Understanding the Molecular Basis of Histologic Grade

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Key Words

Histologic grading • Breast cancer • Gene expression grade index • Gene profiling • Proliferation

Abstract

Histologic grading in breast cancer is based on the evaluation of 3 morphologic features (tubule formation, nuclear pleomorphism and mitotic count), is essentially describing proliferation and differentiation in breast cancer, and is considered an important prognostic factor for this disease. It has been suggested that histologic grade 1 and 3 breast tumors are 2 different diseases that may have distinct molecular origins, pathogenesis and natural history. Different single markers like Ki-67, thymidine labeling index and S phase fraction/flow cytometry have been studied as markers of proliferation, but none of them, with the possible exception of Ki-67, is currently employed routinely in clinical practice. The advent of the powerful microarray technology has enabled scientists to comprehensively study proliferation in breast cancer on a genome-wide scale. A gene expression grade index (GGI) was developed that challenges the existence and clinical relevance of an intermediate grade 2 classification. The GGI could reclassify patients with histologic grade 2 tumors into 2 groups with high versus low risks of recurrence. GGI has also been used to define 2 clinically relevant subgroups in estrogen receptor-positive

breast carcinomas. Finally, in the largest meta-analysis of publicly available gene expression and clinical data, 4 stable molecular subgroups of breast cancer have been identified, namely ER-/HER-, HER2+ and ER+/HER2-, which was divided into 2 subgroups (ER+/low proliferation and ER+/high proliferation). In this same meta-analysis, proliferation was shown to be the common driving force responsible for the performance of various breast cancer prognostic signatures.

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Histologic Grade

Although histologic grade has long been considered as a prognostic factor in breast cancer, it has been the most heavily debated issue considered by the Breast Task Force in revising the AJCC cancer staging and, finally, histologic grade was not included in its staging criteria [1]. Histologic grading was suboptimally reproducible between different institutions. To make grading criteria more quantitative, Elston and Ellis [2] designed a modification of the Bloom and Richardson grading system, namely the Nottingham combined histologic grade. This system was based on the semiquantitative evaluation of 3 morphologic features (percentage of tubule formation, degree of nuclear pleomorphism and accurate mitotic count in a

defined field area). A value of 1–3 was assigned for each feature and a combined score was calculated. A combined score of 3, 4 or 5 was designated as grade 1, a score of 6 or 7 as grade 2 and a score of 8 or 9 as grade 3 cancer. However, even using this improved grading system, concordance between institutions has not exceeded 80% [3]. More importantly, using the Nottingham combined histologic grade, a substantial percentage of tumors (30–60%) are still classified as histologic grade 2, which is not informative for clinical decision making because of intermediate risk of recurrence. Furthermore, in studies that used the Nottingham combined histologic grade, grade 2 has been reported either together with grade 3 or 1, making interpretation of the results extremely difficult and hampering its incorporation in the current TNM system [1].

Mitotic Activity Index

Interestingly, Baak et al. [4] have investigated the value of morphometry as a prognostic factor in breast cancer and have shown that the mitotic count as measured by the Mitotic Activity Index (MAI) was the most important prognostic factor among the 3 morphologic features of the Nottingham combined histologic grade [5]. Tubular formation and nuclear atypia, as constituents of histologic grade were found to have no (tubular formation) or limited (nuclear atypia) additional prognostic value to the MAI in women with node-negative early breast cancer younger than 55 years old [5].

Modified Scarff-Bloom-Richardson Histologic Grading System

Le Doussal et al. [6] have suggested that among the 3 components of histologic grade (percentage of tubule formation, degree of nuclear pleomorphism and accurate mitotic count in a defined field area), it is the nuclear components (nuclear pleomorphism and mitotic count) that are most predictive for clinical outcome when combined into a modified Scarff-Bloom-Richardson (MSBR) grading system in a cohort of 1,262 patients with operable breast cancer. The SBR grade II patient group (55% of patients) was separated into 3 MSBR groups significantly different according to metastasis-free survival. The MSBR was more accurate and predictive than the standard SBR grade and was particularly useful when the nodal status of the patient was negative [6].

Proliferation Markers

Apart from histologic grade, other markers have been used to describe proliferation, such as Ki-67, thymidine labeling index, S phase fraction/flow cytometry, thymidine kinase, cyclins E and D, as well as the cyclin inhibitors p27 and p21 [7].

However, none of the above proliferation markers with the possible exception of Ki-67 is currently used in clinical practice [7]. Ki-67 has been the proliferation marker most extensively studied and a recent meta-analysis has demonstrated that it confers a higher risk of relapse and a worse survival in patients with early breast cancer [8]. However, this meta-analysis could not examine if Ki-67 had independent prognostic value beyond the standard clinicopathological characteristics [8]. Furthermore, Dowsett et al. [9] measured the expression of Ki67 in tumor biopsy samples taken before and after 2 weeks of presurgical treatment with anastrozole or tamoxifen or the combination of anastrozole plus tamoxifen in 158 patients with hormone receptor-positive primary disease. In multivariable analysis, they found that higher Ki-67 expression after 2 weeks of endocrine therapy was statistically significantly associated with lower recurrence-free survival ($p = 0.004$), whereas higher Ki-67 expression at baseline was not.

Microarray Studies about Proliferation

The advent of the powerful microarray technology has enabled scientists to comprehensively study the phenomenon of proliferation in cancer on a genome-wide scale [10–14]. Several studies have compared the gene expression profiles of high- versus low-grade tumors in different cancer types and meta-profiling was performed on 7 ‘undifferentiated versus well-differentiated’ signatures spanning 6 cancer types (overexpressed in undifferentiated cancers relative to well-differentiated cancers [14]). Sixty-nine genes were found to be present in at least 4 of 7 signatures and these genes define a meta-signature of high-grade or undifferentiated cancers. To assess the discriminative power of the undifferentiated meta-signature, Rhodes et al. [14] identified 5 independent data sets that included low- and high-grade cancer samples [15–19]. In 3 of 5 datasets, the meta-signature significantly discriminated between low- and high-grade cancer samples [15–17], whereas in the remaining 2 data sets the signature was not predictive [18, 19]. The authors conclude that this meta-signature is common to undifferentiated breast cancer, ovarian cancer and medulloblastoma [14].

Genomic Grade Index

Another carefully designed study to compare gene expression profiling between high- and low-grade tumors has been performed by our group [12]. A strong rationale for our study has been derived from the study by Roylance et al. [20], who found distinct genetic differences between grade 1 and 3 tumors by applying comparative genomic hybridization. Intriguingly, they observed that 65% of grade 1 tumors lost the long arm of chromosome 16 compared with only 16% of grade 3 tumors. This pattern of loss led the investigators to conclude that the majority of grade 1 tumors do not progress to grade 3 and that grade 1 and 3 breast tumors are 2 different diseases that may have distinct molecular origins, pathogenesis and behavior [20]. Furthermore, we wanted to better characterize the biology and natural history of tumors classified as histologic grade 2 (30–60% of all breast tumors) that were thought to have an intermediate risk of recurrence [12].

We have used primary tumor gene expression profiling to interrogate the existence of grade 2 breast tumors as a separate disease entity. To this end, we used 64 samples of estrogen receptor (ER)-positive tumors in the training set to select genes that were differentially expressed between histologic grade 1 and 3 tumors. Microarray analysis was performed with Affymetrix U133A GeneChips. We used only ER-positive tumors for selecting the genes because of the dependence between ER status and histologic grade; almost all ER-negative tumors were classified as either intermediate or high histologic grade. If we had used all histologic grade 1 and 3 tumors regardless of the ER status in our training set, we would have selected ER-related genes that were spuriously associated with grade. We introduced a score, called the gene expression grade index (GGI), to summarize the similarity between expression profile and tumor grade. A high GGI corresponds to a high grade and vice versa. Although it is possible to use the GGI as a continuous variable, we dichotomized it into high and low genomic grade subgroups for the generation of Kaplan-Meier curves and hazard ratio estimation between risk groups. The cutoff used was chosen to maximize the separation between histologic grades 1 and 3, and no survival information was used to optimize the high- and low-risk groups. Most of the 97 genes of the GGI were overexpressed in grade 3 tumors and had functions that were previously associated with cell cycle progression and proliferation. This is not surprising, since histologic grade is based on mitotic index, nuclear

Fig. 1. Using GGI, histologic grade 2 tumors can be categorized into 2 groups with distinct clinical outcomes similar to grade 1 and 3 tumors, respectively.

pleomorphism and differentiation [2]. To determine whether the gene expression pattern of the 97 genes that we identified in our training set would consistently predict histologic grade in an independent group of tumors, we examined the expression of these genes in 597 independent tumors from 5 different institutions analyzed with different microarray platforms. The gene expression patterns of histologic grade 1 and 3 tumors were similar to those identified in the training set. We used the same gene selection algorithm to compare the gene expression profiles of histologic grade 2 tumors with the profiles from a group of combined histologic grade 1 and 3 tumors. We found no evidence that histologic grade 2 tumors had gene expression profiles that were independent from those that distinguished histologic grade 1 and 3 tumors. Some histologic grade 2 tumors had intermediate GGI values, while some others had extreme indices that were similar to those of histologic grade 1 or 3 tumors. Interestingly, the GGI was able to reclassify patients with histologic grade 2 tumors into 2 groups with distinct clinical outcomes similar to those of histologic grade 1 and 3, respectively (fig. 1) [12]. This observation challenges the existence and clin-

ical relevance of an intermediate-grade classification. In multivariable analysis the GGI had the strongest association with relapse-free survival. When we explored the implications of the joint distribution of ER status and GGI, we found that almost all ER-negative tumors were associated with a high GGI score (high grade), whereas ER-positive tumors were associated with a heterogeneous mixture of GGI values. When we investigated the association between GGI and relapse-free survival in the subset of patients with ER-positive tumors, we found that GGI separated these patients into a high- and a low-risk group. In contrast, among patients with gene expression grade 3 tumors, ER status was not associated with the risk of recurrence. Therefore, when GGI is known, ER status does not provide additional prognostic information, but when ER status is known, GGI can still improve prognostic accuracy [12]. When the prognostic performance of GGI was compared with the 70- and 76-gene signature, we found similar separation in distant metastasis-free survival between low- and high-risk groups by the 3 signatures [12, 21–23].

Definition of Clinically Distinct Molecular Subtypes in ER-Positive Breast Carcinomas through Genomic Grade

Recently, our group has reported that GGI can be used to define 2 clinically relevant molecular subtypes in ER-positive breast cancer [24]. These 2 ER-positive molecular subgroups (high and low genomic grade) were highly comparable to the luminal A and B classification and were associated with distinct clinical outcome in systemically untreated or tamoxifen-only-treated ER-positive breast cancer populations involving more than 650 patients [24]. Moreover, we have shown that the 100 of the 295 tumors from the data set of van de Vijver et al. [25] that could not be confidently assigned to a particular subtype as defined by Sorlie et al. [26] have a statistically distinct clinical outcome based on their classification by GGI. Overall, these results suggest that, particularly for the ER-positive tumors, the GGI values can distinguish clinically relevant subtypes that are highly comparable to those previously described. GGI appeared to be the strongest predictor of clinical outcome, highlighting the prognostic importance of proliferation genes in ER-positive subgroups, as has already been reported [27]. Furthermore, we investigated the prognostic value of ER and progesterone receptor (PR) determined by the microarray expression levels of the corresponding probe sets. For

division into hormone receptor-rich and hormone receptor-poor groups for the survival analyses, the median expression level of these probe sets was used as the cutoff. Interestingly, for both untreated and tamoxifen-treated ER-positive populations, the subgroups produced by high and low expression levels of the ER did not have any prognostic value. In contrast, the subgroups generated by the expression levels of the PR had significant prognostic value. In the multivariate Cox regression analysis, only GGI, PR levels and histologic grade retained significant prognostic value in the untreated data set. For the tamoxifen-treated population, only GGI retained significance in the multivariate model. These results confirm the strong prognostic value of GGI and its prognostic dominance over histologic grade in systemically untreated and tamoxifen-only-treated ER-positive breast cancer.

Because the Oncotype DX® recurrence score (RS) has been validated extensively as a stratification factor for ER-positive breast cancer, we were interested in how the GGI and the RS classifications compared, given that the GGI tracks a single biologic pathway, whereas the RS combines several. We found that the GGI and RS models were significantly correlated ($r = 0.7$; 95% CI 0.63–0.76; $p < 0.0001$). Receiver operating characteristic curves demonstrated that the predictive accuracies of the GGI and RS were similar to each other. This could be explained, since proliferation was 1 of the 5 components associated with the highest weight in the Oncotype DX® RS.

At present, it is unclear whether the high-GGI subgroup will benefit from chemotherapy or aromatase inhibitors. However, in the future, stratification by subtype (high vs. low GGI) in prospective clinical trials may help identify patients who will benefit from the addition of chemotherapy endocrine therapy or patient subgroups who will derive more benefit from one type of endocrine therapy over the other (for example, aromatase inhibitors versus tamoxifen).

Proliferation: The Common Driving Force Responsible for the Performance of the Various Breast Cancer Prognostic Signatures

Breast cancer has been extensively studied by gene expression profiling [28, 29]. However, the different gene expression signatures developed to improve breast cancer prognostication have little overlap in their constituent genes. The inevitable question is whether these prognos-

Fig. 2. Proliferation: the common driving force for the prognostic performance of several gene expression signatures.

tic signatures also have little overlap in the prognostic information they convey. Fan et al. [30] recently compared the prognostic ability of 5 gene expression-based models: intrinsic subtypes [31], 70-gene profile [32], wound response [33], RS [27] and 2-gene ratio [34] in a single dataset of 295 patients and found that 4 of 5 predictors had significant agreement in outcome predictions for individual patients [30]. However, this study was limited to only 1 dataset of 295 patients, examined only 5 gene expression signatures and did not investigate the biology that may drive prognosis among the 5 signatures. To address this issue, a large meta-analysis of publicly available gene-expression and clinical data from 2,833 patients [12, 13, 22, 25, 26, 31, 32, 34–50] with breast cancer was performed by the Swiss Institute of Bioinformatics in collaboration with our group [21]. In this meta-analysis, the concept of coexpression modules (comprehensive list of genes with highly correlated expression) was used to describe important biological processes in breast cancer, like proliferation, ER and HER2 signaling. In this study, we sought to depict the connection between these modules, the previously reported molecular classification, several gene prognostic classifiers and the most established clinicopathological variables. A number of interesting conclusions were drawn from this collaborative effort. First, the disparity of gene lists of the various gene signatures may be attributed to (1) heterogeneity in the patient populations studied, (2) the use of different microarray platforms with dif-

ferent probe sets and different methods for data normalization, and (3) sampling variation due to small sample size relative to the number of genes examined. Second, using these coexpression modules, instead of the 5 molecular portraits reported with the intrinsic genes, breast tumors were now grouped into 3 main subgroups, namely ER-/HER-, HER2+ and ER+/HER2-. Third, the ER-/HER- and HER2+ tumors were characterized by high proliferation, whereas the ER+/HER2- tumors were divided into 2 subgroups, the ER+/low proliferation and the ER+/high proliferation tumors resembling luminal A and B subtypes, respectively. Fourth, 10 prognostic signatures (RS [27], 70-gene signature [32], 76-gene signature [35], wound response [51], p53 signature [36], GGI [12], NCH70 [42], CON52 [50], CCYC [13], ZCOX), despite the disparity in their gene lists, showed similar prognostic performance. It is interesting to note that they all identified as low risk the ER+ subtype associated with low proliferation. Fifth, proliferation was the common driving force responsible for the performance of the various prognostic signatures (fig. 2). Indeed, to further investigate the role of proliferation genes in relation to the different prognostic signatures, we divided each signature into 2 partial signatures, one with only proliferation genes and the other with the complementary nonproliferation genes. Interestingly, when only proliferation genes were used, the total performance was not degraded. In contrast, the nonproliferation partial signatures typically showed degraded

performance. Sixth, combining the signatures did not improve the performance as expected from the high concordance in their classifications. Finally, in multivariate analysis including the genomic predictor derived from coexpression modules, the standard clinicopathological variables, namely tumor size and nodal status, retained their prognostic value.

Conclusions

Proliferation as quantified by gene expression profiling has been shown to outperform the prognostic value of histologic grading. Proliferation was revealed as the driving force responsible for the performance of most breast cancer prognostic signatures and as more useful for determining the risk of recurrence in patients with ER-positive tumors.

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