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Molecular signatures in breast cancer

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ABSTRACT

The use of molecular signatures to add value to standard clinical and pathological parameters has impacted clinical practice in many cancer types, but perhaps most notably in the breast cancer field. This is, in part, due to the considerable complexity of the disease at the clinical, morphological and molecular levels. The adoption of molecular profiling of DNA, RNA and protein continues to reveal important differences in the intrinsic biology between molecular subtypes and has begun to impact the way patients are managed. Several bioinformatic tools have been developed using DNA or RNA-based signatures to stratify the disease into biologically and/or clinically meaningful subgroups. Here, we review the approaches that have been used to develop gene expression signatures into currently available diagnostic assays (e.g., OncotypeDX® and Mammaprint®), plus we describe the latest work on genome sequencing, the methodologies used in the discovery process of mutational signatures, and the potential of these signatures to impact the clinic.

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1. Introduction

Breast cancer is an extremely diverse and complex disease, and is one of the leading causes of death amongst women. There is marked tumour heterogeneity between patients, with specific breast cancer subtypes associated with differing prognoses. Differentiating breast tumour types is a key component of the clinical management process to ensure patients are given the most appropriate type of therapy. In this review we briefly illustrate the best practices in tumour classification from a pathology context, including currently utilised predictive and prognostic biomarkers. We will then highlight the advances made in the molecular arena, which have shed light onto the differences in intrinsic biology between subtypes of the disease and how these have been developed into molecular signatures with clinical utility.

2. Pathological classification of disease

As part of the diagnostic process, a pathologist examines a tissue biopsy or resection specimen. A diagnosis will be made based on key parameters, which include histological type, tumour grade, and tumour stage using criteria outlined by the World Health Organisation (WHO) [1]. There are at least 20 different histological subtypes of breast cancer, which display differences in morphology and growth pattern. The most common is Invasive Carcinoma of No

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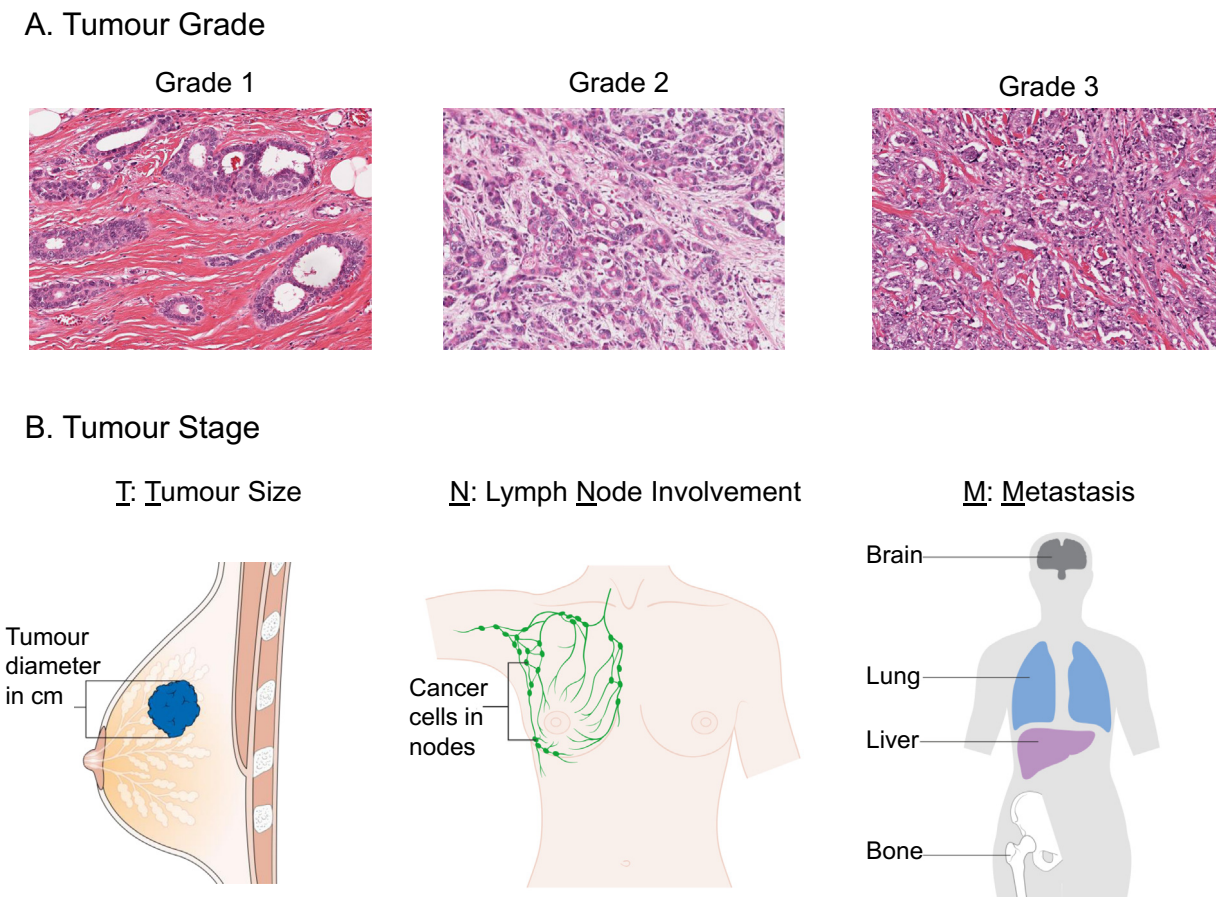

Fig. 1. Conceptual overview of tumour grading and staging. (A) Grading is a measure of tumour cell differentiation, relative to normal cells. Representative histological images of tumours of grade 1, 2 and 3 (see text) are shown, as stained with haemotoxylin and eosin. (B) A pictorial representation of the tumour staging system. The different components of the TNM staging system are highlighted: T represents tumour size and extent of local invasion; N is a measure of tumour spread to regional lymph nodes (N); and M is a clinical assessment to record the extent of cancer metastasis to distant sites, such as the lung, liver, brain and bone.

Special Type (IC-NST; previously called Invasive Ductal Carcinoma (IDC) accounting for 80% of all cases [1]). The remaining are classified as 'special' histological types in that they exhibit unique patterns of growth. Invasive Lobular Carcinoma (ILC) is the most common special type, accounting for between 5% and 15% of cases, with others including medullary, metaplastic, tubular and mucinous subtypes which all have distinctive growth patterns and variable prognoses.

Several diagnostic systems give insight into the behaviour of a tumour, including tumour grade and stage (Fig. 1). *Histological grade* describes how abnormal the tumour appears relative to normal tissue, as a measure of tumour cell differentiation. Grading of breast cancer is performed using the Nottingham grading system [2,3]. This is a three-tiered scoring system, assessing the number of visible mitoses, the presence of tumour cells creating tubule structures and evidence of nuclear pleomorphism. The number of mitoses acts as a surrogate for growth rate, while tubule formation is a measure of whether the tumour tissue resembles normal-like ductal structures. Pleomorphism is a measure of the size, shape and variability of tumour nuclei. The prognostic value of the grading system in predicting behaviour and patient outcome has long been established [4,5]. A histological grade 1, well-differentiated tumour is associated with a significantly better prognosis compared to a grade 3, poorly differentiated tumour.

Tumour Stage is a measure of how far the tumour has spread, and so is also a highly prognostic tool. The American Joint Committee on Cancer (AJCC) TNM staging system is used for most organ systems, including breast. T is a measure of the tumour size (<2 cm, between 2–5 cm and >5 cm) and whether the tumour has invaded the chest wall; N refers to the number of lymph nodes that show evidence of cancer (0, 1–3, 4–9, >10) and the position of the node in the nodal system; and M is a measure of distant metastasis, i.e., if there is a sign of cancer spread beyond the site of the primary tumour.

3. Clinical biomarkers in breast cancer

Biomarkers play important roles in diagnosis and prediction of prognosis, and may also represent therapeutic targets. Key breast cancer biomarkers include Oestrogen Receptor (ER), Progesterone Receptor (PR) and Human Epidermal Growth factor Receptor 2 (HER2/ERBB2); these markers have been reviewed extensively and their expression correlates with differences in tumour behaviour and patient outcome and the potential response to targeted endocrine therapy or HER2 therapy [6]. The protein expression levels of ER, PR and HER2 are assessed using immunohistochemistry and, in addition, the *ERBB2* gene copy number is also quantified using in situ hybridization [6]. If a breast cancer is positive for either ER or PR the breast cancer is termed as Hormone Receptor positive (HR+) and these patients will likely receive endocrine therapy, while patients with HER2+ breast cancers will receive trastuzumab or other HER2 targeted therapies. According to the Surveillance, Epidemiology, and End Results (SEER) survey that