Pathology (February 2017) **49(2)**, pp. 111–119

CURRENT TOPICS IN BREAST PATHOLOGY

Molecular classification of breast cancer: what the pathologist needs to know

EMAD A. RAKHA AND ANDREW R. GREEN

Academic Pathology, Division of Cancer and Stem Cells, School of Medicine, University of Nottingham and Nottingham University Hospital NHS Trust, Nottingham, UK

Summary

Breast cancer is a heterogeneous disease featuring distinct histological, molecular and clinical phenotypes. Although traditional classification systems utilising clinicopathological and few molecular markers are well established and validated, they remain insufficient to reflect the diverse biological and clinical heterogeneity of breast cancer. Advancements in high-throughput molecular techniques and bioinformatics have contributed to the improved understanding of breast cancer biology, refinement of molecular taxonomies and the development of novel prognostic and predictive molecular assays. Application of such technologies is already underway, and is expected to change the way we manage breast cancer. Despite the enormous amount of work that has been carried out to develop and refine breast cancer molecular prognostic and predictive assays, molecular testing is still in evolution. Pathologists should be aware of the new technology and be ready for the challenge. In this review, we provide an update on the application of molecular techniques with regard to breast cancer diagnosis, prognosis and outcome prediction. The current contribution of emerging technology to our understanding of breast cancer is also highlighted.

Key words: Breast cancer; molecular taxonomy; gene expression profiling; luminal; HER2; basal-like; next generation sequencing; molecular prognostic assays.

Received 16 August, accepted 31 October 2016
Available online 28 December 2016

INTRODUCTION

Historically, breast cancer was classified based on clinicopathological features, mainly tumour stage, and grade. Other morphological features such as histological type, proliferation status and lymphovascular invasion are also recognised as important morphological prognostic variables that reflect tumour biology.[1,2] Over time, knowledge about breast cancer biology has significantly increased and led to the understanding that breast cancer represents a heterogeneous group of tumours and that tumour behaviour and response to therapy is determined by the underlying biological features. The expression of oestrogen receptor (ER), progesterone receptor (PgR) and the human epidermal growth factor receptor 2 (HER2) that were originally identified as predictive of response to systemic therapy are now recognised to be the main determinants of breast cancer biology and can be used to refine breast cancer molecular and prognostic taxonomy. More recently, molecular data arising from a variety of high throughput techniques have been used to refine breast cancer stratification and develop prognostic and predictive classification with the aim of individualised therapy.

Although molecular taxonomy of breast cancer based on gene expression profiling, proteomics, DNA copy number alteration and chromosomal changes, mutation status, methylation and microRNAs has been expanding for many years and has increased our knowledge of breast cancer biology, its clinical application remains limited. The introduction of next generation sequencing (NGS) or massively parallel sequencing[3] appears to have opened new avenues for decoding breast cancer molecular complexity, refining molecular classification and identifying new therapeutic targets. These molecular techniques hold promise for improving diagnosis, prediction of outcome and behaviour, and in aiding selection of therapies for individual patients.[4] However, their clinical utility is still under investigation.[5]

Pathologists are currently using conventional and novel molecular techniques in routine practice to help diagnosis of morphologically challenging entities, to assess the expression of hormone receptors and HER2 status on every breast cancer and help oncologists to refine the prognostic stratification of breast cancer and complement the morphological variables with molecular biomarkers. Although immunohistochemistry remains the most commonly used conventional molecular technique, other techniques are increasingly used in routine practice including *in situ* hybridisation (ISH), reverse transcription polymerase chain reaction (RT-PCR), and in some centres NGS and expression microarrays. In the research setting, several other molecular techniques are used including comparative genomic hybridisation (CGH), expanded immunohistochemistry with tissue microarrays and proteomics. In this review, the main applications of molecular techniques on breast cancer are highlighted with emphasis on the practical applications which can be generally divided into three main categories; diagnosis, molecular prognostic and predictive taxonomies.

USING MOLECULAR BIOMARKERS IN THE DIAGNOSIS OF BREAST LESIONS

In addition to prognosis and treatment response prediction, molecular biomarkers are frequently used in the diagnosis of challenging breast lesions; to differentiate between benign and malignant entities, *in situ* and invasive tumours, subtyping of certain lesions and determination of the tissue of

Print ISSN 0031-3025/Online ISSN 1465-3931
DOI: http://dx.doi.org/10.1016/j.pathol.2016.10.012

origin of less differentiated malignant tumours. The most frequent technique utilised in this aspect is immunohistochemistry often using a panel of biomarkers.[6,7] Immunohistochemistry plays a useful role in diagnosing spindle cell lesions, identifying myoepithelial cells, differentiating between ductal and lobular phenotype and between hyperplastic epithelial proliferative process and neoplastic clonal epithelial proliferation, and in the classification of papillary lesions. Cytokeratins can be used to detect small nodal metastases or subtle invasive carcinomas such as invasive lobular carcinomas. Immunohistochemistry also is helpful in recognising metastases to the breast and mammary carcinomas metastasising to extramammary tissues. Different antibodies are useful for different tumours: PAX8 and WT1 for ovarian carcinoma; TTF1 for thyroid and pulmonary adenocarcinoma; melan-A, HMB45 and S100 for melanoma; and lymphoid markers for lymphoma. Specific genetic translocations are also helpful for diagnosis of certain breast lesions (see below) and for exclusion of specific soft tissue tumours when identified on a biopsy as a component of other mammary-specific lesions; for instance pure stromal component of a malignant phyllodes tumour to be differentiated from other soft tissue sarcomas that may have different management strategies.[8]

Companion diagnostics in breast cancer

The ability to predict an individual's response to a specific therapy is the main aim in modern precision medicine. A molecular diagnostic tool in the field of cancer therapy was first used in the 1970s to predict response of breast cancer to the selective ER modulator tamoxifen, based on the expression of ER.[9] Currently, several targeted cancer therapies are utilised in standard oncological care and this field is expanding. As a result, the concept of 'companion diagnostics' has emerged which can be defined as a diagnostic test used as a companion to a therapeutic drug to determine its applicability to a specific patient. Currently, the US Food and Drug Administration (FDA)-approved companion diagnostics are utilised in breast cancer tests for the presence of HER2 protein overexpression or gene amplification. Despite not being considered companion diagnostics by the FDA, ER and PgR testing are mandatory for effective hormone therapy decision making and can be considered as companion diagnostics in breast cancer. Although prognostic multigene assays are not companion diagnostics *per se*, as they are not linked to a particular drug, they can result in changes in clinical decisions and treatment course based on their outcome predictions (Table 1).

Hormone receptor testing

Hormone receptor status is determined by the tumour cells' expression of nuclear receptors for oestrogen (ER) and progesterone (PgR). Biochemical ligand-binding assays were initially used to detect ER and PgR, but they required fresh tissue and were technically challenging and therefore immunohistochemical assays have become routine. Different scoring methods are in use for determining the level of expression but the most widely used systems are the Allred scoring and the histochemical score (H-score) methods which both assess the proportion and intensity of staining that are summed to give an overall score. However, the currently agreed cut-off of positivity of ER and PgR for management purpose relies on proportion scoring and is 1%.[10] Patients with breast cancer showing any nuclear expression of hormone receptor in invasive tumour cells above the cut-off are likely to respond to hormone therapy and therefore are potential candidates for this therapy. However, for a diagnostic purpose, i.e., determination of a mammary origin of a metastatic carcinoma, a more stringent definition of positivity is often used based on the pathologist's discretion. Although current guidelines indicate that immunohistochemistry is used for determination of hormone receptor status[10] in breast cancer, ER and PgR are component genes of some multigene assays including Oncotype DX. Information regarding hormone receptor status using these assays can be used as an additional quality measure for assessment methods. Discrepancy of results should trigger a reflex test.

HER2 testing

HER2 is overexpressed in 12–20% of breast cancers most often because of *HER*2 gene amplification. Because of its predictive value, guideline recommendations for its assessment[11] and updated versions[12,13] have been published to provide guidance on HER2 testing in breast cancer. Key aspects of these guidelines include a recommendation that all breast cancer be tested for HER2 using immunohistochemistry and subsequently with ISH in borderline positive immunohistochemistry cases using a validated test. It should be recognised that both immunohistochemistry and ISH represent an attempt to convert a continuous biological variable into a dichotomous category; borderline or equivocal cases exist and a reflex test is recommended to reduce the proportion of these cases. The use of the updated definition of positivity of HER2 has reduced the proportion of these borderline cases.[12,13]

Ki67 proliferation index

The Ki67 proliferation index has been investigated as a breast cancer prognostic and predictive factor in various settings.[14] Ki67 is assessed in routine practice using immunohistochemistry; however, its analytic validity remains a matter of debate and formal inter-and intra-laboratory standardisation hampers its use in routine practice for management decisions.[15] Ki67 can be used in routine practice to (1) determine the proliferation status in poorly fixed specimens, or (2) stratify grade 2 tumours into two prognostically distinct classes[16] akin to the molecular grade index.[17] Ki67 is also used as a component of some prognostic tools;[18] however, the published 2016 American Society of Clinical Oncology (ASCO) clinical practice guideline on breast cancer[15] recommends that Ki67 labelling index determined by immunohistochemistry should not be used to guide choice on adjuvant chemotherapy with intermediate quality of evidence base and moderate strength of recommendation.

Genetic tests and diagnosis

Some diagnostic microarray-based gene expression tests were developed for identification of cancer tissue of origin. These include the Pathwork Tissue of Origin Test that was developed using a 2000-gene classification model for identification of tumour tissue of origin with an overall accuracy