



Nuclear Receptor Transcription

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We are interested in defining the genomic and proteomic features of oestrogen receptor (ER)-mediated transcription in breast cancer cells. We are specifically interested in understanding how ER activity can cause cancer cells to grow and the basis for responses to drugs, such as selective oestrogen receptor modulators (SERMs).

Introduction

Oestrogen receptor is the defining feature of luminal breast cancers, where it functions as a transcription factor to induce cell cycle progression. One of the most successful anti-cancer treatments to date is tamoxifen, a selective oestrogen receptor modulator. Tamoxifen is a cheap and effective therapy in ER positive breast cancer, which makes up 75% of all breast cancers. However, about a third of women who take tamoxifen will either fail to initially respond to tamoxifen or will develop resistance to tamoxifen over time. Newer ER antagonists are becoming available, including aromatase inhibitors (AI), that function by blocking oestrogen production in post-menopausal women. However AIs are more expensive and have inherent problems including increased arthralgia (joint pain) and bone fractures; furthermore resistance to AIs can occur. Also, AIs are not effective in premenopausal breast cancers, which make up 25% of all breast cancers. As such, there are specific subsets of patients that can benefit from the different types of ER antagonists. Understanding the molecular basis of tamoxifen and AI function and resistance is critical for the development of future therapies.

ER transcriptional activity requires a number of co-factors and co-operating transcription factors that possess enzymatic activity that alters chromatin structure, the outcome of which determines transcriptional activity. It is currently

known that a number of ER co-factors can either assist in transcription (including SRC-1 and AIB-1) or are involved in gene repression by tamoxifen (including N-CoR and SMRT). It is becoming clear that ER co-factors may play a role in tamoxifen resistance.

Recently, using chromatin immunoprecipitation (ChIP) combined with microarrays (termed ChIP-on-chip) we mapped all ER binding sites in a breast cancer cell model after oestrogen treatment and found a number of surprising features about ER biology. These included the observation that of the approximately 3,500 ER binding sites across the human genome, ER rarely regulates genes from promoter regions, but instead utilises distal enhancers. We also identified the role of a 'pioneer factor' called FoxA1, which is critical for ER to function. Our lab is interested in extending on these findings to fully define the cis- and trans-elements that contribute to ER activity in breast cancer cells, with particular emphasis on the factors involved in tamoxifen-mediated growth arrest.

Characterisation of mechanisms of tamoxifen action

Our analysis of the ER binding sites revealed by ChIP-on-chip suggested that motifs for the paired box (Pax) transcription factors were enriched within the ER binding sites. A previous paper had suggested that Pax2 was a tamoxifen induced gene in endometrial cancer cells and that Pax2 was a critical determinant of tamoxifen action in endometrial tissue. We found that Pax2 was recruited with ER to chromatin, but surprisingly, this only occurred with tamoxifen, suggesting that in breast cancer cells, Pax2 was a repressor of activity. We subsequently showed that ErbB2/HER-2 could be repressed by tamoxifen using a cis-regulatory element located within an intron of the ErbB2 gene. A role for Pax2 was shown as a critical repressor of ErbB2 transcription. We found that Pax2 repression of ErbB2 could be inhibited by expression of AIB-1 and that competition between Pax2 and AIB-1 determined ErbB2 expression and tamoxifen sensitivity. We showed that Pax2 expression was lower in tamoxifen resistant breast cancer cells, and that expression of Pax2 could re-sensitise the resistant cells to tamoxifen. In effect, we could reverse

[†]Left during 2008

[‡]with Duncan Odom

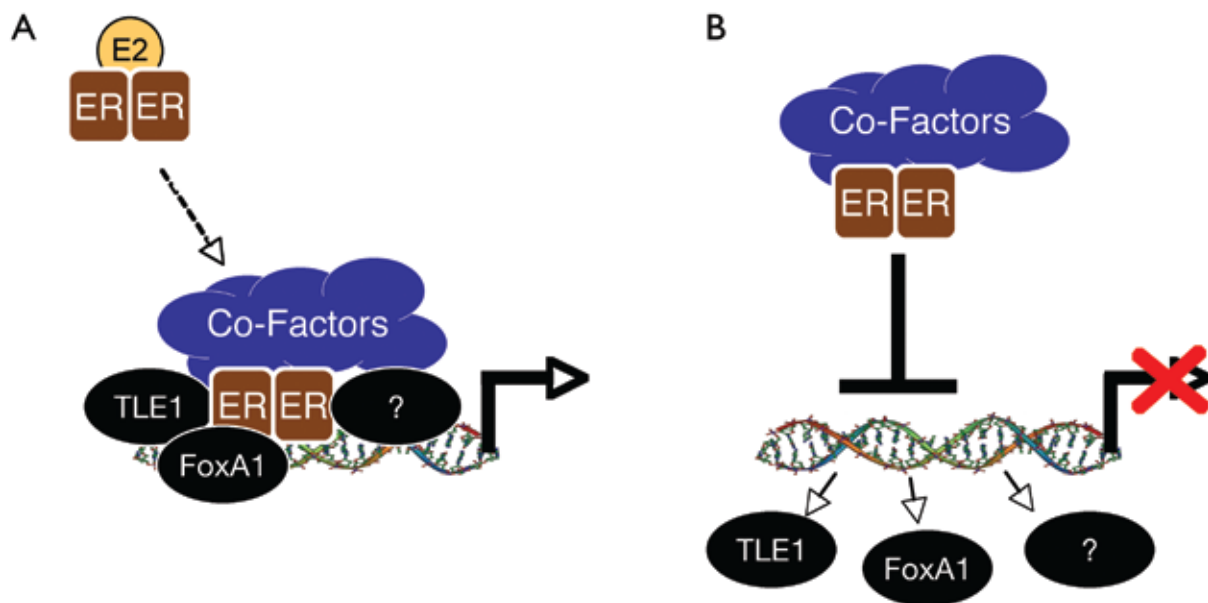


Figure 1. Schematic of pioneer factor function. The pioneer factors stabilise the ER-co-factor complex on the chromatin, permitting gene transcription. If ER requires FoxA1 and/or TLE1 as a pioneer factor to interact with the chromatin (a), mechanisms to block or break this interaction (b) may provide a means to inhibit ER function in tumours that have acquired endocrine resistance and do not respond to treatments.

the tamoxifen resistance in these previously resistant cell lines. We also showed that Pax2 expression was a good predictor of tamoxifen response; high levels of Pax2 protein levels correlated with better outcome in patients. This confirms a direct transcriptional link between the ER and ErbB2 pathways, in which ER, when liganded with tamoxifen, can directly repress transcription of the ErbB2 gene. It appears that this is an essential event for the action of tamoxifen.

Characterisation of the role of pioneer factors in ER biology

We are interested in identifying and characterising the role of pioneer factors in ER activity. We previously showed that FoxA1 was an ER pioneer factor that was required for ER to maintain DNA interactions. As such, we believe that pioneer factors may constitute a new level of ER transcriptional regulation and one that provides the opportunity for blocking ER function. If pioneer factors can be blocked or inhibited, this will inhibit ER-DNA interactions. This is significant given the data that suggest that breast cancers which acquire resistance to endocrine therapies still have functional ER pathways. This implies that these resistant tumours have devised a method of activating transcription even in the presence of an anticancer therapy. These re-activated transcriptional pathways could potentially be blocked by inhibiting ER from interacting with the chromatin (Figure 1).

Previous investigations have shown that the Groucho proteins have properties of pioneer factors, namely that they can bind to heterochromatin independently of other proteins. We have found that Groucho 1/TLE1 is involved in ER biology as a putative pioneer factor. We can show that TLE1 binds

to a number of ER binding sites and that it is important for gene transcription of oestrogen target genes and for oestrogen-mediated cell proliferation. Furthermore, TLE1 appears to predict outcome in patients with breast cancer, although it is specific to ER positive breast cancers. We are currently exploring whether TLE1 is an ER pioneer factor by specifically silencing TLE1 using siRNA and performing genome-wide mapping of ER binding sites using ER chromatin immunoprecipitation in combination with ultra high throughput Solexa sequencing.

These genomic approaches, coupled with focused analyses of important cancer related gene targets, provide a comprehensive analysis of the properties of ER biology.

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