Pioneer factors in hormone-dependent cancers

Kamila M. Jozwik and Jason S. Carroll

Abstract | Pioneer factors are a special class of transcription factor that can associate with compacted chromatin to facilitate the binding of additional transcription factors. The function of pioneer factors was originally described during development; more recently, they have been implicated in hormone-dependent cancers, such as oestrogen receptor-positive breast cancer and androgen receptor-positive prostate cancer. We discuss the importance of pioneer factors in these specific cancers, the discovery of new putative pioneer factors and the interplay between these proteins in mediating nuclear receptor function in cancer.

Pioneer factors belong to a class of proteins that are emerging as crucial transcriptional components during development and, as has been discovered more recently, in cancer biology. They have the capacity to associate with condensed chromatin independently of other factors and can directly modulate chromatin accessibility[1]. Several protein families have been shown to possess these properties, including forkhead box A (FOXA; also known as HNF3)[2], TLE (also known as groucho)[3] and, to a moderate degree, GATA[4]. Although pioneer factors have been implicated in different cancer types (TABLE 1) — for example, PU.1 in acute myeloid leukaemia — important recent developments have been made in understanding their function in hormone-dependent cancers. This Progress article discusses the evolving role of pioneer factors in two of the major solid malignancies in humans — hormone-dependent breast cancer and prostate cancer.

Hormone-dependent cancers

Nuclear receptors have prominent roles as pro-proliferative transcription factors that can drive tumorigenesis. For example, in breast cancer, the expression of oestrogen receptor-α (ERα) is a defining feature of approximately 70% of all breast cancers. A second ER isoform, ERβ, is expressed in the mammary gland, but its role in epithelial cancer cells is unclear, and whether pioneer factors are important for the function of ERβ is currently unknown. Therefore, we refer to ERα using the generic term ER throughout this article.

Similar to ER, the androgen receptor (AR) is a driving factor in androgen-mediated prostate cancer. ER-targeted treatments, such as the selective oestrogen receptor modulator (SERM) tamoxifen and aromatase inhibitors for breast cancer treatment, and AR-specific treatments, such as bicalutamide for prostate cancer treatment, have radically changed the treatment options and survival rates of patients with breast cancer and prostate cancer, respectively. However, a substantial proportion of patients eventually develop resistance to these agents[5,6]. Many mechanisms contribute to resistance in hormone-dependent cancers, and in general these mechanisms differ between breast cancer and prostate cancer. Given the common progression of hormone-driven cancers towards resistance, delineating the mechanisms of ER and AR transcriptional activity to discover more selective and targeted therapeutic approaches is needed for patients who have acquired resistance to current treatments.

It has been known for many years that ER and AR do not function in isolation, but that they also interact with numerous associated proteins that can help to tether the receptor to DNA, that can function as adaptor proteins and that can possess intrinsic enzymatic activity. There has been substantial interest in characterizing proteins that help these nuclear receptors to interact with DNA, and there is an extensive scientific literature describing such a role for AP1 factors (such as JUN and FOS proteins) in promoting ER–DNA interactions. However, these factors are recruited to DNA only when ER interacts with the hormone ligand[7]. As such, they constitute one potential mechanism of promoting nuclear receptor–DNA interactions, as they can form multiprotein complexes between different transcription factors, which, in combination, augment the binding of other transcription factors and prevent chromatin condensation[1]. Another mechanism of facilitating transcription factor interactions with chromatin is through pioneer factors. FOXA proteins are pioneer factors because they can interact with compacted chromatin independently of other proteins and can directly modulate chromatin structure[2,8]. This usually involves 'opening' of the chromatin, but, in specific cases, FOXA1 can also recruit additional factors, such as TLE proteins, to promote chromatin inaccessibility[3]. This unique property of FOXA proteins results from their structural similarity to linker histone proteins[9]. As such, FOXA proteins can interact with DNA between nucleosomes, displacing linker histones and thereby functioning as adaptor proteins between the compacted chromatin and additional transcription factors. Pioneer factors, such as FOXA proteins, limit the requirement for additional factors, allowing

Glossary

Aromatase inhibitors
Therapies used to treat oestrogen receptor$^+$ patients with breast cancer by decreasing circulating levels of oestrogenic compounds.

Bicalutamide
An anti-androgen used for the treatment of prostate cancer.

Luminal breast cancer
Oestrogen receptor$^+$ breast cancer originating from the luminal cells.

Pioneer factors
Proteins that physically interact with compacted chromatin, to rapidly facilitate the binding of additional proteins.

Tamoxifen
Anti-oestrogen drug used for the treatment of oestrogen receptor (ER)$^+$ patients with breast cancer. Tamoxifen binds to and blocks the ligand-binding pocket of ER.

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interactions between transcription factors and chromatin to occur quickly, facilitating rapid transcriptional responses[10].

FOXA1 and nuclear receptors

Coupling chromatin immunoprecipitation (ChIP) to global analytical methods permits the unbiased mapping of transcription factor binding (known as ChIP-on-chip or ChIP–seq). The first global maps of ER and AR binding in the presence of ligand unexpectedly revealed that both nuclear receptors regulate their gene targets from a distance[11–14]. An additional finding from the unbiased mapping approach was that the enrichment of DNA motifs represented the consensus-binding sequence for forkhead transcription factors[11], and the subsequent discovery that FOXA1 bound to approximately 50% of all ER-binding regions[11,15]. Importantly, FOXA1–chromatin interactions were not influenced by oestrogen treatment[15,16], implying that FOXA1 contributes to the preparation of optimal chromatin conditions before oestrogen-mediated recruitment of ER to the chromatin. In ER^+ breast cancer cells, FOXA1 was shown to be essential for almost all ER-binding events in the genome[11,17], including those that may involve indirect ER–chromatin interactions that are mediated by other factors, such as AP1 proteins. FOXA1 was also required for oestrogen-mediated gene expression[11] and for the proliferation of MCF-7 breast cancer cells[17]. The Zaret laboratory had previously described the concept of pioneer factors, showing that FOXA is a potent pioneer factor that is capable of independently associating with and modulating compacted chromatin[2,4]. The link between FOXA1 and ER confirmed that, in hormone-driven cancer cells, nuclear receptors also require pioneer factors, with FOXA1 functioning as an essential link between ER and condensed DNA at potential transcriptional regulatory elements. It should be noted that ER association with DNA is direct and that it is mediated, in most cases, through binding to a specific DNA sequence motif, the oestrogen responsive element (ERE). However, binding of ER to the ERE motif requires the presence of a pioneer factor to promote accessibility to these regions in the chromatin.

Similar results have been observed in prostate cancer cells, with FOXA1 sharing binding events with AR[18]. However, an important distinction exists between the role of FOXA1 with ER in breast cancer cells and its role with AR in prostate cancer cells, as has recently been discussed[19]. In breast cancer cells, specific silencing of FOXA1 results in the global inhibition of ER binding and transcriptional activity[11,16]. When FOXA1 is silenced in prostate cancer cells, AR-binding events are also decreased, but, in addition, numerous new AR-binding events are also acquired[20,21]. This suggests that FOXA1 can mediate some AR-binding events, but can also prevent additional binding events. As such, in prostate cancer cells, silencing of FOXA1 results in a reprogramming of AR and in an altered gene expression programme[20,21]. Similarly, whereas the requirement of FOXA1 for the growth of both wild-type (drug-responsive) and drug-resistant breast cancer cells is clear[16,17,22], its role in modulating prostate cancer cell proliferation is not clear. FOXA1 inhibition results in increased proliferation of wild-type LnCaP prostate cancer cells[20], but, by contrast, inhibition of FOXA1 in a castration-resistant prostate cancer cell line results in growth arrest[23]. Therefore, although FOXA1 is a pioneer factor for both ER and AR, there are differences in the role that FOXA1 has in regulating these two nuclear receptors in their respective cancers. Interestingly, FOXA1 is a well-established liver regulatory factor[24] and was recently shown to be essential for oestrogen- and androgen-mediated hepatocarcinogenesis[25], implying cooperative roles between FOXA1 and nuclear receptor pathways in liver cancer.

Table 1 | **Putative pioneer factors in hormone-dependent cancers**

Putative pioneer factor	Tissue specificity in cancer	Evidence for pioneer factor function
FOXA1	Breast and prostate	Binds to condensed chromatin *in vitro* and is required for ER and AR binding genome-wide
PBX1	Breast	Required for ER binding at tested loci
TLE factors	Breast	TLE1 is required for ER binding genome-wide and TLE3 modulates chromatin
AP2	Breast	Required for ER binding at tested loci
GATA4	Bone	Required for ER binding at tested loci
GATA2	Prostate	Required for AR binding at tested loci
PU.1	Bone marrow	Modulates linker histones and contributes to local histone modifications

AR, androgen receptor; ER, oestrogen receptor; FOXA1, forkhead box A1; TLE, transducin-like enhancer of split.

Additional pioneer factors

There has been interest in finding additional ER pioneer factors that may be important in breast cancer. Because 50% of ER-binding events in the MCF-7 breast cancer genome overlap with a FOXA1-binding event, it was hypothesized that other factors might be contributing as pioneer factors for the remaining ER–chromatin interactions. Several candidate proteins have been suggested, as discussed below.

TLE factors. As TLE factors had previously been shown to associate with compacted chromatin independently of other factors[3], a candidate approach was taken to assess TLE factors that might be used by ER. It was found that TLE1 is required for ER binding at many regions and that it is also essential for ER-mediated gene transcription and cell growth[26]. However, technical limitations restricted the ability to generate a genome-wide map of TLE1 binding, preventing comparative analyses with other factors.

AP2γ. Motifs for AP2 transcription factors are enriched within ER-binding motifs, and this led to the discovery that AP2γ (encoded by *TFAP2C*) is a putative pioneer factor for ER[27]. AP2γ was shown to be essential for almost 60% of all oestrogen-induced genes and is also required for a substantial proportion of repressed genes. AP2γ overlaps with approximately 50% of all ER-binding events in the MCF-7 genome[27], and when AP2γ was silenced, ER binding was diminished on a certain number of tested regions. Interestingly, AP2γ and FOXA1 were shown to be mutually dependent, with the loss of one factor resulting in the decreased expression of the other[27]. AP2γ is also required for the growth of breast cancer cells, although this may be due to its involvement in regulating the expression of *ESR1* (the gene encoding ER)[28].

PBX1. Recently, pre-B cell leukaemia transcription factor 1 (PBX1) was shown to be a putative pioneer factor[29], and it also overlaps with approximately 50% of all ER-binding events in the MCF-7 breast cancer genome. PBX1 is required for cell growth and for ER binding at a few tested regions[29]. It is currently unclear whether AP2γ or PBX1 can directly bind to condensed chromatin independently of other factors, but it is likely that they are bone fide nuclear receptor pioneer factors.

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It is currently not known how much overlap exists between FOXA1, AP2γ and PBX1, or to what degree specific ER-binding regions use one, many or none of the known pioneer factors. To resolve this, we integrated published genome-wide binding information for FOXA1, AP2γ and PBX1, and assessed the overlap between these factors at ER-binding regions within the MCF-7 breast cancer genome (FIG. 1). The greatest proportion (32.3%) of ER-binding events were those that were bound by all three proteins, followed by those bound by FOXA1 plus one of the other factors (30.7%) and those bound by FOXA1 alone (16.3%). Previous reports suggesting that 50% of all ER-binding events overlap with FOXA1-binding events[15,16] may have underestimated the proportion of ER–chromatin interactions that directly involve FOXA1. That said, it is likely that there is a large degree of cooperativity between individual pioneer factors.

In addition, GATA factors might also be involved in hormone-dependent cancer. GATA factors interact with condensed chromatin alone, and can moderately influence chromatin accessibility[4]. GATA3 has a role in modulating differentiation in a mouse model of luminal breast cancer[30], and GATA2 is required for AR binding and cell growth in prostate cancer cells[18].

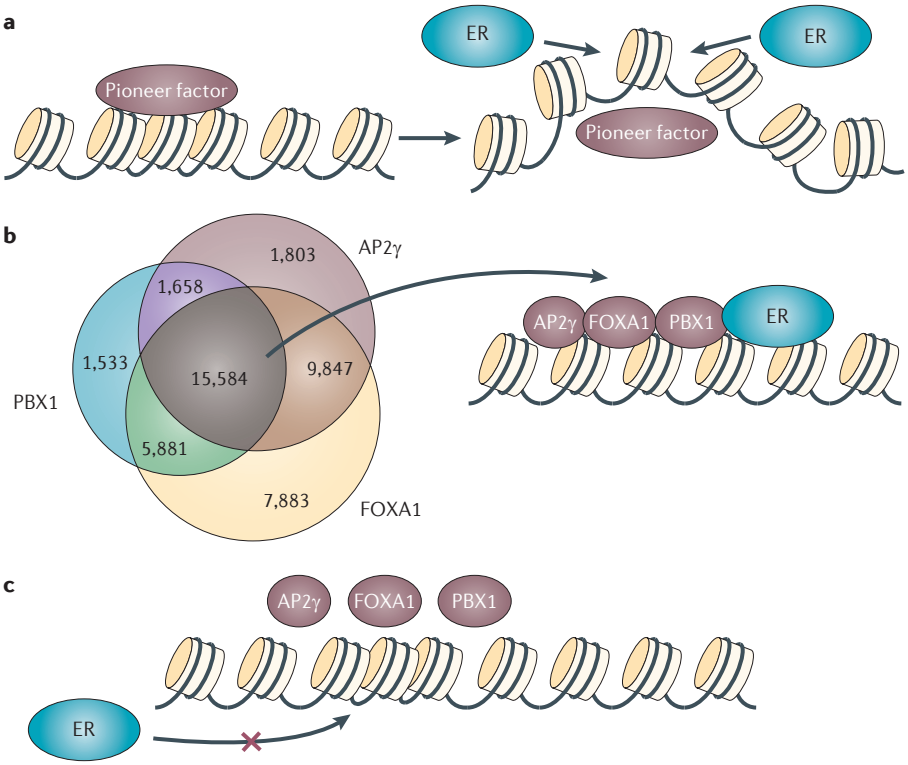

Figure 1 | **Mode of putative ER pioneer factor function. a** | Schematic representation showing the function of pioneer factors. Pioneer factors can directly associate with compacted chromatin on their own, where they provide increased accessibility for subsequent transcription factor recruitment to chromatin. Oestrogen receptor (ER) binding to DNA is mediated, in most cases, via the oestrogen responsive element (ERE) DNA motif, but accessibility to this motif requires pioneer factors to open the chromatin and facilitate ER–chromatin interactions. **b** | Global maps for forkhead box A1 (FOXA1), AP2γ and pre-B cell leukaemia transcription factor 1 (PBX1) have been generated in the same breast cancer cell line — MCF-7 cells[20,28,30]. Using these data, we identified all regions considered to be an ER-binding event in any of the three studies (a total of 48,182 ER-binding regions) and integrated FOXA1, AP2γ and PBX1 binding information within this superset of ER-binding regions. The results are shown in the Venn diagram, and the largest proportion of ER-binding events involved co-occupation by FOXA1, AP2γ and PBX1. **c** | In the absence of crucial pioneer factors, ER cannot associate with chromatin.

The regulation of pioneer factors

Given the evidence that pioneer factors have a role in hormone-dependent cancer progression, it is of paramount importance to understand what regulates the expression of the pioneer factors. Upon oestrogen treatment, FOXA1 protein levels are induced, but *FOXA1* mRNA levels are not appreciably increased[12], implying that a stabilization of the FOXA1 protein is required to increase FOXA1 levels. Recent evidence suggests that insulin-like growth factor 1 (IGFI) can stabilize FOXA1 without affecting mRNA levels[31]. Although the levels of FOXA1 may be differentially regulated depending on environmental and cell type-specific conditions, FOXA1 expression has consistently been found to be a defining feature of ER$^+$ breast cancer[32]. AP2γ is oestrogen-induced[33] through direct ER binding and transcription of *TFAP2C*. PBX1, conversely, seems to be moderately repressed by oestrogen[12,29], although the importance of this is currently unclear. Other than transcription regulation, numerous additional factors may influence levels and activity of pioneer factors, including protein stability, post-translational modifications, microRNA regulation of pioneer factor levels and mutations that alter their function. How relevant these mechanisms are for the modulation of pioneer factor levels and function remains to be clarified.

In addition, indirect parameters influence pioneer factor activity, including changes in chromatin features that either promote or inhibit pioneer factors. Epigenetic properties that influence pioneer factor binding include the methylation and hydroxymethylation of DNA, nucleosome positioning, histone modifications, chromatin looping and higher order chromatin re-organization[1]. It is well established that there are global changes in DNA methylation during tumour progression and, similarly, that the binding profiles of pioneer factors are also altered in hormone-resistant cancers[16,34]. Whether there is a link between these two events is unclear, but FOXA1 binding tends to occur at DNA hypomethylated regions[35]. Furthermore, FOXA1 binding is biased towards regions enriched in histone 3 lysine 4 monomethylation and dimethylation (H3K4me1 and H3K4me2, respectively)[15]. These variables help to explain why there are fewer pioneer factor-binding events than there are potential binding events on the basis of consensus-binding motifs.

Another aspect of pioneer factor modulation arises when considering different pioneer factors that function together, in which case an element of cooperation or equilibrium between pioneer factors ensues. One example is AP2γ and FOXA1, with both proteins stabilizing the binding of the other factor at ER-occupied sites[27]. As the greatest proportion of ER-binding events (32.3%) (FIG. 1) is co-bound by FOXA1, AP2γ and PBX1, it is possible that dominant master pioneer factors regulate other pioneer factors, creating a pioneer factor hierarchy.

Clinical potential of pioneer factors

Pioneer factors as biomarkers. It is clear that ER and AR require pioneer factors for binding to DNA in cell line models. Recent work

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has shown that ER binding is different in primary breast cancer samples from patients who have a good clinical outcome versus tumours from patients with a poor clinical outcome[34]. The differential ER-binding events consistently found in breast cancers that were likely to relapse were enriched for FOXA1 motifs. As such, FOXA1 is likely to be required for ER function even in a drug-resistant context, a finding that is supported by the co-expression of ER and FOXA1 in metastatic samples[34]. In fact, *FOXA1* is a defining gene for ER$^+$ luminal breast cancer[32] and may be a prerequisite for most ER$^+$ breast cancers[32], regardless of outcome. In breast cancer, histological analysis of FOXA1 suggests that its expression is predictive of a positive clinical outcome[36] and that it correlates with favourable prognostic end points[37]. Similar to breast cancer, FOXA1 is expressed in 89% of metastatic prostate cancers[38], suggesting that it may still be functional even in tumours that are refractory to anti-hormone therapies. However, increased FOXA1 levels in prostate cancer correlate with high grade, poor outcome tumours[21,38], a clear distinction from breast cancer.

AP2γ is oestrogen induced and is, therefore, decreased when cells are exposed to anti-oestrogens such as tamoxifen[33], but AP2γ levels remain high in tamoxifen-resistant breast cancer cells[39], suggesting that increased AP2γ levels may increase ER-mediated transcription in the presence of an ER antagonist, contributing to drug resistance. In support of this, high AP2γ protein levels have been shown to be prognostic of poor clinical outcome in patients with breast cancer[39]. Recent work suggests that *PBX1* mRNA levels can be used to discriminate the prognosis of a subset of patients with breast cancer, and future work will confirm whether histological analysis of PBX1 protein levels can be used as a prognostic marker to stratify patients with breast cancer.

It is unclear what the biological importance of increased pioneer factor levels mean for breast and prostate cancer progression. The simplest hypothesis is that increased levels of any pioneer factor in breast or prostate cancer enables increased ER- or AR-mediated transcription and greater proliferative potential, even in the presence of an antagonist ligand or, potentially, in the absence of ligand. This may explain why AP2γ[39] and PBX1 (REF. 29) predict poor outcome in patients with breast cancer and why FOXA1 correlates with poor outcome in prostate cancer[21,38]. An unresolved question is why FOXA1 predicts good outcome in patients with breast cancer[36], but is still expressed in most breast cancer metastases[34]. One possibility is that breast cancers retain ligand dependence during progression and metastasis, and tumours with high FOXA1 represent more ligand- and ER-dependent cancers that are hypersensitive to anti-oestrogens. Prostate cancer, conversely, may obtain ligand independence earlier during disease progression. In the metastatic context, however, both breast and prostate cancer acquire ligand independence, resulting in a nuclear receptor-driven complex that still requires its pioneer factor for effective transcription.

Pioneer factors as potential drug targets. Pioneer factors may constitute attractive therapeutic drug targets for cancer treatment (FIG. 2). It is known that FOXA1 is required for the growth of tamoxifen-resistant MCF-7 cells[16], as well as BT474 cells that are ER$^+$ and that contain amplified ERBB2 (also known as HER2 and neu)[22]. FOXA1 is also expressed in more than 90% of breast cancer metastases, regardless of the site of metastasis[34]. All of these data indicate that FOXA1 is a key nuclear receptor pioneer factor in hormone-resistant cancers. As the majority of metastases that arise from an ER$^+$ breast cancer retain ER and FOXA1 expression[34,40], a specific FOXA1 inhibitor would provide a useful clinical tool for the treatment of ER$^+$ hormone-resistant breast cancer. A precedent has been established for targeting FOX proteins, with the natural product thiostrepton binding directly to and inhibiting FOXM1 in breast cancer cells[41].

PBX1 and AP2γ are also attractive potential drug targets, although future work will need to show that they are expressed in metastases and that they are required for the growth of drug-resistant cell lines. If this can be shown, efforts should be made to generate inhibitors against these factors. In fact, existing therapeutic inhibitors that block homeobox (HOX)–PBX interactions have been shown to be effective in renal cancer cell lines[42] and melanoma[43], although it is not clear whether inhibitors that block the interface between HOX and PBX proteins will be effective in hormone-dependent cancers, in which PBX1 may have a HOX-independent role. Given the role of PBX1 in ER$^+$ breast cancer cells[29], and the observation that it is upregulated in castration-resistant prostate cancer cells[44], PBX1 inhibition may have clinical utility in hormone-dependent cancers.

One consideration when targeting pioneer factors is potential effects in other tissues that express these proteins. Although FOXA1 is an important factor in hormone-dependent cancers[16,45] and is expressed in most breast[34] and prostate cancer[38] metastases, it is also

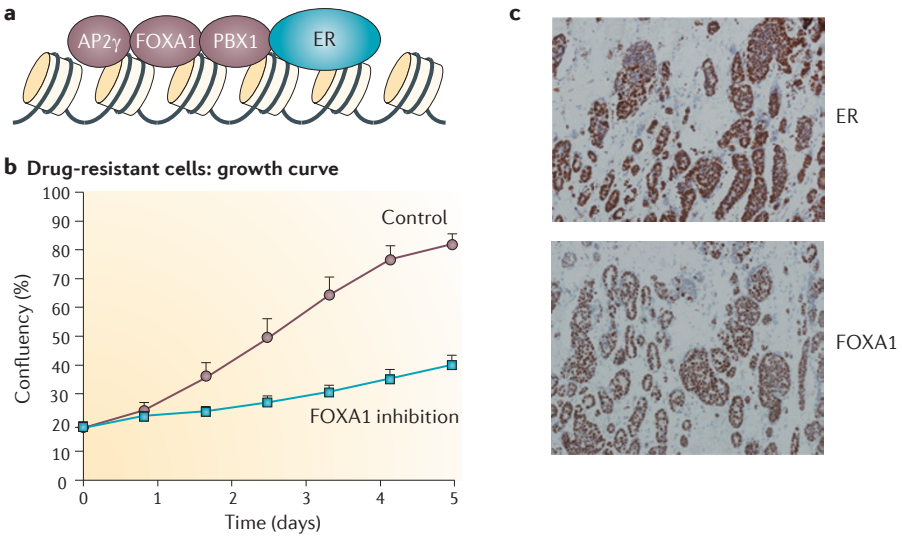

Figure 2 | **Pioneer factor properties that need to be met to constitute a therapeutic target in hormone-dependent cancer.** Crucial pieces of information are required before a pioneer factor can be considered as a potential clinical target. If a pioneer factor is to be pursued as a drug target, mechanistic insight is required to show that the putative pioneer factor can associate with condensed chromatin and that it is essential for global nuclear receptor–chromatin interactions (part **a**). Ideally, expression of the pioneer factor should be restricted to the target tissue of interest, such as the breast or prostate, and should not be a ubiquitous factor. Experimentally, the pioneer factor should be shown to be essential for tumour growth in models of drug-resistant cancer (part **b**). The pioneer factor should be expressed and functional in drug-resistant contexts, such as in metastases (part **c**). ER, oestrogen receptor; FOXA1, forkhead box A1. Part **c** is reproduced, with permission, from REF. 16 © (2011). Macmillan Publishers Ltd. All rights reserved.

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expressed in additional tissues such as the liver. Therefore, selective inhibitors against FOXA1 (or other pioneer factors) may affect FOXA1 expressed in other tissues, indicating the possibility of on target toxicity, such as that seen with tamoxifen. The potential for on target effects that arise from inhibiting pioneer factors will need to be carefully assessed.

Perspectives

Pioneer factors constitute a new level of nuclear receptor regulation, providing the foundation for ER–DNA and AR–DNA interactions. Several aspects of pioneer factor functioning need further exploration, including a better understanding of the interplay between known pioneer factors, a comprehensive identification of all ER and AR pioneer factors, and an appreciation of the heterogeneity within different cells of the same tumour and within different tumours of the same cancer subtype. Even among known breast cancer pioneer factors, there is a significant degree of cooperativity, redundancy and overlap (FIG. 1). The dependency between pioneer factors and the hierarchical interplay needs to be resolved, with particular emphasis on differences that occur in tumours with distinct treatment responses. Developments in imaging technologies make it possible to investigate detailed dynamics of transcription properties in living cells and, as such, it is possible to dissect the kinetics of interactions between different pioneer factors and their associated nuclear receptors by single-molecule approaches and in single-cell contexts. In parallel, systems biology approaches are required for integrating biological, physical and chemical information. In addition, mathematical modelling of pioneer factor and nuclear receptor-binding dynamics will augment understanding of these important cancer pathways. Exploring the detailed mechanisms of pioneer factor function and extrapolating this into cancer biology will prove beneficial for the diagnostic stratification of cancer patients and for the discovery of novel therapeutic targets for drug-resistant or hormone-refractory cancer.

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Competing interests statement

The authors declare no competing financial interests.

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