CHAPTER 1

Overview of Soft Tissue Tumors

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DEFINITION

Soft tissue tumors are generally defined as tumors of connective tissues, including nonosseous sarcomas, benign mesenchymal tumors, and tumor-like proliferations. These tumors are usually considered to include non-osseous tumors of the extremities, trunk wall, intraabdominal and intrathoracic space, and head and neck, although definitions vary. Generally excluded from this definition are non-mesenchymal tumors of the skin, cutaneous melanoma, most primary epithelial tumors, and brain and bone tumors.

In this book, soft tissue is understood broadly to include any important tumors of nonbony tissues of the extremities, trunk wall, retroperitoneum, mediastinum, and head and neck, except organ-specific tumors. Gastrointestinal stromal tumors are included because of their clinical importance and common occurrence as metastatic abdominal masses. Cutaneous nevi and primary cutaneous melanoma and intracranial nerve sheath tumors have been excluded. Metastatic epithelial tumors and metastatic melanoma are included because of their practical importance (Table 1.1). As a subspecialty, soft tissue pathology intersects many other subspecialties of pathology.

CLASSIFICATION

The purpose of classification is to group similar tumors to create an understanding of tumor biology and behavior for developing treatment and follow-up strategies. The study of properly classified tumors also aids in the discovery of pathogenesis, devising biology-based treatments, and perhaps preventing tumors.

Soft tissue tumors continue to be classified according to the cell type that they resemble or have been thought to resemble. The clinical correlations that have already been obtained by the present classification are so numerous that the basis of this classification will probably remain, although cytogenetic, molecular genetic, and gene expression studies continue to refine the classification system. Reaching an ideal classification for this complex group of tumors, one that would be at the same time simple, highly reproducible, and clinically most informative, is not an easy task.

By tumor type, soft tissue tumors comprise a diverse group of benign, malignant, and borderline malignant (intermediate malignant) tumors. Most of them arise from (or show differentiation toward) mesenchymal cells, but some are of neuroectodermal (e.g., Schwann cell tumors), epithelial (metastatic carcinomas), or hematolymphatic (extranodal lymphoid and histiocytic infiltrates and lymphomas) origin (Table 1.2). The generally accepted basis for soft tissue tumor classification is the World Health Organization (WHO) system, last published in 2001.[1]

Malignant mesenchymoma is a sarcoma that displays differentiation toward more than one specific line, except the fibroblastic one. This designation is rarely applied today, because most tumors formerly classified as mesenchymoma are now more preferably diagnosed as liposarcomas or nerve sheath tumors with heterologous differentiation.

Benign tumors generally show the greatest similarity to their normal cell counterparts. For example, lipoma is histologically indistinguishable from normal adipose tissue, and leiomyoma cells greatly resemble normal smooth muscle cells.

Sarcoma cells show varying resemblance to normal cell types, depending on their degree of differentiation. For example, well-differentiated liposarcoma cells greatly resemble fat cells, whereas pleomorphic ones contain more limited numbers of cells with specific fat differentiation. Some sarcoma types, such as synovial, epithelioid, and alveolar soft part sarcoma, have no normal cell counterparts.

HISTOGENESIS

According to current understanding, most tumors are derived from multipotential precursor cells (stem cells) that are preprogrammed to differentiate into various mature cell types. The tumors are thought not to derive from mature cells, such as skeletal muscle and mature adipocytes, because such cells are terminally differentiated and incapable of cellular division.

The preprogrammed nature of many stem cells explains why some sarcomas closely resemble their mature cell types. For example, the cells of leiomyosarcoma closely resemble smooth muscle cells; however, some tumors contain cellular components that have no resemblance to

TABLE 1.1: Summary of Soft Tissue Tumors by Definition

- Primary tumors of different locations
  - Extremities
  - Trunk wall
  - External genitalia
  - Body cavities, including retroperitoneum and mediastinum
  - Head and neck
  - Mesenchymal tumors of the gastrointestinal tract
  - Other organ-based connective tissue tumors
- Tumors of different histogenetic categories
  - Mesenchymal tumors
    - Benign mesenchymal tumors (e.g., lipoma)
    - Malignant mesenchymal tumors (sarcomas)
  - Neuroectodermal tumors (e.g., neurofibroma)
    - Benign: neurofibroma, schwannoma
    - Malignant neuroectodermal tumors
  - Primary and metastatic carcinomas
  - Primary and metastatic malignant melanoma
  - Hematolymphatic neoplasms
  - Miscellaneous tumors and reactive conditions

normal cell types in that location. For example, metaplastic or neoplastic cartilage components can be present in different sarcomas. Similarly, the origin of benign rhabdomyoma as a polyp in the vaginal mucosa and rhabdomyosarcoma of the urinary bladder cannot be understood based on the normal cell types present in these locations.

The tissue origin of soft tissue stem cells is not fully clear, but it seems likely that many of them come from the local, organ-specific pools of stem cells. New data indicate that some soft tissue components are replenished from stem cells of bone marrow origin; these cells also could be the origin of some soft tissue tumors. For example, some regenerating skeletal muscle cells have been shown to have their origins in bone marrow,[2] and a portion of endothelial progenitor cells are of bone marrow stromal origin.[3]

The histogenesis of many sarcomas with no known normal cell counterparts (e.g., synovial sarcoma, alveolar soft part sarcoma) could reflect the unique genetic makeup that has created unprecedented tumor phenotypes that are not comparable to those of any normal tissue.

EPIDEMIOLOGY

Approximately 10,390 people were diagnosed to have a soft tissue sarcoma in the United States in 2008, and of these, 3,680 people (35%) died of these tumors, according to American Cancer Society estimates.[4] The total incidence of sarcomas is higher and might be close to double if organ-based tumors are included, however. In cancer statistics, these tumors customarily are pooled with carcinomas of different organs. Overall, sarcomas are more common in men, although some types (leiomyosarcomas) occur more often in women.

Sarcomas are rare tumors, and non–organ-based sarcomas constitute only 0.7% of all cancers. Their incidence is only about 5% of that of the most common carcinomas (i.e., prostate, breast, and lung), and one half of that of brain tumors and leukemias.[4] The relative rarity of soft tissue sarcomas might be explained by mesenchymal cells being located behind protective epithelial barriers that take most of the carcinogenic hits.

Based on these data, the incidence of sarcomas in the United States is approximately 3.3 per 100,000. This finding is similar to that obtained in the survey of epidemiology and end results (SEERS) program based on a sample population of the United States, where the overall incidence of soft tissue sarcomas was approximately 4 per 100,000 if

TABLE 1.2: Simplified Chart of the Major Types of Primary Soft Tissue Tumors Grouped According to the Cell Types that They Resemble[a]

Cell Type	Benign Tumor	Malignant Tumor
Fibroblast, including myofibroblast	Fibroma, myxoma	Fibrosarcoma, malignant fibrous histiocytoma
Adipocyte	Lipoma	Liposarcoma
Smooth muscle cell	Leiomyoma	Leiomyosarcoma
Skeletal muscle cell	Rhabdomyoma	Rhabdomyosarcoma
Endothelial cell	Hemangioma	Angiosarocoma, Kaposi sarcoma
Schwann cell	Schwannoma, neurofibroma	Some malignant peripheral nerve sheath tumors
Cartilage cell	Chondroma	Chondrosarcoma
Interstitial cell of Cajal of intestines		Gastrointestinal stromal tumors, a spectrum from benign to malignant
Histiocyte	Juvenile xanthogranuloma Rosai-Dorfman disease?	Histiocytic sarcoma (True histiocytic lymphoma)
Lymphoid cells	Benign lymphoid hyperplasia	Extranodal lymphomas in soft tissues
No known normal cell or benign counterparts		Ewing family tumors
		Synovial sarcoma
		Epithelioid sarcoma
		Alveolar soft part sarcoma

[a] Intermediate categories between benign and malignant tumors are excluded for simplicity.

Kaposi's sarcoma is excluded.[5] According to the SEERS data, the incidence of soft tissue sarcomas has increased from the 1960s, although some studies have attributed this increase solely to the Kaposi's sarcoma epidemic.[6]

There might be global differences in sarcoma incidence, according to data from different cancer registries. For example, the incidence per 100,000 was only 0.8 in Osaka, Japan; 1.4 in Bombay, India; and 2.4 in Shanghai, China.[7] These figures are less than those reported for the United States and Europe, where the incidence is between 3 and 4 per 100,000. The apparent variances in incidence could result from differences in diagnosis, coding, and classification, however.

Like most other cancers, most soft tissue sarcomas occur in older adults, who have higher age-specific incidence of these tumors.[7,8] Important subgroups of soft tissue tumors, however, occur predominantly or exclusively in children (e.g., neuroblastoma, embryonal rhabdomyosarcoma, angiomatoid fibrous histiocytoma) and young adults (e.g., Ewing family tumors, alveolar rhabdomyosarcoma, and synovial sarcoma).

The incidence of benign soft tissue tumors is impossible to determine accurately, because benign tumors are underrepresented in hospital materials and usually are not included in tumor registries. As surgical specimens, however, benign soft tissue tumors outnumber their malignant counterparts by a margin of at least 100:1. In major teaching centers and tertiary hospitals with active musculoskeletal tumor surgery, however, the ratio might be closer to 10 or 20:1 because of the relative enrichment of malignant tumors, especially liposarcomas.

ETIOLOGY

The etiology of soft tissue sarcomas is relatively poorly understood, and known causes apply to only a small percentage of these tumors, much less than for many other cancers that are more clearly related to environmental carcinogenesis, for example, lung cancer.

The most important known etiologic factors for soft tissue sarcomas include ionizing radiation, oncogenic viruses, and chemicals. The role of trauma is disputable, although anecdotal cases seem to support it.

All tumors are thought to arise as a result of acquired genetic alterations leading to abnormal quality or quantity of proteins that control cellular proliferation and differentiation. Certain etiologic environmental factors, radiation, certain viruses, and chemicals are known to be capable of causing genetic alterations that can lead to tumorigenesis.

A small percentage of sarcomas arise from host factors. Among them, the most important are hereditary genetic alterations (tumor syndromes), of which the most common by far is neurofibromatosis type 1 (Chapters 4 and 24). Rarely, other host factors are involved, such as immunosuppression and chronic lymphedema.

Radiation

Radiation-induced sarcomas develop in a small minority of patients (<1%) who have undergone therapeutic irradiation, typically 5 to 10 or more years after the radiation. Such postirradiation sarcomas most commonly include undifferentiated tumor types such as fibrosarcoma, malignant fibrous histiocytoma (MFH), osteosarcoma, and, rarely, angiosarcoma and malignant peripheral nerve sheath tumor. The most common locations of postirradiation sarcoma are breast and chest wall in women irradiated for breast carcinoma, and the pelvis and lower abdominal wall in patients irradiated for gynecological or urological cancer.[9–11]

Thorotrast, colloidal thorium oxide–containing radioactive radiologic contrast medium, was used until the 1940s, especially for angiographic studies. This material is permanently deposited to the reticuloendothelial cell system, especially in the liver, and some patients subsequently developed angiosarcoma of the liver or, more commonly, hepatic carcinoma or leukemia.[12]

Viruses

Oncogenic viruses might introduce new genomic material, which encodes for oncogenic proteins that disrupt the regulation of cellular proliferation. These genes are read, and the host makes proteins that alter cell cycle regulation or otherwise promote the viral infection. Two DNA viruses of the herpesvirus family have been linked to specific types of soft tissue sarcomas: human herpesvirus 8 (HHV8) to Kaposi's sarcoma,[13] and Epstein-Barr virus (EBV) to certain leiomyosarcomas.[14] In both instances, the virus-sarcoma connection is more common in immunosuppressed patients.

Most, if not all, Kaposi's sarcomas contain HHV8 sequences. This gamma herpesvirus is parenterally or sexually transmitted and is thought to be etiologically significant for the development of Kaposi's sarcoma, which explains its epidemic nature in HIV-infected populations and higher incidence in populations with higher prevalence of HHV8-infection.[13]

The EBV-associated leiomyosarcomas occur in immunodeficient or immunosuppressed patients, especially in children with HIV infection. Some have been seen in patients under chronic medically induced immunosuppression.[14]

Chemicals

Epidemiologic studies have linked phenoxyacetic acid herbicides to increased incidence of peripheral soft tissue sarcomas in some studies, although others have not confirmed this association. Dioxin contaminants have been suspected as the base of the carcinogenicity of these herbicide preparations.[5]

Sarcomas have developed around permanently retained metal objects, such as shrapnel and implanted surgical devices. These tumors have been mainly angiosarcomas and MFHs in the small numbers of reported cases.[15] Experimental studies support the capability of implanted metal or plastic objects to cause sarcomas. Several types of plastics and metals implanted long term in tissues were shown to induce local sarcoma formation, most commonly MFH or fibrosarcoma in rats. Proliferative mesenchymal lesions, possibly representing preneoplastic changes, were also observed.[16]

Epidemiologic studies have failed to show the association of soft tissue sarcomas and smoking, alcohol use, and organic solvent exposure.[5,8]

Hepatic angiosarcoma is an exceptional sarcoma that is associated with specific chemical agents more commonly than any other sarcoma. According to a large epidemiologic study, such factors were present in the history of approximately 25% of patients with these sarcomas; these factors included vinyl chloride (used in plastic manufacturing), inorganic arsenic (used as a pesticide or historically as a syphilis medicine), thorotrast, and androgenic anabolic steroids (the latter used medicinally or for doping purposes).[17]

Host Factors

Immunosuppression is known to be associated with sarcomas having a viral connection but could also cause other sarcomas. Hereditary or acquired (infection-associated or iatrogenic) lymphedema is a rare cause of extremity-based angiosarcomas, of which postmastectomy angiosarcoma in the lymphedematous arm is the most common example.

GRADING

Grading is an arbitrary estimate of the degree of malignancy. The grading of soft tissue sarcomas by histologic parameters is to provide guidance for prognostic prediction and treatment, especially in relation to the patient's need for adjuvant therapy. Important other factors independent of grade are tumor size, completeness of the surgical excision, and the overall clinical situation.

Low-grade sarcomas are locally aggressive but have a very low metastatic potential and usually a good prognosis. Consequently, they are usually treated with a wide surgical excision whenever possible. High-grade sarcomas have a high risk for both local recurrence and metastasis, and chemotherapy is the main mode of treatment for some high-grade sarcomas, such as childhood rhabdomyosarcoma and Ewing family tumors. Grading is also part of the current staging systems.

None of the grading systems replaces histologic typing, which is very important whenever type can be specified. In fact, the most widely used grading systems include histologic type as a grading variable. Several excellent reviews on sarcoma grading are recommended to the reader.[18–21]

Grading Systems

The most extensively documented and widely used grading system for adult soft tissue sarcomas is the one developed by the French Federation of Cancer Centers (FFCC). This system has evolved over the past 25 years and has been specifically validated for spindle cell sarcomas.[21–24] The FFCC system uses the parameters of tumor differentiation, necrosis, and mitotic activity; the last two parameters were employed differently in the previously introduced grading system of the National Cancer Institute.[24] A comparative study has suggested that the FFCC system might result in a more informative grade, a higher number of high-grade tumors, a lesser number of intermediate-grade tumors, and a higher prognostic predictive value.[25]

The current FFCC system is based on score points obtained as a sum of three factors: differentiation, mitotic rate, and tumor necrosis (Table 1.3). Each soft tissue sarcoma type has a differentiation score assigned, based on the histologic type (Table 1.4).

The pediatric oncology grading system is applicable to nonrhabdomyosarcomatous soft tissue sarcomas of children. This system relies significantly on the histologic type as the basis of the grade, especially for the low-grade (grade 1) and high-grade (grade 3) tumors. It also incorporates necrosis and mitotic activity as grading parameters for some histologic types.[26] This system is summarized in Table 1.5.

A four-tiered system, dividing both low- and high-grade tumors into two grades, has also been suggested.[27] Another proposed grading system was based mainly on mitotic activity,[28] and one system included tumor size, vascular invasion, and microscopic necrosis as prognostic parameters.[29]

Limitations of Grading

Tumor grading applies best to an excision specimen. Limited sampling (e.g., needle biopsy) can give a minimum grade only. Preoperative treatments, such as radiation, chemotherapy, and embolization, often induce tumor necrosis and variable tumor abolition, making grading inapplicable. Grading of tumors that often vary remarkably little from case to case seems to add limited value to the histogenetic diagnosis (e.g., extraskeletal myxoid chondrosarcoma and alveolar soft part sarcoma); these tumors therefore are often considered ungradable. Some grading parameters include a subjective element. For example, the determination of well-differentiated versus conventional or poorly differentiated examples of various tumors can be subjective (Table 1.4).

TABLE 1.3: Grading System of the French Federation of Cancer Centers, Based on Coindre[21]

Tumor differentiation, according to Table 1.4	1–3
Well-differentiated tumors of defined histogenetic types	1
Moderately differentiated tumors of defined histologic types	2
Poorly differentiated tumors and undefined histogenetic types	3
Mitotic count	
0–9/10 HPF[a]	1
10–19/10 HPF	2
20 or more/10 HPF	3
Tumor necrosis[a]	
None	0
<50%	1
≥50%	2
Histologic grade	Sum of the above scores
1	2 or 3
2	4 or 5
3	6, 7, or 8

This grading system formulates the overall grade from total points from scores from tumor differentiation, mitotic rate, and tumor necrosis.

[a] High-power field (HPF) defined as 0.1734 mm^2.

The impact of histologic grading is diluted by the nearly automatic high-grade assignment for some tumor types, because all examples of such entities would result in a high-grade scoring, and because of the lack of grading principles for some tumor types. This was pointed out by the Association of Directors of Anatomic and Surgical Pathology.[30] Even the best grading systems include an element of subjectivity in the assessment of tumor differentiation, mitosis counting, and evaluation of the amount of necrosis. The reliability of grading is probably greater for the more common tumor types, and conversely, the assessment of grading systems for rare tumor types often is based on a

TABLE 1.4: Tumor Differentiation Score According to the Updated Version of the French Federation of Cancer Centers Grading System

Differentiation score 1
Well-differentiated fibro-, lipo-, or leiomyosarcoma
Differentiation score 2
Conventional fibrosarcoma
Myxoid sarcomas (MFH, liposarcoma, chondrosarcoma)
Storiform-pleomorphic MFH
Conventional leiomyosarcoma
Well-differentiated or conventional angiosarcoma
Conventional MPNST
Differentiation score 3
Poorly differentiated fibrosarcoma
MFH with a nonstoriform pattern
Round cell liposarcoma
Pleomorphic sarcomas (liposarcoma, leiomyosarcoma)
Rhabdomyosarcoma (embryonal, alveolar, pleomorphic)
Poorly differentiated and epithelioid angiosarcoma
Triton tumor, epithelioid MPNST
Extraskeletal mesenchymal chondrosarcoma
Osteosarcoma
Ewing family tumors/PNET
Synovial sarcoma
Clear cell sarcoma
Epithelioid sarcoma
Alveolar soft part sarcoma
Malignant rhabdoid tumor
Undifferentiated sarcoma

Modified from Coindre.[21]

TABLE 1.5: The Pediatric Oncology Group Grading System for Nonrhabdomyosarcomatous Soft Tissue Sarcomas of Children

Grade 1
Dermatofibrosarcoma protuberans, deep
Infantile fibrosarcoma, well-differentiated (children not >4 years)
Infantile hemangiopericytoma, well-differentiated
Well-differentiated and myxoid liposarcoma
Well-differentiated MPNST
Extraskeletal myxoid chondrosarcoma
Angiomatoid (malignant) fibrous histiocytoma
Grade 2
Sarcomas not included in grades 1 and 3 with <15% of necrosis with no more than 5 mitoses/10 HPF
No marked atypia, no markedly high cellularity
Includes noninfantile fibrosarcomas, poorly differentiated infantile fibrosarcomas, leiomyosarcomas, and MPNSTs filling the previous criteria
Grade 3
Round cell and pleomorphic liposarcoma
Mesenchymal chondrosarcoma
Extraskeletal osteosarcoma
Malignant triton tumor
Alveolar soft part sarcoma
Sarcomas not included in grade 1 with >15% of necrosis, or with over 5 mitoses per 10 HPF. Marked atypia and cellularity can also result in assignment into grade 3.

Modified and adapted from Parham et al.[26]

TABLE 1.6: Summary of the Current TNM or the American Joint Committee for Cancer Staging System for Soft Tissue Sarcomas

Stage	Histologic Grade (G)	Primary Tumor	Lymph Node Status (N)	Distant Metastasis (M)
I–IV	Low or high	T1 or T2	Negative/Positive	Absent/Present
IA	Low	T1a or T1b	Negative	Absent
IB	Low	T2a or T 2b	Negative	Absent
IIA	High	T1a or T1b	Negative	Absent
IIB	High	T2a	Negative	Absent
III	High	T2b	Negative	Absent
IV	Any	Any	Positive	Absent
	Any	Any	Negative or positive	Present
Grade (G)	An arbitrary determination based on current grading systems. In a three-tier system, intermediate grade (g 2 of 3) is merged with high grade.			
T	Maximum diameter of tumor	T1 = 5 cm or less		T2 = >5 cm
Tumors of each T group are subclassified based on depth or anatomic location or both:				
	a = Superficial tumors of the trunk and extremities not invading in the superficial fascia			
	b = Deep tumors invading, permeating or located below the superficial fascia, or tumors in intraabdominal, retroperitoneal, and intrathoracic location			

very limited number of cases. Ideally, grading for all sarcoma types should be based on studies comparing large numbers of sarcomas of different parameters within a single histologic diagnosis group.

STAGING

The stage is an estimate for the extent or dissemination of a tumor; the current system includes tumor grade as a component. Stage is an important characterization of a tumor for treatment formulation, cooperative clinical trials, and clinicopathologic studies of tumor behavior. The stage is based on clinical and radiologic evaluation of the tumor. The reader is referred to an illustrative review on sarcoma staging systems.[31]

The most widely used staging system is the Union Internationale Contre le Cancer-TNM (UICC-TNM) system.[32] Its current version has merged with the American Joint Committee of Cancer (AJCC) staging system.[33] These two identical systems classify tumors from stages I to IV, in which a low stage represents a small local tumor and stage IV describes metastatic disease (Table 1.6). All low-grade tumors are stage IA or IB, depending on the tumor size. Nonmetastatic high-grade tumors are divided into stages II and III, in which the latter stage is assigned to deep large tumors (>5 cm). This system excludes visceral sarcomas and certain cutaneous tumors such as Kaposi's sarcoma and dermatofibrosarcoma protuberans. Angiosarcoma has been excluded because its common multifocal nature makes the evaluation of tumor size and metastasis problematic.

The current UICC-TNM staging system was developed based on previous systems, especially the one originally suggested by the task force on soft tissue sarcoma of the American Joint Committee for Cancer Staging and End Results Reporting (AJCC). This system incorporated histologic grade into the final stage and was based on evaluation of 1215 cases of 13 types of soft tissue sarcomas, mainly from the extremities. The study documented the value of staging in predicting survival.[34]

The surgical staging system developed by Enneking et al.[35] applies mainly to extremity sarcomas. Like that of the UICC-TNM, this system also incorporates grade as a factor, but it subdivides the stages by the tumor's relationship to the compartments of the extremities, instead of tumor size. In this system, low-grade tumors are stage I, high-grade nonmetastatic tumors stage 2, and metastatic tumors stage 3. Further summary of the radiologic aspects of staging systems is found in Chapter 2.

Many investigators have realized that accurate prognostication must go beyond histologic typing, grading, and staging. Additional informative prognostic parameters include tumor size, tumor depth, anatomic site, and patient age. Based on these parameters and the histologic type, nomograms have been developed to predict potential mortality from sarcoma.[36–38]

EVALUATION OF SOFT TISSUE TUMOR SPECIMENS

The nature of specimens varies, depending on whether punch or needle biopsies are employed, or if incisional biopsies, piecemeal excisions, and complete resection specimens are used.

The trend toward minimally invasive diagnostic procedures has led to increasing use of small specimens, such as needle biopsies. Diagnostic specimens from internal sites, such as intraabdominal and intrathoracic tumors, are commonly needle biopsies. Ultrasound or other radiologically guided procedures have increased the accuracy of lesion sampling.

Success in the definitive diagnosis and typing of tumors varies with needle biopsies. Abdominal tumors that can often be diagnosed reliably on needle biopsy include diffuse large cell lymphoma, well-differentiated liposarcoma, leiomyosarcoma, gastrointestinal stromal tumor, schwannoma, and solitary fibrous tumor. A small biopsy cannot rule out a malignant or high-grade component, however, and also can underestimate the potential of the tumor. Definitive diagnosis of reactive conditions and low-grade lymphomas is often impossible.

Radiologic correlation can enhance the information value of a small specimen by providing additional parameters, such as tumor configuration, relation to surrounding structures, and even tissue composition (e.g., fat and fluid). Magnetic resonance imaging (MRI) studies can help to identify a dedifferentiated liposarcoma, based on the presence of an integral fatty component in a spindle cell or pleomorphic sarcoma.

Open Biopsy and Resection Specimens

Ideally, all tumor specimens should be received fresh immediately from surgery without fixation, because this increases the options for special studies and scientific evaluation. This is not necessary with needle biopsies, however, because the material is limited and optimal fixation can be best assured if the biopsy is immediately placed in the fixative.

Table 1.7 lists the steps that can be taken for the comprehensive analysis of a soft tissue tumor. These steps depend on the clinical environment and the scope of the studies planned in the future. High-quality clinicopathologic evaluation with thorough gross description, evaluation of margins, and histologic sampling must be performed in all cases.

TABLE 1.7: Steps and Parameters in a Comprehensive Analysis of a Soft Tissue Tumor Specimen on Gross Examination

1. Gross photography (preferably fresh tissue, possibly also fixed)
2. Evaluation and inking of margins
3. Gross description and tumor measurements
4. Sampling of tumor and margins for histology (perpendicular recommended to document distance from the margin)
5. Frozen section for diagnostic or triaging purposes
6. Sampling of fresh tissue for further analysis[a]
   - 6.1. Frozen tissue procurement (frozen, in special preservatives) for RNA, DNA, FISH, proteomics, chemical and immunohistochemical analysis. Formalin fixation of adjacent tissues for morphologic documentation of the selections
   - 6.2. Submission of material for cytogenetics or assessment of chemosensitivity for initiation of a short-term cell culture and a possible continuous cell line
   - 6.3. Sampling in special fixatives (alcohol, Carnoy's, B5) for further studies
   - 6.4. Sampling for glutaraldehyde fixation for electron microscopy

[a] Fresh tissue sampling should be performed as soon as possible, preferably first, if possible.

If the diagnosis is unknown when the specimen is received, frozen section is useful for triage purposes, to guide the pathologist in the optimal selection of special studies. In some centers, frozen section is also used as a primary diagnostic mode with many tumors and can be highly accurate with an experienced pathology staff.

Grossing

Small specimens should usually be inked universally, and large specimens selectively, in the areas closest to the tumor. The specimen should then be sliced with 5–10 mm intervals and the tumor measured in three dimensions. The margins are usually best evaluated by sections perpendicular to the specimen surface closest to the tumor. Possible satellite nodules outside the main tumor mass should also be recorded, and the percentage of gross necrosis estimated. Other features to be recorded include color and consistency, as well as the presence of any hemorrhage, calcification, ossification, cysts, and grossly different tumor components. Representative sections should be documented and sampled for microscopy, and small tumors should be submitted entirely (1–2 cm or less). A minimal sampling should include one section per each 1 cm of tumor diameter. A diagnostic pitfall is missing a lipomatous component in dedifferentiated liposarcoma; therefore, surrounding fat should be included in any sarcoma sampling, especially one that is retroperitoneal.

Although smaller tumors can be submitted for tissue processing on the same day that the specimen is received, larger tumors and all lipomatous tumors should be sliced

TABLE 1.8: Suggested Parameters to Be Included in the Surgical Pathology Report of a Sarcoma, as Suggested by the Association of the Directors of Anatomic and Surgical Pathology[30]

Final Report

1. Tumor site, type of biopsy or excision
2. Dept of the tumor (subcutis, fascia, skeletal muscle)
3. Tumor type, possible variant
4. Grade, if possible
5. Tumor size (maximum diameter in cm), plus possible presence of satellite nodules
6. Status of margins (minimal distance to margins) and lymph node status if present
7. Microscopic quantitation of necrosis
8. Vascular invasion (if present)

Addendum Report or Reports (if Studies Cannot be Completed by the Issue of Final Report)

1. Immunohistochemistry
2. Electron microscopy
3. Cytogenetics

and allowed to fix overnight to improve tissue processing and quality of tissue sections.

Gross Photography

Gross photography is an excellent permanent tumor documentation. The ideal photographic documentation includes intact and sliced tumor with overview and close-up views, some of them with a metric scale. Digital photographs can also assist grossing, and annotations can be made on the microscopic sampling.

Reporting

As suggested by the Association of the Directors of Anatomic and Surgical Pathology,[30] the surgical pathology report should accurately document the tumor site, histologic type, grade, tumor size, status or margins, percentage of necrosis, lymph node status, and several other factors (Table 1.8).

TISSUE PROCUREMENT FOR SPECIAL STUDIES

Tissue procurement for special studies is an important part of specimen handling, especially in academic centers, and can usually be accommodated easily without interfering with the diagnostic sampling and evaluation of the margins. It not only relates to scientific studies but also allows the optimal use of advanced diagnostic modalities.

Frozen Tissue

Freezing of tissue is clinically indicated to help perform molecular genetic assays more easily or reliably, and it is also scientifically indicated to build knowledge of genetic and biochemical changes in tumors, compared with normal tissue. Frozen tissue is required for optimal and more effective analysis of nucleic acids. High-molecular-weight DNA and RNA usually can be obtained only from fresh and not from formaldehyde-fixed tissue. Similarly, native proteins can be reliably obtained only from fresh or frozen tissue for proteomics, biochemical microanalysis of the spectrum of cell signaling, and other functionally important proteins.

Freezing in liquid nitrogen is optimal, but long-term storage in a –70°C freezer or liquid nitrogen are both adequate. Well-organized storage compartments and inventory systems are required for optimal retrieval. Liquid nitrogen storage has the advantage of being independent of electric power, which protects the tissue bank from power outages. For liquid nitrogen tanks, automatic refilling systems are available.

Ideally, aliquots of both tumor and normal tissue should be sampled separately. The best way to store the tissue is to freeze small pieces separately in a liquid nitrogen bath and transfer them to the cryovial (allowing the nitrogen to evaporate in –20°C cryostate to prevent the vial's cap popping). Such separately frozen "pearls" of tissue can be easily poured from the cryovial and used one at a time or as needed.

Cytogenetics and Cell Culture

Cytogenetic studies are diagnostically indicated in tumors with specific translocations or other chromosomal morphologic changes (see Chapter 4). They are also indicated to increase scientific knowledge of previously uncharacterized tumor types.

Cytogenetic specimens can be sent to the laboratory in a culture medium and should be preserved in a sterile manner. A very thin slice of well-preserved, non-necrotic tumor tissue should be submitted. Fine-needle aspirates are also suitable.

Short-term cultures needed for karyotyping can be successfully obtained for malignant tumors, but benign tumors can be difficult to grow. Such cultures can also be used for in vitro testing of tumor chemosensitivity.

Long-term cultures and establishing a cell line from a tumor are more challenging, and the success rate is only modest, even with highly experienced investigators. Long-term cultures offer priceless dynamic models for investigating cell biologic, biochemical, and pharmacological characteristics of the tumor, however.

Special Fixatives

Fixation of specimens in special fixatives is often indicated for optimal evaluation of tumors that might be expected to be diagnostically difficult. The tissue aliquots should be small, not exceeding the thickness of a standard coin, to allow the penetration of the fixative prior to autolysis.

TABLE 1.9: Frequent Distinctive Electron Microscopic Findings in Selected Soft Tissue Tumors

Tumor Type	Diagnostic Features
Fibroblastic neoplasms:	Variable myofibroblastic differentiation found in subsets of tumor cells:
Desmoid, fibrosarcoma, MFH	Myofibroblasts with scattered bundles of actin filaments
Smooth muscle tumors, glomus tumor	Cytoplasmic bundles of actin filaments, attachment plaques, basal lamina
Rhabdomyosarcoma	Ribosome myosin complexes, collections of thin and thick filaments, possible sarcomeres and Z-bands
Angiosarcoma	Weibel-Palade bodies (predominantly in well-differentiated tumor cells)
Schwannoma	Spindle cells, complex interdigitating cell processes and prominent basal laminas
Perineurial cell tumors	Spindle cell with long cytoplasmic processes, prominent intermediate filaments, frequent pinocytic vesicles, basal laminas
Melanoma	Melanosomes. Can be sparse and difficult to find.
Paraganglioma	Dense core granules of variable size and morphology, typically abundant
Neuroendocrine carcinoma	Membrane-bound dense core granules of 100–400 nm in diameter, typically abundant, but could be sparse in high-grade tumors
Rhabdoid tumor, sarcomas with rhabdoid cytologic features	Spherical collections of perinuclear collections of cytoplasmic intermediate filaments displacing the cytoplasmic organelles
Alveolar soft part sarcoma	Cytoplasmic rhomboid crystals with 70 Angstrom periodicity
Mesothelioma, differentiated	Long, slender microvilli that are typically 15 times longer than their width
Dendritic reticulum cell sarcoma	Desmosomes, elongated cell processes

Alcohol (absolute ethanol)-fixed tissue can be saved for further studies, such as RNA, DNA, and protein extraction, or they can be embedded in paraffin for tissue section–based studies. Alcohol-fixed tissue can be advantageous for immunohistochemical analysis of some antigens. It can also be suitable for Western blot analysis of proteins and obtaining high-molecular-weight nucleic acids.

Carnoy's fixative is a modified alcohol fixation, added with glacial acetic acid in a ratio of 1:4. Methacarn is an alcoholic fixative using methanol instead of ethanol.

Heavy metal–containing formalin fixative (B5 solution containing mercury chloride) yields superior nuclear detail and is often used for hematopoietic neoplasia. Similarly, zinc salts can be used as a less toxic and more environmentally friendly alternative. Tissues processed with these fixatives, however, are not generally suitable for molecular genetic studies.

A small aliquot of viable tumor, or if necessary, samples of several different areas, should be sampled in 2.5% buffered glutaraldehyde for electron microscopy. The easiest way to prepare a sample is first to cut a thin slice, then section this further into a rod, and then mince the rod into cubes not exceeding 1 mm at the thickest point.

ROLE OF ELECTRON MICROSCOPY

Electron microscopy is very rarely mandatory for diagnosis, but it can have diagnostic potential and therefore should be part of any tissue procurement program. The processed tissue can be saved for future studies, if analysis is not needed immediately for diagnosis. The most useful application of electron microscopy is for those soft tissue tumors with highly distinctive ultrastructural features (Table 1.9).

Ultrastructural details at magnifications ranging from 3,000 to 50,000 can give valuable diagnostic information about selected soft tissue tumors.[39,40] For many groups of tumors, such as lymphomas, melanoma, and undifferentiated tumors, immunohistochemistry has mostly replaced electron microscopy as a diagnostic method; for others it is used only infrequently because of its labor-intensive nature and cost.

Although glutaraldehyde-fixation is optimal for preserving cytoplasmic details, fixation in buffered formalin is also adequate. Cytoplasmic texture and many membranous structures deteriorate during routine formalin fixation, however, and they can be lost during tissue processing and paraffin embedding.

Currently, electron microscopy is practiced extensively only by a small group of dedicated investigators who continue to make interesting ultrastructural observations about tumor cell differentiation and histogenesis.

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