

Part II – Bio-Medical Platforms

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The pathology of human cancers is very complex. Tumors that develop in an organ or from a specific putative progenitor cell invariably consist of multiple types, which are currently best defined by their histological or cytological characteristics and/or clinical behavior. During the last two decades, increasing number of unique genetic abnormalities have been identified and associated with the tumors with specific clinical-pathological features (Vogelstein and Kinzler 2004). This has been most prominent for tumors of mesenchymal and hematopoietic cell origins, or those associated with hereditary syndromes. These discoveries have had significant impacts at the diagnostic and therapeutic levels, since these genetic abnormalities could represent the etiology and pathogenetic mechanisms for the development of these tumors.

The histopathology of most adult cancers is commonly heterogeneous. This is likely a phenotypic reflection of the diverse etiologies and complex genetic abnormalities that these tumors are associated with, most of which remain poorly defined. Nevertheless, there is a strong consensus that future and more effective cancer therapies are based on developing new drugs or therapeutic modalities that target the critical genetic or phenotypic aberrations occurring in the tumors (Arteaga, Khuri et al. 2002; Bild, Yao et al. 2006). Towards this goal, there is a general agreement among biomedical researchers that more precise definitions and classifications of human tumors based on their molecular genotypes and phenotypes are necessary. Molecular definition requires profiling at multiple levels, including at individual gene level (sequences, structure, copy number), expression level (mRNA and protein), as well as tissue organization

and microenvironment level. The most basic requirement and at times the greatest barrier for accomplishing these works are the availability of good quality banked human tumor and the corresponding normal tissue.

Human Tissues Bank

There are many ways that human tissue and cells may be banked, as non-viable or viable tissues/cells. Non-viable tissues may be banked as chemically fixed or snap-frozen tissues. Viable tissue/cells may be banked by cryopreservation, as primary or propagable cell lines, or in the form of living xenograft tumors in immune deficient rodents. Each of these tissue-banking strategies has their respective advantages or disadvantages.

Paraffin embedded tissue bank

Throughout the world, there already exist in the Department of Pathology of every hospital, a very large bank of fixed human tissue representing all types of diseases. These paraffin embedded archival tissues are generally prepared using a standard histopathology protocol, as part of the routine surgical pathology practices. As legally and ethically required for good patient care practice, these blocks are commonly stored for 20 or more years, as required by the local health authorities. Since these tissue blocks are prepared for clinical diagnostic purposes, their processing and fixation protocol usually follows standard practices. In most instances, the protocol requires that tissues be placed immediately in a fixative, or as soon as possible after its resection or biopsy. In most instances, the fixative is a 10% buffered aqueous formaldehyde (formalin) solution. Formalin generates cross-links between proteins and nucleic acids (DNA and RNA), which results in their structural denaturation and fragmentation. This results in limitation for analyses by many quantitative techniques that require preservation of the full length and normal structure of the molecules being analyzed, such as RNA microarrays or proteomics analyses. However, formalin fixation and paraffin

embedding also preserve the tissue, thus allowing them to be kept at low cost and in ambient temperature for many years.

The development of special techniques by microwave treatment to recover the antigenicity of formalin-denatured proteins has greatly enhanced the value of these materials for protein expression studies using the immunohistochemistry technique. The invention of tissue microarray (TMA) has further enhanced the value of paraffin tissue blocks in high throughput validation research on human tumors. In TMA, small (6-15 mm diameter) cores of formalin fixed and paraffin embedded tissue are arrayed into a single paraffin block. This allows the analysis and examination of a large number of tumor cases on a single histology slide and having been subjected to a specific stain. Recent improvement in the designs of microanalytical techniques for nucleic acids (quantitative polymerase chain reactions and microarrays) have also made it possible to perform global genomic and gene expression profiling experiments on paraffin embedded tissue materials.

Snap-frozen tissue bank

Until recently, many quantitative protein and nucleic acid studies that are performed on human tissue require fresh or snap-frozen banked samples. Despite recent improvements in the analytical techniques that allow greater scope of studies on formalin-fixed and paraffin embedded tissues, snap-frozen tissues remains the optimal materials for many studies. Despite this obvious importance of the quality of study samples, there is surprisingly a paucity of standardized protocols for the proper collection, processing and storage of human tissue samples for banking purposes.

Different types of molecules in tissue demonstrate various levels of stability. While RNA is notorious for rapid degradation by RNase, the stability of RNA in biopsy or surgically resected tissues is largely undefined. Based on functional knowledge, it is expected that transcript encoding different classes of genes would demonstrate different half-lives, which would putatively influence their stability and decay rate after vascular devitalization. Blackhall et al. (Blackhall, Pintilie et al. 2004) investigated the stability of gene

expression in surgically resected lung cancer for global expression pattern using cDNA microarray, and for the stability of stress and hypoxia related genes using the reverse transcription and quantitative PCR (RT-qPCR). Fragments of tissues were collected from lung tumors at various intervals up to 120 min after surgical resection. For some cases, several tissue fragments from different areas of the tumor were harvested at a single time point to study gene expression heterogeneity within the tumor. Each sample was snap-frozen after harvesting, and stored in liquid nitrogen until analysis. Remarkably, similar gene expression profiles were obtained for the majority of samples regardless of the time that had elapsed between resection and freezing. It was found that the variations between multiple samplings were significantly greater than those of elapsed time between sampling/freezing (Figure 4). The study concluded that tissue samples snap-frozen within 30-60 minutes of surgical resection are acceptable for gene expression studies, but sampling and pooling from multiple sites of each tumor appears desirable to overcome the molecular heterogeneity present in tumor specimens. Similar finding was reported by Hedley et al. (Hedley, Pintilie et al. 2003), who measured CA-IX in multiple biopsies using a semiautomated fluorescence image analysis technique and observed intratumoral heterogeneity to account for 41% of the variance in the data set.

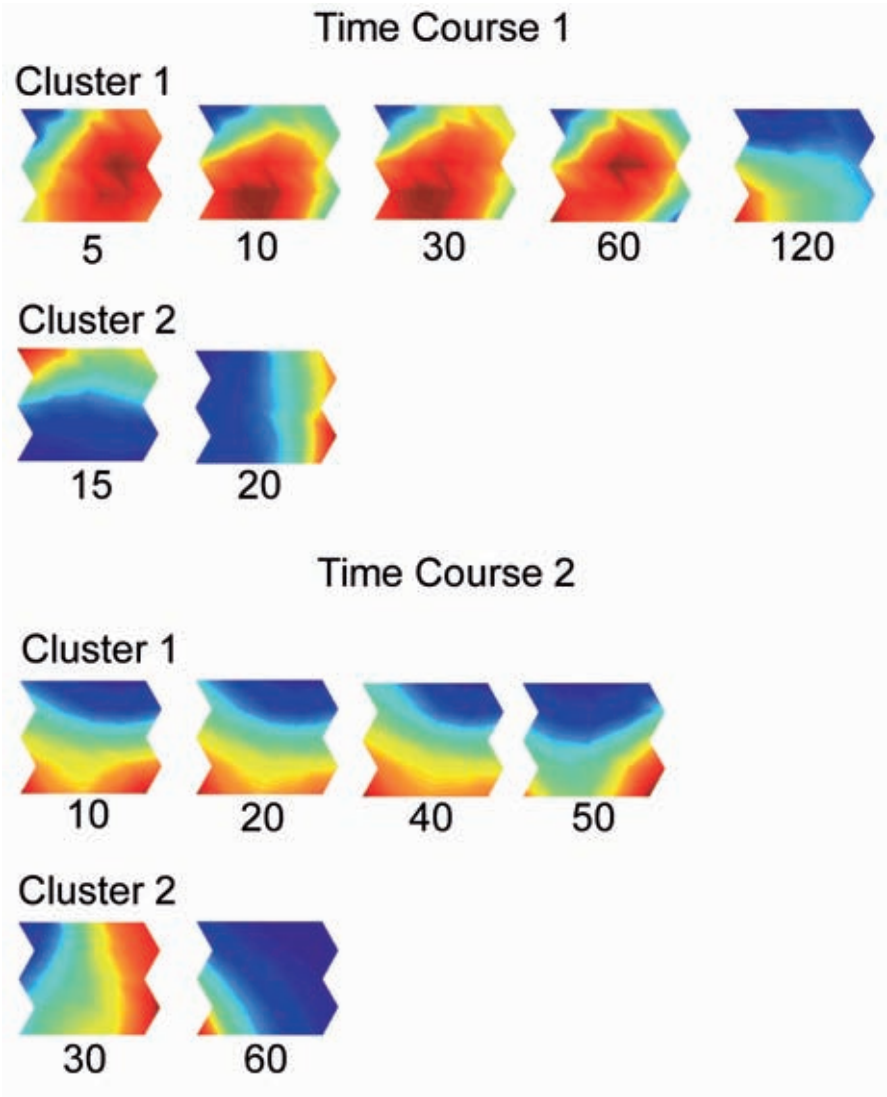


Figure 4. The variations between multiple samplings is significantly greater than those of elapsed time between sampling/freezing (Reprinted with permission from *Neoplasia*; (Blackhall, Pintilie et al. 2004)).

Most tumors are also composed of multiple cell types, including tumor cells, inflammatory cells, stroma fibro and myofibroblasts, and vascular endothelial cells. Heterogeneity in the composition of

these cells may significantly influence the result of analysis performed. Therefore, it is imperative that each tissue that is subjected to molecular profiling study be rigorously quality controlled at histological level. This can be done in 2 ways: frozen section histology or formalin-fixed representative section histology.

Performing frozen sections on the study tissue allows a more accurate sampling of the cells or tissue to be analyzed. The latter can be enriched by microdissection from the stained frozen section slides. The disadvantage is that this is a very time consuming procedure that has to be performed by a very experienced person. The liability of thawed frozen tissue for rapid RNA degradation also represents a serious experimental risk. Frozen sections also do not provide the optimum histology for pathological evaluation of the tissue. Nevertheless, successful expression profiling of tumor tissue using this technique have been reported. An alternate method is to incorporate routinely during the tissue banking, sampling from the frozen tissue sample a representative tissue slice for formalin fixation and paraffin embedding (Figure 5). A regular histology section can then be from the tissue block for histopathological evaluation. An added advantage of this procedure is that the tissue in the paraffin block may also be used for immunohistochemistry studies that require rapid fixation of the tissue sample.

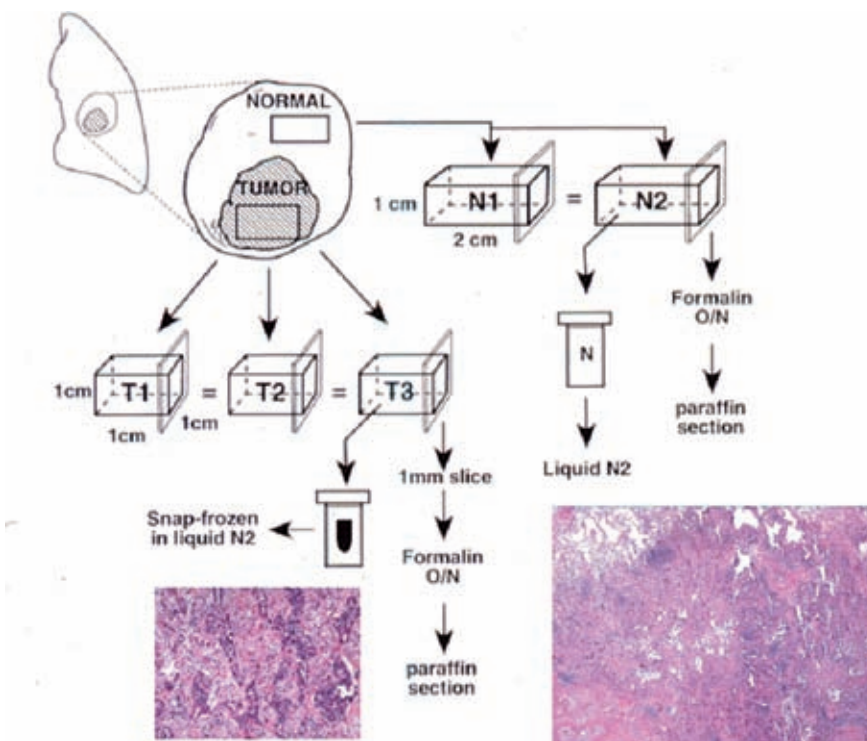


Figure 5. Performing frozen sections. Standard procedure for snap-frozen tissue bank sampling.

Non-frozen and non-chemically denatured tissue bank

Several other methods to preserve tissue in non-frozen condition and thus allowing the preservation of non-denatured molecules have also been tried. These include fixation in ethanol based chemicals or proprietary solutions, such as *RNAlater*® (Ambion). The latter allows the isolation of intact RNA and DNA for profiling studies, but the suitability of tissue fixed in this solution for proteomics analysis is unknown.

Cultured tumor cell lines

Established human tumor cell lines represent the prototype of banking viable tumor cells. Through dedicated efforts of numerous investigators, a large number of propagable cell lines have been derived from most human tumor types or origin. These cell lines have played critical roles in our current understanding on the molecular aberrations and biology of human cancers. However, studies on cell lines present several drawbacks. The ability to establish cell lines from various types of human cancers is variable. Almost all small cell lung cancers when cultured may give rise to cell line. In contrast, only up to 25% of primary non-small cell lung cancer (NSCLC) cultures may lead to the establishment of cell lines. Cell lines appear easier to establish from advanced, poorly differentiated and metastatic cancers. The ability to establish cell line from the tumor has been reported to be a poor prognostic marker in NSCLC patients. Thus, although tumor cell lines demonstrate the genetic aberrations noted also in primary tumors, they may not be representative of the entire spectra of expression changes found in primary human tumors. Genome wide microarray studies have demonstrated that the expression profiles of cell lines tend to segregate separately from that of the primary tumors of same tumor type. However, the expression profiles of xenograft tumors from by these cell lines appear to recapitulate more closely that of the primary tumors.

Primary tumor xenograft tumor lines

Less available than cell lines, human primary tumor xenograft lines represent an alternate method of viable tissue bank. These lines were established by direct implantation of the primary human tumor tissue fragment into the subcutaneous or orthotopic sites of immune deficient mice. Unlike xenograft tumors formed by established cultured cell lines, the tumors formed by primary xenograft lines mostly preserve the histological phenotype of the primary tumors (Figure 6). Furthermore, the success rate of establishing xenograft tumor lines may be higher than that of establishing cultured tumor lines. The

only drawback for setting up primary tumor xenograft tumor lines appear to be the higher cost of maintenance, and their less suitability for genetic manipulation that can be done easily in cultured cell lines.

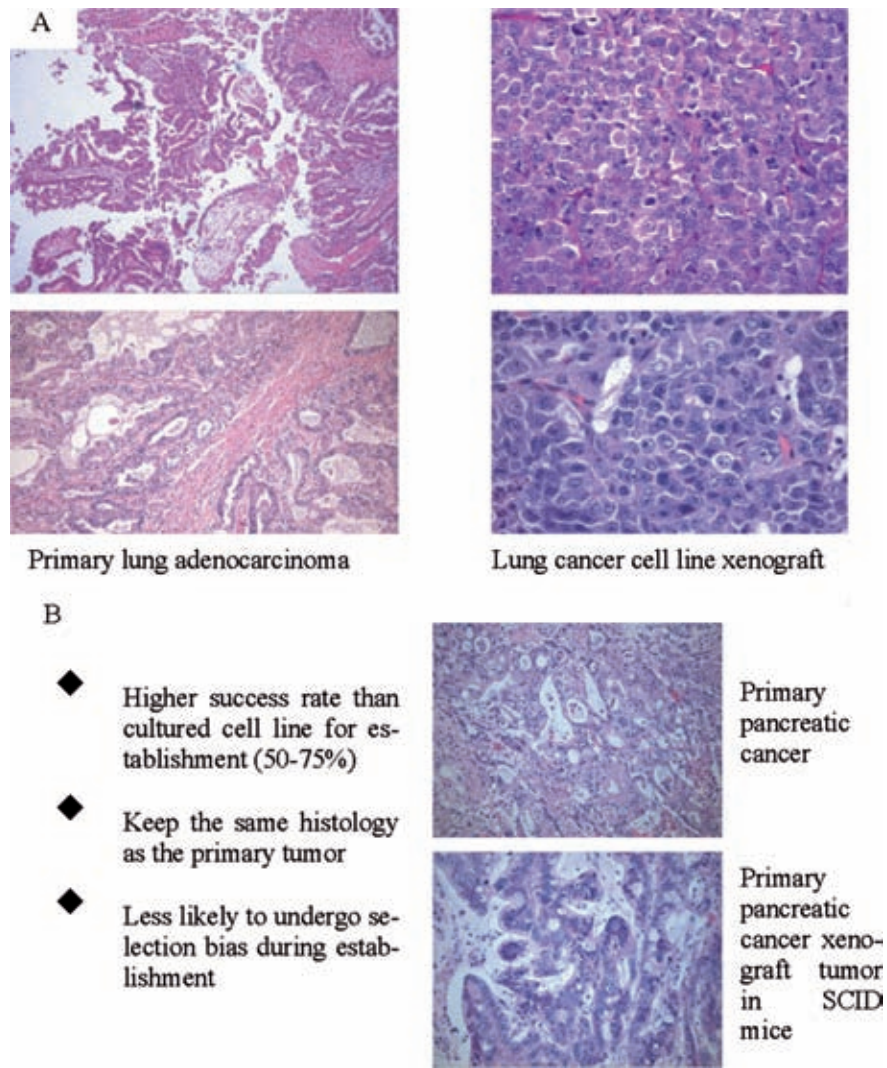


Figure 6. Xenograft tumors formed by established lung adenocarcinoma cell lines (A) and by primary xenograft lines (B).

Cancer Informatics in the Post Genomic Era

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