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 (ER α) 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 ER α 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 ER α cistrome in breast cancer cells relates to expression profiles from ER α -positive primary breast tumors. Additionally, cell-type-specific ER α 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/HNF3 α) plays a central role in AR and ER α 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 (ER α) 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 ER α 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, ER α 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, Fietze *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 ER α 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 ER α 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 ER α - 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 ER α -positive primary breast tumors as well as estrogen target genes in breast cancer cell lines are preferentially surrounded by estrogen-induced ER α -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 ER α 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 ER α cistrome, distinct from the ER α cistrome in breast cancer cells, directly relates to the estrogen-induced expression profile in osteosarcoma

(Krum *et al.* 2008, Fig. 1). Furthermore, ER α -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 ER α or AR, conserved networks of regulatory factors have been defined (Carroll *et al.* 2005, 2006, Laganier *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 ER α - and/or AR-binding sites. GATA3 recognizing the GATA motif was revealed to be part of a positive cross-regulatory loop with ER α in breast cancer cells required for the estrogen-mediated cell proliferation (Eeckhoutte *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 ER α in breast cancer cells regulating key target genes, namely *CCND1* (Cicatiello *et al.* 2004, Carroll *et al.* 2006). More recently, PAX2 co-recruitment with ER α 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

Figure 1 Lineage- and stimuli-specific transcriptional programs are dependent on differential recruitment of ER α . Thousands of putative ER α -binding sites are found across the human genome. This includes over 60 000 estrogen-responsive elements (EREs) and a number of regions recruiting ER α through a tethering mechanism. However, lineage-specific ER α recruitment, as reported between breast and osteosarcoma cancer cell lines, is central to the unique transcriptional program generated in each cell type following estrogen (E₂) treatment. Similarly, the transcriptional program activated through the PI3K/AKT pathway in MCF7 cells expressing a constitutively active AKT (CA-AKT) is dependent on a unique ER α recruitment pattern.

in ER α -binding sites in breast cancer cells behave in a distinct manner. Indeed, instead of being co-recruited with ER α following estrogen stimulation, these transcription factors are bound at the basal state and block ER α 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 ER α recruitment (Holmes *et al.* 2008). Therefore, by defining the cistromes of ER α 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 ER α and AR cistromes (Carroll *et al.* 2005, 2006, Laganier *et al.* 2005, Wang *et al.* 2007). The forkhead family member FoxA1 (HNF3 α) is a key partner for ER α 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 ER α recruitment in breast cancer cells (Carroll *et al.* 2005, Laganier *et al.* 2005, Eeckhoutte *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 ER α - 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, Laganier *et al.* 2005, Eeckhoutte *et al.* 2006). Hence, under hormonal stimulation, ER α 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 ER α signaling, FoxA1 is

Figure 2 Chromatin architecture reprogramming in castration-resistant prostate cancer cells. Similar to ER α in breast cancer cell lines, AR is recruited to a fraction of its putative binding sites in androgen-dependent prostate cancer cells. In castration-resistant prostate cancer cells, the genome-wide AR recruitment pattern is altered. This is dependent on the reprogramming of the chromatin architecture typified by *de novo* methylation of lysine 4 on histone H3 (H3K4me).

typically highly expressed in ER α -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 ER α 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

Figure 3 Lineage-specific distribution of histone H3 methylation on lysine 4 guides FoxA1 recruitment. Differentiated cells are characterized by a unique distribution of epigenetic marks. FoxA1 will specifically be recruited to genomic regions harboring the forkhead motif (FKH) marked by H3K4me1/me2. Through its chromatin-remodeling activity, neighboring chromatin will be further opened and accessible to other transcription factors such as ER α and AR in breast and prostate cancer cells respectively. By contrast, FKH regions lacking H3K4me1/me2 will typically associate with H3K9me2 and be deprived of FoxA1 recruitment. Hence, the lineage-specific distribution of H3K4me1/me2 and H3K9me2 guides FoxA1 binding, which in turn restricts the recruitment of other transcription factors.

prevents FoxA1 recruitment (Lupien *et al.* 2008, Wang *et al.* 2009). This signature is also characteristic of the ER α cistrome not overlapping with FoxA1 (M L and M B unpublished data). In fact, lineage-specific ER α cistromes correlate with the H3K4me1/me2 distribution. Indeed, ER α -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,

Klose *et al.* 2007, Lee *et al.* 2007, Ruthenburg *et al.* 2007, Seward *et al.* 2007, Shi & Whetstone 2007, Tahiliani *et al.* 2007, Yamane *et al.* 2007). Therefore, the methylation state of H3K4 appears to be under tight regulation. Previous reports have indicated that the distribution of H3K4me2 is established early during differentiation (Chambeyron & Bickmore 2004). Similarly, work on promoter regions has revealed a progression from a broad to a more restricted distribution for H3K4me3 in the course of differentiation (Guenther *et al.* 2007, Mikkelsen *et al.* 2007). Interestingly, the bivalent state of histone modifications found at promoters relates to the transitional state of poised to active or repressed promoters. Indeed, in the course of differentiation, promoters harboring both H3K4me3 and H3K27me3 are enriched for H3K4me3 when the associated genes are expressed and for H3K27me3 when the genes are silenced (Azuara *et al.* 2006, Bernstein *et al.* 2006, Guenther *et al.* 2007, Mikkelsen *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, Eeckhoutte *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.

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 ER α and FoxA1 cistromes from breast cancer cells as well as the cistrome of the insulator 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 ER α 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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