Meeting report

International Agency for Research on Cancer Workshop on 'Expression array analyses in breast cancer taxonomy'

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Abstract

In May 2006, a workshop on Expression array analyses in breast cancer taxonomy was held at the International Agency for Research on Cancer (IARC). The workshop covered an array of topics from the validity of the currently defined breast tumor subtypes and other expression profile-based signatures to the technical limitations of expression analysis and the types of platforms on which these omics results will eventually reach clinical practice. Overall, the workshop participants believed firmly that tumor taxonomy is likely to yield improved prognostic and predictive markers. Even so, further standardization and validation are required before clinical trials are set in motion.

Introduction

In December 2005, participants at an International Agency for Research on Cancer (IARC) working group on the molecular pathology of breast cancer spent a considerable time discussing the potential role of expression profiling in tumor classification. Those discussions led to a follow-up meeting, 'Expression array analyses in breast cancer taxonomy', held at IARC in May 2006; the participant list is given at the end of this report. Presentations and discussions covered a wide variety of topics within the general subject and included presentation of results that have not yet been published. There were areas of near total agreement and other areas where views were quite divergent. In this report we summarize six areas of discussion, highlighting areas of agreement and also pointing out key questions to be addressed in future studies.

Detection of breast tumor subtypes

Breast cancer subtypes can clearly be identified by means of expression array analysis [1-3]. With the nearest centroid method, subtypes defined in one sample series can be detected in other independent sample series, and some of the subtypes can be recapitulated by using immunohistochemical or DNA copy-number-based classifiers [2,4-6]. Among the five subtypes originally defined by Sørlie and colleagues [1,2], the basal and luminal-A subtypes are reasonably robust; however, neither these two nor other less robust subtype classifiers are necessarily completely optimized.

Other profile-based signatures

Subtype classification as exemplified by the five-type classifier does not sample all of the signatures available in a large-scale expression profiling analysis. For example, wound healing, proliferation, and tumor grade signatures can be discerned within the data, and these are somewhat independent of the subtypes [7-9]. At a deeper level of analysis, mechanistically defined signatures such as the estrogen receptor (ER)-positive signature and the wound-healing signature can be understood as regulatory modules, often containing biologically informative substructure, whose expression is controlled by the ER, c-Myc, p53, and other key regulatory proteins [10-13].

Prognostic implications

Beyond subtype classification, expression profile signatures clearly have prognostic implications [3,10,14-17]. For example, several research groups have derived signatures that are intended to predict survival and, eventually, whether patients will benefit from adjuvant therapy. Applied to independent data sets, some of these signatures have demonstrated significant power to predict clinical outcome, whereas others have not [17]. At a superficial level, there is a contradiction between efforts to develop breast cancer subtype classifiers and efforts to develop breast cancer prognostic signatures: if breast cancer can indeed be resolved into several independent subtypes, then there is little reason to expect that the same prognostic signature should apply to each subtype [18]. Thus, signatures developed from

ER = estrogen receptor; IARC = International Agency for Research on Cancer; IHC = immunohistochemistry.

patient series that have a particular subtype distribution may lose some of their efficacy when applied to patient series that have a different subtype distribution. In contrast, signatures based on biological phenomena such as wound healing, proliferation index, or p53 status may transcend subtype and even find application to other tumor sites.

Application to genetic and molecular epidemiology

Patients who have an elevated probability of carrying germline susceptibility gene mutations are sometimes detectable [19,20]. In the practise of clinical cancer genetics, mutation screening of high-risk susceptibility genes such as *BRCA1* and *BRCA2* is typically limited to individuals who are high-probability mutation carriers as defined by their personal and family cancer histories. Expression profiling reveals that tumors from *BRCA1* carriers have a relatively clear signature that clusters with the basal subtype, whereas tumors from *BRCA2* carriers have a weaker signature that tends to cluster with the luminal-A subtype. These results are important for two reasons: first, likely mutation carriers can be identified even if they do not have a strong family history, so that unaffected relatives can eventually benefit from a knowledge of carrier status irrespective of family history; and second, there are preliminary indications that tumors from *BRCA1* and *BRCA2* mutation carriers respond quite differently to specific chemotherapies than do tumors from non-*BRCA* carriers [21]. Identifying signatures typical of tumors from *BRCA1* or *BRCA2* mutation carriers can be viewed as an application of molecular breast cancer classification to etiological research. Thus, it may be possible to use this approach to identify homogenous sets of families whose excess risk is explained by other susceptibility genes, or homogenous sets of patients whose tumors have arisen through specific etiological or mechanistic pathways. Ideally, molecular classification of breast cancer should become a standard tool in both genetic epidemiological and molecular epidemiological research on this disease.

Delivery platforms

Breast tumor subtypes and other biological correlates first observed in expression profiling experiments will eventually contribute to the clinical management of breast cancer patients, but the nature of the clinical assays that will be used to harvest the information remains unclear [22]. One possibility is that RNA-based expression profiling of limited panels of validated genes will become an important clinical tool. However, because fine details of sample acquisition and preparation can affect the relative abundance of many transcripts, clinical assays may eventually rest on more stable molecules. For instance, in the omics technology arena, either changes in DNA copy number measured by microarray-based comparative genomic hybridization or patterns of peptide expression measured by mass spectrometry may eventually provide similar information in a more robust assay than expression profiling [6,23]. In contrast, it may be possible to abstract much of the information available from an expression profile into a limited number of carefully chosen immunohistochemistry (IHC) assays and thereby develop evolved IHC panels that match the prognostic or predictive value of an expression profile [5,24]. In addition, translation to clinical practise of medically useful information first gleaned through expression profiling and other omics technologies should be sensitive to the clinical laboratory capabilities in middle-income and low-income countries; this perspective adds weight to the need to develop evolved IHC assays.

Technical limitations

Expression profiling analyses carried out over the past few years are still somewhat confounded by technical challenges, and these challenges are more severe in attempted comparisons between studies than within individual studies. Sample manipulation, analysis of case series selected by means of different ascertainment criteria, and cross-platform inconsistency all contribute to discordant study results. For example, comparative experiments performed on surgical samples that were exposed to open air in the operating room for different lengths of time reveal a small but clearly detectable set of genes whose expression is markedly altered by exposure to air. These genes are often present in published lists of differentially expressed genes and even classifiers, indicating that uncontrolled procedural variables may continue to contribute biologically irrelevant signals to expression profiles. Age distribution and other ascertainment criteria influence the relative prevalence of tumor subtypes in case series, in turn influencing the rank order of fold expression differences in the resulting expression array data. Differences in rank order between studies can lead to classifiers that have little gene overlap, although classifiers developed from one case series may retain prognostic or predictive power when applied to data from another case series. Cross-platform inconsistency operates at two different levels. (1) In comparing a set of differentially expressed genes on different platforms, the rank order of fold expression differences may be systematically different across platforms as a result of differences in probe performance. As above, these differences can lead to classifiers that have little gene overlap. (2) At the other level, there are differences in gene assignment between platforms. If different platforms assign different names to the same gene or assign the same name to different genes, severe cross-platform data incompatibilities will arise. Combined, these technical shortcomings affect the validity of some expression signatures and limit the efficacy of projecting expression signatures between data sets.

Discussion

We look forward to the time when either expression profiling itself or markers and profiles first defined by expression profiling experiments become a standard tool for the management of breast cancer patients. However, several fundamental questions need to be answered before this can take place; the following are four examples of these.

1. Are the provisionally recognized subclasses (basal, luminal-A, etc.) the subclasses or signatures that actually carry the most useful prognostic and differential treatment information, or is much of the relevant information hidden in signatures that have yet to be reproducibly defined and cross-validated?
2. If the different breast tumor subclasses are really recognized as distinct diseases, does it make sense to use a single profile to distinguish between patients who will or will not benefit from adjuvant therapy, or do distinct prognostic profiles need to be developed for each subclass?
3. Is the optimal management of a patient with an ER-negative/basal tumor different from that of a patient with an ER-negative/luminal-B tumor?
4. Is the optimal management of a *BRCA1* carrier with a breast tumor that has basal features different from that of a patient who has wild-type *BRCA1* and *BRCA2* but an otherwise superficially similar basal tumor?

Finally, we believe firmly that tumor taxonomy is likely to yield improved molecular prognostic and predictive markers. However, proper standardization and external validation of signatures are required before clinical trials should be contemplated. As such trials are designed, investigators should be mindful that the efficacy of competing IHC assays will evolve as independently informative markers are cherry-picked from omics discovery studies into more traditional pathology laboratory test formats. The US Program for Assessment of Clinical Cancer Tests (PACCT) has useful guidelines for future studies.

List of Workshop participants

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Competing interests

The authors declare that they have no competing interests.

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