Short communication

The utility of conventional and molecular pathology in managing breast cancer

D Craig Allred

Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO 63110, USA

Corresponding author: D Craig Allred, dcallred@path.wustl.edu

Published: 18 December 2008
This article is online at http://breast-cancer-research.com/content/10/S4/S4

Breast Cancer Research 2008, **10(Suppl 4):**S4 (doi:10.1186/bcr2164)

Pathology currently plays a central role in the interdisciplinary management of breast cancer by establishing the diagnosis, estimating untreated clinical outcome (prognosis), and predicting responsiveness to specific types of therapy (prediction). Establishing the diagnosis of invasive breast cancer (IBC) is the first and most critical responsibility of pathologists. It is primarily based on microscopic evaluation of tissue samples, which most often begins with evaluation of image-guided core biopsies obtained by radiologists. The diagnosis of IBC initiates prompt therapy by other members of the interdisciplinary team. Typically, this first involves surgeons, who excise the tumor (lumpectomy or mastectomy) as well as some (sentinel) or all of the lymph nodes in the ipsilateral axilla. Surgery is usually followed by various types of adjuvant therapy managed by radiation and medial oncologists, as well as social/psychological support by mental health professionals.

Pathologists also evaluate the excised tumor and lymph nodes to determine other important prognostic and predictive features, and the results are essential for deciding on adjuvant therapies. These features include identification of the histologic type of IBC. Five major types are currently recognized [1,2]. Four are characterized by relatively unique/uniform histologic features and favorable clinical outcomes, and thus they are referred to as 'special' histologic types [1,3,4]. Collectively, the special types account for about 25% of all IBCs, and include the so-called invasive lobular, tubular, mucinous, and medullary carcinomas (approximately 15%, 5%, 3%, and 2%, respectively). The remaining 75% of IBCs are histologically and prognostically very heterogeneous, and they are referred to as 'no-special-type' carcinomas or, more commonly, as 'ductal' carcinomas, which is a more historical term [1,2]. Although the prognosis of ductal carcinomas is generally worse than that of the special types (with the exception of lobular carcinomas), this varies across a wide continuum from very good to very poor.

Pathologists use histologic grading systems to estimate the prognosis of ductal carcinomas, although grading is sometimes also applied to special types, but the information is less useful in this setting. There are many systems for histologic grading. Nearly all of them assess the degree of architectural differentiation (tubule/acinus formation), nuclear atypia, and growth (mitotic) rate in tumors, assigning points to each category to obtain a total score. The sum scores are then combined into two or three grades that are directly related to prognosis. The Elston-Ellis modified Scarff-Bloom-Richardson method, which categorizes tumors into low (3 to 5 points), intermediate (6 or 7 points), and high (8 or 9 points) grades, is currently the preferred method because it is relatively simple, reproducible, and has the ability to identify tumors with significantly different prognosis [5-7].

Other important prognostic features determined by pathologists include tumor size, the status of surgical margins, and axillary lymph nodes. Like histologic grade, tumor size is related to prognosis in a direct and highly significant manner [1,8]. It is usually determined by directly measuring the size of the excised mass with a ruler, although small tumors are more accurately measured microscopically in a tissue section on a glass slide. The distance of tumor from the margin of surgical excision is also important because it is inversely related to the likelihood of local recurrence, especially in tumors removed by lumpectomy [9]. Wide margins can be adequately measured with a ruler, whereas close margins require microscopic measurement. The presence of metastatic tumor deposits in axillary lymph nodes is a highly unfavorable prognostic feature, as are a high number of involved nodes and (to a lesser degree) large size of the deposits, which are all determined

ER = estrogen receptor; HER = human epidermal growth factor receptor; IBC = invasive breast cancer; IHC = immunohistochemistry; PCR = polymerase chain reaction; PR = progesterone receptor.

microscopically [8]. Tumor size (T) and nodal status (N) are so powerful prognostically that they comprise two of the three features used in the TNM (where M means distant metastases) breast cancer staging to convey the extent of disease [10], which plays a major role in determining therapy.

The histopathologic evaluation of IBCs by pathologists has a long and impressive history, and it is likely to remain important for the foreseeable future because it provides so much clinically useful information in a fast and inexpensive manner. However, it also has substantial limitations, including an inability to determine the molecular alterations within tumors that, in addition to stage, are the major reasons for differences in prognosis and response to therapy. These limitations have prompted an enormous amount of research to identify and characterize important molecular features. Estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor (HER)2 status are three important molecular features of IBCs that have been identified during the past 30 years, and their evaluation by pathologists is now mandatory. ER and PR are growth-regulating nuclear transcription factors that are usually measured by immunohistochemistry (IHC), and the amount of protein expressed is directly related to responsiveness to endocrine therapy, which is why they are so important [11,12]. HER2 is a growth factor receptor (among other functions) at the cytoplasmic membrane. The level of membrane protein is highly associated with amplification of this oncogene, and thus HER2 status can be measured either at the protein level by IHC or at the DNA level by assessing gene copy number with assays such as fluorescence *in situ* hybridization [13]. Over-expressed and/or amplified HER2 is a relatively weak prognostic factor in untreated patients, but it is a strong predictive factor for responsiveness to targeted therapies such as trastuzumab [14,15], which is the primary reason for measuring it. However, despite their usefulness, ER, PR, and HER2 are still unable to predict response accurately in many patients, which has motivated additional research to find more powerful factors.

Recent studies, based primarily on newer high-throughput technologies, have demonstrated that IBCs are enormously diverse at the molecular level, which suggests that the assessment of multiple molecular features simultaneously may have more prognostic and predictive power than conventional features. The ultimate goal is 'personalized medicine', based on identifying the key molecular defects in each patient's tumor, permitting the use of safe and effective therapies targeted at the specific defects in each patient. During the past several years, many molecular signatures have been identified by pathologists and other investigators. The first important signature, reported in 2000, identified the so-called intrinsic subtypes of breast cancer (luminal, basal, and HER2) [16,17]. It was based on cDNA microarray analysis of several hundred genes, which was later distilled to IHC analysis of five to ten corresponding proteins by IHC [18,19]. MammaPrint® (Agendia, Huntington Beach, CA, USA) came soon thereafter (2002); it assesses the expression of 70 genes by microarray analysis to identify good and poor risk breast cancers [20,21]. OncotypeDX® (Genomic Health, Inc., Redwood City, CA, USA) followed (2004), which measures 21 genes by quantitative PCR, resulting in a continuous recurrence score that is subdivided into low, intermediate, and high risk groups [22]. Then came the Genomic Grade Index (2006), which evaluates 96 genes using microarrays [23], and the Molecular Grade Index (2007), which appears to be as powerful prognostically as the Genomic Grade Index but relies on only five genes, evaluated using quantitative PCR [24]. These are the most notable signatures, but there are many others.

All of these molecular signatures have provided fascinating new insights into cancer biology, and they have more prognostic and predictive power than conventional factors, but surprisingly they are only incrementally more powerful, and we are still far from realizing the dream of truly personalized medicine. For example, in seminal validation studies of OncotypeDX® [22,25] and MammaPrint® [20,21,26], some conventional prognostic factors (for example, histologic grade and tumor size) remained independently significant in multivariate analyses predicting clinical outcome. Thus, combining conventional factors with molecular signatures appears to provide the most useful information. Despite all of the effort invested in measuring multiple gene products, most molecular signatures are reduced to two or three risk categories (for instance, low, intermediate, and high), which seems somewhat counterproductive. They are currently being used primarily to optimize standard endocrine and cytotoxic therapies, although a few ongoing clinical trials are also using them to identify patients who may not need adjuvant chemotherapy at all, which would be a major contribution [27-29]. It is clearly still early in the game.

A recent meta-analysis of 13 major microarray studies comparing breast cancers ($n = 553$) with normal breast tissue ($n = 79$) identified significant differences in the expression of 1,350 genes, but 90% were identified in only one study [30]. On average, the remaining 138 genes were identified in only two studies, and the highest level of confirmation involved upregulation of a single gene (*GATA3*) in only 6 of the 13 studies. It will be important to understand why there is such poor agreement regarding specific molecular alterations between studies and why molecular signatures provide only modest improvements over conventional prognostic and predictive factors. There are probably many contributing factors, including differences in the quality and design of studies, differences in the technology used and, perhaps most importantly, the existence of truly enormous molecular diversity between and within breast cancers.

Initial results from studies sequencing the genome of human breast cancers suggest that an average tumor contains 20

individual mutated genes [31], which translates to about 10^{89} possible combinations if all of the mutations are random or stochastic, and if all 30,000 genes in our genome are cancer genes. This far exceeds the number of stars in the visible universe (estimated at 10^{21}) [32], and is undoubtedly a colossal overestimate, but it does foretell that the magnitude of molecular diversity in breast cancer may be unexpectedly large. Even if there are only 100 cancer-causing genes and three mutations per tumor (and there is compelling evidence that these are realistic or even low estimates), then there are still 10^6 possible combinations of molecular defects in an average human breast cancer. This is still an impossibly large number to identify and design effective treatments against, so - although we must continue to strive for improvement - our expectations should be realistic and molecular signatures may never be perfect.

A recent poll of breast cancer experts from around the world identified the top priorities for future translational research in breast cancer [33]. The top 10 were essentially all directed at improving therapy in patients with breast cancer. Given the apparently enormous molecular complexity of breast cancer, and the corresponding enormous difficulties in treating it, perhaps prevention should also be high on the list, especially because it may be more achievable. It would certainly be preferable.

Competing interests

The author declares that they have no competing interests.

Acknowledgements

This article has been published as part of *Breast Cancer Research* Volume 10 Supplement 4, 2008: Controversies in Breast Cancer 2008. The full contents of the supplement are available online at http://breast-cancer-research.com/supplements/10/S4

References

1. Page DL, Anderson TJ, Sakamotot G: **Infiltrating carcinoma: major histological types.** In *Diagnostic Histopathology of the Breast*, 1st ed. Edited by Anderson TJ, Page DL. Edinburgh, London, and New York: Churchill Livingstone; 1987:193-235.
2. Ellis IO, Schnitt SJ, Sastre-Garau X, Bussolati G, Tavassoli FA, Eusebi V, Peterse JL, Mukai K, Tabar L, Jacquemier J, *et al.*: **Invasive breast carcinoma.** In *Tumours of the Breast and Female Genital Organs.* Edited by Tavassoli FA, Devilee P. Lyon, France: IARC Press; 2003:13-59.
3. Arpino G, Bardou VJ, Clark GM, Elledge RM: **Infiltrating lobular carcinoma of the breast: tumor characteristics and clinical outcome.** *Breast Cancer Res* 2004, **6:**R149-R156.
4. Diab SG, Clark GM, Osborne CK, Libby A, Allred DC, Elledge RM: **Tumor characteristics and clinical outcome of tubular and mucinous breast carcinomas.** *J Clin Oncol* 1999, **17:**1442-1448.
5. Elston CW, Ellis IO: **Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up.** *Histopathology* 1991, **19:**403-410.
6. Elston CW, Ellis IO: **Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up.** *Histopathology* 2002, **41:**154-161.
7. Rakha EA, El-Sayed ME, Lee AH, Elston CW, Grainge MJ, Hodi Z, Blamey RW, Ellis IO: **Prognostic significance of Nottingham histologic grade in invasive breast carcinoma.** *J Clin Oncol* 2008, **26:**3153-3158.
8. Chang JC, Hilsenbeck SG: **Prognostic and predictive markers**. In *Diseases of the Breast*, 3rd ed. Edited by Harris JA, Lippman ME, Morrow M, Osborne CK. Philadelphia, PA: Lippincott Williams and Wilkins; 2004:675-696.
9. Silverstein MJ, Lagios MD, Groshen S, Waisman JR, Lewinsky BS, Martino S, Gamagami P, Colburn WJ: **The influence of margin width on local control of ductal carcinoma in situ of the breast.** *N Engl J Med* 1999, **340:**1455-1461.
10. Singletary SE, Allred C, Ashley P, Bassett LW, Berry D, Bland KI, Borgen PI, Clark GM, Edge SB, Hayes DF, Hughes LL, Hutter RV, Morrow M, Page DL, Recht A, Theriault RL, Thor A, Weaver DL, Wieand HS, Greene FL: **Staging system for breast cancer: revisions for the 6th edition of the AJCC Cancer Staging Manual.** *Surg Clin North Am* 2003, **83:**803-819.
11. Harvey JM, Clark GM, Osborne CK, Allred DC: **Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer.** *J Clin Oncol* 1999, **17:**1474-1481.
12. Mohsin SK, Weiss H, Havighurst T, Clark GM, Berardo M, Roanh le D, To TV, Qian Z, Love RR, Allred DC: **Progesterone receptor by immunohistochemistry and clinical outcome in breast cancer: a validation study.** *Mod Pathol* 2004, **17:**1545-1554.
13. Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, Dowsett M, Fitzgibbons PL, Hanna WM, Langer A, McShane LM, Paik S, Pegram MD, Perez EA, Press MF, Rhodes A, Sturgeon C, Taube SE, Tubbs R, Vance GH, van de Vijver M, Wheeler TM, Hayes DF; American Society of Clinical Oncology; College of American Pathologists: **American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer.** *J Clin Oncol* 2007, **25:**118-145.
14. Ross JS, Fletcher JA, Bloom KJ, Linette GP, Stec J, Symmans WF, Pusztai L, Horobagyi GN: **Targeted therapy in breast cancer: the HER-2/neu gene and protein.** *Mol Cell Proteomics* 2004, **3:**379-398.
15. Tan-Chiu E, Piccart M: **Moving forward: Herceptin in the adjuvant setting.** *Oncology* 2002, **63(suppl 1):**57-63.
16. Perou CM, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, Williams C, Zhu SX, Lønning PE, Børresen-Dale AL, Brown PO, Botstein D: **Molecular portraits of human breast tumours.** *Nature* 2000, **406:**747-752.
17. Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS, Thorsen T, Quist H, Matese JC, Brown PO, Botstein D, Eystein Lønning P, Børresen-Dale AL: **Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications.** *Proc Natl Acad Sci USA* 2001, **98:**10869-10874.
18. Livasy CA, Karaca G, Nanda R, Tretiakova MS, Olopade OI, Moore DT, Perou CM: **Phenotypic evaluation of the basal-like subtype of invasive breast carcinoma.** *Mod Pathol* 2006, **19:**264-271.
19. Livasy CA, Perou CM, Karaca G, Cowan DW, Maia D, Jackson S, Tse CK, Nyante S, Millikan RC: **Identification of a basal-like subtype of breast ductal carcinoma *in situ*.** *Hum Pathol* 2007, **38:**197-204.
20. van de Vijver MJ, He YD, van't Veer LJ, Dai H, Hart AA, Voskuil DW, Schreiber GJ, Peterse JL, Roberts C, Marton MJ, Parrish M, Atsma D, Witteveen A, Glas A, Delahaye L, van der Velde T, Bartelink H, Rodenhuis S, Rutgers ET, Friend SH, Bernards R: **A gene-expression signature as a predictor of survival in breast cancer.** *N Engl J Med* 2002, **347:**1999-2009.
21. van 't Veer LJ, Dai H, van de Vijver MJ, He YD, Hart AA, Mao M, Peterse HL, van der Kooy K, Marton MJ, Witteveen AT, Schreiber GJ, Kerkhoven RM, Roberts C, Linsley PS, Bernards R, Friend SH: **Gene expression profiling predicts clinical outcome of breast cancer.** *Nature* 2002, **415:**530-536.
22. Paik S, Shak S, Tang G, Kim C, Baker J, Cronin M, Baehner FL, Walker MG, Watson D, Park T, Hiller W, Fisher ER, Wickerham DL, Bryant J, Wolmark N: **A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer.** *N Engl J Med* 2004, **351:**2817-2826.
23. Loi S, Haibe-Kains B, Desmedt C, Lallemand F, Tutt AM, Gillet C, Ellis P, Harris A, Bergh J, Foekens JA, Klijn JG, Larsimont D, Buyse M, Bontempi G, Delorenzi M, Piccart MJ, Sotiriou C: **Definition of clinically distinct molecular subtypes in estrogen receptor-positive breast carcinomas through genomic grade.** *J Clin Oncol* 2007, **25:**1239-1246.

24. Ma XJ, Salunga R, Dahiya S, Wang W, Carney E, Durbecq V, Harris A, Goss P, Sotiriou C, Erlander M, Sgroi D: **A five-gene molecular grade index and HOXB13:IL17BR are complementary prognostic factors in early stage breast cancer.** *Clin Cancer Res* 2008, **14:**2601-2608.
25. Paik S, Tang G, Shak S, Kim C, Baker J, Kim W, Cronin M, Baehner FL, Watson D, Bryant J, Costantino JP, Geyer CE Jr, Wickerham DL, Wolmark N: **Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer.** *J Clin Oncol* 2006, **24:**3726-3734.
26. Eden P, Ritz C, Rose C, Ferno M, Peterson C: **'Good old' clinical markers have similar power in breast cancer prognosis as microarray gene expression profilers.** *Eur J Cancer* 2004, **40:**1837-1841.
27. Bogaerts J, Cardoso F, Buyse M, Braga S, Loi S, Harrison JA, Bines J, Mook S, Decker N, Ravdin P, Therasse P, Rutgers E, van 't Veer LJ, Piccart M; TRANSBIG consortium: **Gene signature evaluation as a prognostic tool: challenges in the design of the MINDACT trial.** *Nat Clin Pract Oncol* 2006, **3:**540-551.
28. Koscielny S: **Critical review of microarray-based prognostic tests and trials in breast cancer.** *Curr Opin Obstet Gynecol* 2008, **20:**47-50.
29. Sparano JA, Paik S: **Development of the 21-gene assay and its application in clinical practice and clinical trials.** *J Clin Oncol* 2008, **26:**721-728.
30. Srour N, Reymond MA, Steinert R: **Lost in translation? A systematic database of gene expression in breast cancer.** *Pathobiology* 2008, **75:**112-118.
31. Sjöblom T, Jones S, Wood LD, Parsons DW, Lin J, Barber TD, Mandelker D, Leary RJ, Ptak J, Silliman N, Szabo S, Buckhaults P, Farrell C, Meeh P, Markowitz SD, Willis J, Dawson D, Willson JK, Gazdar AF, Hartigan J, Wu L, Liu C, Parmigiani G, Park BH, Bachman KE, Papadopoulos N, Vogelstein B, Kinzler KW, Velculescu VE: **The consensus coding sequences of human breast and colorectal cancers.** *Science* 2006, **314:**268-274.
32. NASA Goddard Space Flight Center: **How many stars in the universe?** [http://imagine.gsfc.nasa.gov/docs/ask_astro/answers/970115.html]
33. Dowsett M, Goldhirsch A, Hayes DF, Senn HJ, Wood W, Viale G: **International Web-based consultation on priorities for translational breast cancer research.** *Breast Cancer Res* 2007, **9:**R81.