

EDITORIAL

Gene Expression Analyses in Breast Cancer: Sample Matters

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Since the advent of microarray technologies that permit high-throughput gene expression analyses in tumor samples, there has been an explosion of data generated, both through the analysis of archived clinical material and through prospective studies that expressly collect samples for molecular analyses (1–3). Fortunately, much of this has been made publicly available through shared repositories, enabling investigators without direct access to clinical material the opportunity to carry out discovery and validation studies to better characterize the molecular basis of tumor behavior and response to therapies (4,5). The recent efforts of The Cancer Genome Atlas and other groups to generate comprehensive molecular profiles of human cancers have further enriched these data sets, now using the current state-of-the-art RNA-seq technologies (2,3).

There are few areas of cancer research where gene expression profiling has had as great an impact as in breast cancer. Both in basic research and clinical application, gene expression analysis underlies the common molecular classification schemes (eg, intrinsic subtypes), and its importance is second only to the gold standard pathological measurement of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) in defining treatment plans for individual patients (6–11). The development and validation of prognostic gene expression classifiers in early breast cancer (eg, OncotypeDX [12], Prosigna [13], MammaPrint [14], Gene expression Grade Index [15], and others) has substantially refined adjuvant chemotherapy decision-making, permitting optimized delivery of this treatment and sparing thousands of women toxic therapy where it is not required (16).

Given the central role gene expression analyses play in the investigation and management of breast cancer, the report by Gao et al. (17), describing artifactual gene expression changes in serially sampled specimens collected as part of a randomized controlled trial, are particularly relevant to the design and interpretation of gene expression biomarker studies. In their accompanying article,

the authors build on previous reports describing alterations in gene expression related to delays in tissue processing following sampling, including substantial changes in early response genes between diagnostic core biopsies and surgical specimens (18–20). They now analyze and compare whole-genome expression data from samples of ER+ breast cancers obtained by core needle biopsy at baseline and paired surgical specimens obtained after two weeks of preoperative aromatase inhibitor treatment (AI), or control from the POETIC trial (21). The analysis of gene expression alterations in the treated group identified major signaling pathways known to be affected by estrogen deprivation; in addition, as previously reported, substantial changes in some genes are documented in control-treated patients, which are attributed to pre-analytical variables in sample collection and handling. Namely, the baseline core biopsies were processed directly, whereas the surgical specimens were handled routinely following resection (often with a delay for clinical assessment), and core cut samples were obtained from the pathology specimens. This would result in substantial differences in ischemic time that could produce cellular responses and affect sample integrity. Gao et al. showed that many of the genes whose expression is most altered between baseline and surgery in the AI-treated group are also those affected in the control arm (Figure 1) (17). This is a striking finding as it indicates that the genes that could have been attributed to AI treatment were actually due to a confounding factor. The possibility that the expression changes in the surgical samples of the control group were the result of the intervening biopsy causing a wound healing, immune, or other perturbation was addressed by an analysis of a different trial (FAIMoS), where the post-AI samples were collected by a repeat core biopsy prior to surgical resection of the tumor (22). With the benefit of treatment and control groups in POETIC, the authors conclude that the sampling differences in serial specimen collection resulted in the detection of purely artifactual changes in some genes, while also masking real treatment-induced changes in other genes.

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In preclinical settings, researchers have more control over their experiments, consequently reducing the risk of confounding factors biasing the analysis results. For instance, we showed that gene expression profiles of cancer cell lines using large-scale in vitro drug screening initiatives, such as the Genomics of Drug Sensitivity in Cancer (GDSC) (23) and the Cancer Cell Line Encyclopedia (24), were reasonably consistent across studies (25,26). However, intrinsic noise of the pharmacological profiling assay and differences in experimental protocols resulted in marked inconsistencies for the drug sensitivity data (27,28). Similar to Gao et al., the Connectivity Map project investigated the effects of short-term drug treatment on the transcriptomic state of cancer cells in a preclinical setting (29). Although it is not possible to control for all the possible confounding factors, the resulting drug perturbation signatures yielded reasonable consistency across compounds of the same pharmacological class (29–31). These preclinical data indicate that the use of standard operating procedures, notably for sample collection and molecular profiling, resulted in robust pharmacogenomic readouts.

However, such controlled experimental design is often difficult to implement in clinical settings. Prospective studies to evaluate pharmacodynamic effects of novel therapies are often undertaken in early phase clinical trials, which do not always include a control arm (32). In this setting, where analysis of paired samples may be used to adjudicate drug effect, determine dosing, infer potential predictive biomarkers, or identify putative combination partners, care must be taken to account for technical confounders in downstream analyses if technical controls cannot be integrated in the study design. This is especially critical in window of opportunity trials, where no therapeutic effect is expected, or in studies of agents whose biological effect is weaker or less well-defined than the AIs studied in POETIC. In such cases, harmonizing baseline and end-of-treatment sampling procedures (ie, paired biopsies, as used in FAIMOS), as well as inclusion of a control group, is likely advisable, and is typically lacking when assessment of routinely available archival tissue samples is performed.

Retrospective analyses of paired tissue specimens, commonly performed in situations where mature outcome clinical data are required, are even more likely to suffer from technical differences in tissue sampling. Examples include the comparison of residual disease (at surgery) with pretreatment biopsies in the neoadjuvant setting to identify correlates of drug resistance; or metastatic disease (biopsies) to resected primaries (at surgery) to identify features associated with disease progression and dissemination. Attempts to control for potential confounding factors arising from technical issues in retrospective studies require both the recognition that this phenomenon is present and a database of important artifactual changes, as Gao et al. provided in their supplemental data for presurgical ER+ breast cancer. While some of these are likely to be common to other histologies, attention to other settings is necessary to account for disease-specific alterations. Failure to consider and control for such changes can, and undoubtedly will, result in spurious results and misleading conclusions. For those working in this area, take heed: the sample matters.

Notes

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