Gene profiling in breast cancer

Inge Peerlink · Kanchan Kaur · Hemant Singhal

Received: 16 July 2009
Accepted: 9 September 2009
© Indian Association of Surgical Oncology 2010

Abstract
Breast cancer is a heterogeneous disease which shows a great variation in presentation and response to treatment. Currently, the most commonly used prognostic criteria are patient age, tumor size, lymph node status, tumor grade and hormone receptor status. These are however not very accurate. This is partly explained by the fact that they do not demonstrate the inherent genetic variability of breast cancer, which determines the aggressive nature and metastatic potential of the disease. Recent advances in molecular biology have demonstrated that breast cancer is not a single disease. The new diagnostic and prognostic tests based on molecular biology methods have helped identify molecular subtypes of breast cancer that are sensitive to chemotherapy and others that are resistant. This could provide valuable critical information and predict which patients would really benefit from chemo and/or hormonal therapy. Molecular biology will become increasingly important in clinical decision making and as the understanding of molecular processes within cancer cells grow, new targets for therapy will be discovered.

Keywords  Breast cancer · Cancer cells · Microarray chips

Introduction
Breast cancer is a heterogeneous disease which shows a great variation in presentation and response to treatment. Clinicians have constantly explored indices which would aid in assessing the prognosis and at the same time help in tailoring the most appropriate and effective adjuvant treatment in patients with breast cancer. Currently, the most commonly used prognostic criteria are patient age, tumor size, lymph node status, tumor grade and hormone receptor status (Adjuvant Online, St. Gallen criteria). These are however not very accurate. This is partly explained by the fact that they do not demonstrate the inherent genetic variability of breast cancer which determines the aggressive nature and metastatic potential of the disease. More recently, the development of multigene assays or microarray profiling where fragments of DNA are probed for multiple known genes has lead to the manufacturing of microarray chips as diagnostic and prognostic probes. These chips can be used to screen large numbers of normal and cancerous tissues for several individual genes at the same time and determine differences in gene expression. These differences, once identified, can lead to a better understanding of carcinogenesis and to the discovery of new drug targets and prognostic and predictive markers, which would identify patients at risk for relapse or patients that are likely to respond to adjuvant therapy.

Treatment decisions in breast cancer
Breast cancer patients can be treated with surgery alone or with the addition of systemic medical treatment. Prognostic indicators are necessary to determine in which patients the benefits of adjuvant therapy outweigh the risks. Traditionally the choice of treatment is based on clinical, histological and biological parameters. The Nottingham Prognostic Index (NPI) is based on tumor size,
tumor grade and lymph node status. The National Institute of Health (USA) and St. Gallen consensus criteria (EU) use age, tumor size, histological grade, lymphovascular invasion, lymph node status and ER and PR status. Following these criteria up to 90% of young women with node negative breast cancer would be eligible for adjuvant chemo and/or hormonal therapy. A large number of these patients would not have developed distant metastasis without adjuvant therapy and have been ‘over’-treated and unnecessarily been subjected to potential side-effects of systemic therapy. These prognostic indices do not reliably predict true outcome of disease and result in over treatment of many patients. There is a need for more precise stratification of patients into responders and non-responders to therapeutic agents and into good and bad prognostic outcome. The development of novel molecular tools like PCR and microarrays has lead to a better understanding of the molecular differences between histologically similar tumors that would explain different clinical behavior. A molecular classification of breast cancer based on gene expression profiles was proposed. Four subgroups were identified which were shown to have different prognoses: luminal, basal-like, normal-like and erbB2 positive. Basal-like tumors were mostly ER negative, erbB2 positive tumors were mostly Her2 (human epidermal growth factor receptor)-positive and ER negative and luminal-like tumors were ER positive. These various molecular subclasses have different long-term survival rates and different sensitivities to preoperative chemotherapy. There is a need for standardized molecular class predictors that can aid in the decision process to determine which patients would benefit the most from systemic treatment.

**Multigene predictor tests**

In the last few years a number of multigene prognostic and predictive tests have become available to clinicians. A complete review of all the tests that are currently being developed has been written by Ross et al. in *The Oncologist*. Assays can be divided into categories according to the molecular technique that is being used (Table 1).

**Immunohistochemistry-based multigene assays**

Immunohistochemistry (IHC) is a technique that uses antibodies against specific cellular markers. A reporter probe is attached to the antibody that is either an enzyme that can catalyze a color producing reaction or a fluorophore that can emit light when excited. Slides are scored using digital image analysis. IHC has been used for the determination of ER and PR status and cell proliferation status (ki67) and has been a very important development in the management of breast cancer.

**ProEx™** uses five antibodies and an image analysis slide scoring system. The five antibodies used are E2F transcription factor; p21 Ras associated protein, Src kinase protein, secretory leucocyte peptidase inhibitor and proteasome core subunit beta. Over expression of two or more of these genes has been associated with disease relapse in both node negative and node positive patients.

**Mammostrat®** uses a panel of five antibodies: p53 tumor suppressor protein, N-myc downstream regulated gene 1, carcinoembryonic antigen cell adhesion molecule 5, solute carrier family 7 cationic amino acid transporter, y+ system member 5 and HpaII tiny fragments locus 9C with routine slide scoring to score ER positive, lymph node negative tumors into low-, moderate- and high-risk of recurrence if treated with tamoxifen alone.

**Fluorescent in situ hybridization-based multigene assays**

Fluorescent in situ hybridisation (FISH) uses probes that are fragments of DNA, which are tagged with a fluorophore and bind to those parts of the chromosome with which they show a similarity in sequence. FISH has been used routinely in breast cancer for determination of the copy number of the Her-2 gene which helps in the selection of patients that would benefit from anti-Her-2 targeted therapy.

**eXagenBC™** uses fluorescently labelled DNA probes to determine the copy number of three genes for ER positive tumors: cytochrome p450 family 24, programmed cell death 6 interacting protein and baculoviral IAP-repeat containing 5 (survivin) and three genes for ER negative tumors: nuclear receptor subfamily 1, group D, member 1, SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily e, member 1 and BIRC5. Prognostic index is determined using an algorithm. Recurrence rates were significantly higher among high risk patients.

The **oncotypeDX™** RS (recurrence score) assay (www.oncotypedx.com) is a 21-gene assay that determines a 10-year risk for distance recurrence in women with ER positive, lymph node negative tumors and postmenopausal women with node positive, ER positive breast cancer. The likelihood of recurrence or distant metastasis increases continuously with an increase in RS. The score was validated on 668 patients with ER positive, node