Oestrogen receptor–co-factor–chromatin specificity in the transcriptional regulation of breast cancer

This article has been corrected since Advance Online Publication and a corrigendum is also printed in this issue.

Wilbert Zwart^{1,2}, Vasiliki Theodorou²,
Marleen Kok¹, Sander Canisius¹,
Sabine Linn¹ and Jason S Carroll^{2,3,*}

¹The Netherlands Cancer Institute, Amsterdam, The Netherlands,

²Cancer Research UK, Cambridge Research Institute, Cambridge, UK and

³Department of Oncology, University of Cambridge, Cambridge, UK

The complexity of oestrogen receptor α (ER α)-mediated transcription is becoming apparent, but global insight into the co-regulatory proteins that assist ER α transcription is incomplete. Here, we present the most comprehensive chromatin-binding landscape of ER α co-regulatory proteins to date. We map by ChIP-seq the essential p160 co-regulators (SRC1, SRC2 and SRC3), and the histone acetyl transferases p300 and CBP in MCF-7 breast cancer cells. We find a complex network of co-regulator binding, with preferential binding sites for each co-regulator. Unlike previous suggestions, we find SRC recruitment almost exclusively following ligand treatment. Interestingly, we find specific subsets of genes regulated by ligand-dependent and -independent co-regulator recruitment. Co-factor-binding profiles were integrated with expression data from cell lines and primary tumour cohorts, to reveal specific transcriptional networks that influence clinical outcome. Genes that are bound by SRC3, but not other p160 proteins, have predictive value in cohorts of breast cancer patients. By generating a robust and global view of co-factor-binding properties, we discover new levels of co-regulator complexity, but also reveal specific gene networks that may influence endocrine response.

The EMBO Journal (2011) 30, 4764–4776. doi:10.1038/emboj.2011.368; Published online 14 October 2011

Subject Categories: chromatin & transcription; molecular biology of disease

Keywords: breast cancer; ChIP-seq; co-factors; oestrogen receptor; response to treatment

Introduction

Oestrogen receptor α (ER α) is a key transcriptional regulator in the development and homeostasis of female reproductive

organs. In luminal breast cancer, ER α is the causal transcription factor driving cell proliferation and is the main target in endocrine treatment. After ER α binds its natural ligand, estradiol, conformational changes occur within the receptor (Paige *et al*, 1999), activating the co-activator-binding pocket (Shiau *et al*, 1998). SRC (p160) co-activators are among the first factors to be recruited to ER α and this occurs via LXXLL motifs (Heery *et al*, 1997). There are three members of the p160 protein family; SRC1 (NCOA1), SRC2 (NCOA2, Tif2, GRIP1) and SRC3 (NCOA3, AIB1). The association of a p160 co-activator completely occupies the co-activator-binding groove on ER α , preventing other p160 co-activators from binding (Shiau *et al*, 1998; Carraz *et al*, 2009). Along these lines, co-activator recruitment to nuclear receptors has been found to be competitive and mutually exclusive (Ruse *et al*, 2002). These data are supported by the finding that an ER α dimer binds a single p160 (Margeat *et al*, 2001). Heterodimers between SRC1, SRC2 and SRC3 have been described (Zhang *et al*, 2004), but a global understanding of the underlying biological mechanisms and gene-specific interactions are lacking.

P160 co-activators contain multiple functional domains, which enable interaction with nuclear receptors and allow associations with other co-activators (Xu *et al*, 2009), including the histone acetyl transferases (HATs) p300 and CBP (Xu and O'Malley, 2002). Both p300 (Hanstein *et al*, 1996) and CBP (Smith *et al*, 1996) interact with p160 co-regulators as well as with ER α in a ligand-dependent manner (Kobayashi *et al*, 2000), modulating responsive-gene transcription (Kamei *et al*, 1996; Kraus and Kadonaga, 1998). CBP and p300 are highly homologous proteins with a shared domain structure (Goodman and Smolik, 2000).

Differential expression levels of p160 protein family members correlate with response to endocrine treatment in breast cancer (Osborne and Schiff, 2003; Hurtado *et al*, 2008; Su *et al*, 2008; Redmond *et al*, 2009). SRC3 is of particular interest, due to its frequent amplification in breast and ovarian tumours (Anzick *et al*, 1997). Furthermore, increased SRC3 expression correlates, in combination with increased ERBB2 expression, with poor tamoxifen response (Osborne *et al*, 2003; Shou *et al*, 2004; Hurtado *et al*, 2008; Zhao *et al*, 2009). For p300 and CBP expression levels, no direct link with response to treatment has been described in breast cancer, but correlations with tumour grade have been found (Hudelist *et al*, 2003). Although the biological role of these co-regulators is to modulate transcription of downstream targets, the specific regulatory regions and target genes are not completely known.

P300- and CBP-chromatin interactions have been studied on a genome-wide scale in glioblastoma cells, showing similar binding of these two co-regulators (Ramos *et al*,

*Corresponding author. Cancer Research UK, Cambridge Research Institute, Li Ka Shing Centre, Robinson Way, Cambridge CB2 0RE, UK. Tel.: +44 122 340 4510; Fax: +44 122 340 4199; E-mail: jason.carroll@cancer.org.uk

Received: 20 June 2011; accepted: 19 September 2011; published online: 14 October 2011; corrected: 30 November 2011

2010). In addition, SRC3 has been studied on a genome-wide scale in MCF-7 breast cancer cells (Lanz *et al*, 2010), and surprisingly showed E2 independent binding patterns, in contrast with reports focused on co-factor binding at specific *cis*-regulatory elements (Shang *et al*, 2000; Metivier *et al*, 2003).

Since the co-activators of interest have shared transcriptional targets and are thought to functionally compensate for each other and compete for binding to their transcription factors, it is imperative to compare the chromatin associations of all members of the p160 protein family. To this end, we have mapped the chromatin-binding properties of all three p160 co-regulators, plus p300 and CBP in breast cancer cells, in ligand-dependent and -independent conditions, to delineate their relative contributions in ER α -regulated transcription and pathology.

Results

Hormone-dependent recruitment of p160 co-regulators to ER α -binding sites

To determine the genome-wide binding preferences of SRC1, SRC2 and SRC3, we performed ChIP-seq analysis in MCF-7 breast cancer cells (Figure 1A). MCF-7 cells were hormone deprived for 3 days and two conditions were subsequently tested; hormone-independent and estradiol-induced chromatin associations. Maximal p160 recruitment to specific loci was observed ~3 h after estradiol treatment (Metivier *et al*, 2003; and data not shown) and as such, a 3-h time point was chosen. Two biological replicates for each experiment were conducted and only peaks that were called in both replicates were considered.

In the absence of hormone, ER α /chromatin interactions are minimal (Shang *et al*, 2000; Hurtado *et al*, 2011). In line with these data, most chromatin interactions for each p160 co-regulator were hormone induced (Figure 1B). This was in contrast to a previous report on SRC3–chromatin interactions (Lanz *et al*, 2010), but in agreement with single locus studies (Shang *et al*, 2000; Metivier *et al*, 2003). A small number of ligand-independent chromatin interactions were observed for each p160 (Figure 1C and D) and a smaller subset of binding events decreased after hormonal treatment (Figure 1C and D; quantified in Supplementary Figure S1). However, the majority of p160-binding events were ligand induced. To study the functional context of the p160-binding sites, we determined the genomic distributions of co-factor binding (Figure 1E). Relative to ER α -binding profiles (Madak-Erdogan *et al*, 2010), the ligand-independent binding sites for SRC1 and SRC2 were significantly enriched at promoters, while the ligand-independent binding sites for SRC3 were enriched both at promoters and 5' untranslated regions (5'UTRs). Binding sites found at promoters tended to be specific to a certain p160 and showed limited overlap with ER α -binding sites, suggesting that other transcription factors mediate these interactions (Supplementary Figure S2). The E2-induced sites did not show the apparent promoter and/or 5'UTR enrichment, and possessed a genomic distribution that was analogous to ER α (Madak-Erdogan *et al*, 2010).

After hormonal treatment, chromatin associations for SRC1, SRC2 and SRC3 increased (Figure 1B–D). These constituted the vast majority of SRC-binding events and showed considerable overlap with ER α -binding sites. These data were

confirmed by motif analysis, where ESR1 and ESR2 motifs were significantly enriched (Figure 1F). Importantly, these motifs were not found for the vehicle-unique binding events. As expected, FOXA1 and FOXA2 motifs were observed for all three p160 proteins and again were absent from the sites unique in vehicle. Comparing the motifs between the p160-binding sites indicated clear differences between them. While Myc and Max motifs were only observed for SRC3-binding sites, NR2F1 and NR4A2 motifs were exclusively found for SRC2 sites, illustrating that co-activator-specific functions are observed at distinct enriched motifs.

Similarity and specificity of p160-binding sites under estradiol conditions

Next, we determined the interplay between SRC1, SRC2 and SRC3 binding properties (Figure 2). Even though many sites are shared between the p160 co-regulators (Figure 2B), preferential binding events were observed (Figure 2B and exemplified in Figure 2A). Importantly, these preferential sites were still shared with ER α -binding events. In contrast to SRC2 and SRC3, SRC1 showed very few unique binding sites, which does not appear to be a threshold issue (Figure 2C). To determine the defining factors that may dictate the preferential binding patterns, we analysed the enriched motifs (Figure 2D). ESR1 and ESR2 motifs were observed for all other subgroups, while forkhead motifs (FOXA1 and FOXA2) were observed for most. Again, distinct unique motifs were observed between subgroups. Additionally, the genomic distribution of the unique and shared binding events is shown (Figure 2E). The E2-induced SRC unique binding events tend to be biased towards promoter-proximal regions. Collectively, these data illustrate that the majority of p160-binding sites are shared. However, preferential binding sites are found with different sequence composition, suggesting co-factor-specific recruitment by other transcription factors.

Hormone dependency of CBP/p300-chromatin interactions

While p160 co-regulators are directly recruited to the co-activator-binding pocket of ER α , additional co-regulators, such as p300 and CBP can be recruited indirectly (Hanstein *et al*, 1996; Smith *et al*, 1996; Kobayashi *et al*, 2000). To analyse the interplay between p160s and CBP/p300, we determined the E2-induced chromatin interactions of CBP and p300 (Figure 3A) by performing ChIP-seq from duplicate samples. Only peaks observed in both replicates were considered. Both p300 and CBP are, as expected, recruited to many ER α -binding sites and appear to be hormone induced (Figure 3B), while a subset of binding sites for both co-regulators are lost after E2 treatment (Figure 3C; Supplementary Figure S3). In contrast to the p160–chromatin interactions, both p300 and CBP have many binding sites that are not shared with ER α ; nearly half of which are still induced by E2 (Figure 3C). This partial overlap could arise due to an incompleteness of the ER α data set, or due to secondary recruitment of p300/CBP to ER α -induced (but not directly ER α -targeted) genes. Unexpectedly, a considerable fraction of p300/CBP-binding events at ER α -binding regions occurred independently of E2 treatment (Figure 3B and D, right panel) and were prebound to the *cis*-regulatory elements prior to ER α recruitment. To determine the functional implications of

Figure 1 p160-chromatin interactions and their dependence on oestrogen. Hormone-deprived MCF-7 cells were treated with oestrogen (E2) or vehicle, after which ChIP-seq analysis was performed on SRC1 (red), SRC2 (blue) or SRC3 (green). Data were compared with ER α ChIP-seq signal (black) from the same time point of E2 treatment. **(A)** Model of p160 recruitment to the chromatin, where SRC1, SRC2 and SRC3 are capable of binding the same surface on ER α . **(B)** Example of ER α , SRC1, SRC2 and SRC3 ChIP-seq signal in the absence and presence of E2 treatment on the XBP1 locus. Genomic co-ordinates and chromosome number are illustrated. **(C)** Heatmap analysis of p160 ChIP-seq data. Sequence reads were centred on the summit of each peak (highlighted with arrowhead) after which all peaks were vertically aligned. Subgroups illustrate the peaks enriched in vehicle only (I), observed in vehicle and E2 (II) and only observed under E2 conditions (III). Scale bar indicates a 5-kb window around the summit of the peak. **(D)** Venn diagrams showing overlaps between SRC1, SRC2 or SRC3 binding in the presence or absence of E2. Data were integrated with ER α -binding sites. Numbers indicate binding events in each subgroup. **(E)** p160-binding sites and their positions relative to proximal genes. SRC1, SRC2 and SRC3 binding sites were divided in vehicle only, vehicle/E2 or E2 only, and their localization relative to annotated genes was determined. ER α is included as a reference. Genomic distribution is shown. **(F)** Motif analysis for SRC1, SRC2 and SRC3 binding sites. Binding events were categorized as those only observed in vehicle (V), vehicle and E2 (V/E) or E2 (E) alone.

these prebound CBP/p300 regions, we integrated the binding sites with gene expression data from MCF-7 cells, treated for 3 h with E2 (Schmidt *et al*, 2010a). Genes were divided in upregulated or downregulated, after which p300/CBP-binding sites within 20 kb from their transcription start were determined (Figure 3E). A 20-kb window has previously been shown to be an optimal window for ER α enhancer-promoter interactions (Fullwood *et al*, 2009). For both p300 and CBP, there was a clear increase in average binding signal near E2-upregulated genes while pre-existing interaction of p300 and CBP was observed for down-regulated genes. This was also observed for 6 h E2-regulated genes (data not shown). These data were independently verified by ChIP-qPCR analysis on well-described ER α -binding sites at enhancer regions for E2-upregulated genes (XBP1 and RARA) and downregulated genes (TLE1 and ESR1) (Figure 3F). These loci are bound by all five co-factors and ER α (Supplementary Figures S4–S7).

To elucidate the possible mechanisms of the hormone-independent recruitment of p300 and CBP to ER α -down-regulated genes, motif analyses were performed on the binding site subgroups (Figure 3G). The CBP-associated binding sites did not show significant enrichment for forkhead motifs, while the p300-binding sites did. In addition, while the p300-binding sites for both E2-upregulated and -downregulated genes possessed ESR1 and ESR2 motifs (up: ESR1 $P = 1 \times 10^{-30}$, ESR2 $P = 1 \times 10^{-30}$; down: ESR1 $P = 2.5 \times 10^{-4}$, ESR2 $P = 4.7 \times 10^{-4}$), only the CBP-associated upregulated genes showed enrichment for ESR motifs (up: ESR1 $P = 2.9 \times 10^{-6}$, ESR2 $P = 1.9 \times 10^{-6}$; down: ESR1 $P = 0.15$, ESR2 $P = 0.56$). Additional distinct enriched motifs were observed between upregulated and downregulated genes for both p300 and CBP, possibly explaining the observed phenotype.

Collectively, these data indicate that both p300 and CBP are actively recruited to some ER α -binding sites after hormonal treatment, but are already present before treatment at others. The different patterns of binding correlate with specific gene expression properties.

Integration of co-activator chromatin interaction sites

After determining the genome-wide cistrome of SRC1, SRC2, SRC3, p300 and CBP, we integrated the data of all analysed co-regulators. First, CBP- and p300-binding sites were tested for their co-occupancy with ER α (Figure 4A), and a considerable number of shared sites were found. That said, differential binding between CBP and p300 was observed at ER α regions (Figure 4A and C and exemplified in Figure 4B), implying that p300 and CBP are not indistinguishable. We integrated all ChIP-seq data from SRC1, SRC2, SRC3, p300 and CBP in vehicle and E2 (Figure 4D) conditions. In all, 1476 binding sites were shared between all factors in the presence of hormone and only 28 in the absence of E2. These 1476 shared binding regions represent the conserved ER α cis-regulatory elements utilised for gene induction of classic breast cancer genes (Supplementary Table S1) and were almost all shared with ER α -binding events (Figure 4E). These shared binding events were proximal to the highest E2-induced genes (Figure 4F).

The p160 sites were interrogated for the presence of CBP, p300 or both (Figure 4G). For the SRC1 unique sites, many were unbound by p300 or CBP, while occupancy of p300 and CBP was more prominent for SRC2- and SRC3-binding sites.

The sites that were shared by all three p160 proteins were almost always co-occupied by p300 and CBP, with no clear preferential binding between them.

To study the downstream effects of preferential co-activator recruitment on transcription, we performed expression array experiments on MCF-7 cells, treated for 6 h with E2. We found 1822 upregulated and 1813 downregulated genes, which was in line with a previous report (Hurtado *et al*, 2011), see Supplementary Table S2. For all these genes, we interrogated a 20-kb window around the transcription start site for the presence of p160 or CBP/p300-binding sites under vehicle- and E2-conditions (Figure 4H). The ratio of upregulated versus downregulated genes is depicted for each co-regulator. ER α -binding events were enriched near both upregulated (578) and downregulated (519) genes. We found E2-independent p300- and CBP-binding sites enriched at E2-downregulated genes, confirming our early time point data (Figure 3E and F). Comparable data were observed for SRC2, while SRC1-binding sites were mostly found at E2-upregulated genes. These were still mediated by ER α , since similar results were observed when only the ER α shared sites were considered (Supplementary Figure S8). In total, 419 upregulated and 401 downregulated genes possessed a proximal p160-binding event (Figure 4I). When determining the shared p160 (Figure 4I) and CBP/p300 (Figure 4J) proximal genes, almost all SRC1-regulated genes were shared with SRC2 and SRC3, in line with the low number of unique SRC1-binding sites (Figure 2B). For SRC2 and SRC3, a considerable number of genes with preferential binding were found. A complete list of genes can be found in Supplementary Table S3. In total, 790 upregulated and 885 downregulated genes displayed proximal p300- and CBP-binding sites (Figure 4J).

Since many binding events were shared between all three p160 co-regulators, we studied the functional redundancy of SRC1, SRC2 and SRC3 on a subset of shared binding sites (Figure 5A). MCF-7 cells were transfected with siRNA targeting each individual p160 co-regulator, after which we tested whether compensation from the other p160 proteins occurred. P160-specific siRNAs sequences were chosen to ensure knockdown of only the targeted family member (Grenier *et al*, 2004). We could verify these data (Supplementary Figure S9), and only observed a loss of ChIP signal for the targeted p160 (Figure 5A). Interestingly, no compensation between the p160 co-regulators was observed, and the two remaining p160 proteins did not show increased ChIP signal when the other one was knocked down. Next, we determined the mRNA levels of the well-defined E2-responsive genes Myc, PgR and Greb1 (Figure 5B). These genes have proximal p160-binding events that overlap with an ER α -binding site (Supplementary Figure S10) and were also bound by p300 and CBP. Performing siRNA for each individual p160 led to an inhibition of E2-induced transcription of these genes, implying that no functional compensation takes place between them. Next, E2-responsive genes were analysed that possessed a unique p160-binding event proximal to their transcription start sites (Figure 5C), not shared by the other two p160s (Supplementary Figure S11). These regions were not bound by p300 or CBP. Gene induction upon E2 treatment was only lost when the corresponding p160 was knocked down and, again, no compensation was observed.

Preferential co-regulator recruitment and outcome after tamoxifen treatment

Differential co-regulator recruitment appears to dictate the transcriptional effects of ER α action. We determined the clinical consequences of differential expression of SRC1, SRC2, SRC3, CBP and p300-proximal genes. We used a publicly available data set of breast cancer patients who either received tamoxifen treatment or did not receive any adjuvant treatment (Loi *et al*, 2007). The clinical parameters of the patient cohort can be found in Supplementary Table S4. To exclusively study the effect of ER α -target genes, we monitored proximal co-factor binding for differentially expressed genes after 6 h of E2 treatment from MCF-7 cells (Figure 4I and J). Next, expression of the co-regulators in tumours was correlated with global transcript levels of the proximal genes (Figure 6A and B). Of the p160 protein family, only SRC3-unique proximal genes were significantly upregulated in tumours with increased expression levels of SRC3 transcript (Figure 6A). An inverse correlation was found with SRC3 levels for the genes with a shared proximal p160-binding site. For the E2-downregulated genes, increased expression of only SRC3 correlated with a decreased average expression of the associated genes, while SRC1 levels inversely correlated in the shared gene list. These data suggest that variations in SRC3 expression levels correlate with altered expression of its target genes.

Identical analyses were performed for the E2-responsive geneset with proximal p300 or CBP-binding sites (Figure 6B). We found CBP levels to correlate with an enhanced expression of the CBP-unique E2-upregulated geneset and a decreased expression of the E2-downregulated genes. For the genes that were shared between p300 and CBP, a correlation with CBP expression levels was only observed for the ER α -downregulated set.

To correlate co-activator-determined gene expression with prognosis, proximal E2-regulated genes from p160 and CBP/p300-binding signatures were individually correlated with recurrence-free survival of a cohort of breast cancer patients (Sotiriou *et al*, 2006; Loi *et al*, 2007; Figure 6C), who either received adjuvant tamoxifen treatment or did not receive any adjuvant treatment. For most p160- and CBP/p300-binding site proximal genes, no prognostic role was observed in the no-adjuvant cohort (Figure 6C). For the tamoxifen-treated cohort, most gene subsets showed mixed combined response to treatment. This was not the case for SRC3-proximal genes, which predicted a poor response for the upregulated genes and a good response in the downregulated genes. For the CBP and p300-proximal gene lists, all subsets of the upregulated genes showed correlation with poor response to treatment. For the downregulated genes, this was only observed for CBP. A complete list of the gene subsets can be found in Supplementary Table S3.

Action of co-regulators cannot be interpreted as differential expression of single downstream targets, but is represented

by a combined effect of all differentially regulated genes. Therefore, we analysed the average gene expression of all co-activator-unique genesets (i.e. genes specifically bound by only one co-factor) and determined recurrence-free survival, using the combined average gene expression as a classifier (Figure 6D and E). Out of the p160 subgroups, only average expression from SRC3-unique proximal genes correlated with recurrence-free survival for both E2-upregulated ($P=0.0003$; hazard ratio (HR)=2.58) and -downregulated genes ($P=0.0004$; HR=0.39). A similar finding was observed in a second independent breast cancer cohort (Supplementary Figure S12) (Buffa *et al*, 2011).

An identical analysis was performed for genes differentially influenced by p300 and/or CBP (Figure 6E). The E2-upregulated p300-unique ($P=0.0045$; HR=2.106) and CBP-unique ($P=0.0006$; HR=2.464) genesets reached significance for recurrence-free survival. For the ER α -downregulated gene signatures, correlations were found with the CBP-unique set ($P=0.0131$; HR=0.53) and the CBP/p300 shared genes ($P=0.0181$; HR=0.53).

We incorporated both directionalities into one unifying classifier (Figure 6F), only analysing the gene lists that reached significance on an individual basis (Figure 6D and E). Only the unifying classifiers for SRC3-unique genes (univariate: $P<0.0001$; HR=6.54) and the CBP-unique genes (univariate: $P=0.0033$; HR=2.84) correlated with a poor recurrence-free survival. After correction for age, grade, tumour size, ER α and PgR status, the SRC3 classifier still persisted ($P<0.01$; HR=8.82), see Supplementary Table S5. For the CBP classifier, an increased hazard was no longer evident ($P=0.09$; HR=2.39), which could be explained by its possible influence on tumour grade (Hudelist *et al*, 2003).

Taken together, our ChIP-seq data have enabled us to define a subset of E2-responsive genes that are explicitly influenced by SRC3 and CBP and are, as separate networks, capable of identifying breast cancer patients with a poor outcome after tamoxifen treatment.

Discussion

ER α co-activators are essential components of the E2-induced transcription complex. The ER α co-regulator family has many members with a wide range of functions (Tremblay and Giguere, 2002). SRC1, SRC2 and SRC3 contain nuclear receptor interaction domains and CBP/p300 interaction domains (York and O'Malley, 2010). Since CBP and p300 possess HAT activity (Goodman and Smolik, 2000), their functions include enhancing chromatin accessibility and these are general co-factors. SRC1, SRC2 and SRC3 are more specific and are utilised mostly by nuclear receptors. Under physiological conditions, competition between nuclear receptors occurs in recruiting the limited available pools of SRC (Newman *et al*, 2000) and p300 (Speir *et al*, 2000) proteins. We therefore

Figure 2 Comparison between p160-binding regions. (A) Example of unique binding sites for SRC1 (red), SRC2 (blue) and SRC3 (green) that were shared with ER α sites (black). All data are from E2-treated cells. (B) Venn diagram illustrating the overlap of ER α , SRC1, SRC2 and SRC3 binding sites. (C) Heatmap analysis of p160 ChIP-seq data. Subgroups illustrate the peaks shared or preferentially bound by SRC1, SRC2 or SRC3. For all subgroups, ER α data are shown. (D) Motif analyses of binding sites unique or shared by the p160 co-regulators. Motifs were analysed in a 1-kb window around the summit of the peaks (arrowhead) for each subgroup. (E) Genomic distributions of the binding sites exclusively observed for SRC1, SRC2 or SRC3 (top) or binding sites shared between p160 co-regulators (bottom).

Figure 3 Hormone-dependent and -independent chromatin interactions of p300 and CBP. Hormone-deprived MCF-7 cells were treated with E2 or vehicle, after which ChIP-seq analyses were performed on CBP (orange) or p300 (purple). Data were overlaid with ER α ChIP-seq signal (black). **(A)** Model of p300 and CBP recruitment to chromatin-associated ER α . CBP and p300 can bind SRC1, SRC2 and/or SRC3 to become part of the transcription complex. **(B)** Example of genomic regions of hormone-dependent (left panel; GREB1 locus) and hormone-independent (right panel; CCND1 locus) interactions of p300 and CBP that overlap with ER α -binding sites. **(C)** Venn diagram of ER α with p300 (top) and CBP (bottom) binding sites in the absence and presence of hormone. **(D)** Heatmap analysis of CBP/p300/ER α ChIP-seq data. Subgroups illustrate the ligand-dependent and -independent peaks that are shared with ER α . Scale bar indicates a 5-kb window around the summit of the peak. **(E)** All CBP and p300 peaks that were observed within a 20-kb window around the transcription start site of an E2-downregulated (left) or -upregulated (right) gene were analysed for their average intensity in the absence of hormone (black line) and after E2 treatment (red line). **(F)** ChIP-RT-PCR on differentially regulated genes. ChIP was performed for ER α , CBP and p300 and binding was determined at E2-upregulated genes (XBP1 and RARA; grey) and E2-downregulated genes (TLE1 and ESR1; black). Fold enrichment was analysed over a negative control region, and normalized over vehicle signal, that was set on 1. Error bars depict standard deviation. **(G)** Motif analyses of CBP and p300 sites, found within a 20-kb window from the transcription start site of an E2-upregulated or -downregulated gene.

Figure 4 Integration of p160/CBP/p300-binding profiles. **(A)** Venn diagram showing overlap between ER α (black), CBP (orange) and p300 (purple) binding sites. **(B)** Example of unique and shared chromatin interactions of p300 and CBP that overlap with ER α -binding sites. **(C)** Heatmap analysis of CBP/p300/ER α ChIP-seq data. Sequence reads were centred on the summit of each peak (highlighted with arrowhead) after which all peaks were vertically aligned. Subgroups illustrate (partially) shared or unique subsets of binding events. Venn diagrams showing the overlaps of binding events for SRC1, SRC2, SRC3, p300 and CBP under vehicle and E2 conditions, and integrated with ER α -binding events **(E)**. Binding sites that are shared by all proteins are indicated in red. **(F)** The binding events under E2 conditions were integrated with the E2-induced genes expression data. For each subgroup of binding, the average gene expression was determined, and visualized in a false-colour lookup table. **(G)** CBP/p300 occupancy at p160-binding sites. The binding sites that were either unique for one of the p160 proteins or shared between them were interrogated for the presence of CBP, p300 or both. **(H)** E2-upregulated and -downregulated genes were interrogated for p160 and CBP/p300-binding sites within 20 kb from their transcription start site. Genes with proximal co-factor binding events in vehicle (V) or exclusively sites observed after estradiol treatments (E2) were scored and the log ratio of the number of upregulated versus downregulated genes is depicted. **(I)** E2-upregulated genes (left panel) or -downregulated genes (right panel) were analysed for the presence of proximal p160 unique or shared binding sites (<20 kb) as shown in the Venn diagram. Shown are the numbers of genes. **(J)** Identical analyses were performed as described in **(I)**, but for p300 and CBP.

monitored the chromatin interactions of co-regulators in both hormone-deprived cells and after E2, to identify ER α -mediated chromatin interactions. We observed moderate co-factor binding in the absence of hormone, but this was more pronounced for p300 and CBP. This may be due to orphan receptors or other hormone-independent transcription factors being able to recruit these co-factors in the absence of hormone. Our findings that SRC proteins are mostly ligand induced, is in contrast to the recent findings, suggesting that

SRC3 binding, even at classic ER *cis*-regulatory elements is ligand independent (Lanz *et al*, 2010). We do not observe this and this discrepancy may be due to lack of hormone deprivation in the Lanz manuscript or differences in cell line strains. Given the fact that we perform duplicate experiments and see ligand induction in both replicates for all three SRC proteins, plus the fact that this corresponds to published locus-specific experiment, we conclude that SRC binding at ER co-occupied regions are mostly ligand dependent.

Figure 5 Lack of compensation between p160 protein family members on chromatin binding and transcription. **(A)** MCF-7 cells were transfected with siRNA to each p160 co-factor and ChIP of the other two p160 members was performed. Shared binding sites were assessed by real-time PCR. Error bars show standard deviations. A Student's *t*-test was applied, **P* < 0.05. **(B)** E2-responsive genes, possessing a shared proximal p160-binding site (<20 kb) were analysed for the effect of siRNA on individual p160 proteins. Error bars show standard deviations. **(C)** Expression of unique targeted genes and effect of p160 siRNA. Comparable experiments were performed as described in **(B)**, but for a subset of E2-responsive genes, which possess a unique proximal SRC1, SRC2 or SRC3 binding site (<20kb). Error bars show standard deviations.

The small number of SRC-binding events observed in hormone-deprived condition may be influenced by the fact that other ligand-activated nuclear receptors that utilise SRC

proteins, including the androgen receptor (Macedo *et al*, 2006), progesterone receptor and glucocorticoid receptor (Sun *et al*, 1983) are also inhibited by the hormone depriva-

tion conditions of the experiment. The remaining hormone-independent SRC-binding sites were significantly enriched at promoters and showed poor overlap with ER α -binding sites and are likely to be mediated by non-nuclear receptor factors. One possibility is Myc, which is a direct ER α -target gene (Figure 5B) and is implicated in anti-oestrogen resistance (Miller *et al*, 2011). Interestingly, Myc and Max (the dimeric partner of Myc) motifs were enriched in the small number of ligand-independent SRC3-binding regions.

Compared with the SRCs, many more ligand-independent interactions for p300 and CBP were observed. These interactions could either be due to (hormone independent) transcription factor activity, or function as preformed complexes at non-stimulated genes. Since ligand-independent CBP- and p300-binding events were specifically enriched proximal to E2-downregulated genes, the preloaded p300/CBP binding could negatively influence ER α -mediated transcription of these target genes.

Competition occurs not only among transcription factors to recruit co-activators (Newman *et al*, 2000), but also between co-activators themselves (Ruse *et al*, 2002). Interestingly, knocking down individual SRC protein family members did not result in an increased occupancy of the remaining SRC members and no functional compensation occurs between SRC proteins. In contrast to SRC1 and SRC2, expression levels of SRC3 have consistently been found to correlate with tamoxifen resistance (Hudelist *et al*, 2003; Osborne *et al*, 2003; Shou *et al*, 2004; Su *et al*, 2008; Alkner *et al*, 2010). Here, we identify the transcriptional targets that lead to this poor response after endocrine treatment. This genetic signature is represented by the SRC3 targets that are not shared with the other two SRC family members (Figure 6D and F). We found in clinical data sets, that only differential expression of SRC3 consistently correlated with the expression levels of the SRC3-responsive geneset. This possibly explains why SRC3 is the only SRC family member found to be amplified in breast tumours (Anzick *et al*, 1997): SRC1- and SRC2-linked genes do not influence treatment outcome and as such, SRC1 and SRC2 amplified tumours are not selected for during acquisition of endocrine resistance.

Interestingly, the expression of the SRC3- and CBP-bound and regulated genes in patients with poor clinical outcome, suggest that co-factors can determine the transcriptional activity of subsets of ER α -binding events, culminating in distinct biological outcomes. This suggests that a poor response to treatment is partially dictated by binding potential and transcription activity of SRC3 and CBP at ER α -responsive genes.

In transcriptional networks, the relative contribution of individual co-activators is difficult to identify. By identifying the genome-wide chromatin occupancy of two complete families of co-activators in breast cancer, the SRC (p160) protein family and CBP/p300, we can delineate their direct transcriptional target genes and identify their relative contributions to breast cancer biology and outcome after endocrine treatment.

Materials and methods

Cell culture

MCF-7 cells were cultured in DMEM, supplemented with 8% FCS and standard antibiotics. For hormonal deprivation, cells were

cultured for 3 days in phenol-red free DMEM supplemented with 5% charcoal-treated serum and standard antibiotics. E2 treatment was performed for ~3 h at a concentration of 100 nM.

siRNA

siRNA oligo's (Thermo Scientific) used according to the manufacturers' protocol and designed to target SRC1 5'-UGGAAUGUCAAUCCCCGAUU-3' (sense) and 5'-UCGGGAAUUGACAUCCAUU-3' (antisense); SRC2 5'-AGAGCAAACUCAUCCGUUCU-3' (sense) and 5'-GAACGGAUGAGUUUGCUCUUU-3' or SRC3 5'-AUUCCUCCUUGA CCAACUCUU-3' (sense) and 5'-GAGUUGUCAAGGAGGAUUU-3'. AllStars negative control siRNA (Qiagen) was used as a negative control.

ChIP

ChIP experiments were performed as described previously (Carroll *et al*, 2005). Antibodies used were anti-ER α (sc-543), anti-SRC1 (sc-6096), anti-SRC2 (sc-8996), anti-SRC3 (sc-9119), anti-p300 (sc-585) and anti-CBP (sc-369) from Santa Cruz Biotechnologies. Primer sequences for RT-PCR are provided in Supplementary Table S6.

Solexa sequencing and enrichment analysis

ChIP DNA was amplified as described (Schmidt *et al*, 2009). Sequences were generated by the Illumina GAIIX genome analyser (using 36 bp reads), processed by the Illumina analysis pipeline version 1.6.1, and aligned to the Human Reference Genome (assembly hg18, NCBI Build36.1, March 2008) using BWA version 0.5.5. Reads were filtered by removing those with a BWA alignment quality score <15. For each biological replicate, a corresponding set of input sequence reads of similar size was obtained by random sampling from the full set of input sequence reads. Enriched regions of the genome were identified by comparing the ChIP samples to input samples using the MACS peak caller (Zhang *et al*, 2008) version 1.3.7.1. All ChIP-seq data were from intersected peaks, shared between two independent replicates. Details on the number of reads obtained and the percentage of reads aligned, number of peaks called can be found in Supplementary Table S7. ArrayExpress accession numbers for the ChIP-seq and microarray data sets are E-MTAB-785 (high-throughput sequencing experiments) and E-MTAB-788 (microarrays). Control samples for the ChIP-seq experiments were previously submitted to GEO with accessions SRX014075, SRX014076 and GSM798440.

RT-PCR

Hormone-deprived cells were treated for 6 h with E2, after which total RNA was collected and RT-PCR was performed as described (Carroll *et al*, 2006). Primer sequences are provided in Supplementary Table S6.

Microarray analysis

MCF-7 cells were treated with vehicle or E2 for 6 h, after which cells were lysed and mRNA was isolated. Six independent replicates were generated. Gene expression analysis was conducted to identify statistically significant differentially expressed genes (adjusted *P*-value <0.01) as described (Ross-Innes *et al*, 2010). The 3-h E2 treatment expression array data were taken from a publicly available data set (Schmidt *et al*, 2010a).

Motif analysis, heatmaps and genomic distributions of binding events

The motif analysis and heatmap construction from ChIP-seq data were performed as described previously (Schmidt *et al*, 2010b). The genomic distributions of binding sites were analysed using the *cis*-regulatory element annotation system (CEAS) (Ji *et al*, 2006). The genes closest to the binding site on both strands were analysed. If the binding region is within a gene, CEAS software indicates whether it is in a 5'UTR, a 3'UTR, a coding exon, or an intron. Promoter is defined as 1 kb upstream from RefSeq 5' start. If a binding site is >1 kb away from the RefSeq transcription start site, it is considered distal intergenic.

Gene expression data from human breast tumours

Microarray data sets from 268 tamoxifen-treated patients and 125 patients who did not receive adjuvant treatment were obtained from previous investigations (Sotiriou *et al*, 2006; Loi *et al*, 2007). All data have been normalized previously by RMA (Robust Multi-array Analysis). For the correlations of single gene expression with

Figure 6 Co-regulator expression levels in breast cancer patients, their responsive genes and the correlation with outcome after adjuvant tamoxifen treatment. **(A)** E2-upregulated (left panel) and -downregulated (right panel) genes with a proximal p160-binding site (<20 kb) were analysed for their average expression in a cohort of breast cancer patients. The average gene expression values were ranked according to the corresponding p160 levels in the same tumour (bottom 33% (L) versus top 33% (H)). The correlations were performed for unique and shared genes. A Student's *t*-test was applied, **P*<0.05. **(B)** Identical analyses were performed as described in **(A)**, but for genes with proximal p300 or CBP-binding sites. **(C)** Co-activator defined genesets and individual correlations with recurrence-free survival of patients either treated with tamoxifen or received no-adjuvant treatment. Correlations of expression of the genes with recurrence-free survival were scored as good (green), poor (red) or non-significant (white) using a cutoff of *P*=0.05 for each gene, as determined by a log-ranked (Mantel-Cox) test. **(D)** Kaplan-Meier plots for the p160-derived genesets. Recurrence-free survival of tamoxifen-treated patients was correlated with the average gene expression of each subset of p160-influenced genes (SRC1, SRC2 or SRC3-unique or shared by all). Average expression values were ranked (top 33% versus bottom 33%) after which the recurrence-free survival was determined for both groups. *P*-value was determined by a log-ranked (Mantel-Cox) test, HR was determined with the Mantel-Haenszel method. **(E)** Kaplan-Meier plots of the p300/CBP-derived genesets. Identical analyses were performed as described for **(D)**, but for E2-responsive genesets with proximal p300 or CBP-binding sites. **(F)** The co-activator-associated genesets that showed correlations with recurrence-free survival, as depicted in **(D, E)**, were integrated for both upregulated and downregulated genes. The cohort was separated into two groups: either meeting both criteria (E2-upregulated genes within top 33% and E2-downregulated genes within bottom 33%; black line) or the opposite (E2-upregulated genes within bottom 33% and E2-downregulated genes within top 33%; red line). *P*-value was determined by a log-ranked (Mantel-Cox) test, HR was determined with the Mantel-Haenszel method.

recurrence-free survival, recursive partitioning in the R package Party was used on the normalized gene expression data, as performed previously (Ross-Innes *et al*, 2010). A correction for multiple testing is applied. For the integrated expression analyses, average expression of the studied geneset was calculated, ranked and the subgroups were analysed (top 33% versus bottom 33% of average expression values) and recurrence-free survival was determined.

Statistics

Statistical analysis was performed using two-tailed paired *t*-tests. For patient survival analysis, a log-ranked (Mantel-Cox) test was applied. The HR for the Kaplan-Meier plots was calculated using the Mantel-Haenszel method.

Supplementary data

Supplementary data are available at *The EMBO Journal* Online (<http://www.embojournal.org>).

References

- Alkner S, Bendahl PO, Grabau D, Lovgren K, Stal O, Ryden L, Ferno M (2010) AIB1 is a predictive factor for tamoxifen response in premenopausal women. *Ann Oncol* **21**: 238–244
- 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 co-activator amplified in breast and ovarian cancer. *Science* **277**: 965–968
- Buffa FM, Camps C, Winchester L, Snell CE, Gee HE, Sheldon H, Taylor M, Harris AL, Ragoussis J (2011) microRNA associated progression pathways and potential therapeutic targets identified by integrated mRNA and microRNA expression profiling in breast cancer. *Cancer Res* **71**: 5635–5645
- Carraz M, Zwart W, Phan T, Michalides R, Brunsvelde L (2009) Perturbation of estrogen receptor alpha localization with synthetic nona-arginine LXXLL-peptide co-activator binding inhibitors. *Chem Biol* **16**: 702–711
- Carroll JS, Liu XS, Brodsky AS, Li W, Meyer CA, Szary AJ, Eeckhoutte J, Shao W, Hestermann EV, Geistlinger TR, Fox EA, Silver PA, Brown M (2005) Chromosome-wide mapping of estrogen receptor binding reveals long-range regulation requiring the forkhead protein FoxA1. *Cell* **122**: 33–43
- Carroll JS, Meyer CA, Song J, Li W, Geistlinger TR, Eeckhoutte J, Brodsky AS, Keeton EK, Fertuck KC, Hall GF, Wang Q, Bekiranov S, Sementchenko V, Fox EA, Silver PA, Gingeras TR, Liu XS, Brown M (2006) Genome-wide analysis of estrogen receptor binding sites. *Nat Genet* **38**: 1289–1297
- Fullwood MJ, Liu MH, Pan YF, Liu J, Xu H, Mohamed YB, Orlov YL, Velkov S, Ho A, Mei PH, Chew EG, Huang PY, Welboren WJ, Han Y, Ooi HS, Ariyaratne PN, Vega VB, Luo Y, Tan PY, Choy PY *et al* (2009) An oestrogen-receptor- α -bound human chromatin interactome. *Nature* **462**: 58–64
- Goodman RH, Smolik S (2000) CBP/p300 in cell growth, transformation, and development. *Genes Dev* **14**: 1553–1577
- Grenier J, Trousson A, Chauchereau A, Amazit L, Lamirand A, Leclerc P, Guiochon-Mantel A, Schumacher M, Massaad C (2004) Selective recruitment of p160 co-activators on glucocorticoid-regulated promoters in Schwann cells. *Mol Endocrinol* **18**: 2866–2879
- Hanstein B, Eckner R, DiRenzo J, Halachmi S, Liu H, Searcy B, Kurokawa R, Brown M (1996) p300 is a component of an estrogen receptor co-activator complex. *Proc Natl Acad Sci USA* **93**: 11540–11545
- Heery DM, Kalkhoven E, Hoare S, Parker MG (1997) A signature motif in transcriptional co-activators mediates binding to nuclear receptors. *Nature* **387**: 733–736
- Hudelist G, Czerwenka K, Kubista E, Marton E, Pischinger K, Singer CF (2003) Expression of sex steroid receptors and their co-factors in normal and malignant breast tissue: AIB1 is a carcinoma-specific co-activator. *Breast Cancer Res Treat* **78**: 193–204
- 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
- Hurtado A, Holmes KA, Ross-Innes CS, Schmidt D, Carroll JS (2011) FOXA1 is a key determinant of estrogen receptor function and endocrine response. *Nat Genet* **43**: 27–33

Acknowledgements

We thank James Hadfield for Solexa sequencing, Stewart McArthur for motif analyses, Gordon Brown, Rory Stark and Suraj Menon for bioinformatic help, Elizabeth Merrall for statistical analysis, Claudia Kutter for help with data visualization and Jessica Robinson and Antoni Hurtado for critically reading the manuscript. We acknowledge the support of The University of Cambridge, Cancer Research UK, the Dutch Cancer Society and The Netherlands Cancer Institute. JSC is supported by an ERC Starting Grant and an EMBO young investigator grant. WZ is supported by a KWF Dutch Cancer Society fellowship.

Author contributions: WZ and JSC designed all the experiments. WZ conducted all the experiments, with help from VT. MK and SL provided critical clinical information and analysis. SC and WZ conducted all bioinformatics analyses. The paper was written by WZ and JSC, with help from all other authors.

Conflict of interest

The authors declare that they have no conflict of interest.

- Ji X, Li W, Song J, Wei L, Liu XS (2006) CEAS: cis-regulatory element annotation system. *Nucleic Acids Res* **34** (Web Server Issue): W551–W554
- Kamei Y, Xu L, Heinzel T, Torchia J, Kurokawa R, Gloss B, Lin SC, Heyman RA, Rose DW, Glass CK, Rosenfeld MG (1996) A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. *Cell* **85**: 403–414
- Kobayashi Y, Kitamoto T, Masuhiro Y, Watanabe M, Kase T, Metzger D, Yanagisawa J, Kato S (2000) p300 mediates functional synergism between AF-1 and AF-2 of estrogen receptor alpha and beta by interacting directly with the N-terminal A/B domains. *J Biol Chem* **275**: 15645–15651
- Kraus WL, Kadonaga JT (1998) p300 and estrogen receptor cooperatively activate transcription via differential enhancement of initiation and reinitiation. *Genes Dev* **12**: 331–342
- Lanz RB, Bulynko Y, Malovannaya A, Labhart P, Wang L, Li W, Qin J, Harper M, O'Malley BW (2010) Global characterization of transcriptional impact of the SRC-3 coregulator. *Mol Endocrinol* **24**: 859–872
- Loi S, Haibe-Kains B, Desmedt C, Lallemand F, Tutt AM, Gillet C, Ellis P, Harris A, Bergh J, Foekens JA, Klijn JG, Larsimont D, Buyse M, Bontempi G, Delorenzi M, Piccart MJ, Sotiriou C (2007) Definition of clinically distinct molecular subtypes in estrogen receptor-positive breast carcinomas through genomic grade. *J Clin Oncol* **25**: 1239–1246
- Macedo LF, Guo Z, Tilghman SL, Sabnis GJ, Qiu Y, Brodie A (2006) Role of androgens on MCF-7 breast cancer cell growth and on the inhibitory effect of letrozole. *Cancer Res* **66**: 7775–7782
- Madak-Erdogan Z, Lupien M, Stossi F, Brown M, Katzenellenbogen BS (2010) Genomic collaboration of estrogen receptor- α and ERK2 in regulating gene and proliferation programs. *Mol Cell Biol* **31**: 226–236
- Margeat E, Poujol N, Boulahtouf A, Chen Y, Muller JD, Gratton E, Cavaillès V, Royer CA (2001) The human estrogen receptor alpha dimer binds a single SRC-1 co-activator molecule with an affinity dictated by agonist structure. *J Mol Biol* **306**: 433–442
- Metivier R, Penot G, Hubner MR, Reid G, Brand H, Kos M, Gannon F (2003) Estrogen receptor- α directs ordered, cyclical, and combinatorial recruitment of co-factors on a natural target promoter. *Cell* **115**: 751–763
- Miller TW, Balko JM, Ghazoui Z, Dunbier A, Anderson H, Dowsett M, Gonzalez-Angulo AM, Mills GB, Miller WR, Wu H, Shyr Y, Arteaga CL (2011) A Gene expression signature from human breast cancer cells with acquired hormone independence identifies MYC as a mediator of antiestrogen resistance. *Clin Cancer Res* **17**: 2024–2034
- Newman SP, Bates NP, Vernimmen D, Parker MG, Hurst HC (2000) Co-factor competition between the ligand-bound oestrogen receptor and an intron 1 enhancer leads to oestrogen repression of ERBB2 expression in breast cancer. *Oncogene* **19**: 490–497
- Osborne CK, Bardou V, Hopp TA, Chamness GC, Hilsenbeck SG, Fuqua SA, Wong J, Allred DC, Clark GM, Schiff R (2003) Role of the estrogen receptor co-activator AIB1 (SRC-3) and HER-2/neu in tamoxifen resistance in breast cancer. *J Natl Cancer Inst* **95**: 353–361
- 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
- Paige LA, Christensen DJ, Gron H, Norris JD, Gottlin EB, Padilla KM, Chang CY, Ballas LM, Hamilton PT, McDonnell DP, Fowlkes DM (1999) Estrogen receptor (ER) modulators each induce distinct conformational changes in ER alpha and ER beta. *Proc Natl Acad Sci USA* **96**: 3999–4004
- Ramos YF, Hestand MS, Verlaan M, Krabbendam E, Ariyurek Y, van Galen M, van Dam H, van Ommen GJ, den Dunnen JT, Zantema A, t Hoen PA (2010) Genome-wide assessment of differential roles for p300 and CBP in transcription regulation. *Nucleic Acids Res* **38**: 5396–5408
- Redmond AM, Bane FT, Stafford AT, McIlroy M, Dillon MF, Crotty TB, Hill AD, Young LS (2009) Coassociation of estrogen receptor and p160 proteins predicts resistance to endocrine treatment; SRC-1 is an independent predictor of breast cancer recurrence. *Clin Cancer Res* **15**: 2098–2106
- Ross-Innes CS, Stark R, Holmes KA, Schmidt D, Spyrou C, Russell R, Massie CE, Vowler SL, Eldridge M, Carroll JS (2010) Cooperative interaction between retinoic acid receptor- α and estrogen receptor in breast cancer. *Genes Dev* **24**: 171–182
- Ruse Jr MD, Privalsky ML, Sladek FM (2002) Competitive co-factor recruitment by orphan receptor hepatocyte nuclear factor 4 α 1: modulation by the F domain. *Mol Cell Biol* **22**: 1626–1638
- Schmidt D, Schwalie PC, Ross-Innes CS, Hurtado A, Brown GD, Carroll JS, Flicek P, Odom DT (2010a) A CTCF-independent role for cohesin in tissue-specific transcription. *Genome Res* **20**: 578–588
- Schmidt D, Wilson MD, Ballester B, Schwalie PC, Brown GD, Marshall A, Kutter C, Watt S, Martinez-Jimenez CP, Mackay S, Talianidis I, Flicek P, Odom DT (2010b) Five-vertebrate ChIP-seq reveals the evolutionary dynamics of transcription factor binding. *Science* **328**: 1036–1040
- Schmidt D, Wilson MD, Spyrou C, Brown GD, Hadfield J, Odom DT (2009) ChIP-seq: using high-throughput sequencing to discover protein-DNA interactions. *Methods* **48**: 240–248
- Shang Y, Hu X, DiRenzo J, Lazar MA, Brown M (2000) Co-factor dynamics and sufficiency in estrogen receptor-regulated transcription. *Cell* **103**: 843–852
- Shiau AK, Barstad D, Loria PM, Cheng L, Kushner PJ, Agard DA, Greene GL (1998) The structural basis of estrogen receptor/co-activator recognition and the antagonism of this interaction by tamoxifen. *Cell* **95**: 927–937
- 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. *J Natl Cancer Inst* **96**: 926–935
- Smith CL, Onate SA, Tsai MJ, O'Malley BW (1996) CREB binding protein acts synergistically with steroid receptor coactivator-1 to enhance steroid receptor-dependent transcription. *Proc Natl Acad Sci USA* **93**: 8884–8888
- Sotiriou C, Wirapati P, Loi S, Harris A, Fox S, Smeds J, Nordgren H, Farmer P, Praz V, Haibe-Kains B, Desmedt C, Larsimont D, Cardoso F, Peterse H, Nuyten D, Buyse M, Van de Vijver MJ, Bergh J, Piccart M, Delorenzi M (2006) Gene expression profiling in breast cancer: understanding the molecular basis of histologic grade to improve prognosis. *J Natl Cancer Inst* **98**: 262–272
- Speir E, Yu ZX, Takeda K, Ferrans VJ, Cannon III RO (2000) Competition for p300 regulates transcription by estrogen receptors and nuclear factor-kappaB in human coronary smooth muscle cells. *Circ Res* **87**: 1006–1011
- Su Q, Hu S, Gao H, Ma R, Yang Q, Pan Z, Wang T, Li F (2008) Role of AIB1 for tamoxifen resistance in estrogen receptor-positive breast cancer cells. *Oncology* **75**: 159–168
- Sun LH, Pfendner EG, Senior MB, Frankel FR (1983) Progesterone, glucocorticoid and estradiol receptors in MCF-7 cells bind to chromatin. *Mol Cell Endocrinol* **30**: 267–278
- Tremblay GB, Giguere V (2002) Coregulators of estrogen receptor action. *Crit Rev Eukaryot Gene Expr* **12**: 1–22
- Xu J, O'Malley BW (2002) Molecular mechanisms and cellular biology of the steroid receptor co-activator (SRC) family in steroid receptor function. *Rev Endocr Metab Disord* **3**: 185–192
- Xu J, Wu RC, O'Malley BW (2009) Normal and cancer-related functions of the p160 steroid receptor co-activator (SRC) family. *Nat Rev Cancer* **9**: 615–630
- York B, O'Malley BW (2010) Steroid receptor co-activator (SRC) family: masters of systems biology. *J Biol Chem* **285**: 38743–38750
- 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 Dev* **18**: 1753–1765
- Zhang Y, Liu T, Meyer CA, Eeckhoutte J, Johnson DS, Bernstein BE, Nusbaum C, Myers RM, Brown M, Li W, Liu XS (2008) Model-based analysis of ChIP-Seq (MACS). *Genome Biol* **9**: R137
- 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. *Biochem Biophys Res Commun* **380**: 699–704