Gene expression profiling of breast cancer to identify subtypes and to predict local recurrence after breast conserving therapy
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Chapter 8

Discussion
Discussion

The main focus of this thesis is on the identification of gene expression profiles associated with an increased risk of local recurrence after breast conserving therapy. We have found that such a predictive gene expression profile is more difficult to identify than gene expression profiles that predict distant metastasis-free or overall survival in breast cancer. One of the reasons for the difficulty of identifying a gene expression profile associated with local recurrence is that the incidence of a local recurrence is much lower than that of distant metastasis; therefore, it is more difficult to collect sufficiently large series of tumors. Furthermore, it may be that the difference in gene expression profiles are less prominent for local recurrence than for distant metastasis and therefore the number of samples needed to identify this difference will be greater.

It will probably take quite some time until a gene expression profile for local recurrence after breast conserving therapy will be used for clinical decision making. Our last study [1] indicated that there may be such a profile, but it requires a study of larger series of patients to construct a robust gene expression signature. Hopefully, studies like the ongoing “young boost”-trial, that randomizes breast cancer patients under the age of 51 to post-operative radiotherapy of the whole breast followed by a standard “low” boost to a “high” boost, in which tumor material is collected and can be used for gene expression profiling, will bring us closer to a usable gene expression classifier.

Recently, two papers have been published that studied the association between established gene expression profiles and the risk of locoregional recurrence [2,3]. The first paper by Mamounas et al studied the 21-gene OncotypeDX recurrence score assay, that quantifies the risk of distant recurrence in tamoxifen-treated patients with node-negative, estrogen receptor (ER)-positive breast cancer, in relation to the risk of locoregional recurrence in patients with node-negative, ER-positive breast cancer from two National Surgical Adjuvant Breast and Bowel Project (NSABP) trials (NSABP B-14 and B-20) [2]. They found a significant association between the risk of a locoregional recurrence and the recurrence score assay.

The second paper by Voduc et al studied the risk of local and regional relapse associated with each breast cancer molecular subtype in a large cohort of patients with breast cancer (n=2985) [3]. They found, in agreement with our results (chapter 5) that luminal A-like tumors are associated with a low risk of local recurrence and that HER2-like tumors are associated with an increased risk of local recurrence after breast conserving therapy. In contrast with our results, they found that basal-like tumors were associated with a high risk of local recurrence. Both studies give some additional insight in risk factors for local recurrence, but also show that a robust gene expression profile usable for clinical decision making will not be ready soon. In addition, Weigelt et al have recently shown that before molecular classification can be incorporated into routine clinical practice and treatment decision making, stringent standardization of methodologies and definitions for identification of breast cancer subtypes is needed [4].

A potential confounder in our studies is the resection margin status of the lumpectomy specimen. While margin involvement is an
The important risk factor for local recurrence, it will not intrinsically reflect a biological phenomenon. To make absolutely sure that the resection margins are completely free of any breast cancer (including microscopic involvement by DCIS or invasive carcinoma) pathological examination should be done in great detail. The routine examination of surgical breast cancer resection specimens may miss microscopic margin involvement and in addition, microscopic margin involvement is accepted in many Dutch hospitals for patients undergoing breast conserving therapy. Therefore, not for all patients in our studies the surgical margins are free of tumor. Although we believe that this will not confound these studies, as long as involved margins are present in both the cases and controls, it probably will affect the identification of underlying biological mechanisms of local recurrence after breast conserving therapy.

Another possible confounder for a general gene expression signature for local recurrence after breast conserving therapy is that we have specifically studied young patients. Our studies have focused on this group of patients, since young age is a known and strong risk factor for local recurrence and therefore predictive factors are more urgently needed to guide breast conserving therapy in young patients. But young age may be associated with the occurrence of tumors with specific biologic behavior associated with an increased risk of local recurrence. It is of interest to investigate whether the patient’s age is associated to differences in genetic features of the tumor.

Our study on triple negative tumors sheds more light on the relationship between triple negative tumors as identified using immunohistochemistry and basal-like tumors as identified using gene expression profiling. Some have disputed our conclusion that basal-like tumors can also be identified by immunohistochemistry only [5]. It is important to realize that the basal-like breast cancer subtype was initially defined based on the gene expression pattern of the so-called ‘intrinsic gene list’ in only six breast tumors [6]. Since this initial report, the intrinsic gene list that is used to identify basal-like breast tumors has been updated multiple times [6-8]. This shows that a gene-expression-based definition of basal-like breast cancer has its limitations. Rakha and colleagues believed that equating triple negative tumors with basal-like breast cancers is misleading and is not supported by our data [5]. We showed that 93 out of 97 triple negative tumors were allocated to the basal-like subtype and that only 4 samples were allocated to the normal epithelial-like subtype [9,10]. This is a problematic group, as it was originally defined [6] on the basis of samples that did not contain tumor cells after neoadjuvant chemotherapy treatment. Furthermore the normal epithelial-like subtype tumors have many similarities with the basal-like subtype tumors since the “normal epithelial” gene expression pattern is typified by the high expression of genes characteristic of basal epithelial cells and adipose cells, and the low expression of genes characteristic of luminal epithelial cells [6].

The dispute on whether triple negative tumors are identical to basal-like tumors is at present irrelevant for the patient, since these tumors have no specific therapeutic targets. We have shown that triple negative/basal-like tumors are not a homogeneous group of tumors, as assessed by their gene expression profiles and
therefore it is more likely that several different drug targets, rather than one target for all basal-like tumors, have to be identified to treat the whole group of basal-like tumors.

Our study on the use of the natural spline analysis technique indicated that the associations of gene expression levels with disease outcome are not always linear. With relatively simple modifications to the standard statistical models these relations can be found. We think that in future studies on associations of gene expression profiles with disease outcome such additional statistical models should be applied.

Reference List