Rare Tumors: Pathology and Biology Perspectives

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3.1 Introduction

The study of pediatric rare tumors is complicated at many levels. Accurate pathologic identification is essential, yet pediatric expertise is limited. For success, various collaborations are crucial. First, surgeons and pathologists must coordinate their efforts prior to surgery to ensure that sufficient material is obtained and that material is handled appropriately. Second, pathologists must be willing to consult others who may have more expertise. Two examples are a central review team (as developed in pediatric oncology groups) or pathologists with specific skills in adult tumors. Biological studies are also crucial to success in the study of rare pediatric tumors. As the whole-genome project moves forward, clinicians and investigators must be prepared to apply new information and molecular analysis methods to further understand the etiopathogenesis of those tumors. Two successful examples of molecular characterization in pediatrics: the pleurapulmonary blastoma family of diseases (Hill et al. 2009) and midline carcinoma with NUTT gene rearrangement (French et al. 2004). Further progress will be hampered if we do not establish a clear strategy to collect and store precious rare tumor material for future study. In addition, biological data must be fully integrated with data from clinical registries to fully enhance studies on rare pediatric tumors.

3.2 Pathological Diagnosis: Problems of Classification and Impact of Central Pathologic Review

In the last four decades, the 5-year survival rate for childhood cancer patients has improved from 58% to over 80% (Smith et al. 2010). This improvement can

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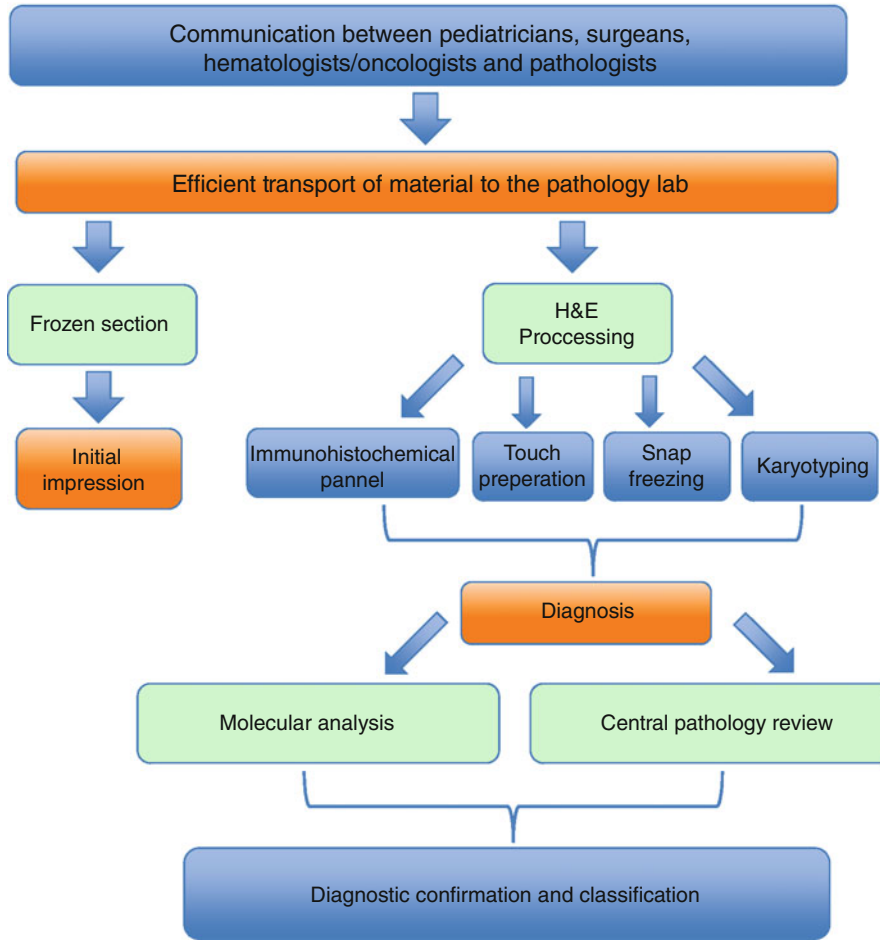

Fig. 3.1 Communication leads to expedited and improved diagnosis

be attributed to the dedicated work of the many national and international pediatric oncology treatment groups and the impact of central pathology review and the classification and subclassification of childhood tumors using advanced immunohistochemical and molecular genetic techniques. Despite the human genome revolution of 2000, this upward trend has reached a plateau. One obstacle to improving the survival rates of childhood cancer patients is imprecise tumor classification based only on morphology. The traditional classification and diagnostic methods of hematoxylin and eosin (H&E), immunohistochemistry, conventional karyotyping, and fluorescence in situ hybridization (FISH) have almost reached their maximum potential. Clinicians and researchers have yet to fully utilize copy number variation analysis, single nucleotide polymorphism (SNP) arrays, methylation analysis, singling pathway analysis, and whole-genome sequencing to create distinct classifications for childhood tumors based on their molecular/genetic basis. The ultimate key to increased survival rate and the future of tumor classification and diagnosis lays in understanding the molecular/genetic basis of childhood tumors (Tschoep et al. 2007).

Departmental cooperation is the first step in ensuring the correct classification of rare childhood tumors. The process is described in Fig. 3.1. First, the referring pediatrician, oncologist, surgeon, and pediatric pathologist must be involved in all aspects of the decision process. This will assure that the appropriate procedures are performed and that tissue is received in a fresh and sterile state (Demeure et al. 2010). Before a lesion is biopsied or a tumor is removed, clinical information about the patient is generally reviewed.

Although this data can help formulate a preoperative clinical differential diagnosis, it is not uncommon for the pathologist to be faced with an unexpected type of tumor. Conflicting preoperative data and pathological findings can ultimately create difficulties in reaching a final diagnosis. The challenge of making a quick and confident diagnosis becomes greater if strict handling of tumor tissue is not followed. Receipt of high-quality tumor specimens will not only aid in the classification and diagnosis of the tumor but it will allow the patient to be enrolled in the appropriate cooperative study (Oosterhuis et al. 2003).

It is extremely imperative that fresh tumor tissue is sent to the pathology department from the operating room as quickly as possible. Upon specimen arrival, initial examination of a preliminary frozen section helps to identify the nature of the tumor and to assure that the material saved in the biorepository is of the highest quality. Touch preparations are then made for future FISH analysis, tissue is snap frozen for permanent banking, and more tissue is sent for karyotyping. In cases where a hematologic malignancy is suspected, additional tissue will be sent to the flowlab. Any delay in tissue banking increases the chance for RNA and DNA degradation which will negatively impact its ability to be used in further research. Therefore, an open line of communication between surgeons and pathologists is fundamentally important to assure expedition of this process and to prevent any unnecessary compromise to the quality of the tumor sample (Oosterhuis et al. 2003).

After tissue is processed and H&E slides are examined, a panel of immunohistochemical stains is ordered to help support the original impression about the nature of the tumor. Karyotyping is also requested to identify any obvious translocations/deletions that might narrow the differential diagnosis. It is not uncommon in cases of rare tumors, even after following theses strict steps, that a final diagnosis cannot be rendered. Microscopically, many childhood tumors look alike. Using these traditional techniques, it is difficult to predict with certainty the histogenesis, phenotype, metastatic potential, genomic alteration, therapeutic response, and outcome of the majority of childhood tumors. In the instance of rare tumors, based on morphology alone, a vague diagnosis such as "sarcoma NOS" and "neoplasm with unknown malignant potential" are often rendered.

Immunohistochemical panels are often also inconclusive. With the implementation of every new immunohistochemical marker, clinicians believed that it would help differentiate between distinct tumor groups. Unfortunately, many immunohistochemical markers have proven to be tumor sensitive but not tumor specific. For example, all specimens labeled as rhabdomyosarcomas that were sent to the central pathology review had the same immunohistochemical panel. Preliminary studies of the rhabdomyosarcoma registry using comparative genomic hybridization (CGH) have shown that the genetic makeup of approximately 15–20% of specimens is not compatible with that of conventional rhabdomyosarcoma. Although the difference in tumor type is now clear, those patients are still enrolled in rhabdomyosarcoma treatment protocols (Morotti et al. 2006). The discovery of the diagnostic inaccuracy in a relatively straightforward case, such as rhabdomyosarcoma, only further elucidates the diagnostic and classification issues with difficulty and rare tumors.

A separate example illustrating the failures of conventional diagnostic tools pertains to a newborn who was clinically diagnosed with stage IV S-neuroblastoma. The histology showed an undifferentiated neoplasm composed of large epitheliod cells, which was not supportive of the clinical diagnosis (Fig. 3.2). Additionally, a large panel of immunohistochemical stains was nonconclusive. After a week, karyotyping results showed at (15:19), and a diagnosis of NUT midline carcinoma was finally reached (French et al. 2004). This case illustrated the inadequacies of histology and immunohistochemical stains while highlighting the need for molecular/genetic analysis. While conventional karyotyping proved to be useful in this instance, it is clear that other undifferentiated tumors can be defined by further molecular/genetic techniques when conventional karyotyping fails (Shehata et al. 2010).

Although conventional karyotyping is helpful in identifying specific translocations for certain tumors, this method misses many tumor genome mutations such as loss of heterozygosity (LOH) events and microdeletions. These mutations can be detected using copy number variation technology or whole-genome mapping. For example, tuberous sclerosis is characterized by the mutation of the *TSC1* or *TSC2* genes, and it is associated with rare renal tumor manifestations (Henske 2005). In several instances, mutations in these genes are not identified by conventional karyotyping. However, they can be seen in SNP copy number array analysis. In children with renal tumors, a quick and

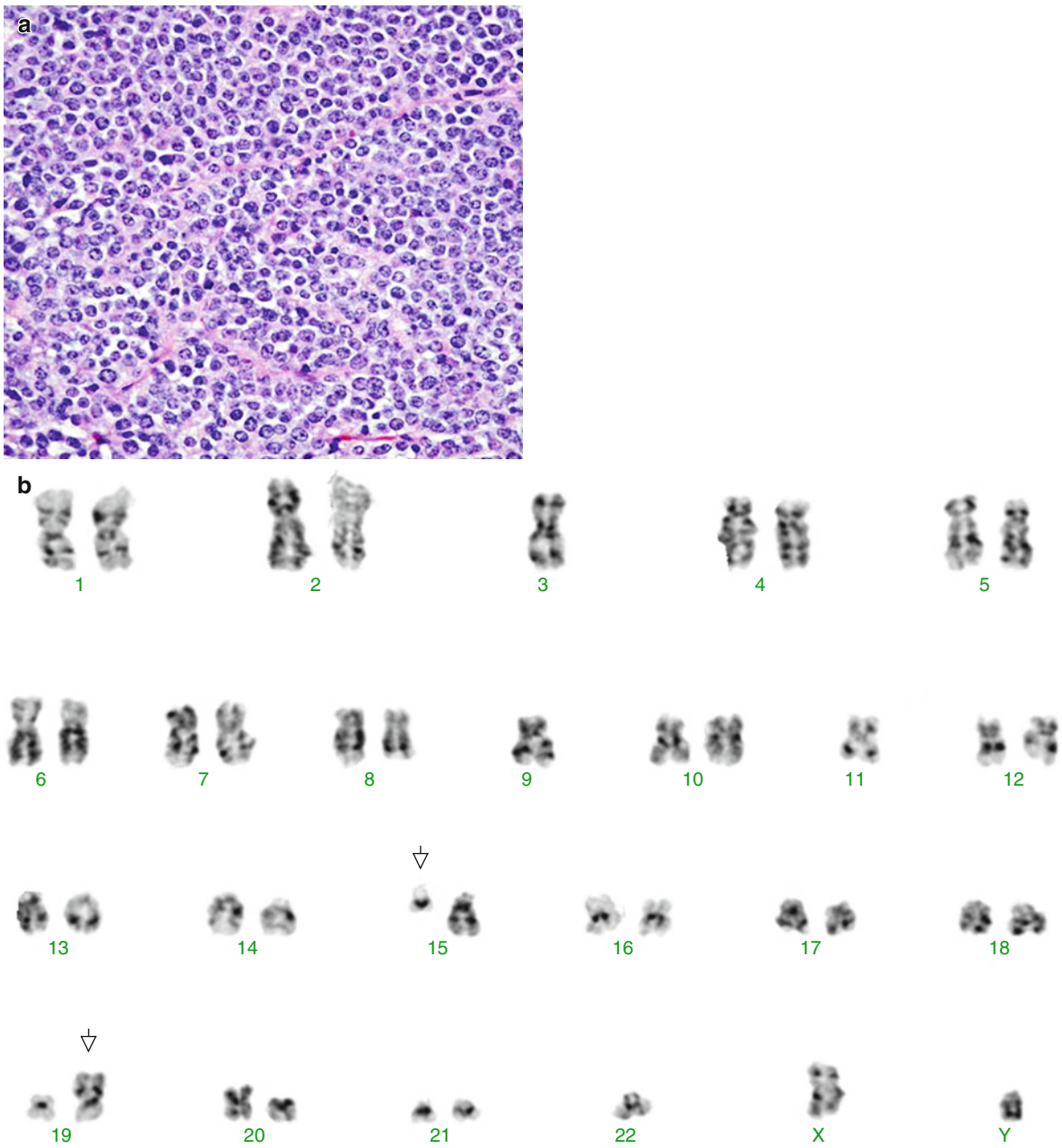

Fig. 3.2 (**a**) Sheets of poorly differentiated epitheliod cells. (**b**) Translocation of chromosome 15 and 19 (NUTT gene rearrangement)

accurate diagnosis of tuberous sclerosis using this technique will ultimately help in the diagnosis of rare associated renal tumors such as angiomyolipoma.

Classification can be further accomplished through the comparative analysis of tumor tissue and non-tumor tissue from each patient. Comparative genomic hybridization cannot only explain why a patient has a specific tumor but it can also provide important data to the survivorship program to have a road map for the follow-up surveillance of this patient. Should the child have a second malignancy, this data can help to explain if the child was susceptible to have the second

malignancy or if it was induced by the treatment regimen. Therefore, collection of tumor and non-tumor tissue is very valuable for the prognosis and follow-up of childhood cancer patients (Weiss et al. 2003).

Overall, in depth, molecular/genetic analysis will allow for greater elucidation about the genetic alterations, present in tumors, which may lead to the development of those particular tumors. Subclassifications of certain tumor types may be more readily formed using these newly developed techniques. This will allow researchers and clinicians to develop more specific treatment protocols based on the prognosis of patients with each subclassification. Therefore, the future of tumor classification and diagnosis, as well as treatment protocol development, lies in molecular/genetic sequencing. However, even the molecular diagnosis of rare childhood tumors remains problematic. The definition of characteristic genetic profiles may be limited by the paucity of cases. While the diagnostic and prognostic impact of chromosomal translocations has been well defined in childhood leukemia, it is almost impossible to perform comparable prospective research in rare solid tumors. If these patients are not registered on therapeutic clinical trials, collection of biological material is unlikely. If a new and recurrent genetic aberration is reported in a rare tumor, the frequency as well as the diagnostic and prognostic impact of the aberration will be elusive for a period of time.

One of the cornerstones of the major success in the treatment of childhood cancers is the impact of central pathology reviewers. In the United States, several groups are charged with reviewing specific tumors including, Wilm's tumor, rhabdomyosarcoma, neuroblastoma, etc. Their ultimate goal is to verify original diagnoses and to assure the collection of biological material for the central biorepositories of the Children's Oncology Group. After reviewing each case, the central pathology reviewers provide feedback to the referring institutions by agreeing, correcting, clarifying, or adding additional information. This collaborative effort is educational and plays a significant role in the management of patients (Teot et al. 2007). In Germany, the vast majority of tumor samples, collected from patients enrolled on therapeutic trials, undergo review at the German Childhood Tumor Registry in Kiel. Brain tumors are reviewed at the German Brain Tumor Registry in Bonn. Pathologic review is mandatory for most protocols, and reference pathologic evaluation is covered financially. Central review ensures uniform diagnosis and classification, but also fosters molecular genetic research on childhood tumors.

Central pathology reviewers are privileged to see a spectrum of cases, giving them the ability to observe specific prognostic factors, which helps in the implementation of standard protocols. Central pathology reviewers also compare the outcomes of specific protocols during the semiannual meetings for COG. This process leads to the initiation of additional studies and the enhancement of existing protocols (Teot et al. 2007). Central review has a more significant impact in the diagnosis and classification of ambiguous rare tumors in comparison to more common childhood tumors. In recent years, the collection of frozen material has been used to bolster the efforts of the molecular analysis of such tumors. The future molecular analysis of rare tumors by the central pathologic review will further help classify these tumors so that individualized treatment protocols can be created. Ultimately, the wealth of the material that they receive will strengthen the classification and reclassification of rare childhood tumors.

Rare tumors have not been well studied. It is difficult to collect sufficient numbers of any particular tumor for biologic studies. Slowly, international collaborations have allowed access to more biological material for some rare pediatric cancers. Rare childhood tumors are not only difficult to classify, but they are also difficult to diagnosis and manage. A more in depth classification of rare childhood tumors will allow for the development of tailored treatment protocols which will help to minimize the rate of relapse and the development of treatment-related malignancies. There has been significant collaboration in the classification of some pediatric cancers, such as rhabdomyosarcoma and neuroblastoma. More collaboration is needed. Ultimately, through the continued work of the central pathology reviewers and the use of molecular/genetic analysis, we can begin to better understand rare childhood tumors and again increase the overall childhood cancer survival rate.

3.3 Tissue Banking

Tremendous improvement in the treatment of childhood cancer and survival of pediatric cancer patients has occurred over the last 30 years as the result of the

use of pediatric cooperative group clinical trials. It is clear that additional significant progress, especially in the treatment of rare tumors, will require an improved and more comprehensive understanding of the molecular genetic basis of pediatric malignancies as well as the specific alterations which underlie resistance to current therapies (Oosterhuis et al. 2003). The National Cancer Institute (NCI) Best Practices for Biospecimen Resources (June 2007) states that the lack of standardized, high-quality biospecimens is widely recognized as a significant roadblock to cancer research. To remove this obstacle, tumor tissue biorepositories need to be created immediately (Demeure et al. 2010).

Tumor banking, while considered a fairly new concept, is still widely known as the most effective method for saving, storing, and delivering high-quality biospecimens for research (Demeure et al. 2010). The current shift from histological means to molecular means in cancer diagnosis, treatment, and research clearly necessitates the development of high-quality tumor banks (Oosterhuis et al. 2003).

Little is known about the etiopathogenesis of childhood tumors. In particular, the infrequent occurrence of rare childhood tumors hinders our ability to garner statistically valid data concerning this subset of tumors. The main goal of pediatric tumor banks is to improve the diagnostic accuracy of pediatric tumors and advance the fundamental knowledge in tumor biology through the preservation of such rare specimens (Oosterhuis et al. 2003). This information will impact the survival rate and long-term quality of life for pediatric cancer patients by providing treating clinicians with more precise diagnoses for targeted therapies and research scientists with high-quality biospecimens (Demeure et al. 2010).

Molecular analysis will help identify candidate genes that denote diagnosis, prognosis, and potential targets for each specific childhood tumor (Tschoep et al. 2007). This will lead to a more accurate pathological diagnosis, and therefore, more precise, targeted molecular therapies (pharmacogenetics) will be made available that can be delivered to tumor cells (Tschoep et al. 2007). These specialized treatments will improve survival and minimize short-term and long-term side effects (Nair 2010). This would represent a huge turning point in pediatric cancer care since the current chemotherapy and radiation therapy treatment methods have many pathological side effects and can impact children's growth and intelligence. This in itself will provide for better survivorship, long-term health, and reduction in second malignancies.

The quality of the results produced by molecular analysis depends on the quality of the tissue samples that are used (Oosterhuis et al. 2003). To ensure a national (or international) standard of tumor biorepositories and the maximization of results, certain tumor bank criteria should be met: the collection of high-quality tumor samples for molecular analysis, the storage of fibroblast cultures and blood to allow for the comparison between genomics of tumor and non-tumor tissue, the standardization of collection and storage to assure high-quality specimens, the continued implementation of standardized protocols based on national standards, and the implementation of statewide educational seminars to strengthen scientific understanding and improve tissue acquisition (Holland et al. 2003; Demeure et al. 2010). A practical approach is detained in Fig. 3.3. The collection of fibroblast cultures is especially important as it allows for the identification of mutations in somatic cells that may explain the current malignancy or any future second malignancies. Although tumor tissue is routinely sent to central suppositories under Children's Oncology Group protocols, the creation of a statewide pediatric tumor banks will give local cancer researchers easy access to substantial numbers of specimens in order to perform their research. Comparable central repositories have also been built in other countries. One example is the BioCase project. Fresh tissue is collected from patients with embryonal tumors (e.g., neuroblastoma) enrolled on prospective clinical trials (Ernestus et al. 2006). The days of attempting to locate specimens piecemeal can be put to bed, and the delays currently experienced for critical research can be made null and void.

According to the National Cancer Institute, there are 11 million cancer survivors alive in the United States. At least, 270,000 survivors were originally diagnosed at age <21 years. Today, approximately 80% of children affected by cancer are alive 5 years after diagnosis (Smith et al. 2010). Collaboration between Pathology Departments, Hematology–Oncology Departments, Surgery Departments, and Clinical Research is crucial for the successful establishment of high-quality tumor banks (Demeure et al. 2010). The implementation of tumor banks around the world can help further our knowledge about rare tumors, increase the number of childhood cancer survivors, and decrease the short-term and long-term side effects of cancer treatment through

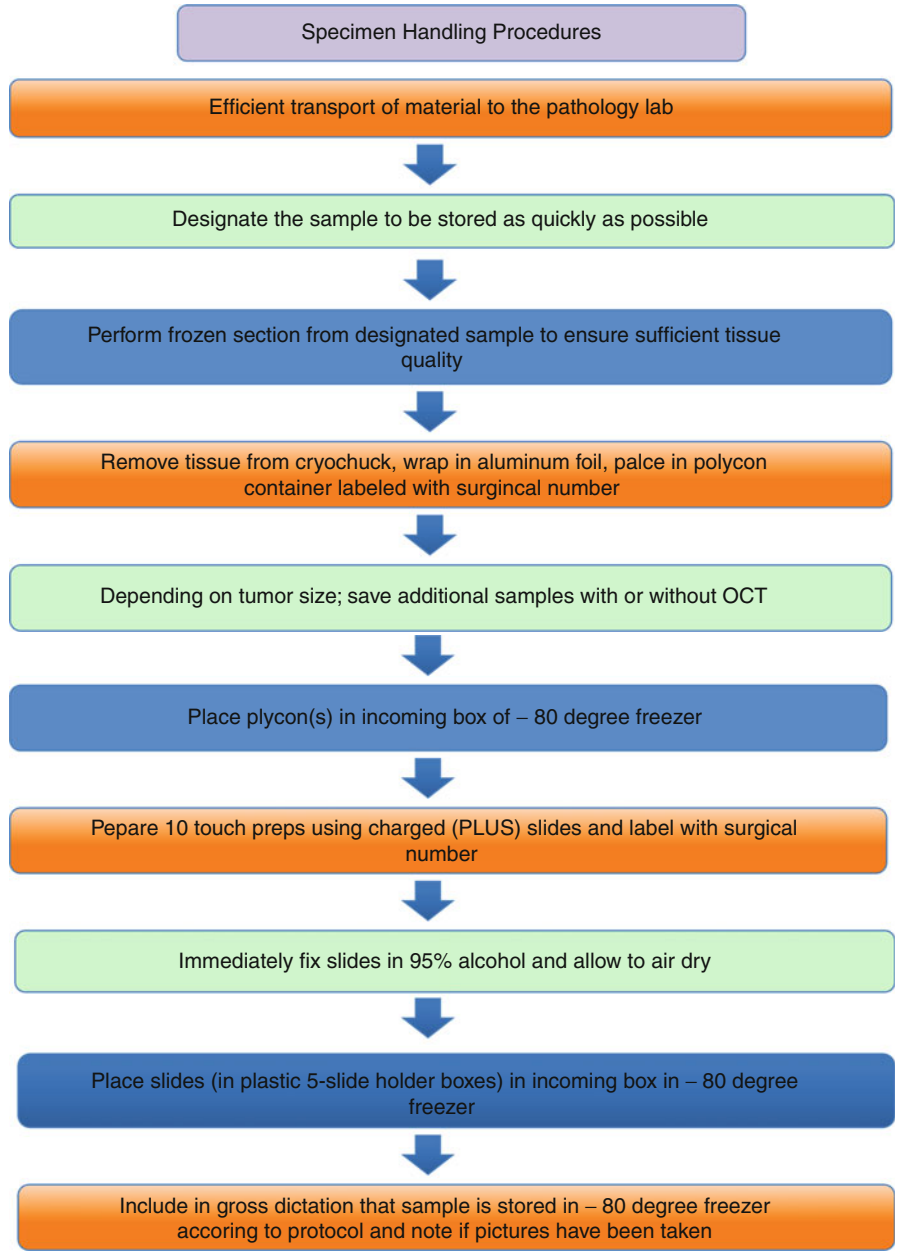

Fig. 3.3 Handling of material for diagnostic studies and tumor banking

the identification of candidate genes and the development of target therapies.

However, several obstacles must be overcome. In particular, complex ethical and legislative issues have urgency. The distribution of tissue samples to "foreign" laboratories may be sanctioned if no specific consent has been previously obtained. It would be preferred that future consent for tissue includes the opportunity that precious material might be shared with international investigators. However, shared samples must be anonymous. Corresponding clinical data must be held at local institution or cooperative group that is authorized, under consent process, to review clinical data. If these issues can be overcome, international cooperation may stimulate molecular genetic research on rare childhood cancers and intensify the development of clinical and

scientific networks, which are essential for the treatment of these patients. Molecular analysis and the characterization of the cancer genome must be performed to reach the ultimate goal of a complete cure for cancer. Tumor banking is the tool necessary to reach this goal.

References

Demeure MJ, Sielaff T et al (2010) Multi-institutional tumor banking: lessons learned from a pancreatic cancer biospecimen repository. Pancreas 39(7):949–954

Ernestus K, Pietsch T et al (2006) Structure, use, and risks of biomaterial repositories of embryonal tumors. Klin Padiatr 218(3):132–138

French CA, Kutok JL et al (2004) Midline carcinoma of children and young adults with NUT rearrangement. J Clin Oncol 22(20):4135–4139

Henske EP (2005) Tuberous sclerosis and the kidney: from mesenchyme to epithelium, and beyond. Pediatr Nephrol 20(7):854–857

Hill DA, Ivanovich J et al (2009) DICER1 mutations in familial pleuropulmonary blastoma. Science 325(5943):965

Holland NT, Smith MT et al (2003) Biological sample collection and processing for molecular epidemiological studies. Mutat Res 543(3):217–234

Morotti RA, Nicol KK et al (2006) An immunohistochemical algorithm to facilitate diagnosis and subtyping of rhabdomyosarcoma: the Children's Oncology Group experience. Am J Surg Pathol 30(8):962–968

Nair SR (2010) Personalized medicine: striding from genes to medicines. Perspect Clin Res 1(4):146–150

Oosterhuis JW, Coebergh JW et al (2003) Tumour banks: well-guarded treasures in the interest of patients. Nat Rev Cancer 3(1):73–77

Shehata BM, Steelman CK et al (2010) NUT midline carcinoma in a newborn with multiorgan disseminated tumor and a 2-year-old with a pancreatic/hepatic primary. Pediatr Dev Pathol 13(6):481–485

Smith MA, Seibel NL et al (2010) Outcomes for children and adolescents with cancer: challenges for the twenty-first century. J Clin Oncol 28(15):2625–2634

Teot LA, Sposto R et al (2007) The problems and promise of central pathology review: development of a standardized procedure for the Children's Oncology Group. Pediatr Dev Pathol 10(3):199–207

Tschoep K, Kohlmann A et al (2007) Gene expression profiling in sarcomas. Crit Rev Oncol Hematol 63(2):111–124

Weiss MM, Kuipers EJ et al (2003) Comparative genomic hybridisation as a supportive tool in diagnostic pathology. J Clin Pathol 56(7):522–527