Commentary

New Tools in Molecular Pathology

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The increasing knowledge of biochemical pathways provides the basis for numerous molecular studies in tumor pathology. Most investigations focus on a single factor or set of factors selected mainly because of our prior knowledge of their function in pathways involved in cell-to-cell communication, signal transduction, cell cycle control, or regulation of programmed cell death. Hence, the success of these approaches relies on the quality of the working hypothesis by which candidate factors are identified.

Genomics Meets Pathology

It is a particular advantage in the tumor world that molecular screening tools for the analysis of genomic alterations can be used to identify new pathogenic factors. Because loss or gain of gene functions by point mutations or chromosome aberrations are crucial for the etiology and development of tumors, recurrence of changes in one locus serves as an indicator of genes playing a pathogenic role. Approaches for the genome-wide screening of such alterations include means of cytogenetics such as chromosome banding,¹ comparative genomic hybridization (CGH),^{2,3} and 24-color chromosome painting by multicolor fluorescence *in situ* hybridization (FISH),^{4,5} as well as means of molecular genetics such as loss of heterozygosity analysis. Thus, the search for new factors is based on a gene's location rather than on its known or assumed function. The recently introduced approach of matrix CGH, whereby chromosome targets are substituted by a set of characterized genomic DNA fragments arrayed on a solid support, allows for genomic profiling at a resolution unprecedented in cytogenetic techniques.⁶ It provides a powerful tool for pinpointing critical genomic subregions that are gained or lost in tumor genomes.^{6,7} More recently, the successful use of arrayed cDNA sequences for the identification of genomic imbalances by CGH has been reported,⁸ offering an even higher resolution of the screening for gene copy number changes.

Genomic versus Expression Profiling

The last years have seen the development and exploitation of new approaches for the identification of tumor-relevant factors based on comprehensive expression analysis. In contrast to genomic studies, where the reference copy number of the normal situation is a known constant (eg, two for disomic loci), the high variability of expression levels requires careful consideration of adequate reference values. The success of such analyses is directly related to the stringency applied to procedures like subtractive hybridization, differential display,⁹ representational difference analysis (originally developed for the comparison of genomes but more successfully used for the comparison of cDNA pools),¹⁰ or hybridization to macro- and microarrays of gene-specific sequences.^{11,12} One approach to circumvent the stringency issue as much as possible is cloning and sequencing of gene-specific subsequences from RNA pools, a method termed serial analysis of gene expression (SAGE).¹³

Although large-scale expression profiling has recently been shown to support tumor classification,^{14,15} identification of unrecognized pathways being activated or inactivated in a tumor type- or stage-specific manner has yet to reach the high level of expectations. This is due in part to the fact that the expression data constitute an average profile of the investigated cell population, which could easily hide alterations in a subset of cells. In analogy to CGH, where the same kind of averaging effect applies on the genomic level, investigation of smaller cell populations, prepared by physical means such as microdissection followed by representative amplification of the nucleic acid pools from this preparation, will likely lead to a wealth of new data in molecular tumor pathology.

As the complexity of expressed proteins, including post-translational modifications, is much higher, large-scale expression profiling on the proteome level is still in its infancy. The community of molecular pathologists is eagerly awaiting new methods for high-throughput proteome analyses to be applied in the world of tumors.

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Tissue Chips, a Powerful Tool in Functional Tumor Genomics

One of the bottlenecks in the identification of tumor-relevant molecular alterations has been the need for analyzing large series of tumors by technically demanding assays on individual samples, requiring a heavy work load. The alteration of interest, such as changes in gene or chromosome copy number or on the expression level, can frequently be scored on a small section of a tumor. Accordingly, the analysis has been greatly facilitated by the development of a miniaturized tool consisting of sets of small tissue sections of different origin arrayed on a glass slide.¹⁶ Such arrays allow the parallel analysis of a large number of tumors by a single *in situ* hybridization or immunodetection experiment. These tissue arrays are not intended to perform diagnosis but rather to screen for a marker on a series of tumors. Obtaining representative tissue pieces depends on the expertise of the pathologist, who selects the site from which the small cylinder of tissue is punched. Although tumor heterogeneity would be missed when using a single piece of tissue, the problem can be circumvented by punching pieces from different sites of the same specimen. Furthermore, the sheer number of tumors testable in a single experiment facilitates the analysis even when some cases will be missed. After the punched cylinders are arrayed in a block, they are subjected to thin sectioning, resulting in more than 100 consecutive sections of the same tumor series. This provides the opportunity to investigate different markers on the same tumor tissue even when different methods must be applied. The groups of Olli Kallioniemi at the National Institutes of Health and Guido Sauter of the Institute of Pathology at the University of Basel, Switzerland, pioneered this procedure and since then have made enormous efforts to produce tissue chips of certain tumor types, chips of tumors from the same organ, and even chips consisting of several thousand different tumors covering the most important types of human cancer. Working through huge archives of the pathology institute, they generated extremely valuable tools to quickly analyze the role of new candidate markers in large tumor series. With all these chips in place, it is now possible to perform comprehensive studies quickly, within weeks. Thus, we can expect many publications on an unprecedented level of completeness. A pivotal study of these laboratories has been published in the September 2000 issue of *The American Journal of Pathology*.¹⁷ As CGH data had identified the cyclin E gene as a candidate for gene amplification in urinary bladder cancer, Richter and coworkers investigated the gene copy number and expression on more than 1500 arrayed bladder cancers. Although they stress the point that their study was accomplished within 2 weeks, this number does not account for the previous efforts to carefully analyze archived material for the generation of the arrays. Nevertheless, the comprehensiveness of the study and the speed with which it was performed are more than impressive. These kind of analyses will certainly change the world of publications in the field of pathology.

Even more important, correlation of molecular markers with other parameters, for example histopathological data, can be performed with an enormous statistical power. Although Richter et al detected amplification of the cyclin E gene by FISH in only 2% of the tumors, the large number of cases analyzed allowed the demonstration of a statistically highly significant association with tumor stage and grade. The impact of this kind of study is further enhanced when clinical data, such as patient survival time, are available. The same paper reports correlations of cyclin E expression not only with tumor stages but also with patient survival, a conclusion that could be underlined with *P* values <0.0001. Thus, the authors offer a complete answer to the question addressed by their study.

Certainly, the strength of this work is based on the size and the quality of the collection of tumor tissues and clinical data at the pathology institute. The combination of such tumor banks with a high-throughput screening using tissue microarrays will greatly accelerate our knowledge about factors and pathways involved in tumor etiology and development as well as about markers of predictive value. Efforts of genomic and expression profiling in tumors will deliver a vastly increasing number of candidate genes. The possible pathogenic role of these genes needs to be tested in an efficient way. Obviously tissue microarray analysis is particularly suited to meet this demand, and integration of the profiling efforts with that approach will be a powerful combination in molecular pathology for the upcoming years.

"Anatomy Chips" to Come?

Although it is obvious that the tissue microarrays are an important tool for the initiative of a cancer anatomy project, possible future applications exist beyond the world of tumors. One goal of those who design DNA chips for expression profiling is to produce chips representing complete biochemical pathways and eventually the whole map of biochemical pathways. In analogy, one could foresee chips representing the different tissues of one organ or even a whole organism. Such "anatomy chips" would allow us to test expression on the level of RNA and proteins in the normal physiological situation.

A number of applications for such a crude screening tool can be envisioned. Organs, which are complex and difficult to access, could be re-created in a coarse miniaturized version on a glass slide. For example, a brain anatomy chip might become generated by using archived and/or biopsy material from different sources. Whole organisms like animal models, which are too big to do whole body sections, could be targeted by series of selected tissue sections that might be arrayed according to their location in the body of that species. This could become a versatile technique to investigate gene expression during the development of larger organisms and possibly even in embryonic and fetal stages of man. Future work will be necessary to demonstrate the potential of such anatomy chips for the elucidation of normal physiology and disease-related alterations.

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