Gene expression profiling in breast cancer. A link between biology and clinical decision making
Hannemann, J.

Citation for published version (APA):

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)
This thesis describes two applications of gene expression profiling in understanding breast cancer biology and behavior: First, the use of gene expression in the clinical setting to identify signatures that predict response to specific chemotherapy regimens. Second, the use of expression profiling to identify global changes between different subgroups of breast tumors and by this to potentially elucidate pathways that are involved in tumor development, progression or sensitivity to treatment.

**Chapter 1** provides a brief general background on breast cancer and an outline for this thesis. Furthermore, this chapter gives a short overview on what microarrays are and how analysis of gene expression data can be performed. Unsupervised and supervised classification are the main methods to analyze the enormous amount of data that are generated by gene expression profiling. Based on gene expression profiles, breast carcinomas can be subdivided in distinct subgroups and mechanisms underlying the differences in clinical behavior of tumors can be studied more specifically.

**Chapter 2** contains a review on the literature of predictive markers for the response to specific chemotherapy regimens. Many markers have been suggested to predict sensitivity of a tumor to specific chemotherapeutic agents, but thus far, none of them has been implemented in clinical routine. A number of prognostic markers have been described for breast cancer, such as estrogen receptor (ER) expression, HER2 amplification and overexpression, or Ki-67 expression. Some of these prognostic markers have also been shown to provide predictive information for response to chemotherapy. Moreover, other markers have been suggested to predict sensitivity to specific chemotherapeutic agents. So far, no single marker has been identified that consistently predicts sensitivity to chemotherapy. Recent research has focussed on the development of multigene predictors by using high-throughput techniques such as quantitative RT-PCR and gene expression profiling by using microarrays.

**Chapter 3** describes a single institute clinical trial that was conducted to identify gene expression patterns that can predict response to neoadjuvant chemotherapy. At present, clinically useful markers predicting response of primary breast carcinomas to specific chemotherapy regimens are lacking. We investigated whether gene expression profiles of the primary tumor could be used to predict treatment response. Within a single-institute randomized phase II trial, patients with locally advanced breast cancer received six courses of either AC (n = 24) or AD (n = 24) neoadjuvant chemotherapy. Gene expression profiles were generated from core-needle biopsies obtained before treatment and correlated with the response of the primary tumor to the chemotherapy administered. Additionally, pretreatment gene expression profiles were compared with those in tumors remaining after chemotherapy. Ten (20%) of 48 patients showed a (near) pathologic complete remission of the primary tumor after treatment. No gene expression pattern correlating with response could be identified for all patients or for the AC or AD groups separately. We could show that the global gene expression of patients with a stable disease stays similar during chemotherapy treatment, whereas the gene expression profile of patients with a partial response changes remarkably during treatment. We conclude that more subtle differences in gene expression are likely to be present but can only be reliably identified by studying a larger group of patients. Response of a breast tumor to neoadjuvant chemotherapy results in alterations in gene expression.

**Chapter 4** describes the continuation of this work. Since trastuzumab had become available as targeted treatment for patients with HER2 positive breast carcinomas and the results of the MRI evaluations showed that patients without an early response to treatment will usually not achieve a complete response after three additional courses of the same treatment, this clinical trial was stopped for ethical reasons. A new trial with an adapted study design was started. Different treatment schedules are applied in patients with HER2 positive and HER2 negative tumors, respectively, to ensure optimal treatment with trastuzumab for HER2 positive tumors. Further-
more, in this new study, treatment schedules were adapted so that patients without a good response after three courses of chemotherapy could be switched to an alternative treatment regimen to improve response.

The pre-treatment gene expression profiles of 63 patients where analyzed. In a preliminary analysis, a classifier of 31 genes could be identified that predicted response to any given chemotherapy with an accuracy of 78%. Another classifier consisting of 22 genes predicts response to treatment with Adriamycin and cyclophosphamide in 20 patients. Based on the so-called “intrinsic genes”, we identified the molecular subtypes of breast cancer in our data set. Thirteen out of 25 patients with a basal-like tumour (52%) showed a complete remission, whereas for the luminal tumours (n = 29) complete remissions were observed in only 7% of the patients. In general, basal-like tumors respond much better to various chemotherapy regimens compared to the other molecular subtypes.

Chapter 5 describes a retrospective analysis of a randomized clinical trial comparing conventional chemotherapy with high dose chemotherapy in high risk breast cancer patients.

Benefit from chemotherapy treatment in breast cancer patients is determined by the molecular make-up of the tumour. In a retrospective analysis of the large Dutch National Study on high dose chemotherapy, we determined the molecular subtypes of breast cancer originally defined by expression microarrays by immunohistochemistry in tumours of patients who took part in a randomised study of adjuvant high-dose chemotherapy in breast cancer. In addition, the topoisomerase IIA (TOP2A) amplification status was determined by fluorescence in situ hybridisation and chromogenic in situ hybridisation. 411 of the 753 tumours (55%) were classified as luminal-like, 137 (18%) as basal-like and 205 (27%) as human epithelial receptor type 2 (HER2) amplified. The basal-like tumours were defined as having no expression of ER and HER2; 98 of them did express epidermal growth factor receptor and/or cytokeratin 5/6. The luminal-like tumours had a significantly better recurrence free and overall survival than the other two groups. From the 194 HER2-positive tumours, 47 (24%) were shown to harbour an amplification of TOP2A. Patients with an HER2-amplified tumour randomised to the high-dose therapy arm did worse than those in the conventional treatment arm, possibly caused by the lower cumulative anthracycline dose in the high-dose arm. The tumours with a TOP2A amplification contributed hardly to this difference, suggesting that TOP2A amplification is not the cause of the steep dose-response curve for anthracyclines in breast cancer. Possibly, the difference of the cumulative dose of only 25% between the treatment arms was insufficient to yield a survival difference.

Chapter 6 describes gene expression profiling of ductal carcinoma in situ (DCIS).

DCIS is characterized by the intraductal proliferation of malignant epithelial cells. Several histological classification systems have been developed, but assessing the histological type/grade of DCIS lesions is still challenging, making treatment decisions based on these features difficult. To obtain insight in the molecular basis of the development of different types of DCIS and its progression to invasive breast cancer, we have studied differences in gene expression between different types of DCIS and between DCIS and invasive breast carcinomas. Gene expression profiling using microarray analysis has been performed on 40 in situ and 40 invasive breast cancer cases. DCIS cases were classified as well- (n = 6), intermediate- (n = 18), and poorly (n = 14) differentiated type. Of the 40 invasive breast cancer samples, five samples were grade I, 11 samples were grade II, and 24 samples were grade III. Using two-dimensional hierarchical clustering, the basal-like type, ERB-B2 type, and the luminal-type tumours originally described for invasive breast cancer could also be identified in DCIS. Using supervised classification, we identified a gene expression classifier of 35 genes, which differed between DCIS and invasive breast cancer; a classifier of 43 genes could be identified separating between well- and poorly differentiated DCIS samples.

Chapter 7 describes the results of in vitro experiments to further elucidate the relationship between genetic alterations and changes in gene expression profiling in breast cancer. Specific genetic alterations in human breast cancer are associated with distinct clinicopathological features. Inactivation of the E-cadherin gene is observed in the majority of lobular carcinomas in situ and invasive lobular carcinomas and is only very rarely observed in other tumor types. HER2 gene amplification is observed in 15–25% of all invasive breast cancers and in over 50% of ductal carcinoma in situ. It is associated with estrogen receptor negativity and poor histological grade. We investigated global gene expression changes in human breast cancer cell lines after siRNA-mediated knock down of HER2 and E-cadherin expression, respectively. These results were correlated to the gene expression profiles of human primary breast cancer datasets hybridized on the same array platform. We silenced HER2 expression in SK-BR-3 cells and E-cadherin expression in MCF-7 and ZR-75-1 cells. Fifteen genes were significantly regulated after...
Summary

Silencing of E-cadherin expression and this signature was used to cluster 255 human breast tumors (34 lobular and 221 ductal carcinomas). Based on the expression pattern of these 15 genes, invasive lobular cancers and other tumor types could not be separated. After silencing HER2/neu expression, 121 genes were significantly regulated. Again, unsupervised hierarchical clustering using these 121 genes did not result in co-clustering of HER2 positive tumors. Comparing inactivation of E-cadherin or HER2 in human breast cancer cell lines does not very accurately reflect the gene expression patterns observed in human breast cancer where these same genes are genetically altered.

In conclusion, microarrays provide a powerful tool that enables us to investigate the expression of thousands of genes simultaneously and to describe gene signatures that are correlated with different biological and clinical observations. On the other hand, gene expression profiling has reminded us of the fact that even apparently clear biological pathways are more complex when studied in detail or when results from *in vitro* experiments have to be translated to studies of human tumors.