Holding the balance; the equilibrium between ERα-activation, epigenetic alterations and chromatin integrity

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Thesis Outline

General introduction

In this thesis we reflect on the effects differential DNA binding of the estrogen receptor α (ERα) can have on the behavior of breast cancer and which factors can contribute to this. Approximately 70% of all breast tumors are derived from the inner lining of cells in the mammary ducts (also known as luminal tumors) and their proliferation is dependent on the activity of (ERα). During the development and homeostasis of the female reproductive organs ERα plays a key role. In ERα-positive breast tumors however, ERα has a causative role in carcinogenesis (1). ERα can be activated by its natural ligand estradiol (2), a steroidal estrogen, which can induce the formation of an activated ERα-dimer. After a conformational change (3), this dimer "opens up" the co-activator-binding pocket (4) resulting in the recruitment of essential co-factors (5) leading to the assembly of the ERα–transcriptional complex.

One of the mainstay endocrine treatment options for ERα-positive breast cancer patients is tamoxifen (6-8), which is an ERα antagonist and competitively inhibits the interaction of ERα and estrogen, thereby repressing ERα activity (9-11). Alternatively aromatase inhibitors can be used to block the synthesis of estrogen, rendering ERα inactive. Despite these therapeutic options, still a significant proportion of patients develop a recurrence. Although cross-resistance between the different therapeutic options does occur, a proportion of patients that relapse on one type of therapy can still benefit from a different treatment modality. This illustrates the existence of multiple resistance mechanisms which can be treatment selective. A better understanding of ERα-biology and the development of drug resistance, cannot only provide us with novel mechanistic insights, but could also lead the way to the discovery of novel biomarkers and potential drug targets, which can further increase patient survival.

Thesis outline

Chapter 1 provides a general overview of ERα-biology and more specifically the mechanistic insights the genome-wide interrogation of ERα-chromatin binding has brought us. Furthermore we discuss where future developments might take us.

Chapter 2 describes a well-known mechanism of tamoxifen-resistance; the PKA-induced phosphorylation of ERα at Serine residue 305 (ERαS305-P). We report on the implications this phosphorylation has on the binding repertoire of ERα and describe the discovery of an altered gene expression profile capable of predicting the outcome of patients treated with tamoxifen.

In **Chapter 3** we provide experimental evidence for a previously unknown ERα-phosphorylation (T594P) and demonstrate how this greatly diminished ERα's binding capacity. Additionally we show that by stabilizing this phosphorylation by administering fusicoccin, ERα-mediated gene transcription was reduced and tumor cell growth was inhibited.

In **Chapter 4** we report on the fact that ERα-function can also be altered by other nuclear receptors and demonstrate that LRH-1 knockdown led to an altered gene expression profile. Additionally we revealed that there is a large overlap between chromatin binding sites of LRH-1 and ERα, and that at these overlapping regions a synergistic stimulation is present.

In **Chapter 5** we computationally refined a 111-gene classifier towards a single gene classifier, revealing that FEN1 levels are predictive of outcome in ERα-positive patients treated with tamoxifen. We describe our findings on the complex regulatory interplay between FEN1 and ERα, and postulate three manners by which FEN1 may modulate ERα activity. Additionally we performed a drug screen which led to the discovery of a FEN1-specific and potent inhibitor. We demonstrate a clear sensitivity of ERα-positive breast cancer cell lines to this inhibitor when compared to ERα-negative cell lines, and an even greater sensitivity of tamoxifen-resistant cell lines. The latter suggesting FEN1 inhibition might be a useful novel therapeutic option in the case of tamoxifen resistant breast.

Chapter 6 contains our findings on the phosphorylation of essential ERα-cofactor SRC3 (SRC3-pS543). This phosphorylation leads to an altered deposition of SRC3 at the chromatin, making it very likely to also alter the gene expression of breast cancer and thereby its phenotype. Furthermore a SRC3-pS543 phospho-specific antibody was capable of identifying patients

with a functional ERα pathway, rendering it a promising novel biomarker for tamoxifen efficacy.

In **Chapter 7** I discuss what the implications of our findings are with regards to a better understanding of ERα-biology and describe the new questions this may invoke. Additionally I discuss how the novel biomarkers described in this thesis could aid in patient stratification and what type of research is still required to successfully implement such a biomarker. With regards to the potential novel drug targets we identified, I describe what the current clinical value of these drug targets would be and what type of evidence still lacks.

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