Research Highlight

# Paradoxical roles for FOXA1 in anti-estrogen resistance and as a luminal differentiation factor in breast cancer

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The authors of a recent paper published in *Nature* [Ross-Innes *et al*. (1)], examined the correlation between the pattern of estrogen receptor-α (ERα) chromatin binding in breast cancers that were resistant to endocrine therapy compared with patients that had a good outcome. The sample populations were derived from eight ER+/progesterone receptor (PR)+/HER2- breast cancers that were clinically confirmed as a good prognosis and seven primary breast cancers from patients with a confirmed poor outcome that were either ER+/PR-/HER2- or ER+/PR+/HER2+.

Using the ChIP-seq technique, ER chromatin binding was confirmed in all tumors demonstrating that ER binding was not lost in the poor outcome samples. Importantly the numbers of same core ER bound regulatory regions was increased in metastases relative to both the poor and good prognosis primary tumor groups and the genes adjacent to the 484 core ER binding events were expressed in most ER+ but not ER- breast cancers in nine different microarray databases.

Clearly the differential activation of specific target genes would be expected to play a role in clinical outcome. To investigate this, the authors performed a differential binding analysis and identified over 1,000 genomic regions in poor outcome samples and about half that number in the good outcome group that demonstrated significantly more ER binding when compared to one another. The forkhead box transcription factor, FOXA1, functions as a "pioneer transcription factor" that opens chromatin to permit transcription. FOXA1 can inhibit both epithelial to mesenchymal transformation (EMT) associated with metastasis and cell growth by regulating E-cadherin (2,3). Together with GATA3 and the ER, FOXA1 contributes to the pattern of gene transcription that establishes luminal cell differentiation (4-6). Expression of FOXA1 correlates with ER positivity (3,7) it is also associated with good prognosis tamoxifen-sensitive Luminal A subtype breast cancers (8-10). Importantly, FOXA1 and has recently been shown to maintain expression of luminal while repressing basal cell transcripts (11). Ross-Innes *et al*. report that estrogen responsive regions clustering in the poor outcome samples also contained FOXA1 sequence motifs suggesting that this class of regulatory region was favoured in tumors that would demonstrate endocrine resistance. These tumors also possessed a genetic signature consistent with luminal B which is the more aggressive of the luminal subtypes. Based on the genes associated with ER binding regions, the group generated a gene expression predictor set for good and poor outcome tumors and applied this to a larger cohort of ER+ tumors. Using metastasis-free survival as the endpoint the gene sets predicted outcome with the strongest significance in the poor outcome group.

Growth factor-mediated ligand-independent activation of the ER is implicated in endocrine resistance. The group treated MCF-7 cells with a mixture of peptide growth factors and chromatin binding was again assessed by ChIP-seq. In this analysis differential binding indicated that more than half of ER binding events induced by growth factors in these experiments involve regulatory elements with a FOXA1 motif. Lastly, the group found that nearly all metastases retained ER expression and FOXA1. They suggest that this dynamic change in ER binding results from rapid "reprogramming" of ER binding mediated by FOXA1 and conclude from these experiments that breast tumors

therefore do not harbour a small selectable population of endocrine-resistant cells. Nevertheless, *in vivo*, such reprogramming would require the acquisition of growth factor signaling which could be either inherent or selected.

Overall, these elegant experiments clearly demonstrate that although ERα target genes in resistant tumors overlap with those in sensitive tumors there exists a separate subset of genes that are differentially and constitutively activated in resistant cells which are associated with FOXA1 DNA elements. The finding that these ER binding events are stimulated by growth factor signaling in vitro suggests that these gene sets are likely to represent the ligand-independent ER targets. The authors suggest that possible explanations for this phenomenon include different expression of cofactors, epigenetic alterations and possible (posttranslational) changes in FOXA1 structure and function. While these are all possibilities, based on the evidence from the experiments with MCF-7 cells, first and foremost a poor outcome ER+ tumor would be predicted to possess an established growth factor signaling pathway(s) resulting from amplification or mutation of endogenous receptors or fed by autocrine or possibly paracrine growth factor mechanisms. Downstream signaling from receptor activation could then modify either FOXA1 or ERα or both to favour interaction with the poor prognosis gene set. Growth factor receptor activation and non-canonical ER signaling can both activate AP-1 response elements (which were also enriched in the resistant tumors). It has long been established that antiestrogens can induce AP-1 elements through the ERβ (12). However, evidence from immunohistochemical analyses suggest that low levels of ERβ expression are associated with tamoxifen resistance (13). Since ERα and ERβ can function as heterodimers, this finding combined with those of the Carroll group could indicate that ERα homodimers preferentially activate genes associated with FOXA1 whereas ERα:ERβ heterodimers may tend to associate with GATA-type promoters.

Phosphorylation of the ER is modulated by growth factor signaling. Interestingly, phosphorylation patterns on the ERα including phospho-T311 have been also shown to predict poor prognosis (14). On the basis of the current findings, it would be intriguing to determine if phospho-T311 or a cohort of poor prognosis phospho-ERα proteins are differentially recruited to FOXA1/ERE motif genes.

While some ER+ tumors that are well defined by PR and ErbB2 status do not respond at all to tamoxifen, about 25% of good prognosis tumors initially respond but then appear to acquire resistance. These tumors may be fundamentally different from the de novo resistant poor prognosis group and acquired resistance may indeed be the result of the selection of a low frequency cancer stem cell or a minority population within the heterogeneous tumor that has the differential ability to increase autocrine growth factor production or has an altered expression ratio of ERα to ERβ. So a good prognosis tumor would regress but then be replaced by the emergence of a poor prognosis tumor following endocrine treatment.

From the clinical standpoint, the findings in this manuscript will likely not impact prognostication, however in the long run, further analysis may provide novel targets for therapies based on the identified subset of primary response genes differentially expressed in poor prognosis breast cancers. However, given the potential importance of FOXA1 in maintaining the less aggressive luminal phenotype, targeting FOXA1 may not be the way to go.

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## References

1. Ross-Innes CS, Stark R, Teschendorff AE, et al. Differential oestrogen receptor binding is associated with clinical outcome in breast cancer. Nature 2012;481:389-93.
2. Carroll JS, Brown M. Estrogen receptor target gene: an evolving concept. Mol Endocrinol 2006;20:1707-14.
3. Wolf I, Bose S, Williamson EA, et al. FOXA1: Growth inhibitor and a favorable prognostic factor in human breast cancer. Int J Cancer 2007;120:1013-22.
4. Kouros-Mehr H, Slorach EM, Sternlicht MD, et al. GATA-3 maintains the differentiation of the luminal cell fate in the mammary gland. Cell 2006;127:1041-55.
5. Asselin-Labat ML, Sutherland KD, Barker H, et al. Gata-3 is an essential regulator of mammary-gland morphogenesis and luminal-cell differentiation. Nat Cell Biol 2007;9:201-9.
6. Ademuyiwa FO, Thorat MA, Jain RK, et al. Expression of Forkhead-box protein A1, a marker of luminal A type breast cancer, parallels low Oncotype DX 21-gene recurrence scores. Mod Pathol 2010;23:270-5.
7. West M, Blanchette C, Dressman H, et al. Predicting the clinical status of human breast cancer by using gene expression profiles. Proc Natl Acad Sci U S A 2001;98:11462-7.
8. Thorat MA, Marchio C, Morimiya A, et al. Forkhead box

 

A1 expression in breast cancer is associated with luminal subtype and good prognosis. J Clin Pathol 2008;61:327-32.
9. Badve S, Turbin D, Thorat MA, et al. FOXA1 expression in breast cancer--correlation with luminal subtype A and survival. Clin Cancer Res 2007;13:4415-21.
10. Mehta RJ, Jain RK, Leung S, et al. FOXA1 is an independent prognostic marker for ER-positive breast cancer. Breast Cancer Res Treat 2012;131:881-90.
11. Bernardo GM, Bebek G, Ginther CL, et al. FOXA1 represses the molecular phenotype of basal breast cancer cells. Oncogene 2012. [Epub ahead of print].
12. Paech K, Webb P, Kuiper GG, et al. Differential ligand activation of estrogen receptors ERalpha and ERbeta at AP1 sites. Science 1997;277:1508-10.
13. Hopp TA, Weiss HL, Parra IS, et al. Low levels of estrogen receptor beta protein predict resistance to tamoxifen therapy in breast cancer. Clin Cancer Res 2004;10:7490-9.
14. Skliris GP, Nugent ZJ, Rowan BG, et al. A phosphorylation code for oestrogen receptor-alpha predicts clinical outcome to endocrine therapy in breast cancer. Endocr Relat Cancer 2010;17:589-97.

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