Endocrine resistance in breast cancer: gene expression profiling and modifications of the estrogen receptor
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Summary

Endocrine treatment has had a groundbreaking contribution to the decline in breast cancer mortality. The estrogen receptor (ER) is expressed in approximately 75% of human breast cancers. Tamoxifen, a Selective ER Modulator (SERM) and one of the first targeted therapies, is part of the clinical management of breast cancer for more than 30 years and has been life saving for many breast cancer patients worldwide. However, still 30% of ER-positive breast cancer patients have no benefit from tamoxifen and endocrine resistance is still a major problem in the treatment of breast cancer in daily clinical practice.

In order to avoid breast cancer death for an individual patient, the choice of adjuvant systemic treatment is crucial. In view of the recent data indicating that postmenopausal patients have most benefit from sequential use of tamoxifen and an aromatase inhibitor (AI) and that in premenopausal patients tamoxifen and an AI (in combination with an LHRH-agonist) are as effective, the current clinical dilemma is to decide whether a patient should start with tamoxifen or an AI (Chapter 1).

Although tamoxifen resistance has been studied in in vitro models and in xenografts in mice for many years, this has not resulted in the clinical implementation of biomarkers that allow the prediction of tamoxifen resistance in ER-positive breast cancer patients. This thesis describes the discovery and validation of biomarkers that may predict response to tamoxifen or outcome in ER-positive breast cancer patients. Basically two approaches are described: first, gene expression profiles of ER-positive breast tumors are determined, and second, modifications of the estrogen receptor are detected.

High throughput analysis of gene expression of breast cancer has increased the insights in ER signaling (reviewed in Chapter 2). Expression of ER and its numerous downstream targets are driving patterns of gene expression and dominate unsupervised analyses of microarray data in breast cancer specimens studied to date. Regarding the genes responding to activation of ER, several lists of either putative ER targets or genes correlating with ER expression have been published. However, currently there is no consensus on the comprehensiveness of these gene sets. While the description of breast cancer phenotypes in distinct molecular subtypes, as first portrayed by Perou and colleagues, has been exciting, further refinement of subdivision of ER-positive breast cancer is needed. Recently, an 81-gene signature, a 21-gene assay (Recurrence Score) and a two-gene-index have been discovered. These genomic tests predict outcome after tamoxifen treatment. In chapter 3 we show that the concordance among these classifiers is relatively low. They classified only 45-61% of the patients in the same category. After adjustment for ER and the progesterone receptor (PR) only the 81-gene signature (hazard ratio (HR) 2.62, p=0.002) and the 21-gene assay (HR 1.94, p=0.048) were significantly associated with outcome after tamoxifen treatment in metastatic breast cancer patients.
Randomized clinical trials such as MINDACT that comprehensively collect frozen material and are designed to correlate gene expression profiles to outcome after endocrine therapy will be crucial to determine the exact predictive value of the gene expression profiles.

The gene signatures described in chapter 3 were initially discovered by selecting genes directly through their association with survival. In contrast, in chapter 4 we started with a specific biological hypothesis. The ‘cancer stem cell’ (CSC) hypothesis states that tumors are initiated and maintained by a small fraction of quiescent, self-renewing cells. Using mammospheres, cancer cells can be enriched for highly tumorigenic cells. In order to get insight into the characteristics of putative CSCs of ER-positive breast tumors, we cultured ER-positive breast cancer cell lines as mammospheres in vitro. Immunohistochemistry and gene expression profiling revealed a significant reduction in the expression of PR, proliferation and cell cycle regulated genes in mammospheres when compared to parental cell lines. Our results suggest that tumor-initiating breast cancer cells grown in mammospheres reside in a quiescent state. Next, we correlated the gene expression profile of ER-positive breast tumors to the gene expression profiles of our mammospheres. Breast tumors with a gene profile similar to mammospheres consistently displayed pathological and molecular features of favorable outcome such as low grade, quiescent Wound Response Signature and good prognosis 70-gene signature. Consequently, ER-positive breast cancers with expression profiles similar to those of mammospheres have a better outcome, providing evidence in support of the concept that outcome of patients with ER-positive disease is for a large part determined by cell cycle and proliferation activity.

Prognostic gene-signatures that maybe helpful in identifying those patients who need adjuvant systemic treatment are urgently needed in the clinic, but it is unclear how information provided by these molecular tests has to be integrated with the conventional predictive markers that are already part of routine clinical practice. In Chapter 5 we present a possible integration of the 70-gene prognosis profile and ER and PR immunohistochemistry. ER and PR were evaluated following St. Gallen Consensus 2007 (Highly Endocrine Responsive: ER and PR ≥ 50%, Incompletely Endocrine Responsive: ER and/or PR low or either one absent). In patients treated with adjuvant tamoxifen, both the 70-gene signature (adjusted for Endocrine Response Categories HR 2.17, 95%CI 1.01-4.66) as well as the Endocrine Response Categories (adjusted for 70-gene signature HR 6.35, 95%CI 1.90-21.3) were associated with breast-cancer-specific-survival (BCSS), suggesting that the 70-gene signature provides additional information on top of the routinely used ER and PR on outcome after tamoxifen for ER-positive breast cancer.
Using biophysical techniques it has been shown that phosphorylation of the ER induces a conformational arrest of the receptor. Using Fluorescence Resonance Energy Transfer (FRET), a phosphorylation of the ER at serine 305 (ER305-P) has been linked to tamoxifen resistance in vitro. Chapter 6 describes the first clinical evaluation of ER305-P using immunohistochemistry in breast carcinomas from premenopausal participants of a randomized trial (n = 248). We showed that adjuvant tamoxifen improved recurrence-free survival (RFS) for ER305-P-negative tumors (multivariate HR = 0.5, p = 0.010), but not for ER305-P-positive tumors (multivariate HR = 1.01, p = 0.99) (interaction p = 0.131). Notably, ER305-P was not significantly associated with RFS in patients not treated with tamoxifen (multivariate HR = 0.64, p = 0.248), indicating that ER305-P is a marker for treatment outcome rather than tumor progression.

Activation of protein kinase A (PKA) results in phosphorylation of ER at serine 305. In addition, ER305-P has been associated with p21-activated kinase (PAK1) expression. In Chapter 7 we show that there is not a significant correlation between PAK1 and ER305-P expression. PKA positivity did, however, correlate with ER305-P positivity, implying a functional relationship between these proteins. More importantly, the combination of the three markers, detecting tamoxifen resistance via two distinct pathways, enabled us to detect a substantial fraction of patients likely to fail to respond to tamoxifen treatment. Tamoxifen effects on RFS differed significantly (interaction, p=0.037) in subgroups defined by the algorithm including PAK1/PKA/ER305-P. Besides ongoing validation of our findings using tumor material of approximately 1000 patients who participated in another randomized trial of adjuvant tamoxifen versus no endocrine therapy, the predictive value of the PAK1/PKA/ER305-P-algorithm will be tested in a prospective trial comparing various endocrine strategies in premenopausal patients with early breast cancer.

The ER can also be phosphorylated at serine 118 (ER118-P). In contrast to ER305-P, ER118-P seems to be required for a proper ER function. Chapter 8 shows that ER118-P can predict tamoxifen response. The predictive value of ER118-P in relation to RFS after tamoxifen treatment was evaluated in premenopausal breast cancer patients who were included in a randomized trial (n=239). Patients with high tumor levels of ER118-P benefited from adjuvant tamoxifen (HR=0.36, 95%CI 0.20-0.65, p=0.001) while patients with low tumor levels of ERS118-P did not (HR=0.87, 95%CI 0.51-1.48). In multivariate analysis, the interaction between ER118-P and treatment was significant (p=0.037). Exploratory analyses suggested that treatment guided by ER118-P might save unnecessary treatment for half of the ER-positive premenopausal breast cancer patients, while approximately maintaining the 10-year RFS.
Besides characteristics of the tumor cells, response to tamoxifen can be influenced by genetic variations in the germline DNA of the patient. Genetic variations in enzymes involved in the metabolism of estrogens or tamoxifen could affect the tumor response to tamoxifen. Tamoxifen is metabolized by cytochrome-P450 (CYP) enzymes. Chapter 9 describes a retrospective multicenter study analyzing genomic DNA of 499 primary breast tumor specimens of patients treated with tamoxifen as first-line therapy for advanced disease. Our data showed a longer time to treatment failure (TTF) for CYP2C19*2 carriers, encoding lower enzymatic activity, compared to non-carriers (HR 0.72, p=0.004). Recently, CYP2D6*4 and CYP2C19*17 genotypes were reported associated with therapy outcome of adjuvant tamoxifen for breast cancer. However, in our study, neither the ultra-active CYP2C19*17 nor the inactive CYP2D6*4 allele were associated with TTF. Demographic factors as well as differences in patient selection (adjuvant setting versus advanced disease) may have contributed to the conflicting results. However, till now the relation between CYP450 genotypes and efficacy of endocrine treatment has not been explored in trials directly designed to study this important question.