

Cancer Biomarkers: Closer to Delivering on their Promise

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Taguchi et al. describe, in this issue of *Cancer Cell*, a quantitative comparative biomarker discovery approach that integrates animal lung cancer models with validation in well-controlled human clinical study sets. This approach overcomes many of the major barriers that have held back the field of cancer biomarkers in the past.

While opinion leaders are exceedingly hopeful that “the ability of biomarkers to improve treatment and reduce health-care costs is potentially greater than in any other area of current medical research,” they have been very pessimistic that “research into biomarkers has not yet delivered on its promise” (Poste, 2011). Poste (2011) recently stated “Technologies such as proteomics and DNA microarrays have contributed a voluminous literature of more than 150,000 papers documenting thousands of claimed biomarkers, but fewer than 100 have been validated for routine clinical practice. This dismal record reflects the failure of researchers to embrace a coordinated systems-based approach.”. Taguchi et al. (2011) now provide fresh optimism that cancer biomarkers are closer to delivering on their promise. This is a landmark paper because it uses a system-based approach to discover candidate biologically relevant biomarkers from animal models and tumor cell lines and then goes on to demonstrate the relevance of these candidate markers in human lung cancer. Biomarker science in the past has been usually limited to a correlation between the level of the marker and the presence of the disease. This study goes beyond correlation to causality.

More than a decade ago, the biomarker field was launched with great enthusiasm because mass spectrometry revealed that blood contained a rich archive of potential biomarkers (Aebbersold and Mann, 2003; Anderson and Anderson, 2002; Petricoin et al, 2006). Since then, the field of protein-based biomarker discovery has been hampered by four major interrelated, overarching barriers (Table 1).

The first barrier has been the inability to mechanistically tie the presence of a candi-

date marker to the biology of the tumor, which made it difficult to have confidence in the marker or to understand its true clinical utility. Ideally, we should link the marker to a functional role in tumorigenesis and have the capability to study the cell biology of the marker in experimental models. Only then can we rationally determine if the marker is best suited for early detection in the general population, high-risk screening, recurrence monitoring, or individualized therapy. The lack of marker biology tie-in also has a tremendous impact on downstream aspects of biomarker development. Invariably, mass spectrometry-based hunts lead to the identification of dozens to hundreds of candidates. Without a firm biologic basis for ranking, most investigators are paralyzed by the sheer numbers in front of them.

The second barrier has been the lack of validation in well-controlled human clinical study sets, especially those where serum/plasma was collected in an asymptomatic group of subjects that were later found to have the cancer under study. The inadequacies of past sample handling methodologies and procedures have created anxiety in the community, as realization has set in that many of our retrospectively collected study sets with long-term follow up are likely fraught with hard-wired biases due to inconsistencies in how samples were collected and stored (Poste, 2011; Service, 2008).

The third barrier has been the fact that many investigators fail to plan for the intended use of the biomarker, and thus omit the appropriate control cohorts. An example is the common use of controls from healthy individuals. Healthy individuals' plasma or sera is not a sufficient control for modern cancer biomarker

research. The proper controls must include patients who are sick with non-cancer illnesses or harbor benign tumors. Cancer nearly always occurs in the background of inflammation, and the aggregate of inflammatory disorders are much more highly prevalent in the test population compared to any single cancer type. Thus, sadly, many cancer markers are not specific enough to be used in the clinic or require re-evaluation in population cohorts more suited for the intended use of the candidate marker.

The fourth major barrier is the lability and extremely low abundance of cancer biomarkers in the blood. Biomarkers emanating from an early stage tumor mass exist at concentrations that are usually in the picogram per ml range, which is ten to fifty fold lower than the sensitivity of current quantitative mass spectrometry (Service, 2008; Brown and Palmer, 2009). Consequently, there exists an ocean of low abundant biomarkers that is invisible to conventional mass spectrometry-based discovery.

Taguchi et al. (2011) describe a workflow that directly addresses the first three of the aforementioned barriers and provide compelling evidence that a mass spectrometry-driven proteomic discovery effort may finally be paying dividends. They overcame the first roadblock using an impressive combination of experimental models that link the biomarker with the cancer biology. The investigators compared the plasma proteins in four mouse models of lung cancer with mouse models of pancreatic, ovarian, prostate, and breast cancer. The series of mouse models independently cover three known aspects of non-small cell lung cancer (NSCLC) tumorigenesis: deregulations in EGFR, KRAS, and p53.

Table 1. Breaking the Barriers to Biomarker Discovery

Barrier to Cancer Biomarker Progress	Emerging Successful Strategies to Break the Barrier
(1) Failure to mechanistically tie a blood biomarker to the tumor itself	(a) Discovery of the biomarker across a series of experimental animal tumor models, (b) Mechanistically showing a role in tumorigenesis or a change after therapy, (c) Validation of the same marker using human samples,
(2) Improper sample handling and tracking; inadequate tissue fixation and body fluid sample preservation that generates bias, false positives, and false negatives	(a) Preservation technologies for tissue and body fluid sample collection. (b) Uniform protocols for collection of tissues and body fluids. (c) Molecular measures to verify the preservation of a biological sample
(3) Lack of independent blinded clinical validation with proper controls for specificity and noncancer diseases	Inclusion of independent epidemiologically credentialed and matched cohorts with inflammatory disease, infectious disease, and benign tumors.
(4) Low analytical sensitivity of mass spectrometry-based detection systems that prevent the detection/identification and measurement of low abundance (<ng/nl) biomarkers emanating from early stage cancer	Nanotechnology-based methods for biomarker capture, preservation, and exclusion of unwanted high abundance proteins such as albumin can amplify mass spectrometry sensitivity 1000 fold

Mutations in EGFR have been shown to be a major driver of NSCLC (Lynch et al., 2004). Taguchi et al. identified EGFR pathway biomarkers in the blood of the mouse NSCLC model and then verified the potential clinical utility of the EGFR protein itself in human study sets. The investigators were able to identify blood-borne biomarkers related to the EGFR-signaling network in the murine system that changed with response to EGFR-targeted therapeutic. Two models of inflammation were also used. This starting point for discovery took advantage of the fact that the animal models could be used to verify that at least some of the potential biomarkers were derived from the cancer cells themselves, and not a reactive product of the host. Using pathway-driving informatics, specific protein expression changes were identified in the plasma that correlated with the underpinning genetic state of the tumor itself. By using multiple models of different cancer types, these investigators directly verified the biomarker tumor type specificity. The inclusion of inflammatory controls provided an important level of assurance that the candidate markers were not just an indicator of inflammation.

Addressing the second and third major barriers, the investigators validated the clinical potential of the biomarker candidates using two very well-controlled human plasma study sets, including a set from presymptomatic patients from the Carotene and Retinol Efficacy Trial (CARET) cohort study. Because the clin-

ical validation was very rigorous, the paper represents a milestone achievement in protein biomarker discovery that transcends the specific candidate markers uncovered.

Moving forward from the example set by Taguchi et al. (2011), the next generation of biomarker discovery platforms will use new technologies such as biomarker-harvesting nanoparticles (Luchini et al., 2008); in one step, in solution, they can concentrate and preserve even the most low abundance proteins, amplifying the sensitivity of mass spectrometry 100 fold or greater. These new nanotechnologies address the fourth (Table 1) and last significant impediment to biomarker discovery.

The ultimate clinical utility of biomarkers described by Taguchi et al. will require further large-scale validation. The biomarkers that change following therapy could be envisioned as potentially useful for recurrence or treatment monitoring in NSCLC patients. While the investigators provide powerful but preliminary evidence that the specific marker sets appear to identify future NSCLC in some of the presymptomatic patients, it is unlikely that this would be the immediate intended use of the markers. In order to be used as a primary screening tool for large populations, a blood biomarker for NSCLC must have extremely high sensitivity and specificity. Work up for suspected NSCLC involves costly procedures such as spiral CT and invasive diagnostic procedures that have associated morbidity and mortality. Given the relatively low overall

specificity of spiral-CT screening for lung cancer (Harders et al., 2011), it is more likely that blood-based lung cancer biomarkers will first be used in conjunction with imaging to improve the specificity of detection for both modalities. This is an important application for biomarkers that could truly reverse the curve against this deadly cancer.

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