Breast Cancer RESEARCH

VIEWPOINT

More on FOX News: FOXA1 on the horizon of estrogen receptor function and endocrine response

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Abstract

Estrogen receptor α (ER) is a major driver of breast cancer and the target of endocrine therapy. Full disclosure of the cofactors regulating ER interactions with chromatin and its transcriptional regulatory activity is still elusive. Novel genome-wide profiling tools have mapped ER binding events in breast cancer cells and delineated cofactors important in ER activity. Among these, the Forkhead protein FOXA1 is emerging as a key factor dictating global chromatin structure and the transcriptional function of ER in breast and non-breast cancer cells. The significance of FOXA1 in the chromatin interactions and transcriptional regulation of both estrogen- and tamoxifen-bound ER, and in supporting tamoxifen-resistant cell growth, may impact current endocrine therapies.

Background

The estrogen receptor α (ER) protein is present in over two-thirds of breast cancers, where it functions in the nucleus as a ligand-dependent transcription factor to drive cell proliferation, survival, and invasiveness. Endocrine therapies to block ER activity are the most important systemic treatments for ER-positive breast cancers, though resistance is prevalent [1]. We need to understand the molecular determinants regulating ER DNA binding and activity to elucidate the mechanisms underlying this resistance.

The advancement of chromatin immunoprecipitation (ChIP)-based technologies, which combine ChIP with microarrays or high throughput sequencing (ChIP-seq), has helped to identify a network of co-regulators and their genome-wide DNA binding sites (known as their cistrome) that cooperate to regulate ER DNA binding and transcriptional activity. These technologies have revealed that, in breast cancer cells, ER mostly binds to distal enhancers that are also enriched for Forkhead motifs [2-4]. Furthermore, the Forkhead protein FOXA1, a favorable prognostic factor that correlates with the luminal A breast cancer subtype and hormonal sensitivity [5], has been shown to act as a pioneer factor, opening chromatin regions for the recruitment of ER to these DNA binding sites [6]. However, how global the importance of FOXA1 is in mediating ER function in breast cancer, as well as in other target tissues and under different ligand conditions, and what are the underlying factors that determine FOXA1 specificity remain open questions.

The article

To more broadly investigate the genome-wide relationship of ER and FOXA1 DNA-binding sites, Hurtado and colleagues [7] first performed ChIP-seq of ER and FOXA1 in three different breast cancer cell lines. FOXA1 binding events were found to be dynamic and cell-line-specific, a phenomenon potentially related to the insulator protein CTCF. Within each cell line, a significant overlap of over 50% was found between ER and FOXA1 sites. FOXA1 was also found to mediate ER function in non-breast cancer cells and to act upstream of ER-chromatin interactions, enabling ER binding at more condensed chromatin regions. Additionally, FOXA1 was required to globally facilitate ER-mediated transcription, since downregulation of FOXA1 affected the transcription of more than 95% of estrogen-regulated genes. Finally, FOXA1 knockdown resulted in significant growth inhibition of MCF7 cells, substantiating the key role of FOXA1 in the estrogen response of breast cancer cells.

To study the ER cistromic profile and the role of FOXA1 in mediating tamoxifen inhibition, estrogen-deprived MCF-7 cells treated with estrogen or tamoxifen were subjected to ER ChIP-seq and gene expression microarray analyses. In contrast to a previous report [8],

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the results demonstrated that tamoxifen induced ER binding events similar to those induced by estrogen. Additionally, estrogen and tamoxifen were found to regulate common genes. FOXA1 knockdown showed that tamoxifen-ER employs similar FOXA1-dependent mechanisms as estrogen to interact with chromatin. However, the experimental setting prevented direct assessment of whether FOXA1 is required for the tamoxifen antiproliferative effects in breast cancer cells. Of note, in tamoxifen-resistant derivatives of MCF-7 cells, chromatin binding profiles of both ER and FOXA1 dramatically differ from those of the wild-type cell line, and the binding occurred independently of tamoxifen treatment. However, ER and FOXA1 binding regions still significantly overlapped and, most importantly, ER chromatin binding and cell proliferation in the tamoxifen-resistant line still required FOXA1.

The viewpoint

Unbiased, genome-wide mapping and profiling of ER interaction with chromatin and its transcriptional regulation activity in breast cancer have recently been established by leading groups in this field [2-4,9,10] and have created a valuable resource to increase our basic understanding of estrogen/ER action and to improve therapeutic strategies. These studies have collectively demonstrated the fundamental role of FOXA1 in guiding and regulating ER chromatin binding events and gene transcription. While the recent report of Hurtado and colleagues [7] strongly substantiates these previous data and notions, its novel insights into the role of FOXA1 in both tamoxifen action and resistance, and into mechanistic aspects of FOXA1 action, are of clinical and biological significance.

The pivotal role of FOXA1 in ER-DNA interaction and transcriptional activity, as well as in the growth of wild type MCF7 cells, points to FOXA1 as a potential therapeutic target for opposing ER activity and tumor growth, either alone or in combination with additional endocrine targets. Conversely, since the tamoxifen-ER interaction with DNA, which is a key component of tamoxifen's inhibitory action on ER-dependent gene expression, was also found to depend tightly on FOXA1, one could argue that co-targeting of FOXA1 might antagonize, instead of enhance, the inhibitory capabilities of anti-estrogens such as tamoxifen. Notably, however, in tamoxifen-resistant cells, the extensively altered pattern of ER binding and resistant cell growth phenotype both required FOXA1, an observation pointing again to FOXA1 as a therapeutic candidate. Interestingly, Lupien and colleagues [11] recently showed that epidermal growth factor stimulation of MCF7 cells induces a ligand-independent distinct profile of ER binding sites that is still enriched for Forkhead binding sites. This growth factor (GF)-specific ER cistrome dictates a unique transcriptional program that correlates with gene expression signatures found in poor-outcome as well as HER2-positive breast cancers [11]. Similarly, a unique ER cistrome and ER transcriptional activation were also documented upon AKT activation [12], and others have shown that blocking this pathway with a PI3K inhibitor can restore the 'classic' pattern of E2-induced gene expression [13]. Acquired tamoxifen resistance of MCF7 cells and xenografts is also associated with increased GF/HER1/2 signaling and a distinct expression profile that correlates with growth factor gene expression signatures [14,15]. As aberrant expression/activation of GF pathways is commonly associated with endocrine resistance [1,15], the data cumulatively imply that the differential ER chromatin binding and subsequently altered transcriptional program that derive from hyperactive GF signaling are fundamental underlying mechanisms of endocrine resistance. The relative contributions to this global molecular switch of ER-chromatin interactions and gene expression provided by (1) non-genomic ER function, (2) additional Forkhead family members, (3) other chromatin remodeling factors and/or ER coregulators, (4) post-translational modification of these key factors, and (5) additional epigenetic modifications are still open questions. However, the critical roles of FOXA1 and ER in this process, as shown by Hurtado and colleagues [7], indeed suggest that co-targeting of FOXA1 together with more potent ER inhibitors or degraders [11] might represent an improved strategy to circumvent endocrine resistance in breast cancer.

Abbreviations

ChIP, chromatin immunoprecipitation; ER, estrogen receptor α; GF, growth factor.

Competing interests

The authors declare that they have no competing interests.

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