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From morphologic to molecular: established and emerging molecular diagnostics for breast carcinoma

Research Paper

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Diagnostics in the field of breast carcinoma are constantly evolving. The recent wave of molecular methodologies, both microscope and non-microscope based, have opened new ways to gain insight into this disease process and have moved clinical diagnostics closer to a 'personalized medicine' approach. In this review we highlight some of the advancements that laboratory medicine technology is making toward guiding the diagnosis, prognosis, and therapy selection for patients affected by breast carcinoma. The content of the article is largely structured by methodology, with a distinct emphasis on both microscope based and non-microscope based diagnostic formats. Where possible, we have attempted to emphasize the potential benefits as well as limitations to each of these technologies. Successful molecular diagnostics, applied in concert within the morphologic context of a patient's tumor, are what will lay the foundation for personalized therapy and allow a more sophisticated approach to clinical trial stratification. The future of breast cancer diagnostics looks challenging, but it is also a field of great opportunity. Never before have there been such a plethora of new tools available for disease investigation or candidate therapy selection.

Introduction

The developing concept of personalized medicine and targeted therapy selection is still relatively new to pathology and laboratory medicine. The challenges associated with this approach are changing the traditionally accepted role for pathology and are forcing a redesign of the conventional microscope-centric pathology service. To address this major revolution in breast cancer diagnosis, new non-microscope based molecular assays are gaining acceptance and new ways of applying molecular methodologies to conventional practice are being established. The widespread use of non-microscope based assays began following the first publications of gene-expression arrays performed on breast tumors. These initial gene-expression assays showed that similar morphologic types of breast cancer can be segregated based on unique mRNA expression patterns [1]. Following this discovery, commercial assays were generated that utilized unique mRNA expression patterns to calculate risk in individual patients for likelihood of recurrence of disease. These assays marked a leap forward from traditional pathology practice and are aiding the transition toward personalized medicine. In addition to gene-expression arrays, newer applications of next-generation sequencing and highly multiplexed Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) offer the potential to further advance the field of breast cancer diagnostics and usher in a true era of personalized care.

Improvements in investigational methodologies and technologies have led to refining our understanding of tumor biology. For example, in breast cancer, the discovery and association of hormone receptor biology (estrogen and progesterone) and roles of signaling pathways linked to cell surface receptor activation (HER2) revolutionized breast cancer diagnostics. Application of this understanding of tumor biology has helped achieve a semi-individualized approach to breast cancer diagnostics and management. Current pathologists and investigators continue to adapt to advancing new technology and materials. For example, newer

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biotechnologies have yielded assays that look beyond simple single gene analysis. Multiplex testing and rapid techniques for whole genome, transcriptome, or proteome screening are starting to migrate from a pure research setting into an environment of clinical assay development [2]. Utilization of these advanced techniques will further aid in obtaining a personalized approach to breast cancer diagnostics. Applying these new molecular assays in concert with the morphologic characterization of a tumor should help identify specific prognostic markers that can reliably predict outcomes while maximizing therapeutic benefit and minimizing toxicity. Perhaps with wide stream application of molecular assays, we will finally be able to deliver an individualized or 'personalized' approach to patient therapy. Therefore, while the future of breast cancer diagnostics still has many challenges to face, it is a field with great opportunity and one that will be driven by a multitude of new tools available for disease investigation or candidate therapy selection.

In this review we highlight some of the advances that laboratory medicine technology is making toward guiding the diagnosis, prognosis, and therapy selection for patients affected by breast carcinoma. The content of the article is largely arranged by diagnostic format with two major areas of focus: microscope based methodologies and non-microscope based molecular methodologies. Where possible, we have attempted to emphasize the potential benefits and limitations to each of these technologies.

The fundamental diagnostic approach

Morphologic characterization

The art of determining the diagnosis, prognosis and predictive outcome of cancer, based on features seen in excised tissue specimens, is a process that is well established. Since the advent of microscopy, early histopathologists have been examining tissue, both normal and diseased, in pursuit of the ability to make clinically significant diagnostic predictions [3–5]. Histology offered early histopathologists a way to gain insight beyond what was possible by studying presenting symptoms and medical history. The pioneers who first utilized histology as a tool for disease discovery and characterization overcame many challenges both technically and philosophically. One significant obstacle included the poor optical quality of early microscopes that produced significant artifacts in the interpretation and published characterization of diseases [6]. The advent and standard application of an achromatic objective circa 1824 yielded the first significant improvements in image quality and reproducibility. The new clarity of observations drew into question published and accepted theories of previously reported morphologic characterizations of disease [3,4]. This improvement and standardization in image quality paired with advances in tissue fixation, tissue sectioning, and use of stains including hematoxylin and eosin were paramount in founding and advancing the role of morphology in diagnosis of disease. Therefore, the technical innovations in microscopy, materials, and the physical sciences established the field of pathology as it is known today and ultimately led to the classification of diseases based on a thorough understanding of morphology.

In regards to tumors of the breast, the objective has long been to accurately predict the pathologic and clinical course of these lesions [7]. While early classification of breast tumors took a simplistic, single tumor/single morphology based approach, subsequent work in the morphologic classification of breast tumors has been particularly challenging due to the recognized wide spectrum of histologic findings and the increasing number of described pre-cancerous lesions [8]. Morphologic findings used to categorize breast carcinomas into meaningful subgroups include histologic grade and histologic type [9]. Histologic grade is an assignment placed upon the tumor indicating the degree of differentiation and includes parameters such as tubule formation, nuclear pleomorphism and mitotic activity while histologic type specifically refers to the architectural growth pattern of the lesion [10,11].

Breast carcinoma, like many other neoplasms, is a heterogeneous disease [12], with variable prognosis and survival rates according to a multitude of characteristics such as age, tumor stage, and the inherent biologic properties of the lesion. In breast tissue, like other solid organs, pathologists have endeavored to characterize and categorize tumors into clinically relevant entities based on the physical properties of the neoplastic cells and the histological degree of differentiation. In breast, this effort has been beset by high inter-observer variability and divergence [13,14], which ultimately led to the employment of standardized classifications and grading algorithms, such as the currently used 'Nottingham combined histologic grade' [15]. While a pure morphologic classification of breast tumors is an attractive starting point for tumor characterization, tumors of mixed histologic type are commonly encountered.

Early taxonomies, such as those based upon the work of Azzopardi, Page, and Anderson, had a strong influence on the scheme that comprises the World Health Organization (WHO) classification of breast tumors. The WHO classification scheme in use currently recognizes 18 histologic types of invasive breast carcinoma (Table 1) [16]. The majority of these tumors belong to the category of invasive ductal carcinoma, not otherwise specified (NOS) with approximately 25% representing special histologic types with unique morphologic features [17]. Prevalence of the different breast cancer morphologic patterns is shown in Table 2 [9,16,18]. Different histologic types of breast cancer were originally presumed to derive from distinct compartments of the breast, however, seminal work by Wellings *et al*. demonstrated that the majority of invasive carcinomas of the breast, and the *in situ* lesions from which they arise, all display histogenesis from the terminal duct lobular unit regardless of the special type [19,20]. Therefore, use of the term 'ductal' and 'lobular' carcinoma does not imply origin from the respective ductal and lobular structures within the breast; rather, these terms simply define the characteristic morphologic features of these entities. The reader is referred to excellent recent reviews of special histologic types of breast carcinoma for additional information on these topics [9,17,21].

The task of making accurate diagnostic and prognostic conclusions based upon morphologic findings is complicated by the observation that approximately one-third of invasive duct carcinomas display mixed morphologic features [22]. On the basis of the recommendations of the WHO classification system, invasive ductal tumors are classified as invasive ductal carcinoma, NOS if this pattern occurs in greater than 50% of the tumor. However, if the invasive ductal carcinoma NOS pattern comprises between 10