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MOLECULAR MARKERS
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Tissue Cork Borer

Richard Peters and Robert Sikorski

Finding new tools for the diagnosis, prognosis, and treatment of cancers is a major goal of cancer research. Tumor markers are particularly useful for both cancer diagnostics and prognosis. New tumor markers based on genetic polymorphisms are routinely identified. The analysis of these markers requires a variety of technologies, from immunofluorescence, to in situ hybridization, to the polymerase chain reaction.

Unfortunately, to date, tumor markers have not gained widespread use in day-to-day clinical practices, although they are used in certain clinical trials to guide therapy. One of the main reasons for this is the paucity of solid population data to show the predictive value of any one marker. Additionally, it is difficult to look at an array of tumor markers simultaneously in single specimens. A multitest survey of tumors is needed to show the predictive relationships among different markers.

A recent report in *Nature Medicine* may well change the medical landscape and speed implementation of such markers in clinical practice. The authors describe a new technique that allows researchers to study up to 1000 tumor biopsies at a time (*1*). The authors built an instrument, which consists of a thin-walled, stainless steel tube, with an inner diameter of 600 μm, sharpened like a cork borer. They used this needle to select punch biopsies 3 to 4 mm in height from fixed tumor samples. Using a solid stainless steel wire, which functions as a stylet, the sample is then emptied into a recipient array block with drilled holes. A digital micrometer moves the system to successive locations in the growing array. The recipient paraffin block (45 mm by 20 mm) can have 200 consecutive 8-μm sections [see figure 1 of (*1*)]. Each of the 200 sections holds up to 1000 tumor samples. The power of this tissue microarray technique is the capability of performing a series of analyses of 1000 specimens in a parallel fashion. Armed with such a research tool, researchers can study vast numbers of tumor samples in a short time and can generate a wealth of data on the application of tumor markers.

As proof of principle, the authors performed immunohistochemical analyses (for protein expression), fluorescence in situ hybridization (for DNA amplification), and RNA/RNA in situ hybridization (for messenger RNA expression). They used 645 breast tumors to create the microarray. They then studied sections of this array with eight markers: estrogen receptor expression, p53 expression, and DNA amplification at the CCND1, ERBB2, MYC, 17q23, 20q13, and MYBL2 loci. Their results match published data, validating their approach. Specifically, at least one of the six DNA loci was amplified in 52% of primary tumors: ERBB2, 18%; MYC, 25%; CCND1, 24%; 17q23, 13%; 20q13, 6%; and MYBL2, 7%. They also reproduced previous findings showing that ERBB2 and MYC were amplified more often in tumors lacking estrogen receptors, whereas the opposite was observed for CCND1.

Finally, p53-positive tumors had a higher frequency of MYC and ERBB2 amplification than p53-negative ones. The technique appears simple, and the authors can process 1000 samples in 3 days time. The major drawback of the approach is the small diameter (0.6 mm) of the punch biopsy. With such a small core, areas of interest are easily missed in tumors that show a significant amount of heterogeneity. Multiple samples from each tumor specimen can reduce this problem. The authors note that increasing the density of the array so that more than 1000 specimens can be housed in the recipient block would extend the throughput of the system. Other technical tricks could be implemented; for instance, researchers could combine immunohistochemical methods with fluorescence in situ hybridization to use multiple detection probes simultaneously.

Variations and improvements on this technique can be expected in the near future. This research tool should have wide application in the field of cancer research, as well as in other fields (such as developmental biology) where molecular markers are especially informative.

References

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A Frankenstein Experiment

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Whenever the process of exchanging reagents among scientists becomes easier, science benefits. Take as an example the community of scientists who experiment with the simple organism, yeast. The simple property that yeast strains can be dried on filter paper disks has aided the distribution of a wide variety of mutants. Dried yeast strains can be maintained indefinitely at room temperature. They can be transported in an envelope for just 32 cents. Imagine, though, if yeast strains needed to be cryopreserved in liquid nitrogen to maintain their viability. The cost alone of exchanging the thousands of mutant yeast strains would be enormous.

Another community of researchers, those studying the mouse, is also trying to manage the ever-increasing numbers of their mutant strains. Mouse strains are usually passed between laboratories in the form of viable animals. Mouse embryos can be frozen, but the process is technically challenging to do in a reproducible way. Also, it is a drain on resources to go through the process of making and saving "libraries" of frozen embryos, merely for distribution. Maintaining a working colony of mice is expensive and time-consuming. Maintaining extra mice for colleagues' requests now adds to the lab budget.

A possible solution for the mouse community, and perhaps others, is in a recent issue of *Nature Biotechnology*. A group from Hawaii has succeeded in bringing freeze-dried mouse sperm back to life (*1*). There have been numerous attempts to develop techniques for low-cost sperm preservation, but they have all yielded the same failing results. At the end of these procedures, the sperm are essentially dead: They do not move and have no plasma membranes.

However, the researchers—led by Ryuzo Yanagimachi—tried a different approach. First, they created vials of freeze-dried sperm samples by freezing sperm in liquid nitrogen and drying them into powder form under a vacuum. No special buffers or technology was used. They then rehydrated the dried sperm and examined them for viability. As before, all sperm looked dead by conventional tests, such as special fluorescence stains.

Next, they performed an experiment a bit like Frankenstein's: They used micromanipulation to remove the freeze-dried sperm heads and to inject them directly into unfertilized mouse oocytes. The idea was to see whether the nucleus was dormant and could be revived within the cytoplasm of a host egg. With this crude process, they achieved rates of fertilization >90%. In the end, 30% of all head-injected oocytes produced viable mouse offspring that appeared completely normal.

The straightforward techniques used by these researchers, micromanipulation and vacuum drying, should be applicable in any major mouse research laboratory. It remains to be seen whether the sperm of other species will be able to withstand