Dr. Gown is a pathologist-scientist recognized as one of the world’s leading experts in the diagnostic and research applications of immunohistochemistry (IHC), and founder, Medical Director and Chief Pathologist of PhenoPath Laboratories, a national reference laboratory specializing in IHC, flow cytometry, fluorescence in situ hybridization (FISH), and other molecular studies. He has developed numerous clinically important monoclonal antibodies used around the world, and continues to be at the forefront of clinical investigative studies employing IHC and other modalities, publishing widely and presenting at national and international conferences. Dr. Gown is a member of the editorial boards of many of the major pathology journals. He is a Clinical Professor of Pathology at the University of British Columbia, Vancouver, BC, and an Affiliate Investigator in the Clinical Research Division of the Fred Hutchinson Cancer Research Center, Seattle, WA. Dr. Gown has contributed extensively to the expanding horizons of immunohistochemistry with over 200 peer-reviewed publications.

Are traditional classification schemes of breast cancer, based upon histology and slide-based techniques such as immunohistochemistry, poised for replacement by molecular-based classification schemes?

Traditionally, the most important information pathologists have provided to breast oncologists regarding a patient’s tumor include the nodal status as well as the tumor’s size, grade, estrogen and progesterone receptor status. More recently, in this “post trastuzumab” era, the assessment of HER2 status of the tumor has become routine. Combining much of these data, it has been possible to assign patients into “low risk” (e.g., tumor size < 1 cm, low grade, node-negative, ER-positive) and “high risk” (e.g., >5 cm, high grade, node-positive, ER-negative) tumors. However, in addition to ignoring a large number of patients with intermediate categories, even within the lower risk group, despite the best treatment, approximately 15% of these patients will recur and die of metastatic disease, and within the high risk group approximately 15% of patients will, somewhat paradoxically, have a favorable outcome. This means that this classification system of high and low risk based upon these traditional factors would result in approximately 15% of patients being mistreated. In the earlier parts of this decade, studies by Perou, et al. (1) and Sorlie, et al. (2) demonstrated that using “heat maps” generated from microarrays scanned for fluorescent signals, the patterns of expression of 426 genes could be analyzed using sophisticated mathematical techniques known as clustering analysis. Emerging from these analyses were at least four subgroups of breast cancer defined by their relatively unique molecular profiles: a luminal type (further subdivided in to variants A and B), a “basal-like,” a HER2-positive, and normal breast-like variant. A striking feature of these analyses was the unique clinical outcomes that could be demonstrated for each of these molecularly-defined groups. In particular, the luminal-type cancers had the best outcome, and the two subgroups with the worst clinical outcomes included the HER2-positive group and the so-called “basal-like” group (2).

Despite the fact that these analyses were unsupervised, the expression of these 426 genes revealed a sub-classification of breast cancers which
actually bears striking similarities to those of immunohistochemically- (and histologically) defined schemes using antibodies to known breast markers. For example: the luminal A breast cancers defined by this methodology correspond largely to the subgroup of low-grade, ER-positive breast cancers; the HER2-positive group corresponds to those demonstrated by immunohistochemistry to overexpress HER2 gene product, and by FISH analysis are found to have amplified HER2 genes. And while there does not appear to be a normal “basal-cell,” several investigators have found that basal-like breast cancers can be distinguished from other types of breast cancers using immunohistochemical techniques by demonstrating expression of proteins such as p63, cytokeratin 5/6, EGFR, c-kit, as well as the p53 gene product (3). Indeed, studies in the laboratory of Dr. Ian Ellis (4) as well as the British Columbia Cancer Agency (5), have shown that the same sort of hierarchical cluster analysis previously applied to gene expression data can also be applied to immunohistochemically-defined protein expression data derived from breast cancers. Indeed, both molecular and immunohistochemical analyses, while not identifying completely identical subgroups, are nonetheless similar in their abilities to define subsets of breast cancer with unique prognostic profiles.

For example, the table (next page) shows a very simplified view of the correspondence of the immunohistochemical with the molecular-defined categories.

A different approach, first taken by van’t Veer and colleagues, also in the earlier part of this decade, was the use of a supervised analysis of gene expression, which involved the determination of, in their case, 70 genes which seemed to separate out “good actors” from “bad actors,” in an initial series of 98 breast cancer patients, all of whom were lymph node-negative at the beginning of the study (6). This analysis correctly identified the outcome in 83% of the study patients, predicting those who were to go on to develop metastatic disease. (Curiously, however, neither estrogen receptor nor HER2 was one of the 70 genes!) This 70 gene assay has now been commercialized and is known as MammaPrint™, the first fully commercialized microarray-based assay of breast cancer, and the first to receive 510(k) clearance from the FDA, in 2007. Validation studies in recent years have demonstrated the ability of this 70 gene prognostic signature, in other cohorts of patients, to also predict outcome, although it is not clear from these published data that the 70 gene signature performs significantly better in terms of sensitivity.

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and specificity of identifying patients who go on to develop metastases and death compared with other prognostic assessments, such as the Adjuvant! Online software, the Nottingham prognostic index, or the St. Gallen criteria (7). There also appears to be an additive quality to the classification systems, with each providing unique information.

Another set of genes determined by supervised analysis includes the 16 gene set which forms the basis of the Oncotype DX™ assay. This set of genes includes a cluster corresponding to proteins involved in cell proliferation, expression of the estrogen receptor protein itself, HER2, and to other non-tumor related genes. The Oncotype DX™ assay features a unique mathematical approach in which a recurrence score is generated based upon a weighted sum of the individual gene expression profiles. In a landmark study of 668 node-negative ER-positive breast cancer patients from the NSABP B-14 trial, this 16 gene expression profile was found to predict the risk of tumor recurrence, in contrast to other single demographic or immunohistochemical assays, including age, size of tumor, status of HER2, and by gene analysis and quantification of ER (8). (Interestingly, however, a poorly differentiated tumor grade showed an even higher hazard ratio than a high recurrence score, at similar p value.). One of the major advantages of the Oncotype DX™ assay is its ability to employ deparaffinized, formalin fixed tissue samples.

It has yet to be systematically investigated, however, as to whether a similarly-weighted score of the results of immunohistochemical analysis of many of the same proteins corresponding to the Oncotype DX™ gene set (including proteins such as ER, HER2, Ki-67, and p53) might yield identical or even superior results to the Oncotype DX™ score in identifying those patients with high or low risk of recurrence. One early attempt at this comparison demonstrated that approximately 66% of the data variability in the Oncotype DX™ score could be reproduced by a multi-variant linear regression model incorporating nuclear grade, mitotic count, ER, PR and HER2 (9). This should prove a fruitful area for future investigation.

Finally, one of the most striking findings has been the fundamental underlying similarity of the tumor subsets identified by various gene expression methodologies and platforms, despite their use of different patient cohorts, microwave platforms, mathematical methods, and sets of genes expressed. In one recent study, when identical individual tumors were re-queried using these different molecular classification techniques, there was a very high level of concordance amongst the various gene expression array platforms. For example, there was agreement in predicting poor versus good outcome in 230 out of 295 or 81% of breast cancer patients between the Oncotype DX™ and MammaPrint™ assays.

Is it possible that the molecular techniques may have, at one level, done nothing more than “reinvent the wheel?” It may well be that supervised gene expression, unsupervised gene expression, immunohistochemistry and even histology behave like “blind men and the elephant,” each identifying different parts of the same entities, in this case subsets of breast cancer with unique outcomes. And it may be some time before molecular classification techniques replace traditional histologic and immunohistochemically-defined groups, and for a number of reasons. While molecular classification of tumors has clearly been demonstrated to be feasible, as this classification incorporates genes expressed both in the tumor and non-tumor elements, it appears that in some ways, these molecular techniques represent a step backwards and are degradative techniques divorced from histology, more analogous to the dextran-coated charcoal or enzyme immunoassays used in determining estrogen receptor in breast cancer, instead of more modern immunohistochemical assays. The most powerful classification schemes for breast cancer may indeed remain those defined by immunohistochemistry, as it has the unique ability to provide comprehensive, and increasingly, quantitative multiplexed analysis of ‘gene expression’ that can be directly linked to histology and all its subtleties.

References