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

Kreike, B.

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

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

UvA-DARE is a service provided by the library of the University of Amsterdam (http://dare.uva.nl)
Summary

Breast cancer is the most frequently diagnosed cancers among women in the Netherlands. When the disease is diagnosed at an early stage the treatment often consists of breast conserving surgery followed by radiotherapy and when indicated followed by adjuvant systemic therapy (i.e. chemotherapy and/or hormonal therapy and/or targeted therapy). It has been shown that despite radical local therapy a subgroup of patients develop a local recurrence.

Chapter 1 contains an introduction describing risk factors for local recurrence after breast conserving therapy; and discussing gene expression profiling studies in breast cancer, aimed at identifying novel prognostic and predictive factors.

In chapter 2 we present the results of a clinical and pathologic risk factor analysis for local recurrence after breast conserving therapy (breast conserving surgery followed by radiotherapy). In 1995 the same cohort of 1,026 patients was analyzed and we have updated the follow-up for this cohort of patients. After a median follow-up of 13.3 years, 114 patients had developed a local recurrence as first event. The local recurrence rate was 9.3% and 13.8%, respectively, at 10 and 15 years of follow-up after treatment of the primary tumor. The increase of local recurrences was continuous without reaching a plateau, even after 15 years. A step-wise proportional hazard Cox regression analysis was performed to identify the risk factors associated with an increased risk of local recurrence after breast conserving therapy at long-term follow-up. Univariate analysis showed that involved surgical resection margins, young age, vascular invasion, and the presence and quantity of an in situ component are risk factors for local recurrence. Multivariate analysis showed that tumor-positive surgical resection margins and the presence of vascular invasion are the most important risk factors for local recurrence after breast conserving therapy.

In chapter 3 we have used gene expression profiling using cDNA microarrays to identify gene expression profiles associated with local recurrence after breast conserving therapy. Using 18K cDNA microarrays, gene expression profiles were obtained from 50 patients who underwent breast conserving therapy. Of these 50 patients, 19 developed a local recurrence; the remaining 31 patients were selected as controls as they were free of local recurrence for at least 11 years after treatment. For 9 of the 19 patients developing a local recurrence, fresh-frozen tumor material of the local recurrence was also available for gene expression profiling. Unsupervised and supervised methods of classification were used to separate patients in groups corresponding to disease outcome. These statistical techniques were also used to compare the gene expression profile of primary tumors and their
recurrences. Hierarchical cluster analysis did not identify subgroups of tumors associated with a high or low local recurrence rate. Supervised analysis revealed no significant set of genes that was able to distinguish recurring tumors from nonrecurring tumors. Paired-data analysis of primary tumors and local recurrences showed a remarkable similarity in gene expression profile between primary tumors and their recurrences. Thus, no significant differences in gene expression between primary breast carcinomas in patients with or without local recurrence after breast conserving therapy were identified. Furthermore, analyses of primary tumors and local recurrences show a preservation of the overall gene expression pattern in the local recurrence, even after radiotherapy up to a dose of at least 50 Gy.

Chapter 4 describes an extension of the study presented in chapter 3, now analyzing gene expression profiling in combination with a hypothesis driven approach to analyze the data. 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. Validation of the different gene lists shows that the wound-response signature is able to separate patients with a high (29%) or low (5%) risk of a local recurrence at 10 years (sensitivity 87.5%, specificity 75%). In multivariable analysis the classifier is an independent predictor for local recurrence. These results indicate that gene expression profiling can identify subgroups of patients at increased risk of developing a local recurrence after breast conserving therapy.

In chapter 5 we extended 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. Both unsupervised and supervised methods of classification were used to separate patients into groups corresponding to disease outcome. In addition, for 15 patients, the gene expression profile in the recurrence was compared with that of the primary tumor. We found that hierarchical cluster analysis of all 165 primary invasive breast carcinomas revealed two main clusters. These main subgroups of tumors identified in this way were not associated with local recurrence. Predefined gene sets (molecular subtypes and the "chromosomal instability" signature) are associated with local recurrence (p=0.0002 and p=0.003, respectively). Using "Significant Analysis of Microarrays" (SAM) analysis, we identified a set of genes that was associated with the development of a local recurrence. This gene set is enriched
for genes associated with cell proliferation. This association was not captured by histologic grading. Using class prediction analysis we constructed a gene classifier, which was successfully validated, cross-platform, on an independent data set of 161 patients (log-rank p=0.041). There were important limitations of the classifier that we identified; as there was a relatively high false positive and low false negative prediction rate. However, 77% of all local recurrences are predicted correctly. Furthermore, a no-local recurrence prediction is very good at reassuring that a patient has a low risk for local recurrence (negative predictive value, 92%).

In addition to the studies on local recurrence after breast conserving therapy, we also studied features of a distinct subgroup of breast carcinomas in chapter 6. Breast cancer is a heterogeneous group of tumors, and can be subdivided on the basis of histopathological features, genetic alterations and gene-expression profiles. One well-defined subtype of breast cancer is characterized by a lack of HER2 gene amplification and lack of estrogen and progesterone receptor expression ('triple-negative tumors'). We examined the histopathological and gene-expression profile of triple-negative tumors to define subgroups with specific characteristics, including risk of developing distant metastases. Ninety-seven triple-negative tumors were selected from the fresh-frozen tissue bank of the Netherlands Cancer Institute, and gene-expression profiles were generated using 35K oligonucleotide microarrays. In addition, histopathological and immunohistochemical characterization was performed, and the findings were associated to clinical features. All triple-negative tumors were classified as basal-like tumors on the basis of their overall gene-expression profile. Hierarchical cluster analysis revealed five distinct subgroups of triple-negative breast cancers. Multivariable analysis showed that a large amount of lymphocytic infiltrate (hazard rate = 0.30, 95% CI 0.09-0.96) and absence of central fibrosis in the tumors (hazard rate = 0.14, 95% CI 0.03-0.62) were associated with distant metastasis-free survival. We concluded that triple-negative tumors are synonymous with basal-like tumors, and can be identified by immunohistochemistry only. Based on gene-expression profiling, basal-like tumors are still heterogeneous and can be subdivided into at least five distinct subgroups. Furthermore, the development of distant metastasis in basal-like tumors is associated with the presence of central fibrosis and a small amount of lymphocytic infiltrate.

Most studies correlating gene expression data to clinical parameters assume a linear increase or decrease of the expression of a gene with the clinical parameter under investigation. In chapter 7 we have used natural spline analysis to study the association between gene expression levels and overall survival. This statistical
Approach also allows for the identification of a non-linear association between gene expression level and disease outcome. Expression data of 16 genes were studied in relation to metastasis-free probability in a cohort of 295 consecutive breast cancer patients treated at The Netherlands Cancer Institute. The independent predictive power for disease outcome of the 16 individual genes was tested in a multivariable model with known clinical and pathological risk factors. There was a linear relationship between increasing expression and a higher or lower hazard for distant metastasis for ESR1, ERBB4, VEGF, CCNE2, EZH2, and UPA; there was no association between expression level and risk of distant metastasis for ERBB2, ERBB3, CCND1, CCNE1, EED, CXCR4, CCR7, SDF1, and PAI1; and for EGFR there appeared to be a non-linear relation between gene expression level and risk of distant metastasis. Both low and high expression of EGFR were associated with a high risk of distant metastasis, whereas intermediate expression levels were associated with a low risk of distant metastasis.

In chapter 8 several subjects of this thesis are discussed.