Fundamental Pathways in Breast Cancer 3: Estrogen Biology

3

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3.1 Introduction

Over the last two decades, it has become evident that breast cancer should be considered as a family of diseases rather than as a unique malignancy. Pathological, molecular, and genetic analysis have revealed the existence of five to ten main subgroups [1–3]. Over 70% of all patients are generally classified by the tumor dependencies on estrogenic compounds [4]. These dependencies are principally mediated by the nuclear receptor estrogen receptor α (ERα) [5, 6]. For all these reasons, ERα remains the key driver in the majority of breast cancers and is commonly used as a molecular biomarker for stratification while serving as the main target for systemic adjuvant chemotherapy. In this chapter we will discuss the molecular mechanisms of ERα activation, focusing on integrative analysis that have recently exposed the intimate link between ERα and chromatin structure.

3.2 Estrogen Signaling and the ERα Underlie a Large Fraction of Breast Cancer Hallmarks

A critical shift in the approaches to studying estrogen biology has occurred in the last decade. The field has gradually moved from the investigation of single genes to the study of model cell lines and finally moving to patient-derived samples. This broadening involved also the molecular toolkit used by scientists through the development of next-generation sequencing and allowed the development of unbiased, genome-wide assays [7]. This transition was critical to refine our understanding and overtake long-standing dogmas. Since then, the field has become aware of the complexities of estrogen signaling and began examining the association between DNA, the scaffolding DNA structure (chromatin), epigenetic and genetic factors, and ERα. Using system biology approaches and genome-wide annotations, we now have also linked ERα to the majority of breast cancer hallmarks thus reemphasizing the importance of this dogmatic transcription factor.

The involvement of estrogen signaling in breast cancer biology was recognized over a century ago, when a causative link between ovariectomy and breast cancer progression was made [4]. Several studies have also linked estrogen and breast cancer etiology. Some of the best-characterized predisposing factors predisposing factors leading to breast cancer breast cancer reflect endogenous estrogenic exposure (reviewed in [8]). In addition, additional exogenous estrogen exposure can also favor the development of luminal breast cancer [9]. Over 5×10^3 studies (source, PubMed) have evaluated the role of estrogen signaling in MCF7 cells, one of the preferred tools to investigate the dynamics of estrogen signaling at a molecular levels. The most investigated aspect of estrogen signaling is without any doubt the sustained growth promoted by activated ERα. Nonetheless, estrogen signaling is also involved in many other cancer hallmarks [10] (Fig. 3.1). Some of the molecular details of how this happens will be discussed in more detail in other sections of this chapter and other chapters as well.

Estrogen signaling has been extensively associated with evasion of cell death [11], invasion and metastasis [12, 13], and inflammation [14] phenotypes. More recently estrogens and ERα have also been associated with angiogenesis [15], genome instability and mutations [16], and deregulation of cellular energetics [17, 18]. It is important to understand that most of these biological features are modulated by activated ERα at the DNA level. In the next sections, we will discuss how breast cancer cells can access such a wide array of cellular response via a single transcription factor.

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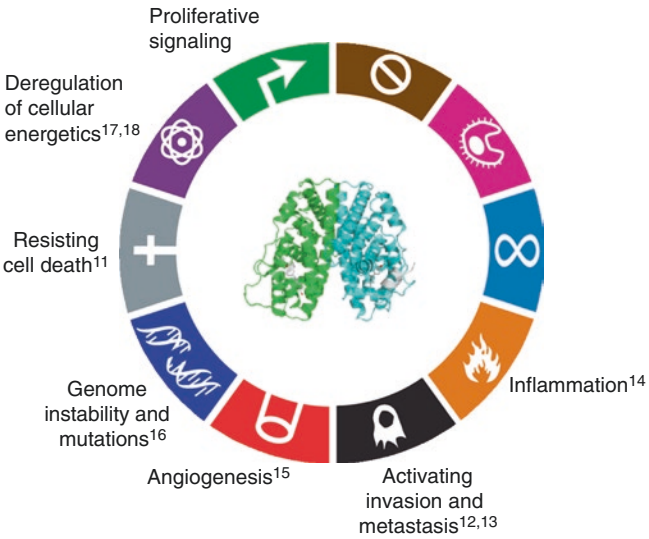

Fig. 3.1 ERα directly controls the majority of cancer hallmarks in breast cancer cells. The image is modified from [10]

3.3 This Must Be the Place: A Brief Introduction of the Chromatin Environment

ERα signaling can be broadly classified into canonic (genomic) and noncanonical (non-genomic). Noncanonical signaling involves ERα activation in the cytoplasm and the subsequent activation of complex signal transduction cascades mediated mostly by kinases. For an in-depth review of the subject, see [19]. An example is provided by EGF-EGFR signaling converging on ERα phosphorylation. Even in these scenarios, ERα ultimately acts via DNA binding [20, 21]. A more controversial line of investigation has addressed the potential role of ERα in the cell membrane [22], the data though have been challenged [23] and the field has not matured a consensus. On the other hand, a large fraction of ERα molecules constantly shuffle between the cytoplasm and the nucleus where they alternate between a free-floating state and a DNA bound state [24, 25]. More importantly, canonic ERα signaling has been associated with the majority of the breast cancer hallmarks discussed above. For all these reasons, we will focus the discussion on canonical signaling.

The full-length ERα contains a ligand binding domain (LBD) and a DNA binding domain (DBD) separated by a hinge domain [26]. Once the ligands contact the LBD, conformational changes occur throughout the entire protein and allow for dimerization and DNA binding [27, 28]. ERα then quickly contacts the DNA at genetically defined DNA sequences called estrogen-responsive elements (EREs) [29]. More than 70,000 EREs are scattered throughout the human genome in addition to regions that harbor half or degenerate EREs which are also permissive to ESR1 recruitment [30]. This poses the question of how many regions ERα binds throughout the genome, how ERα finds these ERE, what are the molecular determinants of ERα binding, and how many ERα are actually functional.

The human genome consists of around 3×10^9 base pairs. Eukaryotes have evolved strategies to compact this vast array of information in the nucleus via higher-ordered packaging (the chromatin). 147–148 bp of the DNA string wrapped around histone proteins is the minimal repeating unit of the chromatin (the nucleosome) [31]. ERα, similarly to 94% of all DNA binding proteins, has higher affinity for nucleosome-free DNA [32, 33]. Thus, chromatin accessibility represents the first barrier to ERα binding. ERα binding is the primary driver of gene expression. Activation of ERα induces the strong transcriptional response that drives breast cancer cell proliferation [34]. ERα orchestrates transcription by binding at critical DNA regions known as regulatory elements [35]. These regions can be broadly classified as promoters and enhancers based on the relative distance from the gene that is controlled (Fig. 3.2). The chromatin environment at regulatory regions is defined by several well-characterized epigenetic features [36]. For example, active promoters and enhancers are typically nucleosome-free and accessible to transcription factors [33]. The nucleosomes surrounding regulatory regions carry special chemical modifications on the histone tails depending on their activity status [36–38]. These modifications, known as histone post-translational modifications (HPMTs), have been extensively used to annotate regulatory regions in the genome by several international consortiums [36, 39, 40]. Promoters are characterized by histone 3 lysine 4 tri-methylation (H3K4me3), while enhancers are generally enriched for histone 3 lysine 4 mono-methylation (H3K4me1) [38]. On the other end of the chromatin spectrum, inactive/repressed regulatory regions carry H3K27me3 and H3K9me3 modifications [41]. Collectively, chromatin accessibility and histone modifications are a constitutive part of the epigenome. Several integrative studies have now dissected the relationship between ERα and the epigenome.

Fine mapping of ERα DNA interaction and integration with epigenetic data were central to remodel a long-standing dogma in ERα biology. For a long time, it was hypothesized that ERα controls transcription by binding to primarily the promoters of target genes. It is now well established that 97% of ERα binding occurs at distal enhancers [35, 42, 43] (Fig. 3.1). These regions are typically enriched for active epigenetic modifications (H3K4me1, H3K27ac) and devoid of repressive marks (H3K27me3 and H3K27me9). These unex-

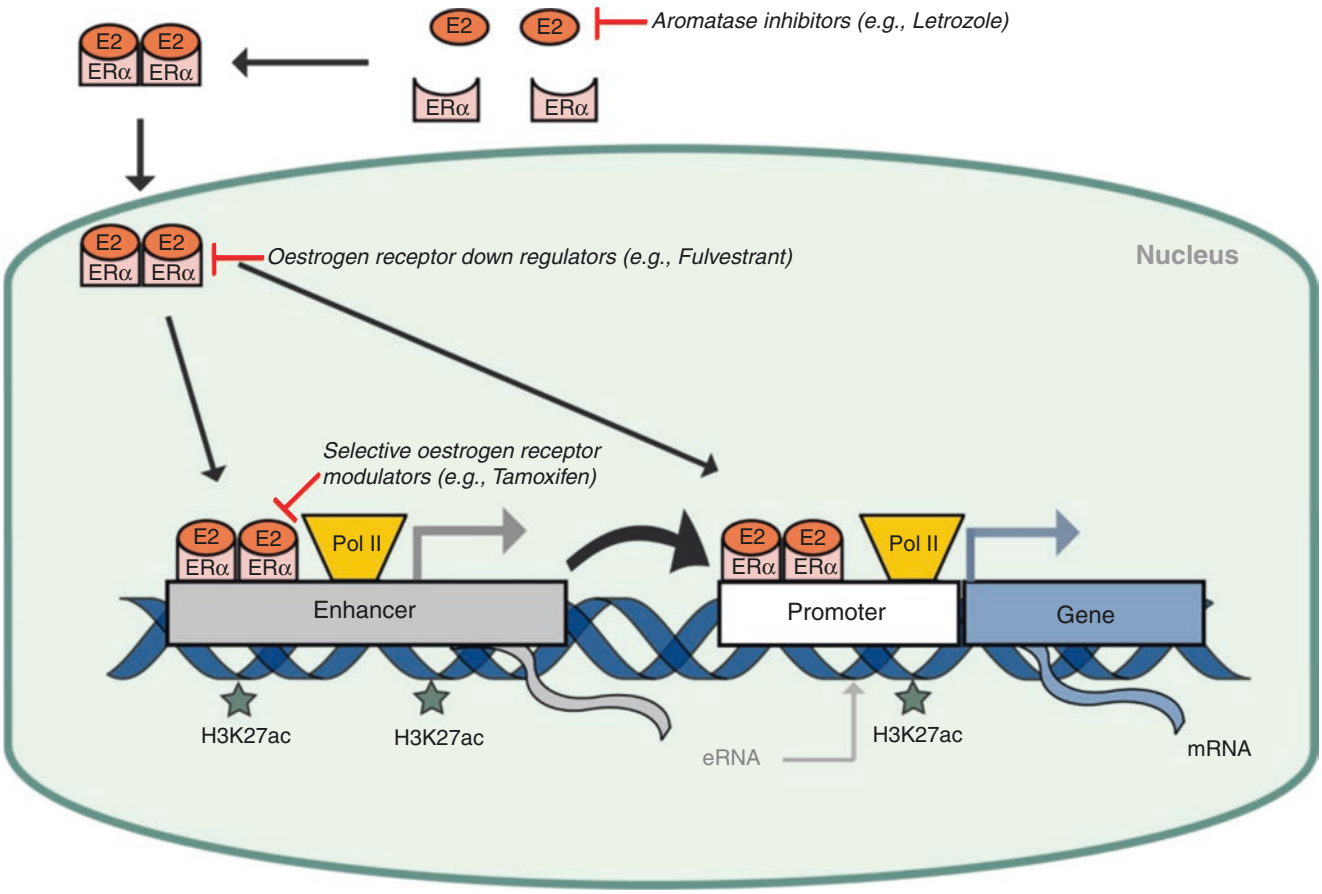

Fig. 3.2 ERα interacts with the chromatin to regulate gene expression. ERα is found both in the cytoplasm and in the nucleus prior to estrogen-mediated activation. Estrogen activation is the main target of endocrine therapies. Once activated, the receptor binding regulatory regions (enhancers and promoters) that contain ERE motif are bookmarked by specific histone modifications. ERα binding potentiates gene transcription by Pol II and leads to the activation of many genes involved in proliferation, invasion, and other cancer hallmarks

pected landmark discoveries have provided the foundations for the last ten years of research looking for the modalities by which ERα mechanistically modulate gene expression.

3.4 Pioneer Factors Are Critical Regulators of ERα Binding to the Chromatin

ERα can bind the DNA only when activated; otherwise, it remains unbound within the cytoplasm/nucleus. Several groups have examined the dynamic properties of ERα binding to the DNA. Biochemical investigation using the average signal from millions of cells have established a paradigm whereby once activated, ERα cyclically binds to target DNA at 45-min intervals [35, 44–46]. Nonetheless, ERα requires nucleosome-free regions for efficient DNA binding. Recent studies have shown that ERα binding sites are maintained in an open chromatin conformation by a specialized set of transcription factors called pioneer factors [47, 48]. While ERα interaction with the DNA is ligand and time dependent, pioneer factors bind near EREs in the absence of external stimuli and are thought to maintain more stable interactions with the chromatin. Two of the best-characterized pioneer factors are FOXA1 [35] and PBX1 [24]. Depletion of FOXA1 and PBX1 results in a dramatic reduction in chromatin accessibility at local EREs [24]. In contrast with other transcription factors, pioneer factor can interact with nucleosomes and bind nucleosome-dense DNA [49] thus increasing chromatin accessibility *de novo* [50]. Pioneer factors also appear to be the link between the epigenome and ERα via nucleosome modifications. Several evidences indicate that pioneer factors might be able to interact with nucleosome modifications. For example, overexpression of a protein involved in erasing the H3K4me2 mark corresponds to a loss of PBX1 and ERα from several enhancers [24]. Furthermore, pioneer factors are found mainly at H3K4me1/2 rich regions [24, 51]. In summary, pioneer factors act as the gatekeepers of potential ERα binding sites by modeling chromatin accessibility and bookmarking a discrete number of genomic locations for ERα (Fig. 3.3).

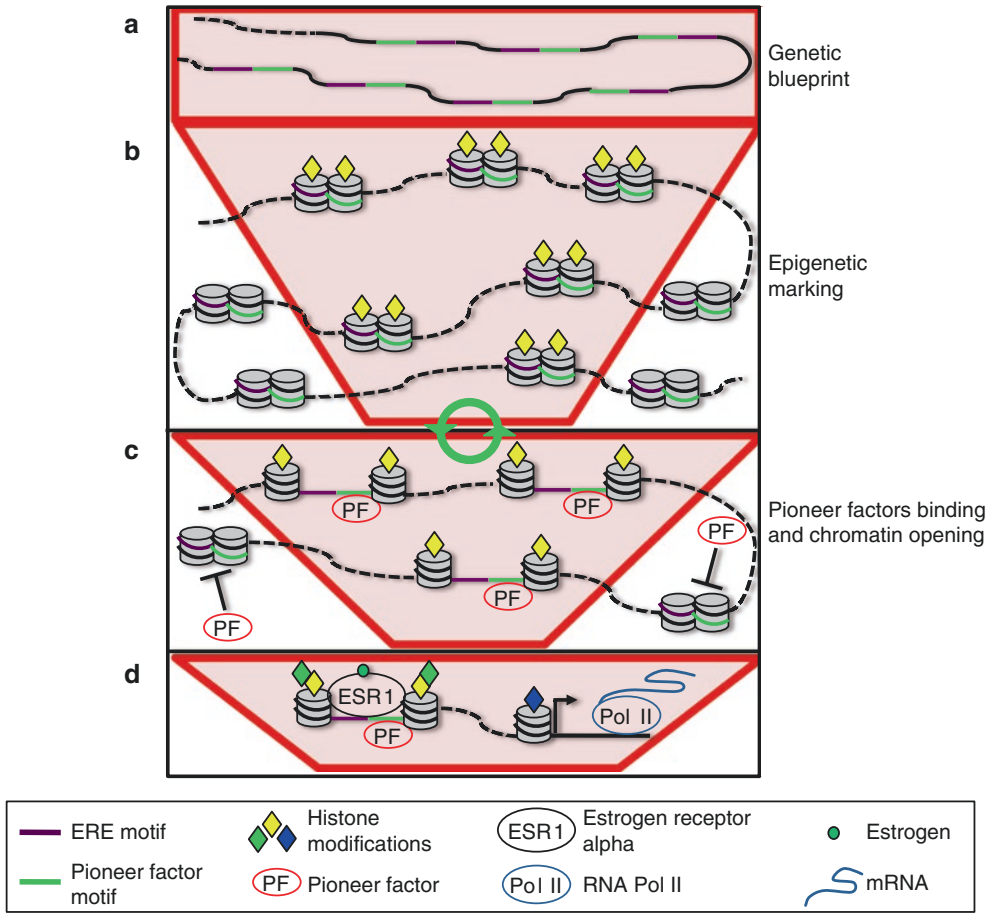

Fig. 3.3 (**a**) The chromatin template mediates ESR1 signaling activity. ERE elements are distributed across the genome and can co-localize near pioneer factor motif elements. (**b** and **c**) Histone modifications (e.g., H3K4me1/2) and pioneer factors cooperate to increase chromatin accessibility at a subset of genomic ERE. (**d**) These events promote competence for successful ESR1 binding in response to external stimuli and mediate ESR1 transcriptional program (reproduced with permission from [6])

3.5 ERα Regulates Transcription via Chromatin Looping

The vast majority of ERα binding occurs at distal regulatory regions (enhancers). How does ERα then activate gene transcription? It is well established that activated ERα is essential to promote efficient RNA polymerase II release from gene promoters and enhancers as well [52–54]. Recent advances in chromatin conformation capture assays have highlighted the tremendous complexity of the 3D organization of the chromatin [55, 56]. These genomic assays characterized thousands of enhancer-promoter interactions partially explaining how distal regulatory regions can mediate transcription. Not surprisingly, ERα was one of the first transcription factors found at interacting chromatin loops [57]. There are, however, some unresolved questions about the formation of these loops. One model postulates that estrogen-activated ERα can drive loop formation [57–59]. However, there is also an indication that ERα might exploit preformed loops that have been set up by pioneer factors with the contribution of epigenetic modifications (reviewed in [60]). It is conceivable that future studies will find that ERα chromatin looping is very context dependent and could include both models of transcriptional activation.

3.6 ERα Regulates Transcription via Protein Recruitment

ERα regulates transcription by modulating RNA polymerase II release from the 5′-prime end of the gene body. Yet, ERα alone is not sufficient for full transcriptional activation. It soon became apparent that ERα recruits several other proteins to promote transcription [46, 61]. These studies also explained how ERα could modulate repression. The proteins recruited by ERα are commonly referred to as coactivators or corepressors. Interestingly, two among the first coactivators to be identified (SRC1 and BRG1) are critical chromatin modulators, further highlighting the strong link between ERα and the chromatin environment. Steroid receptor coactivator-1 (SRC1) is a histone acetyltransferase [62] (Histone 3 lysine 9/14 acetylation), while BRG1 is a chromatin remodeler [63, 64]. Histone 3 lysine 9 acetylation is yet another HPMTs strongly associated with active transcription and has been shown to be important for chromatin relaxation and improved DNA accessibility [65]. On the other hand, histone acetylation provides a docking station for bromodomain proteins, including the chromatin remodeler BRG1. These proteins interact with acetylated histones and are essential to remodel and reposition nucleosomes [66]. These

cofactors mostly lack DNA binding abilities and rely on ERα for recruitment at the correct regulatory regions. Several lines of evidence support this model. For example, it has been noted that BRG1, SRC1, and other cofactors parallel ERα cyclical pattern of recruitment onto the DNA [44]. More importantly, blocking ERα binding is sufficient to abrogate binding for several cofactors [67]. The list of cofactors has been growing dramatically in the last few years. Proteomic-based approach has now identified hundreds of potential coactivators and corepressors [68] including several ERα target genes themselves. Collectively, these examples emphasize the complex transcriptional machinery driving growth in breast cancer cells while underscoring the central role of ERα in coordinating all genomic actions.

3.7 Alternative ERα Binding Programs Correlate with Differential Patient Outcome

ERα binding is modulated by chromatin accessibility, epigenetic modifications, and cofactor recruitment. The combination of these regulatory layers shapes cell type-specific ERα binding. But are alternative ERα binding combinations reflective of different biology? Could alternative ERα binding be used to stratify breast cancer patients *in vivo*? A recent study from the Carroll group have examined, for the first time, the collection of ERα binding (known as cistrome) in several luminal breast cancer patients characterized by distinct outcome [43]. The data suggest that while a lot of ERα binding seems to be patient-specific, there are also clusters unique to good outcome patients and clusters unique to poor outcome patients in addition to a core ERα cistrome common to patients and cell lines as well [43]. Of note, differential ERα binding is potentially correlated with alternative transcriptional programs. Gene expression profiling using putative ERα target genes can also identify subgroups of patients with dramatically different outcome suggesting that ERα can guide both aggressive and nonaggressive breast cancers. An explanation for these patterns can be found in alternative usage of pioneer factors. For example, it was shown that when ERα interacts with PBX1, it can guide transcription of genes associated with aggressive phenotype [24]. On the other hand, ERα interaction with GATA3 [47, 69, 70], another breast cancer pioneer factor, seems to be associated with less aggressive tumors ([43]).

Genetic alteration can also impact ERα recruitment in vivo. Genomic analyses have revealed that about 20% of all luminal breast cancer patients have copy number loss at the progesterone receptor (PGR) locus [71]. PGR is one of the best-characterized ERα target genes and is commonly used to stratify luminal breast cancer patients into luminal A (ER+/PGR+) and luminal B (normally ER+/PGR− or ER+/PR+ and HER2+) subtypes [72]. Nevertheless, PGR has been also described as an ERα cofactor capable of hijacking ERα upon native progesterone stimulations [71]. More importantly, the ERα cistrome obtained from progesterone-treated cells correlate with milder phenotypes and improved outcome. Indeed, patients with PGR copy number loss are characterized by a poorer outcome [71]. In summary, ERα genomic localization has significant effects on tumor biology. These data can be then harnessed clinically by finding practical strategies to reprogram ERα. For example, it has been postulated that native progesterone (but not synthetic progestin) treatment in PGR wild-type patients might carry significant benefits.

3.8 Alternative Means of ERα Activation

Estrogen signaling plays an essential role in driving breast cancer growth at early stages. All approved adjuvant systemic therapies are in fact designed to block estrogen signaling (for an updated review see [73]) (Fig. 3.2). Targeting estrogen signaling lowers the rate of relapse by about 50% in ERα-positive patients [74]. However, it is becoming apparent that estrogen signaling remains central at later stages of the disease as well. In the last three years, it has been shown how ERα-positive breast cancer cells develop alternative strategies to activate the receptor in later stages of the disease. There are two main mechanisms through which this can happen. The first involves activating mutations targeting the LBD [75, 76]. Two independent studies found that metastatic breast cancer patients with a history of luminal disease have a significant prevalence (~20%) of mutations targeting the LBD of ERα. These mutations appear to activate the receptor in the absence of estrogens through conformational changes. This results in a constitutively active form of the receptor that cannot be turned off by conventional chemotherapy. It remains unclear at what stage of the disease these mutations arise, since the patients in which they were identified received an extensive array of treatments [75, 76].

The second mechanism involves the activation of cholesterol biosynthesis in estrogen-independent ERα breast cancer (i.e., letrozole resistant) [18]. In this case, ERα cancer cells develop the ability to synthesize *de novo* an alternative ERα ligand (27-hydroxycholesterol) [77]. This in consequence allows estrogen-independent, ERα-dependent proliferation [18]. Moreover, 27-hydroxycholesterol was previously shown to stimulate an invasive phenotype in ERα breast cancer mouse models [12]. Ultimately, estrogen signaling might become redundant as the disease approaches the later stages despite breast cancer cells remaining frequently ERα positive [78]. This is also reflected clinically by the limited benefit of ERα downregulators such as Faslodex [79]. In summary, estrogen signaling and ERα continue to play a key role throughout the patients' entire journey.

3.9 Novel Insight in the Genomic Activity of ERα

Estrogen signaling is essential to promote growth, invasion, and survival of breast cancer cells. In addition, recent studies have also linked ERα and estrogen signaling to genetic instability and mutational burden. One of the most recently identified ERα cofactors is the cytosine deaminase APOBEC3B [16]. APOBEC3B was previously linked to a specific mutational signature (C to T) in breast cancer patients [80, 81]. Interestingly, APOBEC3B is temporarily co-recruited on the chromatin along with ERα and depletion of ERα results in loss of APOBEC3B recruitment [16]. One of the key findings however is that APOBEC3B is essential for ERα transcriptional activity. Moreover, estrogen stimulation in ERα-positive cell lines was sufficient to jump-start DNA repair mechanisms and the accumulation of double-strand breaks at ERα binding sites [16]. Why estrogen activity induces risky double-strand breaks? Mechanistically, these findings fit with the idea of chromatin remodeling at ERα regulatory regions. While the transcriptional machinery advances, it might require relax and unwounded DNA [82, 83]. Nonetheless, cells with inefficient DNA repair might then have an increased mutational burden at regulatory elements. Altogether these data suggest that estrogen signaling and ERα might also contribute to the mutational signature found in ERα breast cancer patients.

Conclusions

In this chapter, we have discussed some of the critical roles of estrogen signaling in breast cancer cells. By using integrative analysis, we are finally addressing the question we are finally addressing the question as to why ERα is so dominant in breast cancer cells. Yet, some aspects remain uncertain. For example, is ERα binding important in the context of breast cancer predisposition? A recent study found that single nucleotide polymorphisms (SNPs) associated with increased breast cancer risk have a significant tendency toward EREs and FOXA1 binding sites [84]. Possibly, these SNPs act by modulating ERα and other pioneer factors binding to DNA [85]. It is fascinating how then the ERα might evolve during the patient journey. If ERα is involved in increasing the mutational burden, it is then easy to speculate that some of these mutations might increase affinity for ERα, while others might decrease it. Consequently, the ERα cistrome might change at high frequency allowing the tumor to transform during progression and activate or adapt many of the cancer hallmarks in response to change in tissue, therapy, and many other physiological parameters.

References

1. Cancer Genome Atlas Network, Koboldt DC, Fulton RS, McLellan MD, Schmidt H, Kalicki-Veizer J, McMichael JF, Fulton LL, Dooling DJ, Ding L et al (2012) Comprehensive molecular portraits of human breast tumours. Nature 490:61–70
2. Curtis C, Shah SP, Chin S-F, Turashvili G, Rueda OM, Dunning MJ, Speed D, Lynch AG, Samarajiwa S, Yuan Y et al (2012) The genomic and transcriptomic architecture of 2000 breast tumours reveals novel subgroups. Nature 486:346–352
3. Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS et al (2001) Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci 98:10869–10874
4. Beatson G (1896) On the treatment of inoperable cases of carcinoma of the mamma: suggestions for a new method of treatment, with illustrative cases.1. Lancet 148:104–107
5. Ali S, Coombes RC (2002) Endocrine-responsive breast cancer and strategies for combating resistance. Nat Rev Cancer 2:101–112
6. Magnani L, Lupien M (2014) Chromatin and epigenetic determinants of estrogen receptor alpha (ESR1) signaling. Mol Cell Endocrinol 382:633–641
7. Magnani L, Carroll J, Zwart W, Palmieri C (2012) ChIPing away at breast cancer. Lancet Oncol 13:1185–1187
8. Collaborative Group on Hormonal Factors in Breast Cancer (2012) Menarche, menopause, and breast cancer risk: individual participant meta-analysis, including 118 964 women with breast cancer from 117 epidemiological studies. Lancet Oncol 13:1141–1151
9. Colditz GA (1998) Relationship between estrogen levels, use of hormone replacement therapy, and breast cancer. JNCI J Natl Cancer Inst 90:814–823
10. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144:646–674
11. Lewis-Wambi JS, Jordan VC (2009) Estrogen regulation of apoptosis: how can one hormone stimulate and inhibit? Breast Cancer Res 11:206
12. Nelson ER, Wardell SE, Jasper JS, Park S, Suchindran S, Howe MK, Carver NJ, Pillai RV, Sullivan PM, Sondhi V et al (2013) 27-Hydroxycholesterol links hypercholesterolemia and breast cancer pathophysiology. Science 342:1094–1098
13. Platet N, Cathiard AM, Gleizes M, Garcia M (2004) Estrogens and their receptors in breast cancer progression: a dual role in cancer proliferation and invasion. Crit Rev Oncol Hematol 51:55–67
14. Franco HL, Nagari A, Kraus WL (2015) TNFa signaling exposes latent estrogen receptor binding sites to alter the breast cancer cell transcriptome. Mol Cell 58:21–34
15. Syed Khaja AS, Dizeyi N, Kopparapu PK, Anagnostaki L, Härkönen P, Persson JL (2013) Cyclin A1 modulates the expression of vascular endothelial growth factor and promotes hormone-dependent growth and angiogenesis of breast cancer. PLoS One 8:e72210
16. Periyasamy M, Patel H, Lai C-F, Nguyen VTM, Nevedomskaya E, Harrod A, Russell R, Remenyi J, Ochocka AM, Thomas RS et al (2015) APOBEC3B-mediated cytidine deamination is required for estrogen receptor action in breast cancer. Cell Rep 13:108–121
17. Faulds MH, Zhao C, Dahlman-Wright K, Gustafsson JA (2011) The diversity of sex steroid action: regulation of metabolism by estrogen signaling. J Endocrinol 212:3–12
18. Nguyen VTM, Barozzi I, Faronato M, Lombardo Y, Steel JH, Patel N, Darbre P, Castellano L, Gyorffy B, Woodley L et al (2015) Differential epigenetic reprogramming in response to specific endocrine therapies promotes cholesterol biosynthesis and cellular invasion. Nat Commun 6:10044

19. Björnström L, Sjöberg M (2005) Mechanisms of estrogen receptor signaling: convergence of genomic and nongenomic actions on target genes. Mol Endocrinol 19:833–842
20. Lupien M, Meyer CA, Bailey ST, Eeckhoute J, Cook J, Westerling T, Zhang X, Carroll JS, Rhodes DR, Liu XS et al (2010) Growth factor stimulation induces a distinct ER cistrome underlying breast cancer endocrine resistance. Genes Dev 24:2219–2227
21. Magnani L, Patten DK, Nguyen VTM, Hong S-P, Steel JH, Patel N, Lombardo Y, Faronato M, Gomes AR, Woodley L et al (2015) The pioneer factor PBX1 is a novel driver of metastatic progression in ERα-positive breast cancer. Oncotarget 6:21878–21891
22. Revankar CM, Cimino DF, Sklar LA, Arterburn JB, Prossnitz ER (2005) A transmembrane intracellular estrogen receptor mediates rapid cell signaling. Science 307:1625–1630
23. Pedram A, Razandi M, Levin ER (2006) Nature of functional estrogen receptors at the plasma membrane. Mol Endocrinol 20:1996–2009
24. Magnani L, Ballantyne EB, Zhang X, Lupien M (2011) PBX1 genomic pioneer function drives ERα signaling underlying progression in breast cancer. PLoS Genet 7:e1002368
25. Zhu J, Zhao C, Kharman-Biz A, Zhuang T, Jonsson P, Liang N, Williams C, Lin CY, Qiao Y, Zendehdel K et al (2014) The atypical ubiquitin ligase RNF31 stabilizes estrogen receptor |[alpha]| and modulates estrogen-stimulated breast cancer cell proliferation. Oncogene 33:4340–4351
26. Kumar V, Green S, Stack G, Berry M, Jin J-R, Chambon P (1987) Functional domains of the human estrogen receptor. Cell 51:941–951
27. Beekman JM, Allan GF, Tsai SY, Tsai MJ, O'Malley BW (1993) Transcriptional activation by the estrogen receptor requires a conformational change in the ligand binding domain. Mol Endocrinol 7:1266–1274
28. Paige LA, Christensen DJ, Grøn H, Norris JD, Gottlin EB, Padilla KM, Chang CY, Ballas LM, Hamilton PT, McDonnell DP et al (1999) Estrogen receptor (ER) modulators each induce distinct conformational changes in ER alpha and ER beta. Proc Natl Acad Sci 96:3999–4004
29. Klinge CM (2001) Estrogen receptor interaction with estrogen response elements. Nucleic Acids Res 29:2905–2919
30. Bourdeau V, Deschênes J, Métivier R, Nagai Y, Nguyen D, Bretschneider N, Gannon F, White JH, Mader S (2004) Genome-wide identification of high-affinity estrogen response elements in human and mouse. Mol Endocrinol 18:1411–1427
31. Luger K, Dechassa ML, Tremethick DJ (2012) New insights into nucleosome and chromatin structure: an ordered state or a disordered affair? Nat Rev Mol Cell Biol 13:436–447
32. Kittler R, Zhou J, Hua S, Ma L, Liu Y, Pendleton E, Cheng C, Gerstein M, White KP (2013) A comprehensive nuclear receptor network for breast cancer cells. Cell Rep 3:538–551
33. Thurman RE, Rynes E, Humbert R, Vierstra J, Maurano MT, Haugen E, Sheffield NC, Stergachis AB, Wang H, Vernot B et al (2012) The accessible chromatin landscape of the human genome. Nature 489:75–82
34. Hah N, Kraus WL (2013) Hormone-regulated transcriptomes: lessons learned from estrogen signaling pathways in breast cancer cells. Mol Cell Endocrinol 382:652–664
35. Carroll JS, Liu XS, Brodsky AS, Li W, Meyer CA, Szary AJ, Eeckhoute J, Shao W, Hestermann EV, Geistlinger TR et al (2005) Chromosome-wide mapping of estrogen receptor binding reveals long-range regulation requiring the forkhead protein FoxA1. Cell 122:33–43
36. Consortium TEP, Data Analysis Coordination OC, Data Production DPL, Data Analysis LA, Group W, Scientific Management NPM, Steering Committee PI, Boise State University and University of North Carolina at Chapel Hill Proteomics Groups (data production and analysis), Broad Institute Group (data production and analysis), Cold Spring Harbor, University of Geneva, Center for Genomic Regulation, Barcelona, RIKEN, Sanger Institute, University of Lausanne, Genome Institute of Singapore Group (data production and analysis) et al (2012) An integrated encyclopedia of DNA elements in the human genome. Nature 488:57–74
37. Ernst J, Kellis M (2010) Discovery and characterization of chromatin states for systematic annotation of the human genome. Nat Biotechnol 28:817–838
38. Ernst J, Kheradpour P, Mikkelsen TS, Shoresh N, Ward LD, Epstein CB, Zhang X, Wang L, Issner R, Coyne M et al (2011) Mapping and analysis of chromatin state dynamics in nine human cell types. Nature 473:43–49
39. Gerstein MB, Rozowsky J, Yan K-K, Wang D, Cheng C, Brown JB, Davis CA, Hillier L, Sisu C, Li JJ et al (2014) Comparative analysis of the transcriptome across distant species. Nature 512:445–448
40. Roadmap Epigenomics Consortium, Kundaje A, Meuleman W, Ernst J, Bilenky M, Yen A, Heravi-Moussavi A, Kheradpour P, Zhang Z, Wang J et al (2015) Integrative analysis of 111 reference human epigenomes. Nature 518:317–330
41. Di Croce L, Helin K (2013) Transcriptional regulation by Polycomb group proteins. Nat Struct Mol Biol 20:1147–1155
42. Lin C-Y, Vega VBB, Thomsen JS, Zhang T, Kong SLL, Xie M, Chiu K-PP, Lipovich L, Barnett DH, Stossi F et al (2005) Whole-genome cartography of estrogen receptorα binding sites. PLoS Genet e87, preprint
43. Ross-Innes CS, Stark R, Teschendorff AE, Holmes KA, Ali HR, Dunning MJ, Brown GD, Gojis O, Ellis IO, Green AR et al (2012) Differential oestrogen receptor binding is associated with clinical outcome in breast cancer. Nature 481:389–393
44. Métivier R, Penot G, Hübner MR, Reid G, Brand H, Kos M, Gannon F (2003) Estrogen receptor-alpha directs ordered, cyclical, and combinatorial recruitment of cofactors on a natural target promoter. Cell 115:751–763
45. Reid G, Hübner MR, Métivier R, Brand H, Denger S, Manu D, Beaudouin J, Ellenberg J, Gannon F (2003) Cyclic, proteasome-mediated turnover of unliganded and liganded ERalpha on responsive promoters is an integral feature of estrogen signaling. Mol Cell 11:695–707
46. Shang Y, Hu X, DiRenzo J, Lazar MA, Brown M (2000) Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription. Cell 103:843–852
47. Magnani L, Eeckhoute J, Lupien M (2011) Pioneer factors: directing transcriptional regulators within the chromatin environment. Trends Genet 27:465–474
48. Zaret KS, Carroll JS (2011) Pioneer transcription factors: establishing competence for gene expression. Genes Dev 25:2227–2241
49. Sekiya T, Muthurajan UM, Luger K, Tulin AV, Zaret KS (2009) Nucleosome-binding affinity as a primary determinant of the nuclear mobility of the pioneer transcription factor FoxA. Genes Dev 23:804–809
50. Sherwood RI, Hashimoto T, O'Donnell CW, Lewis S, Barkal AA, van Hoff JP, Karun V, Jaakkola T, Gifford DK (2014) Discovery of directional and nondirectional pioneer transcription factors by modeling DNase profile magnitude and shape. Nat Biotechnol 32:171–178
51. Lupien M, Eeckhoute J, Meyer CA, Wang Q, Zhang Y, Li W, Carroll JS, Liu XS, Brown M (2008) FoxA1 translates epigenetic signatures into enhancer-driven lineage-specific transcription. Cell 132:958–970
52. Hah N, Danko CG, Core L, Waterfall JJ, Siepel A, Lis JT, Kraus WL (2011) A rapid, extensive, and transient transcriptional response to estrogen signaling in breast cancer cells. Cell 145:622–634
53. Hah N, Murakami S, Nagari A, Danko CG, Kraus WL (2013) Enhancer transcripts mark active estrogen receptor binding sites. Genome Res 23:1210–1223

54. Li W, Notani D, Ma Q, Tanasa B, Nunez E, Chen AY, Merkurjev D, Zhang J, Ohgi K, Song X et al (2013) Functional roles of enhancer RNAs for oestrogen-dependent transcriptional activation. Nature 498:516–520
55. Lieberman-Aiden E, van Berkum NL, Williams L, Imakaev M, Ragoczy T, Telling A, Amit I, Lajoie BR, Sabo PJ, Dorschner MO et al (2009) Comprehensive mapping of long-range interactions reveals folding principles of the human genome. Science 326:289–293
56. Rao SSP, Huntley MH, Durand NC, Stamenova EK, Bochkov ID, Robinson JT, Sanborn AL, Machol I, Omer AD, Lander ES et al (2014) A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. Cell 159:1665–1680
57. Fullwood MJ, Liu MH, Pan YF, Liu J, Xu H, Bin Mohamed Y, Orlov YL, Velkov S, Ho A, Mei PH et al (2009) An oestrogen-receptor. Nature 461:58–64
58. Perillo B, Ombra MN, Bertoni A, Cuozzo C, Sacchetti S, Sasso A, Chiariotti L, Malorni A, Abbondanza C, Avvedimento EV (2008) DNA oxidation as triggered by H3K9me2 demethylation drives estrogen-induced gene expression. Science 319:202–206
59. Tan SK, Lin ZH, Chang CW, Varang V, Chng KR, Pan YF, Yong EL, Sung W-K, Sung WK, Cheung E (2011) AP-2γ regulates oestrogen receptor-mediated long-range chromatin interaction and gene transcription. EMBO J 30:2569–2581
60. Levine M, Cattoglio C, Tjian R (2014) Looping back to leap forward: transcription enters a new era. Cell 157:13–25
61. Oñate SA, Tsai SY, Tsai MJ, O'Malley BW (1995) Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. Science 270:1354–1357
62. Spencer TE, Jenster G, Burcin MM, Allis CD, Zhou J, Mizzen CA, McKenna NJ, Oñate SA, Tsai SY, Tsai MJ et al (1997) Steroid receptor coactivator-1 is a histone acetyltransferase. Nature 389:194–198
63. DiRenzo J, Shang Y, Phelan M, Sif S, Myers M, Kingston R, Brown M (2000) BRG-1 is recruited to estrogen-responsive promoters and cooperates with factors involved in histone acetylation. Mol Cell Biol 20:7541–7549
64. Sif S, Saurin AJ, Imbalzano AN, Kingston RE (2001) Purification and characterization of mSin3A-containing Brg1 and hBrm chromatin remodeling complexes. Genes Dev 15:603–618
65. Eberharter A (2002) Histone acetylation: a switch between repressive and permissive chromatin: second in review series on chromatin dynamics. EMBO Rep 3:224–229
66. Narlikar GJ, Sundaramoorthy R, Owen-Hughes T (2013) Mechanisms and functions of ATP-dependent chromatin-remodeling enzymes. Cell 154:490–503
67. Liu Z, Merkurjev D, Yang F, Li W, Oh S, Friedman MJ, Song X, Zhang F, Ma Q, Ohgi KA et al (2014) Enhancer activation requires trans-recruitment of a megatranscription factor complex. Cell 159:358–373
68. Mohammed H, D'Santos C, Serandour AA, Ali HR, Brown GD, Atkins A, Rueda OM, Holmes KA, Theodorou V, Robinson JLL et al (2013) Endogenous purification reveals GREB1 as a key estrogen receptor regulatory factor. Cell Rep 3:342–349
69. Jozwik KM, Carroll JS (2012) Pioneer factors in hormone-dependent cancers. Nat Rev Cancer 12:381–385
70. Theodorou V, Stark R, Menon S, Carroll JS (2013) GATA3 acts upstream of FOXA1 in mediating ESR1 binding by shaping enhancer accessibility. Genome Res 23:12–22
71. Mohammed H, Russell IA, Stark R, Rueda OM, Hickey TE, Tarulli GA, Serandour AA, Serandour AAA, Birrell SN, Bruna A et al (2015) Progesterone receptor modulates ERα action in breast cancer. Nature 523:313–317
72. Koboldt DC, Cancer Genome Atlas Network, Fulton RS, McLellan MD, Schmidt H, Kalicki-Veizer J, McMichael JF, Fulton LL, Dooling DJ, Ding L et al (2012) Comprehensive molecular portraits of human breast tumours. Nature 490:61–70
73. Palmieri C, Patten DK, Januszewski A, Zucchini G, Howell SJ (2014) Breast cancer: current and future endocrine therapies. Mol Cell Endocrinol 382:695–723
74. Early Breast Cancer Trialists' Collaborative Group (EBCTCG) (2015) Aromatase inhibitors versus tamoxifen in early breast cancer: patient-level meta-analysis of the randomised trials. Lancet 386:1341–1352
75. Robinson DR, Wu Y-M, Vats P, Su F, Lonigro RJ, Cao X, Kalyana-Sundaram S, Wang R, Ning Y, Hodges L et al (2013) Activating ESR1 mutations in hormone-resistant metastatic breast cancer. Nat Genet 45:1446–1451
76. Toy W, Shen Y, Won H, Green B, Sakr RA, Will M, Li Z, Gala K, Fanning S, King TA et al (2013) ESR1 ligand-binding domain mutations in hormone-resistant breast cancer. Nat Genet 45:1439–1445
77. Umetani M, Domoto H, Gormley AK, Yuhanna IS, Cummins CL, Javitt NB, Korach KS, Shaul PW, Mangelsdorf DJ (2007) 27-hydroxycholesterol is an endogenous SERM that inhibits the cardiovascular effects of estrogen. Nat Med 13:1185–1192
78. Magnani L, Stoeck A, Zhang X, Lánczky A, Mirabella AC, Wang T-L, Gyorffy B, Lupien M (2013) Genome-wide reprogramming of the chromatin landscape underlies endocrine therapy resistance in breast cancer. Proc Natl Acad Sci U S A 110:E1490–E1499
79. Di Leo A, Jerusalem G, Petruzelka L, Torres R, Bondarenko IN, Khasanov R, Verhoeven D, Pedrini JL, Smirnova I, Lichinitser MR et al (2010) Results of the CONFIRM phase III trial comparing fulvestrant 250 mg with fulvestrant 500 mg in postmenopausal women with estrogen receptor-positive advanced breast cancer. J Clin Oncol 28:4594–4600
80. Burns MB, Lackey L, Carpenter MA, Rathore A, Land AM, Leonard B, Refsland EW, Kotandeniya D, Tretyakova N, Nikas JB et al (2013) APOBEC3B is an enzymatic source of mutation in breast cancer. Nature 494:366–370
81. Nik-Zainal S, Wedge DC, Alexandrov LB, Petljak M, Butler AP, Bolli N, Davies HR, Knappskog S, Martin S, Papaemmanuil E et al (2014) Association of a germline copy number polymorphism of APOBEC3A and APOBEC3B with burden of putative APOBEC-dependent mutations in breast cancer. Nat Genet 46:487–491
82. Ju B-G, Lunyak VV, Perissi V, Garcia-Bassets I, Rose DW, Glass CK, Rosenfeld MG (2006) A topoisomerase IIbeta-mediated dsDNA break required for regulated transcription. Science 312:1798–1802
83. Li W, Hu Y, Oh S, Ma Q, Merkurjev D, Song X, Zhou X, Liu Z, Tanasa B, He X et al (2015) Condensin I and II complexes license full estrogen receptor α-dependent enhancer activation. Mol Cell 59:188–202
84. Cowper-Sal lari R, Zhang X, Wright JB, Bailey SD, Cole MD, Eeckhoute J, Moore JH, Lupien M (2012) Breast cancer risk-associated SNPs modulate the affinity of chromatin for FOXA1 and alter gene expression. Nat Genet 44:1191–1198
85. Zhang X, Cowper-Sal middle dot lari R, Bailey SD, Moore JH, Lupien M (2012) Integrative functional genomics identifies an enhancer looping to the SOX9 gene disrupted by the 17q24.3 prostate cancer risk locus. Genome Res 22:1437–1446