Cistromics of hormone-dependent cancer

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Abstract

Alterations in transcription programs are a fundamental feature of cancer. Nuclear receptors, such as the estrogen receptor alpha (ERα) and androgen receptors (ARs), are central in this process as they can directly impact gene expression through interaction with the chromatin and subsequent association with coregulators and the transcriptional machinery. Unbiased genome-wide investigations have demonstrated the predominant recruitment of both ERα and AR to distant (non-promoter)-regulatory elements. Furthermore, these studies revealed a clear relationship between sites of transcription factor recruitment and gene regulation. Indeed, expression profiles from AR-positive primary prostate tumors and cell lines directly relate to the AR cistrome in prostate cancer cells, while the ERα cistrome in breast cancer cells relates to expression profiles from ERα-positive primary breast tumors. Additionally, cell-type-specific ERα cistromes are linked to lineage-specific estrogen-induced expression profiles in different cell types, for example osteosarcoma and breast cancer cells. The pioneer factor forkhead box A1 (FoxA1/HNF3α) plays a central role in AR and ERα signaling. It is recruited in a lineage-specific manner translating the epigenetic signature consisting of mono- and dimethylated histone H3 on lysine 4 (H3K4me1/me2) into functional regulatory elements. Hence, through the interplay between the pioneer factor, namely FoxA1, and epigenetic events, the transcriptional potential of a given cell lineage is predefined. Since this directly impacts signaling through nuclear receptors, these discoveries should significantly impact the development of novel therapeutic strategies directed against multiple types of cancer.

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Characteristics of nuclear receptor cistromes

Gene expression profiling of cancer has led to important new insights both in terms of classification and outcome. This is exemplified in breast cancer through studies where different subtypes of primary breast tumors have been identified (Sorlie et al. 2001, 2003), which also correspond to different disease/treatment outcomes (Sorlie et al. 2003). Similarly, prostate cancer development associates with transcriptional programs distinct from normal tissues (Welsh et al. 2001, Lapointe et al. 2004, Yu et al. 2004). Therefore, understanding the mechanisms that lead to these altered expression profiles is fundamental to the development of effective therapeutic intervention against cancers.

Nuclear receptors are central to the development of both breast and prostate cancer. The estrogen receptor alpha (ERα) is a fundamental feature of more than two-thirds of breast cancers (Sorlie et al. 2001, 2003), while prostate cancer is highly dependent on the actions of the androgen receptor (AR; Heinlein & Chang 2004). Both ERα and AR are ligand-dependent transcription factors recruited directly to the chromatin through the estrogen-responsive elements (EREs) and androgen-responsive elements (AREs) respectively. They are also indirectly recruited to other genomic regions through a tethering mechanism involving other transcription factors such as AP-1 and Sp1 (Sanchez et al. 2002). Through their interplay with coregulators, ERα and AR regulate the expression of genes central to breast and prostate cancer development, including CCND1, E2F1, Myc as well as TMPRSS2, and PSA respectively (Prall et al. 1998, Balk et al. 2003, Demichelis & Rubin 2007, Stender et al. 2007, Frietze et al. 2008, Setlur et al. 2008).
Recent technological advancements have allowed the mapping of the regulatory regions recruiting either directly or through tethering mechanisms these receptors on a genome-wide scale defining their cistromes (Lupien et al. 2008). Indeed, upon stimulation ERα and AR are recruited to 1000 of sites in breast and prostate cancer cell lines respectively (Carroll et al. 2005, 2006, Lin et al. 2007, Wang et al. 2007, Hua et al. 2008, Hurtado et al. 2008, Liu et al. 2008, Lupien et al. 2008). These unbiased genome-wide studies have revealed the preferential recruitment of both ERα and AR with non-promoter-regulatory elements. This pattern of promoter-distant recruitment is also typical of other transcription factors in various systems such as forkhead box A1 (FoxA1), RelA (p65), NRSF, SRA, GABP, and many more (Carroll et al. 2006, Johnson et al. 2007, Lim et al. 2007, Lupien et al. 2008, Valouev et al. 2008). This contrasts with the distribution of other transcription factors such as E2F family members that are primarily recruited to promoters (Bieda et al. 2006, Xu et al. 2007). This highlights the complexity of the transcriptional response in higher order eukaryotes as regulatory elements can be found hundreds of kilobases (kb) away from their target genes, but still be brought into promoter close proximity through chromosome looping (Dekker 2008). In fact, this process has been previously reported on a limited subset of ERα- and AR-binding sites for regions up to 140 kb away from the target gene (Carroll et al. 2005, Deschenes et al. 2007, Wang et al. 2007). Hence, a key milestone in transcriptional biology will consist of establishing the sum of all chromosome loops guiding transcriptional responses on a genome-wide scale.

From nuclear receptor cistrome to transcription

The comparison of nuclear receptor cistromes and hormone-regulated expression profiles reveals a clear relationship between the two. Indeed, genes over-expressed in ERα-positive primary breast tumors as well as estrogen target genes in breast cancer cell lines are preferentially surrounded by estrogen-induced ERα-binding sites found in this same system (Carroll et al. 2006, Lupien et al. 2008, Fig. 1). Similarly, MCF7 cells overexpressing AKT induce a unique ERα cistrome that relates to the AKT-dependent expression profile (Bhat-Nakshatri et al. 2008, Fig. 1). This is also observed in osteosarcoma where the estrogen-induced ERα cistrome, distinct from the ERα cistrome in breast cancer cells, directly relates to the estrogen-induced expression profile in osteosarcoma (Krum et al. 2008, Fig. 1). Furthermore, ERα-binding sites cluster around these regulated genes (Krum et al. 2008). Similarly, the AR cistrome in androgen-dependent prostate cancer cells relates to the androgen-induced transcriptional program in these same cells as well as to the expression profile from primary prostate tumors (Wang et al. 2007, 2009, Lupien et al. 2008, Fig. 2). Furthermore, as prostate cancer cells become castration resistant following androgen-deprivation therapy, they acquire an altered expression program accompanied by a related novel AR cistrome (Wang et al. 2009). Hence, the capacity to establish specific cistromes under distinct activation and in different lineages is central to the implementation of transcriptional programs that define the nature of cellular identity.

Coregulators central to nuclear receptor cistromes

Through sequence analysis of regulatory regions recruiting either ERα or AR, conserved networks of regulatory factors have been defined (Carroll et al. 2005, 2006, Laganiere et al. 2005, Green & Carroll 2007, Wang et al. 2007, Hurtado et al. 2008). Noteworthy, the GATA, OCT, PAX, NKX, and LEF motifs are significantly enriched near the center of ERα- and/or AR-binding sites. GATA3 recognizing the GATA motif was revealed to be part of a positive cross-regulatory loop with ERα in breast cancer cells required for the estrogen-mediated cell proliferation (Eeckhoute et al. 2007). In prostate cancer cells, GATA2 also recognizing the GATA motif was found to interact with AR and potentiates its regulation of target genes (Perez-Stable et al. 2000, Wang et al. 2007). Similarly, Oct-1 recognizing the OCT motif was shown to physically interact with AR and its expression is required for AR-mediated transcriptional regulation in prostate cancer cells (Gonzalez & Robins 2001, Wang et al. 2007). Furthermore, Oct-1 is co-recruited with ERα in breast cancer cells regulating key target genes, namely CCND1 (Cicatiello et al. 2004, Carroll et al. 2006). More recently, PAX2 co-recruitment with ERα to the ERBB2-regulatory element has revealed its central role as a transcriptional repressor required for inhibition of ERBB2 expression in breast cancer cells (Hurtado et al. 2008). Accordingly, loss of PAX2 recruitment allowed for ERBB2 expression in the presence of the anti-estrogen tamoxifen conferring anti-estrogen-resistant-like properties to normally anti-estrogen-sensitive breast cancer cells (Hurtado et al. 2008). Other factors such as LEF-1 and Nkx3-1 whose DNA recognition motif is enriched
in ERα-binding sites in breast cancer cells behave in a distinct manner. Indeed, instead of being co-recruited with ERα following estrogen stimulation, these transcription factors are bound at the basal state and block ERα recruitment abrogating estrogen growth-promoting properties (Holmes et al. 2008). Since LEF-1 and Nkx3-1 can associate with the histone deacetylase HDAC1, increased chromatin condensation is thought to be fundamental to block ERα recruitment (Holmes et al. 2008). Therefore, by defining the cistromes of ERα and AR, the intricate interplay between transcription factors and their network of coregulatory proteins taking place at the chromatin is gradually being revealed.

**Pioneer factors as mediator of lineage-specific transcriptional programs**

The FKH motif is an additional motif highly enriched in both ERα and AR cistromes (Carroll et al. 2005, 2006, Laganiere et al. 2005, Wang et al. 2007). The forkhead family member FoxA1 (HNF3α) is a key partner for ERα and AR transcriptional activity in breast and prostate cancer respectively, recognizing the FKH motif. It was first characterized as a pioneer factor in liver tissue (Gualdi et al. 1996, Cirillo et al. 1998, Bossard & Zaret 2000). More recently, its ATP-independent chromatin-remodeling activity, distinguishing it from the classical SWI/SNF complex, has shown to be central for ERα recruitment in breast cancer cells (Carroll et al. 2005, Laganiere et al. 2005, Eeckhoute et al. 2006), while it was found to physically interact with AR in prostate cancer cells (Gao et al. 2003, Wang et al. 2007). Present on the chromatin at the basal state, FoxA1 is found at more than 60% of ERα- and AR-binding sites driving the transcriptional response in breast and prostate cancer cells respectively (Lupien et al. 2008). In fact, through its chromatin-remodeling activity, FoxA1 allows for the opening of specific genomic regions in the absence of hormone (Carroll et al. 2005, Laganiere et al. 2005, Eeckhoute et al. 2006). Hence, under hormonal stimulation, ERα and AR are recruited to FoxA1 sites harboring permissive sequences such as EREs and AREs (Lupien et al. 2008, Fig. 3). In accordance with its predominant role in ERα signaling, FoxA1 is
typically highly expressed in ERα-positive primary breast tumors and is an important marker of breast cancer subtype and prognosis (Habashy et al. 2008, Thorat et al. 2008). FoxA1 is also highly expressed in prostate cancer where it is believed to contribute to the establishment of specific gene expression programs (Mirosevich et al. 2006). However, the comparison of FoxA1 cistromes between breast and prostate cancer cells reveals its cell-type-specific recruitment (Lupien et al. 2008). Indeed, less than 40% of FoxA1-binding sites are shared between these two cell lines supporting the notion that FoxA1 is recruited in a lineage-specific fashion. Importantly, because FoxA1 guides the recruitment of transcription factors, such as ERα and AR, lineage-specific transcriptional programs dependent on these transcription factors are directly affected by FoxA1’s cell-type-specific recruitment (Fig. 3).

**Epigenetic signatures define lineage-specific functional regulatory elements**

The requirement for lineage-specific recruitment of the pioneer factor FoxA1 highlights the importance of understanding how such differential recruitment takes place. Recently, specific epigenetic signatures distinguishing non-promoter from promoter-regulatory elements have been reported (Santos-Rosa et al. 2002, Ng et al. 2003, Schneider et al. 2004, Schubeler et al. 2004, Bernstein et al. 2005, Pokholok et al. 2005, Heintzman et al. 2007). This signature, characterized by different methylation states (mono-, di-, or tri-) of lysine 4 on histone H3 (H3K4me1, me2, or me3), was also found associated with distinct chromatin regions permissive for transgene expression (Yan & Boyd 2006). Specifically, H3K4me1 and H3K4me2 were associated with non-promoter-regulatory elements, while H3K4me3 was found at promoter regions (Heintzman et al. 2007). Furthermore, regions enriched in H3K4me2 or me3 associate with DNase I hypersensitivity, a marker of active-regulatory regions (Xi et al. 2007). Therefore, this supports the notion that H3K4me2 and H3K4me3 are specific for functional non-promoter and promoter-regulatory elements respectively.

In agreement, the cell-type-specific cistromes for the pioneer factor FoxA1 in breast and prostate cancer cells are dependent on H3K4me1/me2 distribution (Lupien et al. 2008). In reality, H3K4me1/me2 defines in a lineage-specific manner through which regulatory elements are able to recruit FoxA1 (Fig. 3). Indeed, removal of this epigenetic signature through over-expression of the lysine demethylase KDM1/LSD1
prevents FoxA1 recruitment (Lupien et al. 2008, Wang et al. 2009). This signature is also characteristic of the ERα cistrome not overlapping with FoxA1 (M L and M B unpublished data). In fact, lineage-specific ERα cistromes correlate with the H3K4me1/me2 distribution. Indeed, ERα-binding sites specific to breast cancer or osteosarcoma cells relate to the unique distribution of H3K4me1/me2 in these respective cell lines, regardless of FoxA1 status (Krum et al. 2008).

Similarly, the recruitment of AR to novel sites in castration-resistant prostate cancer cells is dependent on the de novo H3K4 mono- and dimethylation (Wang et al. 2009). Removal of H3K4 mono- and dimethylation through KDM1 overexpression in this model also suppressed FoxA1 and AR recruitment (Wang et al. 2009).

Similar to the role of FoxA1 as a pioneer factor that translates the H3K4me1/me2 signature at non-promoter-regulatory elements, specific chromatin-remodeling components are recruited to H3K4me3-marked promoters. The ATP-dependent chromatin-remodeling enzyme CHD1 and the ATPase SNF2H are recruited to H3K4-methylated promoters (Santos-Rosa et al. 2003, Flanagan et al. 2005, Sims et al. 2005). This suggests that epigenetic marks are insufficient for transcription factor recruitment. Therefore, it appears that the interplay between epigenetic marks and chromatin-remodeling factors is required to open chromatin in specific genomic locations to guide transcription factor recruitment both at promoter and non-promoter-regulatory elements.

Understanding how the epigenetic signatures, such as the methylated-H3K4-based signature, are established in the course of normal and disease development is of central interest. To date, up to ten histone methyltransferases specific to H3K4 have been characterized and a growing number of histone demethylases are being identified (Christensen et al. 2007, Eissenberg et al. 2007, Iwase et al. 2007,
events may have on epigenetic components. Regulation as well as the contribution of transcriptional mechanisms fine tuning the actions of ERα and AR. Considering the increasingly recognized role of epigenetic components in cancer development and progression, a better understanding of their function in transcriptional regulation will prove fundamental in the elaboration of novel therapeutic strategies to breast, prostate, and other cancers.

Conclusion

The wealth of information derived from cistrome-based studies is already revealing core concepts of transcriptional regulation. Recently, a predictive model based on the ERα and FoxA1 cistromes from breast cancer cells as well as the cistrome of the insulin protein CCCTC-binding factor (CTCF) has predicted up to 70% of estrogen-regulated genes (Chan & Song 2008). Pursuing this analysis to coregulatory factors and epigenetic components should reveal intricate mechanisms fine tuning the actions of ERα and AR. Considering the increasingly recognized role of epigenetic components in cancer development and progression, a better understanding of their function in transcriptional regulation will prove fundamental in the elaboration of novel therapeutic strategies to breast, prostate, and other cancers.

Declaration of interest

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

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References


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Klose et al. 2007, Lee et al. 2007, Ruthenburg et al. 2007, Seward et al. 2007, Shi & Whetstine 2007, Tahlilian et al. 2007, Yamane et al. 2007). Therefore, the methylation state of H3K4me3 has been found enriched at inaccessible promoter-distant regulatory elements (Lupien et al. 2008). Furthermore, H3K4me1/me2 and H3K9me2 occurred together on more condensed FoxA1-binding sites as defined by DNaseI hypersensitivity and Formaldehyde-Assisted Isolation of Regulatory Elements, an additional method that delineates DNA accessibility (Giresi et al. 2007, Bernstein et al. 2006, Guenther et al. 2007, Mikkel森 et al. 2007). Although there is no clear indication of a similar signature at non-promoter-regulatory elements, H3K9me2 has recently been found enriched at inaccessible promoter-distant regulatory elements (Lupien et al. 2008). Furthermore, H3K4me1/me2 and H3K9me2 occurred together on more condensed FoxA1-binding sites as defined by DNaseI hypersensitivity and Formaldehyde-Assisted Isolation of Regulatory Elements, an additional method that delineates DNA accessibility (Giresi et al. 2007, Eeckhoute et al. 2009). Therefore, additional studies are needed to reveal fundamental components of the role played by chromatin structure in transcriptional regulation as well as the contribution transcriptional events may have on epigenetic components.

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References


Gonzalez MI & Robins DM 2001 Oct-1 preferentially interacts with androgen receptor in a DNA-dependent manner that facilitates recruitment of SRC-1. Journal of Biological Chemistry 276 6420–6428.
Heinlein CA & Chang C 2004 Androgen receptor in prostate cancer. Endocrine Reviews 25 276–308.


Prall OW, Rogan EM, Musgrove EA, Watts CK & Sutherland RL 1998 c-Myc or cyclin D1 mimics estrogen effects on cyclin E-Cdk2 activation and cell cycle reentry. *Molecular and Cellular Biology* 18 4499–4508.


Sims RJ III, Chen CF, Santos-Rosa H, Kouzarides T, Patel SS & Reinberg D 2005 Human but not yeast CHD1 binds
directly and selectively to histone H3 methylated at lysine 4 via its tandem chromodomains. Journal of Biological Chemistry 280 41789–41792.


