E9. Critical issues in the molecular analysis of tumours

J. Costa

Yale University School of Medicine, and Yale Comprehensive Cancer Center, New Haven, CT, USA

Tissue analysis, an activity that is crucial for the diagnosis and management of cancer patients, has traditionally consisted of histopathological examination. The purely histological approach has been augmented, during the last 25 years, by immuno-histochemistry (IHC) and molecular analysis of the samples obtained under the guidance of gross or microscopic examination. The invention of new technologies that make possible the comprehensive analysis of the set of proteins found in the cell (proteomics), of the sets of mRNA expressed (transcriptome and functional genomics) and of the structural genetic alterations present in abnormal cells (structural genomics) are about to alter how we investigate tumour tissue samples. Furthermore, the synergies of biotechnology, nano-technology and information-technology accelerate the progress in tissue analysis and in oncology. Instead of studying one gene or protein at a time, we are now able to examine large segments of the regulatory circuits operating in the cell. Instead of taking one marker and then exploiting it for diagnosis and prognosis, and perhaps for therapy (e.g. Her2neu), we now examine constellations of genes or proteins and are beginning to design specific therapeutic interventions to repair disabled regulatory pathways. Diagnosis and therapy have become more intimately linked. The ways in which we obtain knowledge in medicine have also changed. The success of the reductionistic approach has now been complemented by a systems approach that enables investigators in the biomedical sciences to treat complexity. We continue to conduct hypothesis-driven studies, but "scientific discovery" is made possible by high through-put (htp) technologies and computational biology.

The critical issues concerning the molecular analysis of tumours can be grouped under the following headings: (i) how to procure tissue; (ii) how to investigate that tissue and (iii) how to obtain, organise and display data. The molecular analysis of tissue is still too often performed in a research mode, without thought to the clinical applicability. The field moves forward with such a momentum that many modalities of analysis are not incorporated into standard medical practice. If this is to change, the issues concerning procurement of tissue for research purposes will have to become incorporated into the standard operating procedures of the Pathology laboratories. Issues of concern include patient's informed consent, tissue banking, tissue-derived products (e.g. sections, tissue microarrays, DNA). The incorporation of molecular analysis into standard clinical practice is forcing changes in the ways that tissues are handled. Many molecular techniques rely on exquisite preservation of the tissue constituents. Freezing the tissue immediately after biopsy calls for the presence of a specialised technician, often with the professional on-site supervision of a pathologist, to insure the quality and the optimal use of tissue obtained in the operating room. This also applies to cytological samples obtained by fine-needle aspiration under radiological guidance. Depending on the questions to be resolved, special fixatives that preserve nucleic acids may be called for in order to make molecular techniques and routine histo-pathology possible on the same tissue sample. Judgement is called for to divide precious tissue in ways that will insure that the maximum information can be obtained for the patient's benefit. Storage and preservation of specimen collections pose new challenges (e.g. storing frozen samples vs paraffin blocks), but also provide invaluable collections of well characterised tissues to validate new discoveries or to serve as the substrates for new inquiries. The acquisition of tissue has been refined by micro-dissection. Several laser-enabled instruments allow the procurement of microscopic lesions. This has the advantage of avoiding contamination of the sample to be analysed by tissue that is irrelevant to the question addressed by the test. Microscopic procurement is enhanced by targeting the cells to be obtained with immunological reagents. In this way, sub-populations of cells with specific phenotypes can be selected and characterised at the molecular level. Specific therapies that target molecular lesions will require assessment of tumour cell heterogeneity with respect to the molecular pathways that are disrupted. This will give practical relevance to the study of tumour cell sub-populations which will be obtained by micro-dissection or htp cell sorting.

The number of available tools for investigating tissue at the molecular level is increasing very rapidly and keeping up with the pace of technological development has become a challenge for clinical laboratories. Some of the comprehensive modalities for tissue analysis have been recently reviewed [1,2]. Few of these have found robust applications and have been reduced to practice outside of the research institutions. The value of htp technologies is

enhanced if the tissue architecture is taken into account when extracting information. In practice, preserving the context, as opposed to averaging by obliteration of the micro-structure, means techniques of *in-situ* analysis, or application of htp approaches to micro-dissected samples. The *in-situ* approach requires a high sensitivity of detection (ideally a single molecule!) and a process for tissue analysis that preserves the structure and the molecular targets to be analysed. The most widely used *in situ* technique is immuno-histochemistry. Replacing the usual chromogens with fluorophores will make multiplexing and quantitation possible and it is likely that many studies using comprehensive gene expression analysis on cDNA micro-arrays will be transposed to multiplex immuno-assays *in situ*. Improvements in detection such as Rolling Circle Amplification [3] and tags used in a bar-code mode (e.g. Quantum dots) are likely to speed up these practices. Combining *in situ* protein analysis with *in situ* fluorescent *in situ* hybridisation (FISH) will be a powerful way to delineate subpopulations of cells in solid tumours based on their genetic structural features and complement of proteins. The multiplexing of FISH will make the present methods [4] easier and susceptible of combination with *in situ* point mutation or limited tissue proteomics.

The alternative way to extract information without losing the architectural context is to apply htp technologies to specific cell populations obtained by micro-dissection. The necessary step, barring extraordinary sensitivity, is amplification of the target to be analysed. Amplification of proteins is not possible, but the polymerase chain reaction (PCR) provides an eloquent example of the power of amplification techniques for nucleic acids. Epigenetic alterations such as methylation are now within the scope of PCR technology. Progress in biochemistry promises amplification of genomic material in ways that render it suitable for the analysis of subtle gains and losses of genetic sequences (unpublished data). At present, we are able to perform comparative genomic hybridisation (CGH) starting with 500 cells using isothermal single-strand displacement reactions.

One of the limiting factors in the utilisation of *in situ* technologies is the need for human analysis of the results and capture of the data that are derived from quantitative *in situ* measurements. Not only is there is an issue of speed in looking at tissues, but also of quantitation of the findings. Progress in both hardware and software for htp image analysis and storage will accelerate the implementation of *in situ* tissue analysis. Slides with tissue micro-arrays can be probed and digitised to store, process and display the information at will. Without the progress in computational biology stimulated by the genome project and progress in information technology, we would be unable to harvest the benefit of new insights and of htp's. The need to deal with large data-sets can only be met by a strong information technology infrastructure and by sophisticated statistical treatment of the data. In addition, dealing cogently with large data-sets requires expertise in the mathematical techniques used in the field of artificial intelligence.

It is clear that physician scientists will need to work with physicists, chemists, bio-engineers and mathematicians to keep the momentum in the clinic. It is possible to think that improved modalities of tissue analysis will contribute to change cancer medicine from a reactive mode to a predictive one.

References

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