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Breast Cancer RESEARCH

VIEWPOINT

To bind or not to bind - FoxA1 determines estrogen receptor action in breast cancer progression

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Abstract

Chromatin immunoprecipitation followed by massively parallel sequencing (ChIP-seq) is rapidly enabling the comprehensive characterization of genome-wide transcription factor-binding sites, thus defining the cistrome (cis-acting DNA targets of a trans-acting factor). Estrogen receptor (ER) ChIP-seq studies have been performed mainly in cell lines, but Ross-Innes and colleagues have now completed the first such study in clinical breast cancer samples. The study aimed at determining the dynamics of ER binding and differences between more and less aggressive primary breast tumors and metastases. The authors found that ER bound to DNA in both aggressive and drug-resistant tumors but to different sites and with different affinities. Given previous findings from cell lines, FoxA1 appears to play a critical role in this reprogramming of ER binding.

Background

Patients with breast cancer that express estrogen receptor-alpha (ERα$^+$) are candidates for endocrine therapies. Although endocrine therapies are among the most successful targeted therapies in oncology, a significant subset of ER$^+$ breast cancers have become resistant to them. The activation of growth factor receptor (GFR) pathways has been identified as a possible culprit, and although ER is rarely mutated in endocrine-resistant tumors, it can be aberrantly activated by GFR signaling in a ligand-independent manner [1].

Over the last few years, the application of chromatin immunoprecipitation (ChIP) coupled with massively parallel sequencing (ChIP-seq) enabled the identification of the ER cistrome in breast cancer cells [2]. By showing the following, the results brought an end to the dogma that ER binds primarily to the proximal promoters: (a) ER frequently binds distal enhancers [3], (b) the forkhead protein FoxA1 is necessary for ER-chromatin interactions [3-6], and (c) activation of GFR signaling results in the redirection of ER binding [7]. However, all previous studies, though highly informative, were performed in cell lines (primarily MCF-7 cells). Obtaining ChIP-seq results from primary breast tumors was the next step that everyone was eagerly awaiting.

The article

Ross-Innes and colleagues [8] analyzed ER ChIP-seq data from 15 ER$^+$ tumors (eight with a good prognosis and seven with a poor prognosis) and three distant metastases. The authors found a core set of 484 ER-binding events present in at least 75% of all ER$^+$ tumors (but not in the ER$^-$ controls). Intriguingly, ER-binding signal intensity was highest in metastatic samples and lowest in patients with good outcomes, suggesting that binding intensity may correspond to disease progression. Differential binding analysis found 1,192 ER-binding events that were stronger in the poor prognosis/metastasis group in comparison with the good outcome samples and found 599 binding events more prevalent in the good outcome tumors. Motif analysis revealed the presence of estrogen response element (ERE) sites in all three groups but a unique enrichment of FoxA1 sites in the poor prognosis/metastatic tumors. An important finding was that ER still bound DNA in tumors resistant to hormonal therapies but was recruited to novel sites in the genome. These sites are functionally and biologically relevant since a gene expression predictor based on genes within a 20-kb window of the binding sites was associated with survival in ER$^+$ data sets.

Treatment of breast cancer cells (whose ER cistrome closely overlaps with that of poor prognosis/metastatic tumors, presumably because the cell lines were isolated from metastases) with a mitogenic cocktail also resulted in rapid enrichment of novel ER-binding sites.

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Intriguingly, half of these binding sites occurred in regions to which FoxA1 was already bound or to which FoxA1 was recruited in response to mitogenic stimulus. High and correlated expression of ER and FoxA1 in metastatic samples further supports the idea that FoxA1 might direct the reprogramming of ER binding in advanced disease.

Viewpoint

This study presents an important and exciting milestone in our efforts to understand the plasticity of ER function in breast tumors. Although the numbers of samples are small, the data strongly suggest that differential ER binding is associated with the outcome of patients with breast cancer. Increasing the number of samples will allow the analysis of poor outcome tumors separately from metastases, a critical question given the lack of knowledge about the role of ER in metastasis.

An interesting finding of the study is the observation that the average ER-binding signal was highest in the metastatic samples. Additional studies are necessary to determine whether this is a general phenomenon and, if so, the underlying mechanisms. This could include altered levels or post-translational modification of ER (or both) or altered interaction of ER with co-regulator proteins. Alternatively, this may be simply a result of increased tumor cellularity or decreased tumor cell heterogeneity in metastatic samples or both. In any case, this is a curious finding and deserves further study.

For us, however, the most interesting observation is that reprogramming of ER binding seems to be associated with co-recruitment of FoxA1 and frequently with recruitment to sites pre-bound by FoxA1. Although this study does not include FoxA1 ChIP-seq data from clinical samples, the *in vitro* data point toward a unique mechanism of FoxA1 binding to 'pre-marked' DNA in poor outcome tumors, and this may have critical clinical relevance. The further use of frozen tumor specimens for ChIP-seq studies and the use of paraffin-embedded samples as recently described by Fanelli and colleagues [9,10] will undoubtedly shed more light on these questions.

Abbreviations

ChIP, chromatin immunoprecipitation; ChIP-seq, chromatin immunoprecipitation coupled with massively parallel sequencing; ER, estrogen receptor; GFR, growth factor receptor.

Competing interests

The authors declare that they have no competing interests.

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