The handle http://hdl.handle.net/1887/49509 holds various files of this Leiden University dissertation

Author: Droog, Marjolein
Title: The effects of breast cancer therapy on estrogen receptor signaling throughout the body
Issue Date: 2017-06-08
Chapter 6

General Discussion
Why do the majority of breast cancer patients use hormone targeted therapy?

Each year, breast cancer affects the health of 1.67 million women, and causes over half a million deaths. In Caucasian populations, roughly 75% of breast cancers express the steroid hormone receptor Estrogen Receptor (ER)α. Physicians treat these women with agents that target this receptor. Although multiple endocrine treatments exist, this thesis focuses on tamoxifen, which is one of the most applied therapies that targets ERα.

In breast cancer cells, hormones activate ERα, which then binds the chromatin and directs gene transcription to enable cell proliferation. Tamoxifen competitively inhibits the hormone-induced estrogen receptor pathway in breast cells. But some tissues that express ERα respond differently to this endocrine intervention, as I described in chapter 1 of this thesis.

Physicians aim to stop breast cancer cells from growing while keeping healthy cells intact. Many breast cancer patients benefit from tamoxifen-treatment as it prevents recurrence. Furthermore, it benefits postmenopausal women by increasing bone density, protecting against cardiovascular disease, and improving immunosuppressive functions in regard to autoimmunity.

What are the adverse side effects of tamoxifen?

Tamoxifen benefits many breast cancer patients but it has side-effects, such as hot flushes, and increased uterine in postmenopausal patients. The most alarming side-effect however, is the association with increased endometrial cancer risk in postmenopausal women.

Endometrial cancer is the sixth most common cancer in women worldwide, with 320.000 new cases diagnosed each year and 76.000 deaths. The 5-year survival-rate, as determined by tumor-stage at time of diagnosis, ranges from 90%-15%. In breast cancer patients, the risk of developing endometrial cancer is 2-7 fold increased by tamoxifen, depending on duration of treatment.

In a study that pooled case-patient data from three large case-control studies, 25% of 1104 deaths in 3750 tamoxifen-associated endometrial cancer patients died due to endometrial cancer. Initial histological studies reported a good prognosis for patients with a tamoxifen-associated endometrial tumor. More recent studies however, show tamoxifen-associated endometrial tumors are more often of the morphologically less favorable subtypes than endometrial cancers of non-users.
What do we know on the development of tamoxifen-associated endometrial cancer in postmenopausal breast cancer patients?

Tamoxifen has an agonistic effect on endometrial cell proliferation, similar to estrogen\textsuperscript{21-23}, but it remains elusive whether this relates to the development of endometrial cancer, and if so, by what mechanism. The increased endometrial cancer risk upon tamoxifen exposure could be due to an imbalance between apoptosis and proliferation, epigenetic disruption that alters gene regulation, or due to mutational dis-regulation of tumor suppressor genes and oncogenes. Besides the ER\textsubscript{α}, tamoxifen also interacts with other proteins, such as protein kinase C, calmodulin, and c-myc\textsuperscript{32-34}. In the first chapters\textsuperscript{1-3} of this thesis we focused on ER\textsubscript{α} as a target of tamoxifen, but later in chapter 4 we also reveal data on the effects of tamoxifen that are not directly related to ER\textsubscript{α}.

Throughout our work we were careful to use the term ‘tamoxifen-associated endometrial cancer’ as not to imply that tumors were induced by tamoxifen. However likely it is that most of them were indeed caused due to tamoxifen use, there might have been patients in our studies that would have developed an endometrial tumor irrespective of treatment\textsuperscript{35}.

To understand the molecular mechanism of tamoxifen-associated endometrial cancer requires studies that focus on long-term effects of tamoxifen exposure. What makes it hard to uncover the cause of tamoxifen-associated endometrial cancer is the lack of models. Past studies have looked at tamoxifen’s effects in the endometrial cancer cell line Ishikawa\textsuperscript{36} or in murine models\textsuperscript{37,38}. Although, these models show the effects of tamoxifen on the endometrium, they are unusable to investigate tamoxifen-associated endometrial cancer because they developed without tamoxifen exposure.

From a molecular point-of-view, we need a model that mimics the development tamoxifen-associated endometrial tumors if we want to understand it better. This means we need a model in which endometrial cancer developed upon prolonged exposure to tamoxifen. These exist only in patients.

A pre-treatment endometrial model (healthy) and a post-treatment endometrial model (cancerous) from the same individual would be ideal because then we can compare changes in tamoxifen-related targets. A pre-treatment model from an individual that will develop endometrial cancer is impossible to obtain however, because we cannot predict yet who these few patients are. But the post-treatment models are available, and described in this thesis on the TAMARISK study (a study in which breast cancer patients, half of whom received tamoxifen, developed an endometrial cancer)\textsuperscript{29,39}. 
Chapter 6

Does tamoxifen affect the ERα cistrome of the endometrium?

In chapter 2 we reveal that the ERα cistrome differs between endometrial cancers that developed in postmenopausal women who were on tamoxifen to treat their breast cancer compared with postmenopausal breast cancer patients who never received tamoxifen. Why ERα sites differ between endometrial tumors of tamoxifen-users versus non-users remains unknown. Two possibilities exist: 1, the ERα cistrome was already like that before tamoxifen-treatment due to heterogeneity in active enhancers; or 2, Tamoxifen shifts the ERα cistrome upon tamoxifen treatment in the endometrium.

If the ERα cistrome is similar before and after tamoxifen treatment, it would be likely that the cofactors changed, causing the gene transcription to be altered. In breast cancer it is well known that cofactors of ERα at the chromatin change upon different stimulations (40) due to a conformational change in ERα’s helix1241,42.

In the well-known breast cancer cell line MCF-7, hormone-deprived cells stimulated with tamoxifen started to proliferate again after several months43. Compared to the original MCF-7 cells, the ERα cistrome had shifted44. The exact reason for the shift of ERα binding sites remains elusive: Was it due to tamoxifen? Or due to the lack of other hormones? This could be determined by comparing the ERα cistrome of these tamoxifen-resistant MCF-7 cells with the ERα cistrome in existing so called Long Term Estrogen Deprived MCF-7 cells, as these cells are solely hormone-deprived. An answer to this question might indicate if tamoxifen is capable to shift the ERα cistrome.

We know from literature that the progesterone receptor can redirect ERα to other binding sites when both are activated by their ligands45. And although blocking the ERα pathway inhibits the expression of the progesterone receptor, other nuclear receptors might have similar capacities to bind ERα upon ligand-activation. Possibly, by depleting the cell’s growth medium of all hormones, interaction partners no longer lead ERα to the binding sites it occupied in the original MCF-7 cells.

In MCF-7 cells that were hormone depleted and treated with tamoxifen for six months, it is unknown when the ERα cistrome shifted. Perhaps this already occurred after days, but the mechanism through which the cells started proliferating again, and became tamoxifen-resistant, simply took months46. If the ERα cistrome shifted months before the cells became tamoxifen resistant, this might indicate a non-hormonal pathway is inducing cell proliferation. To answer this question requires more measurements at different time points.
What did we learn from ERα comparative cistromics between tamoxifen-associated endometrial tumors and breast tumors?

ERα binds mostly enhancer elements at the DNA, both in breast cancer and in endometrial cancer. Literature describes enhancer-usage to be tissue-specific\(^47\). Our data in chapter 2 and 3 shows enhancer-usage by ERα in tamoxifen-associated endometrial cancer and breast cancer resemble each other. Perhaps the enhancer elements in breast cancer and tamoxifen-associated endometrial cancer are more similar because hormones influence their cellular’s epigenetic program. Vice versa, it is possible that specific enhancers with poised ERα binding sites, which are used upon hormonal stimulation, make these patients more susceptible to develop endometrial cancer.

In chapter 3 we show that endometrial tumor cells express FOXA1, serving the classical ERα-pioneer factor role as was originally identified in breast cancer\(^15\). In contrast to breast cancer however, where FOXA1 facilitates tamoxifen to block the ERα pathway, FOXA1 enables tamoxifen’s stimulatory potential in the endometrium of postmenopausal women. This makes FOXA1 an interesting drug target because disabling its function blocks the ERα pathway in both breast and endometrial tissues while keeping other ERα-positive tissues, that lack FOXA1, unaffected.

Various molecular studies revealed crosstalk between the ERα pathway and mitogen-activating signaling pathways in breast cancer cells\(^48\text{-}50\). When simultaneously treated with estradiol, mitogens remodeled ERα’s cistrome because it regulated binding of forkhead box protein A1 (FOXA1) at the chromatin, which subsequently changed gene expression. These data highlight the potential for signals other than (anti)hormones to influence ERα’s cistrome through FOXA1. These signals might differ between breast cancer and endometrial cancer and therefore explain tamoxifen’s opposite influence on these tissues.

How can prognostic biomarkers improve breast cancer treatment?

Many postmenopausal women with ERα-positive breast cancer receive tamoxifen, but not everyone will benefit from the treatment. Finding out who will benefit from tamoxifen, and who will not, is an important step in tailor made cancer therapy. Tamoxifen has been published to inhibit cell proliferation in ERα-negative breast cancer cells\(^51\), and is described to be associated with the c-Jun N-terminal Kinase (JNK)\(^52\) and p38 cascade\(^53\), involved in the induction of apoptosis. Thus, tamoxifen affects signaling cascades other than just the ERα pathway.
In chapter 4 we show that knockdown of ATF-2 decreases the inhibitory effect of tamoxifen on cell proliferation in MCF-7 cells, indicating it has a key role in tamoxifen-treatment of breast cancer. We further revealed that the levels of phosphorylation at the activating domain of ATF-2 increased upon tamoxifen treatment (which associated with increased phosphorylation levels of both p38, JNK, and ERK). Any crosstalk with the ERα pathway however, remains elusive. Does tamoxifen for instance induce phosphorylation of ATF-2 within ERα-negative cells?

Interestingly, we also show that phosphorylation of ATF-2 at Thr71 predicts for improved outcome for ERα -positive breast cancer patients who receive tamoxifen. These data suggest that phosphorylation of ATF-2, which is required for transcriptional activation\textsuperscript{54,55}, by tamoxifen is essential for tamoxifen’s ability to block breast tumor growth. Hence, we reveal a little bit of the molecular mechanism that tamoxifen affects.

Clinical researchers are up against an accumulating mass of molecular tested biomarkers from which they can only test so many in cohort studies. Although many women benefit from tamoxifen, a subset of women are over-treated, whereas others are wrongly treated and require a different strategy\textsuperscript{56}. Deciding which biomarkers are worth testing in the clinic is an important step as described in chapter 5.

**What can we conclude from this thesis?**

Although the benefits of tamoxifen in the treatment of breast cancer far outweighs its adverse effects such as endometrial cancer\textsuperscript{24}, biomolecular markers could prevent or at least diminish the risk. In this thesis we have tackled several issues on the effects of tamoxifen: (1) Breast cancer therapy in the form of tamoxifen affects ERα throughout the body, not just in breast tumors; (2) To identify strategies that lower tamoxifen-associated endometrial cancer risk, requires an understanding of the molecular mechanisms that tamoxifen affects; (3) It is important to distinguish who will benefit from a drug such as tamoxifen so that clinicians may treat each person for the tumor that they have.
References


