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Breast Cancer Genomics: Challenges in Interpretation and Application

CATHY M. KELLY,[a] W. FRASER SYMMANS,[b] ELENI ANDREOPOULOU,[c] GIAMPAOLO BIANCHINI[d]

[a]Mater Misericordiae University Hospital, Dublin, Ireland; [b]Department of Pathology, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA; [c]Department of Medical Oncology, Montefiore Medical Center, The University Hospital for Albert Einstein College of Medicine, New York, New York, USA; [d]San Raffaele Cancer Center, Milan, Italy

Disclosures of potential conflicts of interest may be found at the end of this article.

Section Editors' Note: 'If you design a perfect key for a door, don't be surprised if it will not work in any other door'…This article highlights the problems of overfitting and multiple testing in genomics and the resultant exponential increase in statistical interactions, which were recently discussed at an International Breast Cancer Research Meeting held in Dublin (September 2012).

INTRODUCTION

Genomic DNA copy number arrays, exome sequencing, messenger RNA arrays, microRNA sequencing and reverse-phase protein arrays are some of the many new high-throughput technologies facilitating a deeper understanding of the molecular characteristics of breast cancers. However, with these technologies come new statistical challenges, in particular the ubiquitous problem of statistical interaction.

A statistical interaction occurs when the effect of one independent variable(s) on the dependent variable depends on the value of another variable. In other words, the relationship between two genes or proteins is modified by the value of a third gene or protein. In a dataset containing thousands of genes, the more analyses performed to identify genes or groups of genes to predict the risk of disease relapse or response to a specific therapy, the greater the chance of introducing statistical interactions that may bias the results and their interpretation. Many statistical methods have been developed to reduce false positive and false negative findings, but some assumptions persist. A common assumption is "additivity", where we assume that the effect of one independent variable(s) on the dependent variable does not depend on the value of another independent variable(s).

BREAST CANCER AND THE INFLUENCE OF TIME-DEPENDENT VARIABLES

Another type of interaction of importance in endocrine-treated breast cancer is the time varying effects of biomarkers of proliferation and estrogen-related gene activity on outcome. Survival analyses using the Cox proportional hazards model are based on the assumption that the effects of different variables on survival are additive in a particular scale and the effects of different variables on survival are constant over time. Examples of two variables obeying this rule are tumour size (T stage) and nodal status (N stage). Both are prognostic, and the hazard ratios for both markers are constant over time by the test for proportional hazards in tamoxifen-treated patients.

There are a number of multigene tests that are employed to assess prognosis in early stage breast cancer [1–3]. Tests such as the 21-gene recurrence score assay Onco*type* DX (Genomic Health, Redwood City, CA, http://www.oncotypedx.com/) are used by clinicians to assist in decision making regarding the requirement for chemotherapy in addition to endocrine therapy for lymph node-negative, estrogen receptor (ER)-positive, human epidermal growth factor-2 (HER2) receptor-negative breast cancer [3, 4] However a violation of the proportional hazards assumption was reported for both the Oncotype DX recurrence score and for the PAM50 intrinsic subtype classifier [5, 6]. These tests were prognostic in the first 5 years but not in the time period from 5 to 10 years; however, the initial strong effect persisted over the entire period. Bianchini et al. recently examined whether a different combination of markers of proliferation (mitotic kinase score [MKS]) and estrogen-related (estrogen-related score [ERS]) genes could predict the time course of relapse in women with ER-positive breast cancer treated with tamoxifen [7]. Four biomarker groups were examined (i.e., high MKS and high ERS, high MKS and low ERS, low MKS and high ERS, and low MKS and high ERS). Patterns of distant recurrence were assessed in clinically relevant time intervals; under 5 years of tamoxifen (0 to 5 years), upfront therapy (0 to 2.5 years), switch to an aromatase inhibitor after 2.5 years (2.5 to 5 years), and extended therapy (5 to 10 years).

The results were interesting and clinically relevant. The majority of the relapses in the "upfront" 2.5-year period occurred in the high proliferation/low estrogen-related score group. The authors concluded that these cancers are enriched in tumours intrinsically resistant to tamoxifen and, possibly, aromatase inhibitors. Individuals with breast cancers with high proliferation/high estrogen-related gene expression were at higher risk of recurrences beyond 5 years of tamoxifen. This group may benefit from extended therapy beyond 5 years of tamoxifen. The group with low proliferation and low estrogen-related gene expression were also at high risk of relapse after 5 years of tamoxifen, and possibly, aromatase inhibitors or new approaches (e.g.,

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Correspondence: Cathy M. Kelly, M.D., Department of Medical Oncology, Mater Misericordiae University Hospital, Eccles St, Dublin 7. Telephone: 01 803 4447; E-Mail: ckelly@mater.ie Received December 7, 2012; accepted for publication April 2, 2013. http://dx.doi.org/10.1634/theoncologist.2013-0116

mTOR inhibitors, CDK inhibitors) will benefit these patients.

Chemosensitivity, Endocrine Sensitivity, and the Concept of Sequential Synergy

The concept of chemotherapy sensitivity and endocrine sensitivity as mutually exclusive within ER-positive breast cancer was challenged by the results of a recent study [8]. The sensitivity to endocrine therapy (SET) index measures the transcriptional activity of 165 ER-related genes and predicts the risk of distant relapse in tamoxifen-treated patients. Breast cancers are classified into low, intermediate, and high, depending on their predicted sensitivity to endocrine therapy. In their initial study, the authors found that lymph node status was independently prognostic in tamoxifen-treated patients.

Two recent studies showed that patients with node-positive and ER-positive breast cancer had clinically significant (>10%) risk of relapse for any 21-gene recurrence score class [5, 9]. In one study, the low or intermediate recurrence score identified a subset of patients for whom chemotherapy offered no significant benefit over tamoxifen alone, but the recurrence score failed to identify any subset with excellent survival from either treatment arm [5]. Similarly, the SET index failed to identify a group of patients with lymph node-positive, ER-positive breast cancer that had a risk of distant recurrence of <20% from adjuvant endocrine therapy alone [8]. So what do we do with these patients? The SET index did identify patients with high or intermediate SET index with an excellent survival with an anthracycline-taxane based chemotherapy (T-FAC) regimen followed by endocrine therapy. Interestingly, those patients with a high SET index had an excellent outcome if they had some response to prior neoadjuvant chemotherapy (T-FAC). However, the prognosis of those with chemoresistant disease (high residual cancer burden) remained poor, irrespective of endocrine sensitivity (SET). The authors concluded that partial or better response to chemotherapy in a tumour with intrinsic endocrine sensitivity can facilitate further benefit from adjuvant endocrine therapy, introducing the concept of sequential synergy [8].

In conclusion, this era of personalised medicine has led to the application of a number of multigene approaches to help predict therapeutic response and risk of recurrence in breast cancer. Although these approaches are proving useful to the practicing breast cancer physician, it is important to ensure that appropriate statistical rigour is applied to ensure correct interpretation of gene expression profile results.

Disclosures

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