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

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English summary

What is this Thesis about?

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. ERα is a transcription factor than can drive tumor cell proliferation and approximately 70% off all breast tumors is thought to be dependent on the activity of this hormone-mediated transcription factor. After stimulation of ERα a wild variety of co-factors are recruited, leading to the assembly of a transcriptional complex. Although there are multiple ways of targeting the action of ERα and thereby inhibiting tumor growth, still a significant proportion of patients develop a recurrence. Cross-resistance between the different endocrine therapy options can occur, but a proportion of patients that relapse on one type of therapy can still benefit from a different treatment modality, illustrating the existence of multiple resistance mechanisms which can be treatment selective. A better understanding of ERα-biology and the development of endocrine therapy resistance, can lead the way to the discovery of novel biomarkers and potential drug targets, that can further increase patient survival.

Endocrine resistance prediction

In **Chapter 2** we demonstrated that tamoxifen-resistance by PKA-induced phosphorylation of ERα at Serine residue 305 (ERαS305-P), positions ERα at different sites of the genome, resulting in differential gene expression. We were able to translate this altered gene expression profile into a gene signature that was able to predict the outcome of patients treated with tamoxifen. Besides this gene profile, we also describe the discovery of two single gene classifiers; FEN1 and SRC3-pS543. As described in **Chapter 5**, we found that ERα-coregulator FEN1 correlated with patient outcome in ERα-positive, but not ERα-negative patients. More importantly, FEN1 levels were predictive of outcome in ERα-positive patients receiving adjuvant tamoxifen. Additionally **Chapter 6** describes our findings on a SRC3-pS543 phospho-specific antibody which was able to identify patients with a functional ERα pathway, which is indicative of a favorable outcome in the absence of adjuvant therapy and therefore a lack of tamoxifen efficacy. All together we elaborate on our findings of three types of biomarkers with the potential to predict the treatment response of a patients on an individual bases. These biomarkers could aid in the process of decision-making on endocrine treatment regimens when

first-in-line treatment tamoxifen is not likely to result in a benefit for the patient, by directly administrating an alternative treatment.

Differential ERα-chromatin interactions

As briefly described above, post-translational modifications of ERα, such as phosphorylations, can modify its activity and can redirect ERα to different places on the DNA, thereby altering its binding repertoire (also known as cistrome). This differential cistrome can have a major impact on the genes that are transcribed and the resulting phenotypic behavior of a cell. Besides the above mentioned ERαS305-P, we provided experimental evidence in **Chapter 3** for a previously unknown ERα-phosphorylation (T594P) and demonstrate that by direct interaction of 14-3-3 proteins with ERα, its DNA binding capacity is greatly diminished. By shielding the T594 phosphorylation site with fusicoccin (FC) we were able to stabilize and induce this T594P, ultimately resulting in decreased gene transcription and inhibited cell growth.

Besides ERα itself, the phosphorylation of essential ERα-cofactors can also redirect the cistromic repertoire, as described in **Chapter 6**. Herein we demonstrated that S543-phosphorylation of SRC3 resulted in an increased deposition at promoter regions, while normally SRC3 is predominately found together with ERα at distal enhancers and introns. This changed cistromic profile, together with the above described findings that SRC3-pS543 expression was associated with a poor response to tamoxifen treatment, makes it very likely that pS543 can also alter the gene expression of breast cancer and thereby ultimately its phenotype.

Novel drug targets

Minimization of predictive gene-profiles can not only make a classifier more easily implementable in the clinic, but can also be used to identify novel drug targets by eluting the driving genes in a predictive profile. In **Chapter 5** we demonstrated an example of this in the discovery of FEN1 as a crucial ERα-coregulator by minimization of a 111-genes profile. We showed that altering FEN1 protein levels by knockdown or overexpression results in differential ERα-activity, implying FEN1 might be a promising drug target in ERα-positive breast cancer. We performed a small-compound screen for FEN1 inhibition, which ultimately led to the discovery of a FEN1-specific and potent inhibitor. We investigated its potential as novel therapeutic option by assessing its efficacy in breast cancer cell lines, demonstrating clear sensitivity of ERα-positive breast cancer cell lines to this inhibitor when compared to ERα-negative cell lines. The even greater sensitivity of tamoxifen-resistant

derivatives of the ERα-positive cell lines, suggests FEN1 inhibition might be useful in tamoxifen resistant breast cancer patients as an alternative therapy. Although promising, at the moment it is still too early to state definitively whether FEN1 inhibition would be a realistic therapeutic option on its own.

Additionally the stabilization of ERα-T594P by FC we describe in **Chapter 3**, might also yield a novel therapeutic option. We demonstrated that FC administration leads to diminished transactivation of ERα and subsequently resulted in inhibition of cell proliferation. At the moment however, the relatively low binding affinity of FC might hinder further pre-clinical development, making further research into identifying fusicoccanes with an higher binding affinity necessary in order to further pursue its therapeutic options.

Novel mechanistic insights

As discussed in **Chapter 1**, ERα-function is not only influenced by its coregulators, but also by other nuclear receptors. In **Chapter 4** we demonstrate an example of this in the case of liver receptor homolog-1 (LRH-1). Knockdown of LRH-1 levels led to a subset of 222 differentially expressed genes, known for their estrogen responsiveness. We revealed that there is a large overlap between the chromatin interactions of LRH-1 and ERα. At these shared regions both receptors stimulated each other's recruitment, leading to increased recruitment of ERα co-regulators and altered gene expression. To date, the exact mechanism behind this synergistic stimulation remains unknown.

An additional novel insight in ERα-biology is described in **Chapter 5**, where we provide evidence that FEN1 is an ERα co-regulator capable of modulating ERα-activity in multiple ways. 1) Mapping the methylome (the parts of the DNA that contain methylation modifications) after ERα-stimulation with and without FEN1-inhibitor was suggestive of a key role for FEN1 in active DNA demethylation, thereby alleviating some local epigenetic repression. 2) Inhibition of FEN1 resulted in reduced recruitment of chromatin remodeling factor BRG1 to sites of ERα-chromatin interactions, thereby decreasing the activating nature of ERα-induced chromatin remodeling. 3) Inhibition of the proteasome prevented the FEN1 inhibition-induced reduction of ERα-chromatin interactions. This suggests that FEN1 can stabilize ERα-chromatin interactions by preventing the proteasome-mediated degradation of ERα.