of physiological extrapolation. Chromatin interaction analysis by paired-end tag (ChIA-PET) sequencing analyses, which enable the identification of long-range chromatin interactions, illustrated that the distal enhancer-associated ER α -binding sites were found to loop to anchor genes through connections with proximal ER α -binding sites, suggesting that ER α functions by bringing genes together for coordinated transcriptional regulation by extensive chromatin looping (Fullwood *et al.* 2009). At the GREB1 and TFF1 loci, this chromatin looping was dependent on ER α expression and was inducible by estradiol stimulation (Pan *et al.* 2008, Fullwood *et al.* 2009). Probing the 3D architecture of the genome by coupling proximity-based ligation with massively parallel sequencing (Hi-C; Lieberman-Aiden *et al.* 2009) yielded similar ER α -mediated enhancer-promoter interactions (Mourad *et al.* 2014). These sites of chromatin looping highly correlated with CCCTC-binding factor (CTCF)-binding sites, suggesting CTCF to play a key role defining the boundaries of chromosomal territories and influence gene expression through cross talk between promoters and regulatory elements (Splinter *et al.* 2006, Botta *et al.* 2010, Handoko *et al.* 2011). Besides ER α , these chromatin loops have also been observed for other nuclear transcription factors, including AR (Wang *et al.* 2005) and GR (Hakim *et al.* 2009).

On the transcriptomic level, the use of global nuclear run-on sequencing (GRO-seq; [Core et al. 2008](#)) analysis increased our understanding of ER α -regulated transcription by identifying primary and immediate estrogen-induced effects as opposed to steady-state transcript level analyses ([Hah et al. 2011](#)). GRO-seq demonstrated that estrogen is able to regulate the activity of all three RNA polymerases and led to the discovery of previously undetected estrogen-regulated intergenic transcripts ([Hah et al. 2011](#)). Transcription profiling by GRO-seq could be used for the prediction of *de novo* enhancers across various cell types ([Hah et al. 2013](#)). In combination with RNA-seq, GRO-seq was able to annotate long noncoding RNAs (lncRNAs) and characterized the lncRNA transcriptome in MCF-7 breast cancer cells, including over 700 previously unannotated lncRNAs ([Sun et al. 2015](#)). Furthermore, GRO-seq analysis at ER α enhancers revealed the existence of estradiol-induced unidirectional and bidirectional eRNAs, which were strongly correlated with enhancer–promoter looping ([Hah et al. 2013](#)). The described role of these intergenic transcripts in enhancer–promoter looping ([Fullwood et al. 2009](#), [Schmidt et al. 2010](#), [Li et al. 2013](#)) and the fact that one promoter can be involved in multiple enhancer-associated loops ([Fullwood et al. 2009](#), [Mourad et al. 2014](#)) might explain the seemingly large discrepancy between the number of ER α -regulated genes (approximately 2000; [Zwart et al. 2011](#)) in relation to the number of ER α -binding sites in the same cell line (>10,000; [Welboren et al. 2009](#), [Hurtado et al. 2011](#)).

Due to technical limitations in the ChIP-seq protocol, the resolution of DNA-binding analyses is typically quite limited with events being mapped with ± 300 base pairs. Further refinement of the ChIP-seq procedure has led to the implementation of lambda exonuclease digestion in the protocol (ChIP-exo), enabling high-resolution mapping of chromatin binding and identification of unique transcription factor binding sites that could not be identified by ChIP-seq ([Rhee & Pugh 2011, 2012](#), [Serandour et al. 2013](#)). The addition of exonucleases also results in the degradation of contaminating DNA, effectively lowering the required depth of sequencing coverage.

Apart from forming the foundations of *cis*-regulatory gene regulation, chromatin looping and eRNA action, genome-wide profiling analyses of ER α sites can also lead to the identification of additional transcription factor motifs often co-enriched at ER α sites and proximal to estrogen response elements. These motif analyses revealed the presence of Forkhead binding motifs at roughly

50% of ER α -bindings sites ([Carroll et al. 2005](#)). This observation led to the discovery that FOXA1 is essential for chromatin accessibility at ER α sites and crucial for ER α binding and functionality ([Carroll et al. 2005](#), [Hurtado et al. 2011](#)). More recently, this same approach has been used to identify other putative pioneer factors for ER α , including PBX1 that can guide ER α to a specific subset of sites ([Magnani et al. 2011](#)). When investigating the motifs of ER α -bindings sites identified by ChIA-PET, Tan and coworkers found that approximately 40% of these binding sites contained the AP-2 motif ([Tan et al. 2011](#)). They next demonstrated that transcription factor AP-2 γ can bind to these ER α -bindings sites in a ligand-independent manner and there is a functional interplay between AP-2 γ and FOXA1 ([Tan et al. 2011](#)).

Besides the interplay between ER α and its pioneer factors and coregulators, it is becoming increasingly apparent that a complex interplay exists between different steroid hormone receptor family members. The AR, a transcription factor classically known for its oncogenic role in prostate cancer, is expressed in 84–95% of the ER α -positive breast cancers ([Niemeier et al. 2010](#), [Qi et al. 2012](#), [Chia et al. 2015](#)) and is usually associated with a favorable outcome ([Peters et al. 2009](#), [Castellano et al. 2010](#), [Hu et al. 2011](#)). Exogenous overexpression of AR inhibits ER α transactivation activity and estrogen-induced cell growth ([Ando et al. 2002](#), [Peters et al. 2009](#)), which may be explained by a direct competition between ER α and AR at binding the same genomic regions ([Peters et al. 2009](#)). This notion was further strengthened by ChIP analysis showing AR recruitment to the progesterone receptor (PR) promoter in T47D cells ([Peters et al. 2009](#)).

Another steroid hormone receptor family member known for its coexpression and favorable outcome in ER α -positive breast cancers is the PR ([Pichon et al. 1980](#), [Blows et al. 2010](#)). Progesterone induces the association of PR with ER α , thereby regulating ER α –chromatin interactions and transcriptional activity, providing mechanistic insights behind the clinical implications of PR status in ER α -positive tumors ([Mohammed et al. 2015](#)).

The GR, in the presence of dexamethasone, is able to associate with similar binding regions as ER α , and GR stimulation leads to reduced transcription of key ER α target genes ([Meyer et al. 1989](#), [Karmakar et al. 2013](#)). This direct protein–protein interaction between GR and ER α can play an important role in the GR-mediated growth inhibition of ER α -positive cells ([Karmakar et al. 2013](#)). Besides this general inhibitory role of GR, gene-specific regulation with both cooperation and antagonism

has also been described (Bolt *et al.* 2013). Apart from direct physical interactions between nuclear receptors, nuclear receptors can also inhibit each other's activity through cross-interference ("squenching"), where direct competition for cofactor recruitment can inhibit nuclear receptor activity without associating with the same genomic regions (Cahill *et al.* 1994, Lopez *et al.* 1999).

Cistromics of ER α coregulators

To date, several studies have compiled an overview of ER α coregulators and interacting proteins, with numbers varying around 17 (Foulds *et al.* 2013) to 108 (Mohammed *et al.* 2013). The p160 protein family members are reproducibly and consistently identified as part of the ER α complex, for which a level of mutual exclusivity has been described for ER α binding (Shiau *et al.* 1998, Margeat *et al.* 2001, Carraz *et al.* 2009). With the recent finding that an activated ER α dimer can bind one p300 protein (Yi *et al.* 2015) and p300 and CBP have a substantial overlap of ~70% in binding sites (Zwart *et al.* 2011), it is not unlikely that a level of mutual exclusivity between p300 and CBP also exists. As a direct consequence thereof, the composition of ER α complexes can differ between different sites on a genome-wide scale, with potentially far-reaching consequences on gene expression profiles. Cistromic analyses of the p160 family members illustrated that even though most genomic sites are shared among SRC1, SRC2, and SRC3, distinct subsets of sites were identified where gene expression was selectively responsive to one specific p160 protein, as part of the ER α complex (Zwart *et al.* 2011). Interestingly, the gene profile under the control of ER α with exclusively SRC3 binding (devoid of SRC1 or SRC2) had prognostic potential and enabled the identification of breast cancer patients with a poor outcome after tamoxifen treatment (Zwart *et al.* 2011). This link between SRC3 gene targets and tamoxifen treatment is in line with previous reports describing increased SRC3 expression, in combination with increased ERBB2 expression, to correlate with a poor tamoxifen response (Osborne & Schiff 2003, Shou *et al.* 2004, Hurtado *et al.* 2008, Zhao *et al.* 2009). Another ER α -interacting protein that can affect ER α complex formation and gene expression is the transcriptional regulator RIP140 (Rosell *et al.* 2014). Genes under the specific control of RIP140 (identified by siRNA experiments) could be used to classify tamoxifen-treated patients on clinical outcome (Rosell *et al.* 2014). Both RIP140 and the p160 family members further stipulate the observation that

the composition of the transcriptional complex may differ on a genome-wide scale, which could have direct physiological consequences on the level of transcriptional output and clinical response (Fig. 2).

ER α phosphorylations and genome-wide effects on ER α action

Besides the composition of the transcriptional complex, phosphorylations on ER α can also regulate the transcriptional activity of the receptor and play a crucial role in endocrine resistance (Joel *et al.* 1998, de Leeuw *et al.* 2013). These phosphorylation events mainly revolve around serine residues 104/106 (Thomas *et al.* 2008), 118 (Kok *et al.* 2009), 167 (Yamashita *et al.* 2005), 236 (Atriku *et al.* 2009), and 305 (Michalides *et al.* 2004). The kinases involved in phosphorylation of ER α at s104/106 include CDK2 and ERK1/2 (Rogatsky *et al.* 1999, Thomas *et al.* 2008); at s118 ERK1/2, EGFR, and IGF1R (Park *et al.* 2005, Santen *et al.* 2009); at s167 AKT and CK2 (Arnold *et al.* 1994, Campbell *et al.* 2001); at s236 PKA (Chen *et al.* 1999b); and at s305 PAK1 and PKA (Wang *et al.* 2002, Michalides *et al.* 2004). The clinical implications of these phosphorylations remain not fully understood, where higher expressions of s118 and s167 phosphorylations are generally but not uniformly associated with a favorable outcome in patients on tamoxifen therapy (Murphy *et al.* 2004, 2011, Jiang *et al.* 2007, Yamashita *et al.* 2005, 2008), whereas the s305 phosphorylation is associated with a poor clinical outcome (Kok *et al.* 2011, Murphy *et al.* 2011). Furthermore, s118 phosphorylation expression appears to be a predictive biomarker for tamoxifen response (Murphy *et al.* 2004, Kok *et al.* 2009). Recently, the phosphorylation on the 594 threonine (t594) residue of ER α was found to play a key role in the regulatory interaction of ER α with 14-3-3 proteins (De Vries-van Leeuwen *et al.* 2013). This t594 phosphorylation resulted in decreased estradiol-stimulated ER α dimerization, reduced ER α -chromatin interactions, and reduced gene expression (De Vries-van Leeuwen *et al.* 2013).

The spectrum of ER α phosphorylation events appears to be able to dictate differential transcriptional programs of ER α , as exemplified by the PKA-induced s305 phosphorylation that redirects ER α to differential transcriptional start sites, translating into a 26-gene expression classifier that identified patients with a poor clinical outcome after tamoxifen treatment (de Leeuw *et al.* 2013). Additionally, it was found that stimulation of ER α by EGF, which induces s118 phosphorylation

(Bunone *et al.* 1996), led to a distinct cistromic landscape and induced a unique set of genes, compared to estradiol stimulation (Lupien *et al.* 2010). Stimulation of ER α by AKT, capable of inducing S167 phosphorylation (Campbell *et al.* 2001), also mediated changes in ER α chromatin binding and altered its transcriptional output (Bhat-Nakshatri *et al.* 2008), further indicating that specific phosphorylations of ER α may yield distinct genomic actions and may target unique locations throughout the genome (Fig. 2). Although the binding patterns of some of the phosphorylated ER α forms are known, a complete and comparative overview is still lacking. Furthermore, multiple reports have studied ER α cistromics upon activation of a specific cellular signaling cascade, including the previously mentioned AKT or EGF, where it still remains elusive which specific variable is actually responsible for the altered ER α behavior.

Besides the effect direct ER α phosphorylations can have on ER α 's genomic landscape and transcriptional activity, posttranslational modifications of coregulators can also influence ER α action. Where ER α -bound SRC3 binding is predominantly enhancer bound, phosphorylated SRC3 at Ser543 (pSRC3) was selectively found at promoters of ER α -regulated genes (Zwart *et al.* 2015). pSRC3 functioned as an independent prognostic factor as well as a predictive marker for tamoxifen treatment, potentially enabling the identification of patients with a good clinical outcome without receiving adjuvant therapy (Zwart *et al.* 2015). Additionally, SRC2 can be phosphorylated at Ser736 through the MAPK pathway, increasing SRC2 interactions with p300 and CBP, further facilitating SRC2 recruitment to the ER α complex (Lopez *et al.* 2001). These posttranslational modifications on coregulators further illustrate the intrinsic complexity and flexibility of ER α transcription complex formation, where multiple cell signaling cascades converge to collaboratively fine-tune ER α action on a genome-wide scale (Fig. 2).

Cistromic analyses in clinical samples and potential clinical applications

Over recent years, the transition has been made from studying ER α cistromics in cell lines toward genomic interrogation of ER α sites in clinical specimens. Obviously, in contrast to cell lines, clinical samples cannot be readily manipulated and represent heterogeneous populations of multiple cell types. Even with this difference between tumors and cell lines, the cistromic information obtained from both settings yields quite similar conclusions. When

looking at ER α , most well-described ER α -binding sites found in MCF-7 cells (Carroll *et al.* 2005, Welboren *et al.* 2009) such as enhancer regions proximal to RARA, GREB1, XBP1, and TFF1 are also observed in tumor specimens (Ross-Innes *et al.* 2012). Not only for ER α but also for its coregulators, the overlap of chromatin binding in cell lines versus clinical specimens was considerably high. For example, SRC3-pS543 ChIP-seq analyses showed 51% overlap in binding sites between MCF-7 cells and an ER+/PR+ breast tumor, being on the same order of magnitude as found between two tumor samples (61% overlap; Zwart *et al.* 2015).

The first analyses of ER α -binding patterns in clinical samples directly illustrated the added value of assessing ER α binding in clinical specimens (Ross-Innes *et al.* 2012), where differential ER α -binding sites found between tumors could stratify patients on outcome (Ross-Innes *et al.* 2012). A more recent study identified ER α -chromatin-binding patterns in primary breast tumors that enabled patient classification on their response to aromatase inhibition in the metastatic setting (Jansen *et al.* 2013). This same report analyzed profiles for H3K27me3, resulting in a gene classifier that seemed to outperform other prognostic classifiers, including Oncotype DX (Cobleigh *et al.* 2005) and PAM50 (Parker *et al.* 2009). As the classification potential of these genes was only partially preserved in a cohort of tamoxifen-treated patients, this suggests some treatment selectivity for patient classification. Both studies demonstrate clear advantages of studying ER α cistromic analyses in clinical specimens, with the potential to facilitate tailored therapy selection and enable patient stratification on outcome.

Although these cistromic classifiers made use of associated gene profiles, it remains largely unknown which genes in these classifiers are now the driving force behind any prognostic or predictive effect. Fine-tuning these classifiers toward optimized gene sets and further biological investigation of these genes could reveal the biologically most relevant genes for disease progression and might lead to novel biological insights in ER α biology as well as potentially novel drug targets.

As the main function of ER α is to activate its downstream target genes involved in tumor progression, ER α cistromic analyses may yield novel drug targets. A key example for this line of thought can be found in Myc, representing one of the best-studied ER α -responsive genes (Dubik *et al.* 1987, Dubik & Shiu 1988, Watson *et al.* 1991) and widely accepted as a potent novel drug target in cancer (Soucek *et al.* 2008, Albihn *et al.* 2010).

Besides targeting ER α -regulated genes to inhibit its stimulatory effect, ER α cofactors also receive increasing attention as potential drug targets. Small-molecule inhibitors against both SRC1 and SRC3 (Wang *et al.* 2011b, 2014) or SRC3 alone (Yan *et al.* 2014), as well as a stimulator for SRC3 activity (Wang *et al.* 2015), have been recently identified and proved successful in inhibiting breast cancer cell proliferation *in vitro* as well as in xenograft mouse models. Such novel therapeutic options could revolutionize endocrine therapeutic drug design, not aiming at blocking the receptor itself, but targeting the proteins required for receptor action. As in case of endocrine therapy resistance ER α can still remain a driver (Vergote *et al.* 2006, Wang *et al.* 2009, Yoo *et al.* 2011), such novel inhibitors have the potency to remain effective after progression on currently available endocrine therapies.

Even though promising, at the moment there are no cistromic classifiers being used in the clinic. One of the major practical limitations is the typically low amounts of available tumor tissue. Although initially challenging, continuing technical developments, including single-tube linear DNA amplification method (Shankaranarayanan *et al.* 2011) and the combination of a high-sensitivity ChIP assay with new library preparation procedures (Adli *et al.* 2010), have now greatly increased the applicability of ChIP-seq on limited amounts of tissue. Another example of these developments is the incorporation of carrier chromatin that can be removed before library preparation, improving ChIP efficiency while limiting background signal (Zwart *et al.* 2013). Furthermore, a great promise for the future of ChIP-seq on limited tumor material might be found in the combination of microfluidics and DNA barcoding and sequencing, which have recently enabled the generation of ChIP-seq data at a single-cell resolution (Rotem *et al.* 2015).

Discussion

Within 10 years, ER α genomics has gone from single locus to genome wide and toward single cell. Initial reports on ER α cistromics in breast cancer have revolutionized the way we think about ER α action and ER α -responsive genes. By far, most transcriptional effects found regulated by ER α are represented as eRNAs. With conflicting reports about the role of eRNAs in chromosomal looping, a comprehensive overview of eRNA action, and with this to a certain degree a functional overview of ER α -enhancer action, is currently lacking. As ER α seems to function

mostly through chromatin loops, it is not unlikely that ER α enhancers and a subset of responsive eRNAs are functionally involved in such looping structures.

In ER α -positive breast cancer cell lines and tumors, many thousands of ER α -binding sites can be found, of which a large number are shared between them. This could imply a selection pressure throughout human evolution for the maintenance of these ER α sites throughout the human genome. As technological development continues, future studies will further elucidate the functional relevance of all these ER α sites and identify the genomic regions responsible for proliferative potential.

Clearly, our knowledge on ER α genomic regulation in breast cancer has increased exponentially over the last decade. A major factor in this is the parallel development of novel technologies and computational tools, which not only enable us to generate genomic data with an unprecedented level of data richness and detail but also enable us to process and understand the data. Now, with novel technologies on genome editing (*e.g.* CRISPR Cas9) and single-cell ChIP-seq analyses, the second decade of cistromics in breast cancer will no doubt unveil another layer of unprecedented complexity in breast cancer and may lead us toward a comprehensive understanding of the disease with its full genomic complexity and diversity.

Declaration of interest

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

Funding

This work did not receive any specific grant from any funding agency in the public, commercial, or not-for-profit sector.

Acknowledgments

The authors thank the Dutch Cancer Society KWF, Alpe d'HuZes, the Netherlands Organisation for Scientific Research (NWO), and A Sister's Hope for financial support.

References

- Adli M, Zhu J & Bernstein BE 2010 Genome-wide chromatin maps derived from limited numbers of hematopoietic progenitors. *Nature Methods* **7** 615–618. (doi:10.1038/nmeth.1478)
- Albihn A, Johnsen JI & Henriksson MA 2010 MYC in oncogenesis and as a target for cancer therapies. *Advances in Cancer Research* **107** 163–224. (doi:10.1016/S0065-230X(10)07006-5)
- Ando S, De Amicis F, Rago V, Carpino A, Maggiolini M, Panno ML & Lanzino M 2002 Breast cancer: from estrogen to androgen receptor.

- Molecular and Cellular Endocrinology* **193** 121–128. (doi:10.1016/S0303-7207(02)00105-3)
- Anzick SL, Kononen J, Walker RL, Azorsa DO, Tanner MM, Guan XY, Sauter G, Kallioniemi OP, Trent JM & Meltzer PS 1997 AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer. *Science* **277** 965–968. (doi:10.1126/science.277.5328.965)
- Arnold SE, Obourn JD, Jaffe H & Notides AC 1994 Serine 167 is the major estradiol-induced phosphorylation site on the human estrogen receptor. *Molecular Endocrinology* **8** 1208–1214.
- Arpino G, De Angelis C, Giuliano M, Giordano A, Falato C, De Laurentiis M & De Placido S 2009 Molecular mechanism and clinical implications of endocrine therapy resistance in breast cancer. *Oncology* **77** (Supplement 1) 23–37. (doi:10.1016/S0960-9776(11)70293-4)
- Atsriku C, Britton DJ, Held JM, Schilling B, Scott GK, Gibson BW, Benz CC & Baldwin MA 2009 Systematic mapping of posttranslational modifications in human estrogen receptor- α with emphasis on novel phosphorylation sites. *Molecular & Cellular Proteomics* **8** 467–480. (doi:10.1074/mcp.M800282-MCP200)
- Beatson G 1896 On treatment of inoperable cases of carcinoma of the mamma: suggestions for a new method of treatment, with illustrative cases. *Lancet* **2** 104–107. (doi:10.1016/S0140-6736(01)72307-0)
- Belandia B, Orford RL, Hurst HC & Parker MG 2002 Targeting of SWI/SNF chromatin remodelling complexes to estrogen-responsive genes. *EMBO Journal* **21** 4094–4103. (doi:10.1093/emboj/cdf412)
- Bhat-Nakshatri P, Wang G, Appaiah H, Luktuke N, Carroll JS, Geistlinger TR, Brown M, Badve S, Liu Y & Nakshatri H 2008 AKT alters genome-wide estrogen receptor α binding and impacts estrogen signaling in breast cancer. *Molecular and Cellular Biology* **28** 7487–7503. (doi:10.1128/MCB.00799-08)
- Blows FM, Driver KE, Schmidt MK, Broeks A, van Leeuwen FE, Wesseling J, Cheang MC, Gelmon K, Nielsen TO, Blomqvist C, et al. 2010 Subtyping of breast cancer by immunohistochemistry to investigate a relationship between subtype and short and long term survival: a collaborative analysis of data for 10,159 cases from 12 studies. *PLoS Medicine* **7** e1000279. (doi:10.1371/journal.pmed.1000279)
- Bolt MJ, Stossi F, Newberg JY, Orjalo A, Johansson HE & Mancini MA 2013 Coactivators enable glucocorticoid receptor recruitment to fine-tune estrogen receptor transcriptional responses. *Nucleic Acids Research* **41** 4036–4048. (doi:10.1093/nar/gkt100)
- Botta M, Haider S, Leung IX, Lio P & Mozziconacci J 2010 Intra- and inter-chromosomal interactions correlate with CTCF binding genome wide. *Molecular Systems Biology* **6** 426. (doi:10.1038/msb.2010.79)
- Bunone G, Briand PA, Miksic RJ & Picard D 1996 Activation of the unliganded estrogen receptor by EGF involves the MAP kinase pathway and direct phosphorylation. *EMBO Journal* **15** 2174–2183.
- Cahill MA, Ernst WH, Janknecht R & Nordheim A 1994 Regulatory squelching. *FEBS Letters* **344** 105–108. (doi:10.1016/0014-5793(94)00320-3)
- Campbell RA, Bhat-Nakshatri P, Patel NM, Constantinidou D, Ali S & Nakshatri H 2001 Phosphatidylinositol 3-kinase/AKT-mediated activation of estrogen receptor α : a new model for anti-estrogen resistance. *Journal of Biological Chemistry* **276** 9817–9824. (doi:10.1074/jbc.M010840200)
- Carraz M, Zwart W, Phan T, Michalides R & Brunsvelde L 2009 Perturbation of estrogen receptor α localization with synthetic nona-arginine LXXLL-peptide coactivator binding inhibitors. *Chemistry & Biology* **16** 702–711. (doi:10.1016/j.chembiol.2009.06.009)
- Carroll JS, Liu XS, Brodsky AS, Li W, Meyer CA, Szary AJ, Eeckhoutte J, Shao W, Hestermann EV, Geistlinger TR, et al. 2005 Chromosome-wide mapping of estrogen receptor binding reveals long-range regulation requiring the forkhead protein FoxA1. *Cell* **122** 33–43. (doi:10.1016/j.cell.2005.05.008)
- Castellano I, Allia E, Accortanzo V, Vandone AM, Chiusa L, Arisio R, Durando A, Donadio M, Bussolati G, Coates AS, et al. 2010 Androgen receptor expression is a significant prognostic factor in estrogen receptor positive breast cancers. *Breast Cancer Research and Treatment* **124** 607–617. (doi:10.1007/s10549-010-0761-y)
- Catelli MG, Binart N, Jung-Testas I, Renoir JM, Baulieu EE, Feramisco JR & Welch WJ 1985 The common 90-kd protein component of non-transformed '8S' steroid receptors is a heat-shock protein. *EMBO Journal* **4** 3131–3135.
- Chen H, Lin RJ, Schiltz RL, Chakravarti D, Nash A, Nagy L, Privalsky ML, Nakatani Y & Evans RM 1997 Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300. *Cell* **90** 569–580. (doi:10.1016/S0092-8674(00)80516-4)
- Chen D, Ma H, Hong H, Koh SS, Huang SM, Schurter BT, Aswad DW & Stallcup MR 1999a Regulation of transcription by a protein methyltransferase. *Science* **284** 2174–2177.
- Chen D, Pace PE, Coombes RC & Ali S 1999b Phosphorylation of human estrogen receptor α by protein kinase A regulates dimerization. *Molecular and Cellular Biology* **19** 1002–1015.
- Cheung E, Acevedo ML, Cole PA & Kraus WL 2005 Altered pharmacology and distinct coactivator usage for estrogen receptor-dependent transcription through activating protein-1. *PNAS* **102** 559–564. (doi:10.1073/pnas.0407113102)
- Chia K, O'Brien M, Brown M & Lim E 2015 Targeting the androgen receptor in breast cancer. *Current Oncology Reports* **17** 4. (doi:10.1007/s11912-014-0427-8)
- Cobleigh MA, Tabesh B, Bitterman P, Baker J, Cronin M, Liu ML, Borchik R, Mosquera JM, Walker MG & Shak S 2005 Tumor gene expression and prognosis in breast cancer patients with 10 or more positive lymph nodes. *Clinical Cancer Research* **11** 8623–8631. (doi:10.1158/1078-0432.CCR-05-0735)
- Core LJ, Waterfall JJ & Lis JT 2008 Nascent RNA sequencing reveals widespread pausing and divergent initiation at human promoters. *Science* **322** 1845–1848. (doi:10.1126/science.1162228)
- Dahlman-Wright K, Cavailles V, Fuqua SA, Jordan VC, Katzenellenbogen JA, Korach KS, Maggi A, Muramatsu M, Parker MG & Gustafsson JA 2006 International Union of Pharmacology. LXIV. Estrogen receptors. *Pharmacological Reviews* **58** 773–781. (doi:10.1124/pr.58.4.8)
- de Leeuw R, Flach K, Bentin Toaldo C, Alexi X, Canisius S, Neefjes J, Michalides R & Zwart W 2013 PKA phosphorylation redirects ER α to promoters of a unique gene set to induce tamoxifen resistance. *Oncogene* **32** 3543–3551. (doi:10.1038/onc.2012.361)
- De Vries-van Leeuwen IJ, da Costa Pereira D, Flach KD, Piersma SR, Haase C, Bier D, Yalcin Z, Michalides R, Feenstra KA, Jimenez CR, et al. 2013 Interaction of 14-3-3 proteins with the estrogen receptor α F domain provides a drug target interface. *PNAS* **110** 8894–8899. (doi:10.1073/pnas.1220809110)
- Devin-Leclerc J, Meng X, Delahaye F, Leclerc P, Baulieu EE & Catelli MG 1998 Interaction and dissociation by ligands of estrogen receptor and Hsp90: the antiestrogen RU 58668 induces a protein synthesis-dependent clustering of the receptor in the cytoplasm. *Molecular Endocrinology* **12** 842–854. (doi:10.1210/mend.12.6.0121)
- Dubik D, Dembinski TC & Shiu RP 1987 Stimulation of c-myc oncogene expression associated with estrogen-induced proliferation of human breast cancer cells. *Cancer Research* **47** 6517–6521.
- Dubik D & Shiu RP 1988 Transcriptional regulation of c-myc oncogene expression by estrogen in hormone-responsive human breast cancer cells. *Journal of Biological Chemistry* **263** 12705–12708.
- Early Breast Cancer Trialists' Collaborative Group, Davies C, Godwin J, Gray R, Clarke M, Cutter D, Darby S, McGale P, Pan HC, Taylor C, et al. 2011 Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. *Lancet* **378** 771–784. (doi:10.1016/S0140-6736(11)60993-8)

- Fabian CJ 2007 The what, why and how of aromatase inhibitors: hormonal agents for treatment and prevention of breast cancer. *International Journal of Clinical Practice* **61** 2051–2063. (doi:10.1111/j.1742-1241.2007.01587.x)
- Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D & Bray F 2015 Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *International Journal of Cancer* **136** E359–E386. (doi:10.1002/ijc.29210)
- Foulds CE, Feng Q, Ding C, Bailey S, Hunsaker TL, Malovannaya A, Hamilton RA, Gates LA, Zhang Z, Li C, et al. 2013 Proteomic analysis of coregulators bound to ERalpha on DNA and nucleosomes reveals coregulator dynamics. *Molecular Cell* **51** 185–199. (doi:10.1016/j.molcel.2013.06.007)
- Fullwood MJ, Liu MH, Pan YF, Liu J, Xu H, Mohamed YB, Orlov YL, Velkov S, Ho A, Mei PH, et al. 2009 An oestrogen-receptor-alpha-bound human chromatin interactome. *Nature* **462** 58–64. (doi:10.1038/nature08497)
- Glass CK, Rose DW & Rosenfeld MG 1997 Nuclear receptor coactivators. *Current Opinion in Cell Biology* **9** 222–232. (doi:10.1016/S0955-0674(97)80066-X)
- Greene GL, Gilna P, Waterfield M, Baker A, Hort Y & Shine J 1986 Sequence and expression of human estrogen receptor complementary DNA. *Science* **231** 1150–1154. (doi:10.1126/science.3753802)
- Hah N, Danko CG, Core L, Waterfall JJ, Siepel A, Lis JT & Kraus WL 2011 A rapid, extensive, and transient transcriptional response to estrogen signaling in breast cancer cells. *Cell* **145** 622–634. (doi:10.1016/j.cell.2011.03.042)
- Hah N, Murakami S, Nagari A, Danko CG & Kraus WL 2013 Enhancer transcripts mark active estrogen receptor binding sites. *Genome Research* **23** 1210–1223. (doi:10.1101/gr.152306.112)
- Hakim O, John S, Ling JQ, Biddie SC, Hoffman AR & Hager GL 2009 Glucocorticoid receptor activation of the Ciz1-Lcn2 locus by long range interactions. *Journal of Biological Chemistry* **284** 6048–6052. (doi:10.1074/jbc.C800212200)
- Handoko L, Xu H, Li G, Ngan CY, Chew E, Schnapp M, Lee CW, Ye C, Ping JL, Mulawadi F, et al. 2011 CTCF-mediated functional chromatin interactome in pluripotent cells. *Nature Genetics* **43** 630–638. (doi:10.1038/ng.857)
- Hayashi SI, Eguchi H, Tanimoto K, Yoshida T, Omoto Y, Inoue A, Yoshida N & Yamaguchi Y 2003 The expression and function of estrogen receptor alpha and beta in human breast cancer and its clinical application. *Endocrine-Related Cancer* **10** 193–202. (doi:10.1677/erc.0.0100193)
- Hong H, Kohli K, Garabedian MJ & Stallcup MR 1997 GRIP1, a transcriptional coactivator for the AF-2 transactivation domain of steroid, thyroid, retinoid, and vitamin D receptors. *Molecular and Cellular Biology* **17** 2735–2744. (doi:10.1128/MCB.17.5.2735)
- Hu R, Dawood S, Holmes MD, Collins LC, Schnitt SJ, Cole K, Marotti JD, Hankinson SE, Colditz GA & Tamimi RM 2011 Androgen receptor expression and breast cancer survival in postmenopausal women. *Clinical Cancer Research* **17** 1867–1874. (doi:10.1158/1078-0432.CCR-10-2021)
- Hurtado A, Holmes KA, Geistlinger TR, Hutcheson IR, Nicholson RI, Brown M, Jiang J, Howat WJ, Ali S & Carroll JS 2008 Regulation of ERBB2 by oestrogen receptor-PAX2 determines response to tamoxifen. *Nature* **456** 663–666. (doi:10.1038/nature07483)
- Hurtado A, Holmes KA, Ross-Innes CS, Schmidt D & Carroll JS 2011 FOXA1 is a key determinant of estrogen receptor function and endocrine response. *Nature Genetics* **43** 27–33. (doi:10.1038/ng.730)
- Jansen MP, Knijnenburg T, Reijm EA, Simon I, Kerkhoven R, Droog M, Velds A, van Laere S, Dirix L, Alexi X, et al. 2013 Hallmarks of aromatase inhibitor drug resistance revealed by epigenetic profiling in breast cancer. *Cancer Research* **73** 6632–6641. (doi:10.1158/0008-5472.CAN-13-0704)
- Jensen EV & DeSombre ER 1973 Estrogen-receptor interaction. *Science* **182** 126–134. (doi:10.1126/science.182.4108.126)
- Jiang J, Sarwar N, Peston D, Kulinskaya E, Shousha S, Coombes RC & Ali S 2007 Phosphorylation of estrogen receptor-alpha at Ser167 is indicative of longer disease-free and overall survival in breast cancer patients. *Clinical Cancer Research* **13** 5769–5776. (doi:10.1158/1078-0432.CCR-07-0822)
- Joel PB, Smith J, Sturgill TW, Fisher TL, Blenis J & Lannigan DA 1998 pp90orsk1 regulates estrogen receptor-mediated transcription through phosphorylation of Ser-167. *Molecular and Cellular Biology* **18** 1978–1984. (doi:10.1128/MCB.18.4.1978)
- Jordan VC & Murphy CS 1990 Endocrine pharmacology of antiestrogens as antitumor agents. *Endocrine Reviews* **11** 578–610. (doi:10.1210/edrv-11-4-578)
- Jozwik KM & Carroll JS 2012 Pioneer factors in hormone-dependent cancers. *Nature Reviews. Cancer* **12** 381–385. (doi:10.1038/nrc3263)
- Karmakar S, Jin Y & Nagaich AK 2013 Interaction of glucocorticoid receptor (GR) with estrogen receptor (ER) alpha and activator protein 1 (AP1) in dexamethasone-mediated interference of ERalpha activity. *Journal of Biological Chemistry* **288** 24020–24034. (doi:10.1074/jbc.M113.473819)
- Katzenellenbogen BS, Miller MA, Mullick A & Sheen YY 1985 Antiestrogen action in breast cancer cells: modulation of proliferation and protein synthesis, and interaction with estrogen receptors and additional antiestrogen binding sites. *Breast Cancer Research and Treatment* **5** 231–243. (doi:10.1007/BF01806018)
- Kawazu M, Saso K, Tong KI, McQuire T, Goto K, Son DO, Wakeham A, Miyagishi M, Mak TW & Okada H 2011 Histone demethylase JMJD2B functions as a co-factor of estrogen receptor in breast cancer proliferation and mammary gland development. *PLoS One* **6** e17830. (doi:10.1371/journal.pone.0017830)
- Kok M, Holm-Wigerup C, Hauptmann M, Michalides R, Stal O, Linn S & Landberg G 2009 Estrogen receptor-alpha phosphorylation at serine-118 and tamoxifen response in breast cancer. *Journal of the National Cancer Institute* **101** 1725–1729. (doi:10.1093/jnci/djp412)
- Kok M, Zwart W, Holm C, Fles R, Hauptmann M, Van't Veer LJ, Wessels LF, Neefjes J, Stal O, Linn SC, et al. 2011 PKA-induced phosphorylation of ERalpha at serine 305 and high PAK1 levels is associated with sensitivity to tamoxifen in ER-positive breast cancer. *Breast Cancer Research and Treatment* **125** 1–12. (doi:10.1007/s10549-010-0798-y)
- Kong SL, Li G, Loh SL, Sung WK & Liu ET 2011 Cellular reprogramming by the conjoint action of ERalpha, FOXA1, and GATA3 to a ligand-inducible growth state. *Molecular Systems Biology* **7** 526. (doi:10.1038/msb.2011.59)
- Korkmaz G, Lopes R, Ugalde AP, Nevedomskaya E, Han R, Myacheva K, Zwart W, Elkon R & Agami R 2016 Functional genetic screens for enhancer elements in the human genome using CRISPR-Cas9. *Nature Biotechnology* **34** 192–198. (doi:10.1038/nbt.3450)
- Kumar V & Chambon P 1988 The estrogen receptor binds tightly to its responsive element as a ligand-induced homodimer. *Cell* **55** 145–156.
- Kushner PJ, Agard DA, Greene GL, Scanlan TS, Shiau AK, Uht RM & Webb P 2000 Estrogen receptor pathways to AP-1. *Journal of Steroid Biochemistry and Molecular Biology* **74** 311–317. (doi:10.1016/S0960-0760(00)00108-4)
- Li W, Notani D, Ma Q, Tanasa B, Nunez E, Chen AY, Merkurjev D, Zhang J, Ohgi K, Song X, et al. 2013 Functional roles of enhancer RNAs for oestrogen-dependent transcriptional activation. *Nature* **498** 516–520. (doi:10.1038/nature12210)
- Lieberman-Aiden E, van Berkum NL, Williams L, Imakaev M, Ragoczy T, Telling A, Amit I, Lajoie BR, Sabo PJ, Dorschner MO, et al. 2009 Comprehensive mapping of long-range interactions reveals folding principles of the human genome. *Science* **326** 289–293. (doi:10.1126/science.1181369)
- Lopez GN, Webb P, Shinsako JH, Baxter JD, Greene GL & Kushner PJ 1999 Titration by estrogen receptor activation function-2 of targets that are

- downstream from coactivators. *Molecular Endocrinology* **13** 897–909. (doi:10.1210/mend.13.6.0283)
- Lopez GN, Turck CW, Schauele F, Stallcup MR & Kushner PJ 2001 Growth factors signal to steroid receptors through mitogen-activated protein kinase regulation of p160 coactivator activity. *Journal of Biological Chemistry* **276** 22177–22182. (doi:10.1074/jbc.M010718200)
- Lupien M, Eeckhoutte J, Meyer CA, Wang Q, Zhang Y, Li W, Carroll JS, Liu XS & Brown M 2008 FoxA1 translates epigenetic signatures into enhancer-driven lineage-specific transcription. *Cell* **132** 958–970. (doi:10.1016/j.cell.2008.01.018)
- Lupien M & Brown M 2009 Cistromics of hormone-dependent cancer. *Endocrine-Related Cancer* **16** 381–389. (doi:10.1677/ERC-09-0038)
- Lupien M, Meyer CA, Bailey ST, Eeckhoutte J, Cook J, Westerling T, Zhang X, Carroll JS, Rhodes DR, Liu XS, *et al.* 2010 Growth factor stimulation induces a distinct ER(alpha) cistrome underlying breast cancer endocrine resistance. *Genes and Development* **24** 2219–2227. (doi:10.1101/gad.1944810)
- Magnani L, Ballantyne EB, Zhang X & Lupien M 2011 PBX1 genomic pioneer function drives ERalpha signaling underlying progression in breast cancer. *PLoS Genetics* **7** e1002368. (doi:10.1371/journal.pgen.1002368)
- Margeat E, Poujol N, Boulahtouf A, Chen Y, Muller JD, Gratton E, Cavailles V & Royer CA 2001 The human estrogen receptor alpha dimer binds a single SRC-1 coactivator molecule with an affinity dictated by agonist structure. *Journal of Molecular Biology* **306** 433–442. (doi:10.1006/jmbi.2000.4418)
- McKenna NJ, Xu J, Nawaz Z, Tsai SY, Tsai MJ & O'Malley BW 1999 Nuclear receptor coactivators: multiple enzymes, multiple complexes, multiple functions. *Journal of Steroid Biochemistry and Molecular Biology* **69** 3–12. (doi:10.1016/S0960-0760(98)00144-7)
- Melo CA, Drost J, Wijchers PJ, van de Werken H, de Wit E, Oude Vrielink JA, Elkon R, Melo SA, Leveille N, Kalluri R, *et al.* 2013 eRNAs are required for p53-dependent enhancer activity and gene transcription. *Molecular Cell* **49** 524–535. (doi:10.1016/j.molcel.2012.11.021)
- Metivier R, Penot G, Hubner MR, Reid G, Brand H, Kos M & Gannon F 2003 Estrogen receptor-alpha directs ordered, cyclical, and combinatorial recruitment of cofactors on a natural target promoter. *Cell* **115** 751–763. (doi:10.1016/S0092-8674(03)00934-6)
- Meyer ME, Gronemeyer H, Turcotte B, Bocquel MT, Tasset D & Chambon P 1989 Steroid hormone receptors compete for factors that mediate their enhancer function. *Cell* **57** 433–442. (doi:10.1016/0092-8674(89)90918-5)
- Michalides R, Griekspoor A, Balkenende A, Verwoerd D, Janssen L, Jalink K, Floore A, Velds A, van't Veer L & Neeftjes J 2004 Tamoxifen resistance by a conformational arrest of the estrogen receptor alpha after PKA activation in breast cancer. *Cancer Cell* **5** 597–605. (doi:10.1016/j.ccr.2004.05.016)
- Mohammed H, D'Santos C, Serandour AA, Ali HR, Brown GD, Atkins A, Rueda OM, Holmes KA, Theodorou V, Robinson JL, *et al.* 2013 Endogenous purification reveals GREB1 as a key estrogen receptor regulatory factor. *Cell Reports* **3** 342–349. (doi:10.1016/j.celrep.2013.01.010)
- Mohammed H, Russell IA, Stark R, Rueda OM, Hickey TE, Tarulli GA, Serandour AA, Birrell SN, Bruna A, Saadi A, *et al.* 2015 Progesterone receptor modulates ERalpha action in breast cancer. *Nature* **523** 313–317. (doi:10.1038/nature14583)
- Mourad R, Hsu PY, Juan L, Shen C, Koneru P, Lin H, Liu Y, Nephew K, Huang TH & Li L 2014 Estrogen induces global reorganization of chromatin structure in human breast cancer cells. *PLoS One* **9** e113354. (doi:10.1371/journal.pone.0113354)
- Mousavi K, Zare H, Dell'orso S, Grontved L, Gutierrez-Cruz G, Derfoul A, Hager GL & Sartorelli V 2013 eRNAs promote transcription by establishing chromatin accessibility at defined genomic loci. *Molecular Cell* **51** 606–617. (doi:10.1016/j.molcel.2013.07.022)
- Murphy LC, Niu Y, Snell L & Watson P 2004 Phospho-serine-118 estrogen receptor-alpha expression is associated with better disease outcome in women treated with tamoxifen. *Clinical Cancer Research* **10** 5902–5906. (doi:10.1158/1078-0432.CCR-04-0191)
- Murphy LC, Seekallu SV & Watson PH 2011 Clinical significance of estrogen receptor phosphorylation. *Endocrine-Related Cancer* **18** R1–R14. (doi:10.1677/erc-10-0070)
- Niemeier LA, Dabbs DJ, Beriwal S, Striebel JM & Bhargava R 2010 Androgen receptor in breast cancer: expression in estrogen receptor-positive tumors and in estrogen receptor-negative tumors with apocrine differentiation. *Modern Pathology* **23** 205–212. (doi:10.1038/modpathol.2009.159)
- Ogryzko VV, Schiltz RL, Russanova V, Howard BH & Nakatani Y 1996 The transcriptional coactivators p300 and CBP are histone acetyltransferases. *Cell* **87** 953–959. (doi:10.1016/S0092-8674(00)82001-2)
- Onate SA, Tsai SY, Tsai MJ & O'Malley BW 1995 Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. *Science* **270** 1354–1357. (doi:10.1126/science.270.5240.1354)
- Osborne CK & Schiff R 2003 Growth factor receptor cross-talk with estrogen receptor as a mechanism for tamoxifen resistance in breast cancer. *Breast* **12** 362–367. (doi:10.1016/S0960-9776(03)00137-1)
- Paige LA, Christensen DJ, Gron H, Norris JD, Gottlin EB, Padilla KM, Chang CY, Ballas LM, Hamilton PT, McDonnell DP, *et al.* 1999 Estrogen receptor (ER) modulators each induce distinct conformational changes in ER alpha and ER beta. *PNAS* **96** 3999–4004. (doi:10.1073/pnas.96.7.3999)
- Pan YE, Wansa KD, Liu MH, Zhao B, Hong SZ, Tan PY, Lim KS, Bourque G, Liu ET & Cheung E 2008 Regulation of estrogen receptor-mediated long range transcription via evolutionarily conserved distal response elements. *Journal of Biological Chemistry* **283** 32977–32988. (doi:10.1074/jbc.M802024200)
- Park KJ, Krishnan V, O'Malley BW, Yamamoto Y & Gaynor RB 2005 Formation of an IKKalpha-dependent transcription complex is required for estrogen receptor-mediated gene activation. *Molecular Cell* **18** 71–82. (doi:10.1016/j.molcel.2005.03.006)
- Parker JS, Mullins M, Cheang MC, Leung S, Voduc D, Vickery T, Davies S, Fauron C, He X, Hu Z, *et al.* 2009 Supervised risk predictor of breast cancer based on intrinsic subtypes. *Journal of Clinical Oncology* **27** 1160–1167. (doi:10.1200/JCO.2008.18.1370)
- Peters AA, Buchanan G, Ricciardelli C, Bianco-Miotto T, Centenera MM, Harris JM, Jindal S, Segara D, Jia L, Moore NL, *et al.* 2009 Androgen receptor inhibits estrogen receptor-alpha activity and is prognostic in breast cancer. *Cancer Research* **69** 6131–6140. (doi:10.1158/0008-5472.CAN-09-0452)
- Pichon MF, Pallud C, Brunet M & Milgrom E 1980 Relationship of presence of progesterone receptors to prognosis in early breast cancer. *Cancer Research* **40** 3357–3360.
- Pratt WB & Toft DO 1997 Steroid receptor interactions with heat shock protein and immunophilin chaperones. *Endocrine Reviews* **18** 306–360.
- Qi JP, Yang YL, Zhu H, Wang J, Jia Y, Liu N, Song YJ, Zan LK, Zhang X, Zhou M, *et al.* 2012 Expression of the androgen receptor and its correlation with molecular subtypes in 980 chinese breast cancer patients. *Breast Cancer* **6** 1–8. (doi:10.4137/BCBCR.S8323)
- Reddy TE, Pauli F, Sprouse RO, Neff NE, Newberry KM, Garabedian MJ & Myers RM 2009 Genomic determination of the glucocorticoid response reveals unexpected mechanisms of gene regulation. *Genome Research* **19** 2163–2171. (doi:10.1101/gr.097022.109)
- Rhee HS & Pugh BF 2011 Comprehensive genome-wide protein-DNA interactions detected at single-nucleotide resolution. *Cell* **147** 1408–1419. (doi:10.1016/j.cell.2011.11.013)
- Rhee HS & Pugh BF 2012 ChIP-exo method for identifying genomic location of DNA-binding proteins with near-single-nucleotide accuracy. *Current Protocols in Molecular Biology* Chapter **21** Unit 21.24. (doi:10.1002/0471142727.mb2124s100)

- Rogatsky I, Trowbridge JM & Garabedian MJ 1999 Potentiation of human estrogen receptor alpha transcriptional activation through phosphorylation of serines 104 and 106 by the cyclin A-CDK2 complex. *Journal of Biological Chemistry* **274** 22296–22302. (doi:10.1074/jbc.274.32.22296)
- Rosell M, Nevedomskaya E, Stelloo S, Nautiyal J, Poliandri A, Steel JH, Wessels LF, Carroll JS, Parker MG & Zwart W 2014 Complex formation and function of estrogen receptor alpha in transcription requires RIP140. *Cancer Research* **74** 5469–5479. (doi:10.1158/0008-5472.CAN-13-3429)
- Ross-Innes CS, Stark R, Teschendorff AE, Holmes KA, Ali HR, Dunning MJ, Brown GD, Gojis O, Ellis IO, Green AR, *et al.* 2012 Differential oestrogen receptor binding is associated with clinical outcome in breast cancer. *Nature* **481** 389–393. (doi:10.1038/nature10730)
- Rotem A, Ram O, Shores N, Sperling RA, Goren A, Weitz DA & Bernstein BE 2015 Single-cell ChIP-seq reveals cell subpopulations defined by chromatin state. *Nature Biotechnology* **33** 1165–1172. (doi:10.1038/nbt.3383)
- Santen RJ, Fan P, Zhang Z, Bao Y, Song RX & Yue W 2009 Estrogen signals via an extra-nuclear pathway involving IGF-1R and EGFR in tamoxifen-sensitive and -resistant breast cancer cells. *Steroids* **74** 586–594. (doi:10.1016/j.steroids.2008.11.020)
- Schmidt D, Schwalie PC, Ross-Innes CS, Hurtado A, Brown GD, Carroll JS, Flicek P & Odom DT 2010 A CTCF-independent role for cohesin in tissue-specific transcription. *Genome Research* **20** 578–588. (doi:10.1101/gr.100479.109)
- Serandour AA, Brown GD, Cohen JD & Carroll JS 2013 Development of an Illumina-based ChIP-exonuclease method provides insight into FoxA1-DNA binding properties. *Genome Biology* **14** R147. (doi:10.1186/gb-2013-14-12-r147)
- Shang Y, Hu X, DiRenzo J, Lazar MA & Brown M 2000 Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription. *Cell* **103** 843–852. (doi:10.1016/S0092-8674(00)00188-4)
- Shankaranarayanan P, Mendoza-Parra MA, Walia M, Wang L, Li N, Trindade LM & Gronemeyer H 2011 Single-tube linear DNA amplification (LinDA) for robust ChIP-seq. *Nature Methods* **8** 565–567. (doi:10.1038/nmeth.1626)
- Sharma NL, Massie CE, Ramos-Montoya A, Zecchini V, Scott HE, Lamb AD, MacArthur S, Stark R, Warren AY, Mills IG, *et al.* 2013 The androgen receptor induces a distinct transcriptional program in castration-resistant prostate cancer in man. *Cancer Cell* **23** 35–47. (doi:10.1016/j.ccr.2012.11.010)
- Shi L, Sun L, Li Q, Liang J, Yu W, Yi X, Yang X, Li Y, Han X, Zhang Y, *et al.* 2011 Histone demethylase JMJD2B coordinates H3K4/H3K9 methylation and promotes hormonally responsive breast carcinogenesis. *PNAS* **108** 7541–7546. (doi:10.1073/pnas.1017374108)
- Shiau AK, Barstad D, Loria PM, Cheng L, Kushner PJ, Agard DA & Greene GL 1998 The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. *Cell* **95** 927–937. (doi:10.1016/S0092-8674(00)81717-1)
- Shou J, Massarweh S, Osborne CK, Wakeling AE, Ali S, Weiss H & Schiff R 2004 Mechanisms of tamoxifen resistance: increased estrogen receptor-HER2/neu cross-talk in ER/HER2-positive breast cancer. *Journal of the National Cancer Institute* **96** 926–935. (doi:10.1093/jnci/djh166)
- Soucek L, Whitfield J, Martins CP, Finch AJ, Murphy DJ, Sodir NM, Karnezis AN, Swigart LB, Nasi S & Evan GI 2008 Modelling Myc inhibition as a cancer therapy. *Nature* **455** 679–683. (doi:10.1038/nature07260)
- Splinter E, Heath H, Kooren J, Palstra RJ, Klous P, Grosveld F, Galjart N & de Laat W 2006 CTCF mediates long-range chromatin looping and local histone modification in the beta-globin locus. *Genes and Development* **20** 2349–2354. (doi:10.1101/gad.399506)
- Stallcup MR, Chen D, Koh SS, Ma H, Lee YH, Li H, Schurter BT & Aswad DW 2000 Co-operation between protein-acetylating and protein-methylating co-activators in transcriptional activation. *Biochemical Society Transactions* **28** 415–418. (doi:10.1042/bst0280415)
- Stender JD, Kim K, Charn TH, Komm B, Chang KC, Kraus WL, Benner C, Glass CK & Katzenellenbogen BS 2010 Genome-wide analysis of estrogen receptor alpha DNA binding and tethering mechanisms identifies Runx1 as a novel tethering factor in receptor-mediated transcriptional activation. *Molecular and Cellular Biology* **30** 3943–3955. (doi:10.1128/MCB.00118-10)
- Suen CS, Berrodin TJ, Mastroeni R, Cheskis BJ, Lyttle CR & Frail DE 1998 A transcriptional coactivator, steroid receptor coactivator-3, selectively augments steroid receptor transcriptional activity. *Journal of Biological Chemistry* **273** 27645–27653.
- Sun M, Gadad SS, Kim DS & Kraus WL 2015 Discovery, annotation, and functional analysis of long noncoding RNAs controlling cell-cycle gene expression and proliferation in breast cancer cells. *Molecular Cell* **59** 698–711. (doi:10.1016/j.molcel.2015.06.023)
- Tan SK, Lin ZH, Chang CW, Varang V, Chng KR, Pan YF, Yong EL, Sung WK & Cheung E 2011 AP-2gamma regulates oestrogen receptor-mediated long-range chromatin interaction and gene transcription. *EMBO Journal* **30** 2569–2581. (doi:10.1038/emboj.2011.151)
- Theodorou V, Stark R, Menon S & Carroll JS 2013 GATA3 acts upstream of FOXA1 in mediating ESR1 binding by shaping enhancer accessibility. *Genome Research* **23** 12–22. (doi:10.1101/gr.139469.112)
- Thomson A 1902 Analysis of cases in which oophorectomy was performed for inoperable carcinoma of the breast. *BMJ* **2** 1538–1541. (doi:10.1136/bmj.2.2184.1538)
- Thomas RS, Sarwar N, Phoenix F, Coombes RC & Ali S 2008 Phosphorylation at serines 104 and 106 by Erk1/2 MAPK is important for estrogen receptor-alpha activity. *Journal of Molecular Endocrinology* **40** 173–184. (doi:10.1677/JME-07-0165)
- Torchia J, Rose DW, Inostroza J, Kamei Y, Westin S, Glass CK & Rosenfeld MG 1997 The transcriptional co-activator p/CIP binds CBP and mediates nuclear-receptor function. *Nature* **387** 677–684. (doi:10.1038/42652)
- Umayahara Y, Kawamori R, Watada H, Imano E, Iwama N, Morishima T, Yamasaki Y, Kajimoto Y & Kamada T 1994 Estrogen regulation of the insulin-like growth factor I gene transcription involves an AP-1 enhancer. *Journal of Biological Chemistry* **269** 16433–16442.
- Vergote I, Amant F, Leunen K, Van Gorp T, Berteloot P & Neven P 2006 Metastatic breast cancer: sequencing hormonal therapy and positioning of fulvestrant. *International Journal of Gynecological Cancer* **16** (Supplement 2) 524–526.
- Voegel JJ, Heine MJ, Zechel C, Chambon P & Gronemeyer H 1996 TIF2, a 160 kDa transcriptional mediator for the ligand-dependent activation function AF-2 of nuclear receptors. *EMBO Journal* **15** 3667–3675.
- Walter P, Green S, Greene G, Krust A, Bornert JM, Jeltsch JM, Staub A, Jensen E, Scragge G, Waterfield M *et al.* 1985 Cloning of the human estrogen receptor cDNA. *PNAS* **82** 7889–7893.
- Wang RA, Mazumdar A, Vadlamudi RK & Kumar R 2002 P21-activated kinase-1 phosphorylates and transactivates estrogen receptor-alpha and promotes hyperplasia in mammary epithelium. *EMBO Journal* **21** 5437–5447. (doi:10.1093/emboj/cdf543)
- Wang Q, Carroll JS & Brown M 2005 Spatial and temporal recruitment of androgen receptor and its coactivators involves chromosomal looping and polymerase tracking. *Molecular Cell* **19** 631–642. (doi:10.1016/j.molcel.2005.07.018)
- Wang J, Jain S, Coombes CR & Palmieri C 2009 Fulvestrant in advanced breast cancer following tamoxifen and aromatase inhibition: a single center experience. *Breast Journal* **15** 247–253. (doi:10.1111/j.1524-4741.2009.00713.x)
- Wang D, Garcia-Bassets I, Benner C, Li W, Su X, Zhou Y, Qiu J, Liu W, Kaikkonen MU, Ohgi KA, *et al.* 2011a Reprogramming transcription

- by distinct classes of enhancers functionally defined by eRNA. *Nature* **474** 390–394. (doi:10.1038/nature10006)
- Wang Y, Lonard DM, Yu Y, Chow DC, Palzkill TG & O'Malley BW 2011b Small molecule inhibition of the steroid receptor coactivators, SRC-3 and SRC-1. *Molecular Endocrinology* **25** 2041–2053. (doi:10.1210/me.2011-1222)
- Wang Y, Lonard DM, Yu Y, Chow DC, Palzkill TG, Wang J, Qi R, Matzuk AJ, Song X, Madoux F, *et al.* 2014 Bufalin is a potent small-molecule inhibitor of the steroid receptor coactivators SRC-3 and SRC-1. *Cancer Research* **74** 1506–1517. (doi:10.1158/0008-5472.CAN-13-2939)
- Wang L, Yu Y, Chow DC, Yan F, Hsu CC, Stossi F, Mancini MA, Palzkill T, Liao L, Zhou S, *et al.* 2015 Characterization of a steroid receptor coactivator small molecule stimulator that overstimulates cancer cells and leads to cell stress and death. *Cancer Cell* **28** 240–252. (doi:10.1016/j.ccell.2015.07.005)
- Watson PH, Pon RT & Shiu RP 1991 Inhibition of c-myc expression by phosphorothioate antisense oligonucleotide identifies a critical role for c-myc in the growth of human breast cancer. *Cancer Research* **51** 3996–4000.
- Welboren WJ, van Driel MA, Janssen-Megens EM, van Heeringen SJ, Sweep FC, Span PN & Stunnenberg HG 2009 ChIP-Seq of ERalpha and RNA polymerase II defines genes differentially responding to ligands. *EMBO Journal* **28** 1418–1428. (doi:10.1038/emboj.2009.88)
- Yamashita H, Nishio M, Kobayashi S, Ando Y, Sugiura H, Zhang Z, Hamaguchi M, Mita K, Fujii Y & Iwase H 2005 Phosphorylation of estrogen receptor alpha serine 167 is predictive of response to endocrine therapy and increases postrelapse survival in metastatic breast cancer. *Breast Cancer Research* **7** R753–R764.
- Yamashita H, Nishio M, Toyama T, Sugiura H, Kondo N, Kobayashi S, Fujii Y & Iwase H 2008 Low phosphorylation of estrogen receptor alpha (ERalpha) serine 118 and high phosphorylation of ERalpha serine 167 improve survival in ER-positive breast cancer. *Endocrine-Related Cancer* **15** 755–763. (doi:10.1677/ERC-08-0078)
- Yan F, Yu Y, Chow DC, Palzkill T, Madoux F, Hodder P, Chase P, Griffin PR, O'Malley BW & Lonard DM 2014 Identification of verrucarin A as a potent and selective steroid receptor coactivator-3 small molecule inhibitor. *PLoS One* **9** e95243. (doi:10.1371/journal.pone.0095243)
- Yi P, Wang Z, Feng Q, Pintilie GD, Foulds CE, Lanz RB, Ludtke SJ, Schmid MF, Chiu W & O'Malley BW 2015 Structure of a biologically active estrogen receptor-coactivator complex on DNA. *Molecular Cell* **57** 1047–1058. (doi:10.1016/j.molcel.2015.01.025)
- Yoo C, Kim SB, Ahn JH, Jung KH, Ahn Y, Gong G, Kim HH, Kim HJ, Son BH & Ahn SH 2011 Efficacy of fulvestrant in heavily pretreated postmenopausal women with advanced breast cancer: a preliminary report. *Journal of Breast Cancer* **14** 135–139. (doi:10.4048/jbc.2011.14.2.135)
- Yu J, Yu J, Mani RS, Cao Q, Brenner CJ, Cao X, Wang X, Wu L, Li J, Hu M, *et al.* 2010 An integrated network of androgen receptor, polycomb, and TMPRSS2-ERG gene fusions in prostate cancer progression. *Cancer Cell* **17** 443–454. (doi:10.1016/j.ccr.2010.03.018)
- Zaret KS & Carroll JS 2011 Pioneer transcription factors: establishing competence for gene expression. *Genes and Development* **25** 2227–2241. (doi:10.1101/gad.176826.111)
- Zhang H, Yi X, Sun X, Yin N, Shi B, Wu H, Wang D, Wu G & Shang Y 2004 Differential gene regulation by the SRC family of coactivators. *Genes and Development* **18** 1753–1765. (doi:10.1101/gad.1194704)
- Zhao W, Zhang Q, Kang X, Jin S & Lou C 2009 AIB1 is required for the acquisition of epithelial growth factor receptor-mediated tamoxifen resistance in breast cancer cells. *Biochemical and Biophysical Research Communications* **380** 699–704. (doi:10.1016/j.bbrc.2009.01.155)
- Zwart W, Theodorou V, Kok M, Canisius S, Linn S & Carroll JS 2011 Oestrogen receptor-co-factor-chromatin specificity in the transcriptional regulation of breast cancer. *EMBO Journal* **30** 4764–4776. (doi:10.1038/emboj.2011.368)
- Zwart W, Koornstra R, Wesseling J, Rutgers E, Linn S & Carroll JS 2013 A carrier-assisted ChIP-seq method for estrogen receptor-chromatin interactions from breast cancer core needle biopsy samples. *BMC Genomics* **14** 232. (doi:10.1186/1471-2164-14-232)
- Zwart W, Flach KD, Rudraraju B, Abdel-Fatah TM, Gojis O, Moore D, Nevedomskaya E, Opdam M, Droog M, Hofland I, *et al.* 2015 SRC3 phosphorylation at Serine 543 is a positive independent prognostic factor in ER positive breast cancer. *Clinical Cancer Research* **22** 479–491. (doi:10.1158/1078-0432.CCR-14-3277)

Received in final form 11 February 2016

Accepted 23 February 2016

Accepted Preprint published online 23 February 2016