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Hypothesis driven gene expression profiling in breast cancer

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Chapter 1

Introduction

Introduction

Breast cancer is the most common form of cancer among women in Western Europe and North America reaching a lifetime incidence of 1 in 9. In the Netherlands 12.495 women were diagnosed with breast cancer in 2006 and 3350 died of this disease (1). Much progress has been made in optimizing local and systemic treatment. Local treatment consisted of radical mastectomy in the 1st half of the 20th century; then gradually shifted to modified radical mastectomy; and in the last 20-30 years to breast conserving therapy for two thirds of all breast cancer patients (2;3). A less radical local treatment leads to reduced morbidity and better cosmetic outcome for patients. Quality of local treatment, measured by the rate of local recurrences after therapy, has improved dramatically over the last 25 years. Especially after breast conserving therapy local recurrence rates have dropped from 1-1.5% per year to 0.5% per year (4;5). All treatment modalities have contributed to the decrease in incidence of local recurrences. Surgeons, together with pathologists, have focused on the margins status, e.g. tumor involvement of the surgical resection margin. By making sure the tumor is excised radically, e.g. no tumor cells are left at the resection margin, the local recurrence rate has decreased. Also, better imaging techniques, including preoperative breast MRI (6;7), enable better localization of the tumor before surgery. After surgery, whole breast radiation therapy completes the breast conserving treatment. Whole breast radiation reduces the risk of a local recurrence by 50-60% (4). The next step in further reducing the risk of local recurrence came from (radiation) dose escalation. The EORTC conducted a large phase III randomized trial showing that an additional dose of 16 Gy to the post surgery tumor site, further reduced the risk of a local recurrence by 50% (8). Other improvements in radiation treatment included better treatment planning and tumor site localization. Lastly, the wide use of adjuvant systemic treatment (hormonal treatment, chemotherapy and ((HER2-)targeted therapy) also reduces the risk of local recurrence significantly (9;10). The reduction of local recurrences has been shown to impact survival in the long term. At 15 years, for every four local recurrences that have been prevented, one additional patient is cured from breast cancer (4).

Larger improvements in overall survival have been established by the wide spread use of adjuvant systemic therapy, first hormonal treatment and chemotherapy (11;12) and more recently targeted therapy (most notably directed against the HER2 protein) (13). Up to the early nineties only patients with metastasis to the loco-regional lymph nodes (lymph node positive disease) were considered to be eligible for adjuvant systemic treatment. Emerging evidence from large clinical trials also showed a small but significant benefit for patients with lymph node negative disease (12). At the present time, the majority of patients undergo adjuvant systemic treatment with hormonal therapy, chemotherapy, targeted therapy or a combination. However, the absolute survival benefit from adjuvant systemic treatment for lymph node negative patients is roughly 5 to 10 %, which translates into an overtreatment rate of 90 to 95% (12). E.g. to cure 5 patients from breast cancer, 100 patients are treated with adjuvant systemic therapy, while 95 patients are exposed to the short and long term side effects of systemic treatment without benefiting. These side effects include the rare but life

threatening risk of leukemia and chronic heart failure (14-16). Numerous guidelines have been developed for adjuvant systemic treatment for breast cancer, e.g. NIH, St. Gallen, NPI (Nottingham Prognostic Index) (17-20). These guidelines are based on a combination of clinical and pathological risk factors that have been shown to have an impact on prognosis (risk of disease recurrence and death due to breast cancer). Established prognostic factors are patient's age, tumor size, tumor grade and lymph node status (21). A commonly used tool is the online program Adjuvant! Online (22;23). Based on clinical and pathological risk factors, the program calculates the 10 year probability of disease specific and overall survival and estimates the absolute risk reduction that can be achieved with adjuvant systemic treatment.

In addition to the improvement of therapies per se, an important gain in treatment outcome is to be expected from better selection of patients for specific therapies. This includes radiation therapy, especially identifying patients that might not need whole breast radiation because their baseline risk for developing a local recurrence is very low and identifying patients that benefit from a higher dose of radiation, e.g. for whom the additional risk of site effects of radiation treatment is offset by the expected additional reduction in local recurrence rate when offered a higher dose of radiation.

To guide systemic treatment in individual patients, the first step is to assess prognosis; what is the risk that this patient would develop a local or distant disease recurrence. The second step is to assess predictive factors: for local treatment the question is what the radiation sensitivity of the tumor is, based on which the appropriate dose needs to be adapted; and, if indicated, what adjuvant systemic therapy should be given.

While prognostic factors determine the risk of developing distant metastases, predictive factors are needed to determine the responsiveness to systemic therapy. A limited number of predictive factors to guide adjuvant systemic therapy is currently available. The hormone receptor status of a tumor, ER (estrogen receptor) and PR (progesterone receptor) is assessed routinely and dictates whether or not a patient should undergo adjuvant hormonal treatment (e.g. tamoxifen, anastrozole, letrozole, exemestane). It should be kept in mind that not all ER positive tumors will respond to adjuvant hormonal therapy, as it has been shown that the predictive value of a positive estrogen receptor status for response to hormonal treatment in the metastatic setting is only 21-32% (24;25). The HER2 status is a predictive marker for response to HER2-targeted therapy; currently trastuzumab (Herceptin[®]) is approved for treatment of HER2-positive breast cancer patients in both the adjuvant and metastatic setting (in combination with chemotherapy) (26-28) and after trastuzumab failure, lapatinib (Tykerb[®]) is approved in the metastatic setting (29). For patients whose tumors overexpress HER2, targeted therapy is routinely combined with chemotherapy. For patients whose tumors express ER and/ or PR, hormonal therapy can be given as sole therapy or chemotherapy can be given first, followed by hormonal therapy.

Patients presenting with a so-called "triple-negative" breast cancer, i.e. a tumor lacking expression of ER, PR and HER2, can only be treated with chemotherapy. The designation of tumors as triple-negative refers to the lack of expression of three established predictive markers, this does not indicate that these patient form a homogeneous group.

A number of investigators have attempted to identify predictive factors for the response to various chemotherapy regimens (30-34); however, at present, no validated predictive test is available to guide chemotherapy selection for an individual patient. Two tests (OncotypeDX[®] and MammaPrint[®]) that are commercially available do not only stratify patients by risk of recurrence, but the high risk groups also have been shown to have a greater absolute benefit when treated with chemotherapy (35), compared to patients with “low risk” tumors. However, these tests do not guide which treatment regimen should be selected; OncotypeDX[®] has been developed for ER-positive patients who also undergo tamoxifen treatment (36).

A technical advancement that may help in the identification of prognostic and predictive factors by gene expression profiling is microarray analysis. This technique, described in detail in chapter two of this thesis, enables researchers to analyze gene expression patterns in tumor samples for ten thousands of genes in one single experiment (37-39). This information can not only be used for prognostication (36;40-48), but also to dissect the underlying biology of tumors and to better understand mechanisms that lead to poor prognosis (40;42;47;49-52). In addition, microarray analysis can be used in studies of the sensitivity to various treatments (30;31;33;53-56). Thirdly, microarray analysis has the potential to select patients that have a higher likelihood of developing radiation induced toxicity (57;58).

The aim of this thesis is to investigate the use of gene expression profiling using microarray analysis of breast tumors in trying to improve prognostication of breast cancer, understanding biological mechanisms underlying good and poor prognosis and using this information for preliminary drug sensitivity mapping of subtypes of tumors. The most important overlying method we have used is the so-called hypothesis driven gene expression analysis. Starting with a hypothesis that captures a biological process that potentially plays an important role in cancer, for this process an in vitro model is constructed. The in vitro model reveals a specific gene expression pattern that is captured by using microarray analysis. The derived gene expression pattern is subsequently tested in human breast tumors and these tumors are grouped according to different stages of the model.

Chapter 2 represents a review of microarray analysis focusing on applicability in breast cancer and radiation oncology. We review different approaches that are utilized in the analysis of breast cancer gene expression profiles (e.g. unsupervised, data driven of supervised and hypothesis driven analysis) as well as the applicability to predict normal tissue toxicity in patients treated with radiation therapy.

In chapter 3, we describe an example of hypothesis driven gene expression profiling utilizing the Wound-response Signature. We show how this in-vitro based signature can be used to predict distant metastasis free and overall survival in breast cancer patients. Furthermore we show a comparative analysis with clinical and pathological variables as a tool for outcome prediction, as well as a comparison with established guidelines for treatment decision-making. Lastly, we show how the wound signature can be optimized in predicting a specific clinical end point (e.g. distant metastasis) by incorporating clinical data into a training and validation model.

In chapter 4 we aim to build a gene expression classifier to predict local recurrence after breast conserving treatment. We apply different methods of analysis, e.g. a data driven or supervised approach and optimization of previously established (hypothesis-driven and supervised) gene expression profiles.

Chapter 5 deals with the translation of established gene expression signatures into a tool for individualized treatment decision-making. We show how correlations between gene expression signatures and predefined modules (e.g. sets of genes representing a pathway) can lead to drug target discovery. We apply this method and provide preliminary evidence of a link between the Wound-response Signature and the proteasome pathway. Subsequently, we test this link in an in vitro model using Wound-response Signature activated and quiescent cells and the proteasome inhibitor bortezomib.

In Chapter 6 we combine the Wound-response Signature with a second hypothesis driven gene expression signature, the Hypoxia-response Signature. We show how this combination improves outcome prediction in breast cancer patients compared to the individual signatures, clinical and pathological variables and another combination signature. Furthermore, we show that the hypothesis driven approach can serve as an alternative to a data driven approach.

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