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Cancer

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Diagnosing Cancer: A Genomics and Proteomics Approach


Detecting tumors earlier and integrating diagnostics more closely with therapeutic decisions should improve survival

| [By Tom Hollon](#)

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In 1996, Jeff Trent and colleagues published the first paper describing DNA microarrays as tools for pinpointing gene variants underlying various tumor properties.¹ Now, as president and scientific director of Translational Genomics Research Institute (TGEN), in Phoenix, Trent is using microarrays to look for gene expression patterns that can be applied to developing diagnostics.

The role of microarrays, Trent says, "will be on the discovery side. Testing all 30,000 genes against a diagnostic specimen is prohibitively expensive." Therefore, thousands of genes will be winnowed down to small sets. These, says Trent, "are very strong discriminators of whatever you're interested in," such as distinguishing tumors that will respond to a drug from those that won't, or diagnosing stage I versus stage II, or invasive versus noninvasive.

Prediction based on just one gene--higher expression for response, perhaps, lower for nonresponse--is impossible, Trent says. "One gene is incapable of having enough information for something as complex as

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response. You need to identify a pattern of several genes that separates response versus nonresponse."

Sifting such enormous amounts of data takes Trent "to an entirely new level of statistical and computational challenges." Suppose, he says, that a researcher has expression data for 600 genes and looks for a two-gene pattern that discriminates between the two groups. That means testing almost 180,000 patterns. Three genes translates into more than 35 million patterns, and so on. Trent's response to the challenge: supercomputing, for which he has enlisted collaborators at IBM Life Sciences.

DIAGNOSTICS BY TISSUE MICROARRAYS One way to validate diagnostic gene patterns is to study levels of corresponding proteins using tissue microarrays, which allow researchers to spot hundreds of tissue samples on just one glass slide.² Despite caveats--protein levels may not match mRNA levels, for example--"there's no doubt that it's useful," says Trent.

Among those researchers combining DNA and tissue microarrays is Arul Chinnaiyan of the University of Michigan in Ann Arbor. One of his goals is to find gene patterns that detect prostate cancer more accurately than PSA (prostate specific antigen) level. While PSA is a "reasonable first screen," it lacks specificity, Chinnaiyan says. About 20% of men with mildly elevated PSA do not have prostate cancer; about one-third of prostate cancers have normal levels.

Chinnaiyan is searching for gene expression patterns that signal whether a tumor is indolent, or in about 15% of cases, aggressive. To understand better a potential marker gene found with DNA microarrays, he uses these tissue biochips to correlate expression of the gene's protein with indolence or aggression. They're assembled from cores extracted from pathologists' archived paraffin-block tissue slides. Most are five to 10 years old, with extensive records of the course of disease and response to treatment.

"Tissue microarrays are not likely to be used in a diagnostic setting," says David L. Rimm, director of Yale Medical School's Tissue Microarray Facility. They are a discovery tool, he says, and one that he is making more quantitative. Rimm and his colleagues have developed software

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called AQUA for automated, quantitative analysis of immunohistochemical stains in tissue microarrays. A recent AQUA study of HER2 protein levels in a tissue microarray of 300 breast tumors found that tumors with normal HER2 levels are as aggressive as tumors overexpressing *HER2*,³ which stands for human epidermal growth factor receptor.

Pathologists usually judge protein expression, based on the intensity of peroxidase-staining immunoassays, by sight. "And the tests have done OK; for breast cancer you can do HER2 or estrogen receptor testing by eye. However, with an automated device, we're finding that you can more accurately predict survival," says Rimm.

Quantitative information "has the potential to change the way that we look at tissue," Rimm says, invaluable enhancing traditional diagnosis according to morphological patterns in tissue slices. "In the future, it will be equally important to know the expression levels of various proteins as it will be to make that primary morphologic pattern-recognition diagnosis."

When that happens, pathologists can move closer to what ought to be their real role, Rimm says: "to serve as consultants to physicians." Sometimes a pathologist will only read a tissue and provide a diagnosis, "but other times what you really want is to tell the doctor which therapy the patient will respond to. To do that, a morphologic pattern won't help you. You need to measure levels of protein to decide whether the patient will respond to Rituxan, Iressa, or other drugs."

Like Chinnaiyan, Rimm makes tissue microarrays from archived samples. At Yale, samples going back to the 1950s are logged into a database along with corresponding treatments and outcomes. The database makes it easy for Rimm to locate the tissues he needs for studies. And so far, he has not found an age beyond which samples are too old. Core samples from 1960s tissue blocks "are still fine for analysis."

Ultimately, pharmaceutical companies need to conduct clinical trials that have an associated diagnostic, Rimm says. Researchers would measure levels of various proteins in patients' tumor samples for correlation to outcome. A few clinical trials are doing this, but

pathologists are still judging protein levels by eye. The big payoffs, he predicts, will come when studies become quantitative.

METHYLATION PATTERN DIAGNOSTICS Measuring mRNA levels is not the only way to bring DNA microarrays to bear on diagnosis, says Alexander Olek, CEO of Epigenomics, in Berlin, Germany and Seattle, Wash. Epigenomics develops microarray-based assays that diagnose cancers by their patterns of methylated CpG dinucleotides.

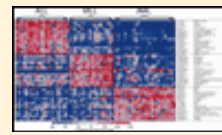
More than 40 tumor types have signature patterns of altered cytosine methylation. "It always holds," Olek says. "It is a general phenomenon." Patterns within tumor classes also correlate to stage, aggressiveness, and drug response.


Methylation pattern diagnosis exploits the fact that, beginning in early stages, tumor cells shed genomic DNA into body fluids, where, unlike mRNA, it is relatively stable.⁴ Detection begins with PCR amplification of tumor DNA, followed by methylation of cytosines into uridines. Amplified DNA then becomes a hybridization probe for detecting single nucleotide polymorphisms (SNPs) on a microarray. These SNPs, called methylated sequence tags, represent sequences from CpG islands associated with gene promoters. Epigenomics has verified that methylation pattern detection in this manner is an indirect indicator--"a mirror image," Olek calls it--of tumor gene expression.

To find methylated sequence tags, Epigenomics scans whole genomes of many tumors of the same type, identifying hundreds of SNPs linked to cytosine methylation. Only SNPs that reliably distinguish DNA from cancerous and normal cells are kept. On the drawing board are diagnostics for early detection of colon, breast, and prostate cancers.

PROTEOMIC OCEANOGRAPHY If all goes well, Quest Diagnostics and LabCorps, two diagnostic services providers, will offer high-risk screening for ovarian cancer by year's end, say Lance A. Liotta and Emanuel Petricoin, co-directors of the National Institutes of Health-Food and Drug Administration clinical proteomics program. This, says Liotta, will validate that "proteomics may have clinical benefit before genomics."

The NIH-FDA program mirrors Trent's in presuming that diagnosis and treatment response are too complex for a single biomarker. The ovarian cancer test uses mass spectrometry to measure the levels of a small set of serum proteins, which were found only after Correllogic Systems, a Bethesda-based bioinformatics company, figured out how to handle the same astronomical numbers of pattern combinations that challenge Trent.



 DNA microarrays reveal a new type of leukemia.

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When ovarian cancer is detected before metastasis, surgery is 90% curative; otherwise prognosis is poor. The test's 99% accuracy "is still not good enough for general population screening," says Liotta, "but we do think the test would be useful for any woman who has any abnormal finding." To verify this, a clinical trial will examine women with any ovarian cancer risk factor, such as an imaging or physical finding or a family history.

The hunt for diagnostic sets of protein biomarkers (called proteomic fingerprints) continues for cancers of the prostate,⁵ breast, pancreas, and lung. Results seem to verify the program's founding idea. "Our basic hypothesis," says Liotta, "was that the serum contains proteome information that potentially reflects what goes on in every tissue in the body. What people might think of as a dumping ground for protein fragments and residual cellular metabolic products, we see as a gold mine of diagnostic information--an ocean of biomarkers that no one has bothered to look at before."

REALITY CHECK How far will genomics and proteomics go in directing therapy decisions? While the answer of course is that no one knows, Gregory J. Tsongalis, director of molecular pathology at Hartford Hospital in Hartford, Conn., has a thoughtful perspective on the challenges ahead⁶ seen through the lens of deciding whether to treat breast cancer with Herceptin.

About a third of patients have breast tumors overexpressing the *HER2* gene, whose protein product is the monoclonal antibody's target. Binding of Herceptin to *HER2* protein on the surface of a tumor cell activates the immune system to destroy the cell. At Hartford, a pathologist's report that *HER2* is overexpressed does not make

Herceptin the drug of first choice. "If the physicians can use something else that is a milder treatment that's just as effective, I think they'd rather save Herceptin in reserve, in case there is a recurrence," says Tsongalis. They hesitate for fear of cardiac toxicity, because *HER2* is also expressed in cardiac muscle.

The natural heterogeneity of tumor cell populations, evident under a microscope, also influences treatment choice. "A lot of solid tumors are composed of different cell types, different stages of disease, and different grades of disease. If a woman has an overexpressed *HER2* gene," Tsongalis continues, "would the other types of cells in her tumor influence a Doctor's decision to give her Herceptin? The answer is yes." For example, although most breast tumors descend from a single cancerous progenitor, "there are women with different clonal populations, potentially two or three different primary tumors, some of which overexpress *HER2* and others that do not." Herceptin might kill one population only to create an environment in which others explode.

Tsongalis expects positive change as researchers continue to identify the best drugs for individual patients. He says that he does not expect microscopes for staging and grading to go away, because he doesn't expect the challenge of dealing with the tissues to go away. "Breast cancer is not a cut-and-dried disease by any means," he cautions. Drug choice decisions in the future, "will still be made in the context of the disease and how well the patient is doing."

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