The effects of breast cancer therapy on estrogen receptor signaling throughout the body

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Cover: Marjolein Droog. The cover depicts the effects of tamoxifen on the Estrogen Receptor alpha cistrome in endometrial tumors. The use of the color black is a tribute to the “Zwartlabbers”, the members of the Zwart lab.

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Preface: Thesis outline

For over a century, estrogen hormones have been studied for their effects in both healthy and pathological contexts. Healthy physiological processes include breast maturation, ovulation, and endometrial thickening, whereas pathogenic phenomena include breast cancer and endometrial cancer. Estrogens affect these tissues by targeting a steroid hormone receptor that serves as a transcription factor: Estrogen receptor (ER)α.

The bloodstream carries estrogens to various tissues, where they may diffuse through cell membranes and physically bind to ERα, thereby activating it. Upon activation, ERα binds the chromatin. The regions that ERα binds are generally already made accessible by the action of other proteins, named pioneer factors. After ERα has bound the chromatin, it recruits a transcriptional complex to regulate gene expression, which induces cell proliferation.

To block cell proliferation in ERα-positive breast cancer, clinicians aim to target this pathway using hormonal therapies. Although these therapies benefit many breast cancer patients, they can cause adverse side-effects. In addition, some women lack the desired response and relapse, despite treatment. To determine strategies that optimize treatment requires an understanding of the molecular mechanisms that tamoxifen affects.

Thesis outline

This thesis focuses on a chemical agent, named tamoxifen, that intervenes with the ERα pathway and is therefore often used to treat breast cancer. We specifically focus on tamoxifen's most alarming side effect: increased endometrial cancer risk (chapters 1-3), and also on new prognostic biomarkers for breast cancer in relation to tamoxifen-response (chapter 4-5). In chapter 6, I discuss the results of our studies.

Chapter 1 reviews literature on the effects of estrogen and tamoxifen in several tissues such as breast, endometrium, and bone. We focus on the DNA binding sites (cistrome) of ERα in specific cells of these tissues. This chapter further discusses how different ligands, and also ligand-independent conformational changes of ERα, may affect the ERα cistrome.

Chapter 2 reports differential binding sites of ERα in endometrial tumors between women who used tamoxifen to treat their breast cancer, versus women who never received tamoxifen to treat their breast cancer. These differential binding sites associate with differential gene expression between these two groups. Strikingly, we also found that tamoxifen-associated endometrial tumors, more than endometrial tumors of non-users, resembled breast cancers in regard to the ERα cistrome.

In **Chapter 3**, we compared the ERα cistrome between tamoxifen-associated endometrial tumors to breast tumors, and identified a common

pioneer factor: forkheadbox protein A1 (FOXA1). We showed that cross-talk between ERα and FOXA1 is greatly preserved between these tissues. In addition, we investigated the difference in interval time, of tamoxifen-users versus non-users, between breast cancer and endometrial cancer. Only tamoxifen-users displayed a longer interval time between breast cancer and endometrial cancer, if their endometrial tumor lacked both ERα and FOXA1.

Chapter 4 reveals that activating transcription factor 2 (ATF-2) plays a key role in tamoxifen-response in breast cancer as knockdown of ATF-2 reduced tamoxifen's inhibitory effects on cell proliferation. We further show that tamoxifen phosphorylates ATF-2, and that phosphorylation of ATF-2 at its amino acid Threonine-71 predicts for improved outcome for ERα-positive breast cancer patients who receive tamoxifen.

Despite hormonal treatment, there are patients whose breast tumors recur. In **chapter 5**, we therefore review literature on components of the ERα-transcription complex, as well as components of growth factor receptor signaling pathways, to highlight their potential to serve as biomarkers in hormonal therapy response. With this, we aim to bridge the gap between the molecular scientist and the clinical scientist.

Finally, in **chapter 6**, I discuss the new insights that our work has provided and the new questions that this thesis invokes.