Gene expression profiling of breast cancer to identify subtypes and to predict local recurrence after breast conserving therapy

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Citation for published version (APA):
Kreike, B. (2011). Gene expression profiling of breast cancer to identify subtypes and to predict local recurrence after breast conserving therapy

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Chapter 1

Introduction and outline of the thesis
Introduction

This thesis focuses primarily on gene expression profiling studies of breast cancer, with emphasis on the identification of novel risk factors for local recurrence after breast conserving therapy; novel methods for the analysis of gene expression data; and analysis of specific subgroups of breast carcinomas. In this general introduction these topics are discussed.

Local recurrence and breast conserving therapy
Breast cancer is the most common cancer type among Dutch women. Approximately 12% of Dutch women develop breast cancer during their lifetime and about 4% die of this disease [1]. The local treatment for early stage breast cancer can consist of mastectomy or breast conserving therapy. Mastectomy comprises complete removal of the breast, whereas breast conserving therapy involves surgical removal of only the part of the breast where the tumor is located surrounded with a margin of healthy breast tissue, followed by whole breast irradiation.

In clinical studies it has been found that when a conservative surgical approach is used, radiation therapy is mandatory to achieve acceptable local control rates [2-6]. Six prospective randomised trials [7-12], comparing mastectomy with breast conserving therapy for the treatment of stage I-II breast cancer showed no significant difference in terms of loco-regional control and distant metastasis with limited follow-up, but publications of the same trials with longer follow-up showed poorer local-control after breast conserving therapy as compared to mastectomy [4,13-16]. These trials also demonstrated no significant difference in distant metastasis or long-term survival for both treatment approaches. Based on the results of these large clinical trials the choice for the primary treatment modality is often breast conserving therapy, unless there are risk factor that indicate that there is a high risk of local recurrence.

The importance of preventing a local recurrence is highlighted by the updated analyses of the Early Breast Cancer Trialists’ Collaborative Group showing that local recurrence is associated with poorer survival [3] and by Voogd et al who suggested that early detection of local recurrence could improve survival [17]. Thus the identification of robust risk factors for local recurrence is of great clinical significance.

Several risk factors for local recurrence after breast conserving therapy have been identified [18-34]. The majority of these risk factors reflect factors that are associated with a high likelihood that a significant amount of cancer tissue has been left behind, including involvement of the surgical margins by invasive carcinoma (Hazard ratio (HR) ranging from 2.8-3.9) and/or extensive ductal carcinoma in-situ (HR 2.5-4.2), vascular invasion (HR 2.0-2.9) and tumor multicentricity (HR 1.8-3.3). In addition, young age (HR 2.4-9.2) has been associated with a high risk of local recurrence after breast conserving therapy; and at present it is poorly understood what the biological mechanism for this association is. The addition
of a radiation boost after whole breast irradiation and adjuvant systemic therapy reduces local recurrence rates by 40-60% [18,22,26,31,33,34]. Despite these known risk factors and their use to guide therapy, local recurrence after breast conserving therapy still occurs and a recurrence rate of 5-10% after 10 years follow-up is generally considered as clinically acceptable for T1-2 N0-1 breast cancers.

Gene expression profiling technology
In the past, individual gene expression levels or protein expression levels, usually assessed with immunohistochemistry, have been correlated to disease outcome, but it was not possible to screen the entire genome (~25,000 genes) in one single experiment. Microarray technology has made it possible to analyze the entire gene expression profile of a tumor in a single experiment. This technology evolved from similar hybridization principles as Southern blot analysis [35], a technique developed in 1975. Using microarray analysis the expression levels of genes is measured using a device that holds several thousands of DNA fragments, corresponding to known genes, in an ordered fashion (the array). mRNA from (tumor)cells is isolated, processed, labeled and subsequently hybridized onto this array. When genes are highly expressed in the (tumor)sample, a higher accumulation of labeled RNA molecules will be present on the spot of these genes on the array compared to the spot corresponding to genes that are expressed at lower levels. The result of a hybridization experiment of labeled RNA to an array can be transformed into a database containing the expression level of each gene in a sample.

When multiple (tumor)samples are analyzed using this technique, the resulting lists of expression levels for each gene can be compared. Samples can be grouped together when their genes share a similar pattern of gene expression. These groups of tumors with similar expression patterns can be correlated to pathological and clinical features, such as tumor type, histological grade, proliferation status, metastasis-free survival, death or local recurrence. When an association between a particular gene expression pattern and disease outcome is identified, this gene expression pattern may be used as a clinical risk factor to help guide treatment strategies.

Clinical implementation of gene expression profiling
Gene expression microarray technology has proven to be a valuable tool in characterizing numerous tumor types [36-43]. In breast cancer several studies have shown the existence of gene expression profiles predictive for distant metastasis-free survival [44-47]. Some of these studies have led to clinical studies where the gene expression profiles are used to guide treatment decisions, especially for adjuvant systemic therapy [48]. Until 2006, no studies were published on the association of gene expression patterns of breast cancers with local recurrence after breast conserving therapy. Gene expression based predictors of risk of local recurrence after breast conserving therapy will be helpful in the decision making for local therapy (mastectomy versus breast conserving therapy), i.e. when the expression profile indicates a high risk for local recurrence, patients can be advised to undergo mastectomy or breast conserving therapy with
a higher radiotherapy dose. It may also be possible to identify patients at such a low risk of local recurrence that adjuvant radiotherapy after breast conserving surgery is not required. In addition to using gene expression profiling in breast cancer to identify novel prognostic and predictive factors that can be used to guide therapy, it has proven to be a valuable tool in better characterization and understanding of the disease. The World Health Organization has defined a wide range of histopathological subtypes of invasive breast cancer and classified these carcinomas into 19 categories [49], most of which are quite rare [50]. Perou et al. and Sorlie et al. were the first to show that breast carcinomas can also be subdivided based on gene expression profiles [41,44,51,52]. They have used hierarchical cluster analysis to derive a subset of genes (so-called ‘intrinsic gene subset’) that can distinguish several relatively homogenous groups of breast carcinomas. The largest difference in the overall gene-expression profile is observed between tumors that are estrogen receptor (ER) positive and those that are ER-negative. These ER-negative tumors are further sub-divided into tumors with gene expression characteristics of HER2-positive tumors, normal breast tissue and basal epithelial/myoepithelial cells. It is believed that basal-like tumors constitute a homogenous sub-group of breast carcinomas and are defined using gene expression profiling [41,44,51-54].

Brief introduction to the chapters of this thesis
Chapter 2 describes the results of a risk factor analysis for local recurrence after breast conserving therapy. In 1995 a cohort of 1,026 patients was analyzed for the same risk factor analysis. For this chapter we have updated the follow-up for this cohort of patients. Chapter 3 describes the results of our study to identify novel risk factors for local recurrence after breast conserving therapy. We used gene expression profiling using cDNA microarrays to identify gene expression profiles associated with local recurrence. Using 18K cDNA microarrays, gene expression profiles were obtained from 50 patients who underwent breast conserving therapy. Chapter 4 describes subsequent studies to identify biological markers predictive of a local recurrence after breast conserving therapy. We used previously established gene expression profiles with proven value in predicting metastasis-free and overall survival (the wound-response signature, the 70-gene prognosis profile and the hypoxia-induced profile). We re-trained these signatures towards an optimal prediction of local recurrences and validated the obtained signatures on an independent dataset.

In chapter 5 we extended the work on our initial group of primary breast carcinomas to compare the gene expression profiles of 56 primary invasive breast carcinomas from patients who developed a local recurrence after breast conserving therapy with profiles of 109 tumors from patients who did not develop a local recurrence after breast conserving therapy. Gene expression profiles were generated using 35K oligonucleotide microarrays. Both unsupervised and supervised methods of classification were used to separate patients into groups corresponding to disease outcome.

In chapter 6 we examined the histopathological and gene-expression profile of triple-negative tumors to define subgroups with specific
characteristics, including risk of developing distant metastases. In chapter 7 we have used natural spline analysis to study the association between gene expression levels and overall survival. Most studies correlating gene expression data to clinical parameters assume a linear increase or decrease of the clinical parameter under investigation with the expression level of a gene. Our statistical approach will also allow for the identification of a non-linear association between gene expression level and disease outcome.

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