3 Tissue Collection for Molecular Genetic Analysis

Completion of the Human Genome Project will soon result in the identification of more than tens of thousands of new genes. Insight into the function and complex interaction of these genes is of more than just academic interest. Indeed, an understanding of the molecular genetic underpinning of human disease will fundamentally change the practice of surgical pathology.

In the past, a major role of the prosector was to submit well-fixed tissue sections for traditional light microscopic examination. Toward this end, the routine handling of specimens generally involved refrigeration for variable periods of time, fixation in formalin or other denaturing solutions, sampling for microscopic evaluation, and ultimate disposal of excess tissues. The role of the prosector is clearly evolving. These changes will first affect research hospitals, but the pace of change is so great that soon everyone practicing surgical pathology will have to be familiar with tissue collection for molecular genetic analysis. In this new era of functional genomics, there is a new emphasis on rapid collection of fresh, unfixed tissues to optimize preservation of undegraded DNA and RNA for genomic studies. Toward this end, handling of specimens now emphasizes prompt dissection, avoidance of formalin and denaturing solutions, multiplex processing for diverse diagnostic assays, and long-term storage of excess fresh tissues. A flow diagram for the increasingly complex and ever-evolving nature of tissue distribution is shown in Figure 3–1.

The bane of genomic studies is the degradation of RNA and DNA, and thus the major aim when securing tissue is to do so as quickly as possible. Degradation of DNA and RNA begins at the moment the blood supply to a tissue has been interrupted. Therefore, rapid tissue collection involves a coordinated network that begins with punctual delivery of specimens from the operating room to the pathology laboratory and ends with prompt processing of the specimen in the surgical pathology suite. The time allowed from surgical resection to specimen processing depends on a host of factors, but as a rule of thumb: *the faster the better*. In busy surgical pathology laboratories, rapid tissue collection requires prioritization of specimens potentially requiring molecular genetic evaluation over specimens that do not. Hence, be on the lookout for hematopoietic tumors and primitive tumors (i.e., "small round blue cell tumors") of children and young adults, as the molecular genetic profile of these tumors already plays a central role in tumor characterization and patient treatment. Chromosome analysis, molecular cytogenetics, and molecular assays are becoming increasingly useful in the diagnosis of other tumors as well. Table 3–1 provides a partial list of heterogeneous mesenchymal lesions where the identification of certain specific chromosomal translocations now permits more thorough and accurate classification. If any one of these lesions is considered in the differential diagnosis, the specimen should be targeted for rapid tissue collection.

The optimal way to process tissues for molecular genetic studies obviously depends on the nature and methodology of the analysis. Flow cytometry, cytogenetics, and other studies that entail the growth of living cell cultures require fresh sterile tissue samples. These samples should be collected as 0.5- to 1.0-cm cubes of tissue. A balanced physiologic solution such as Roswell Park Memorial Institute (RPMI) medium serves as an excellent medium for short-term storage and

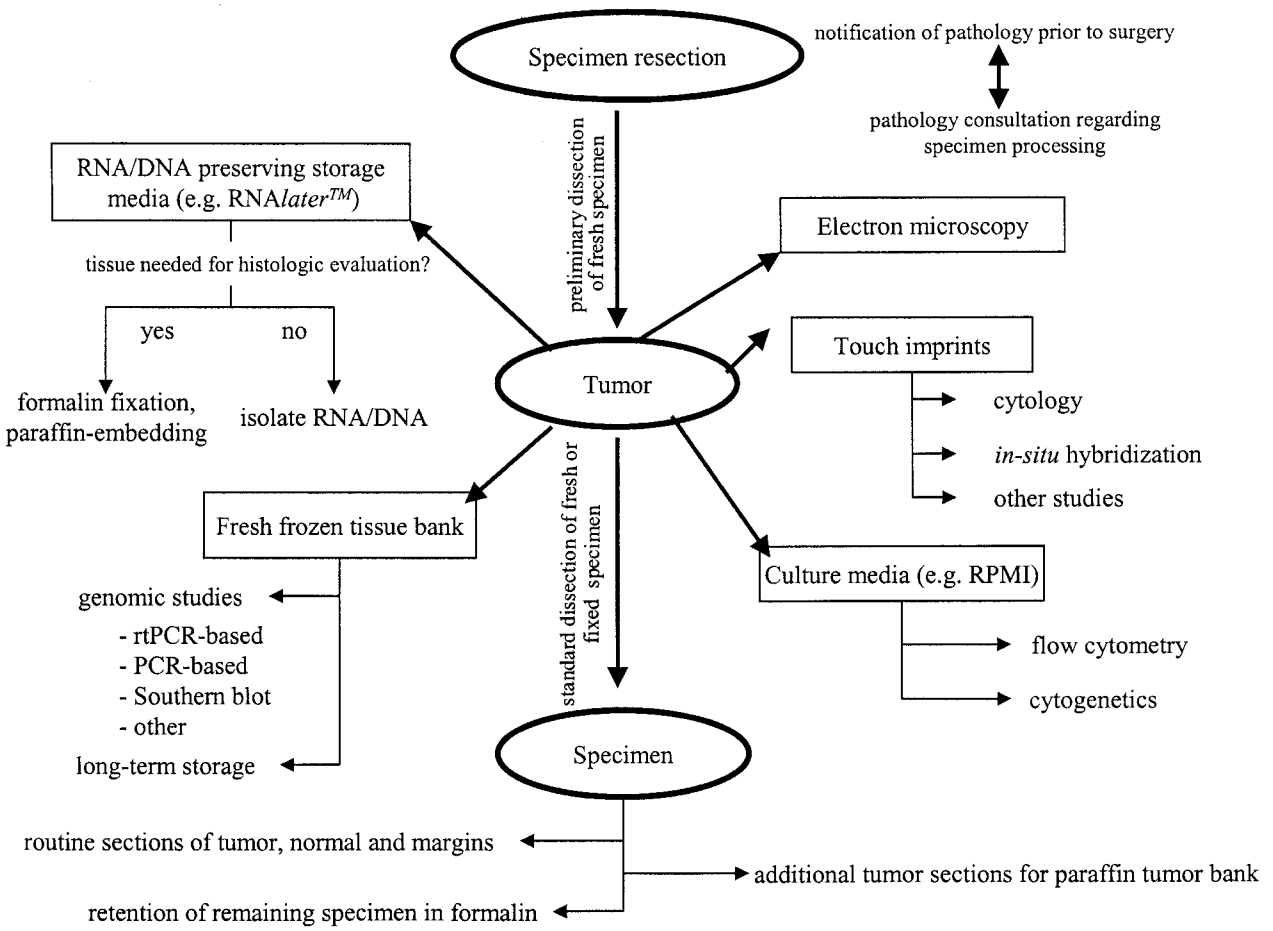

Fig. 3-1. Flow diagram of tissue distribution. (Modified from Florell SR, Coffin CM, Holden JA, et al. Preservation of RNA for functional genomic studies: a multidisciplinary tumor bank protocol. Mod Pathol 2001; 14: 116–128,[1] with permission.)

TABLE 3–1. Tumor-specific chromosomal translocations helpful in classifying mesenchymal lesions.

Tumor type	Chromosomal translocation
Alveolar soft part sarcoma	der(17)t(X;17)(p11.2;q25)
Myxoid/round cell liposarcoma	t(12;16)(q13;p11)
Extraskeletal myxoid chondrosarcoma	t(9;22)(q22;q12)
Dermatofibrosarcoma proteberans	t(17;22)(q22;q13)
Giant cell fibroblastoma	t(17;22)(q22;q13)
Infantile fibrosarcoma	t(12;15)(p13;q25)
Synovial sarcoma	t(X;18)(p11.2;q11.2)
Clear cell sarcoma of tendon sheeth	t(12;22)(q13;q12)

transportation. Although various methods are being developed to optimize DNA and RNA extraction from formalin-fixed tissues, polymerase chain reaction (PCR)-based techniques looking for DNA and RNA alterations are best performed on fresh, unfixed tissues. This tissue can be snap-frozen in liquid nitrogen and stored for long periods at −80°C. Advances are being made in the development of more versatile tissue media (e.g., RNA*later*™) that preserve the integrity of DNA and RNA for molecular analysis and at the same time maintain the histologic and immunohistochemical properties of the tissue. Remember that PCR-based techniques are highly sensitive for detecting rare abnormal cells among large numbers of normal cells. Careful attention to cleanliness, such as the use of fresh cutting utensils and changing gloves between specimens, is therefore critical if one is to avoid the effects of specimen contamination.

Given the breakneck pace at which genomic studies are finding increasing diagnostic and therapeutic applications, it is often prudent to store excess fresh tissue in a repository should it be needed for future analysis. This need to collect, store, process, and distribute well-characterized human tissues for diagnostic and investigative purposes has resulted in the emergence of *tissue banks*. Unlike traditional archival banks where the tissues are stored as formalin-fixed and paraffin-embedded blocks, the tissues in tissue banks are generally stored at −80°C in an unprocessed state or as pellets of extracted DNA or RNA.

Not only may these banked fresh frozen tissues be utilized for present and future diagnostic studies, they are of considerable value as resources for molecular genetic translational research. A few guidelines should be kept in mind when collecting and distributing tissues for investigative purposes. First, the pathology laboratory should not distribute tissue for research purposes without prior documentation of approval from the local Institutional Review Board (IRB). IRBs have been established to define the obligations of researchers and to ensure that the use of human tissues conform to federal regulations. Second, patient care must always come first. There may be instances when it is simply not possible to submit tissues for investigative studies without compromising your ability to optimize patient care. In the case of limited specimens (e.g., biopsies), there may not be enough tissue to support microscopic examination and research studies; and for anatomically complex specimens, where it is vital to maintain the integrity of the specimen for proper orientation and evaluation of margins, it may not be prudent to violate the specimen to obtain fresh tissue when formalin fixation is necessary. What ever the scenario, whenever patient care collides with basic science requirements, patient care must win.

Molecular genetic analysis of human tissues is a constantly changing field. New and exciting techniques are being developed every day. Surgical pathology prosectors familiar with the latest developments in molecular diagnoses are best prepared to handle resected and biopsied tissues appropriately.