

## THE EVOLVING CONCEPT OF HEMANGIOENDOTHELIOMA

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### **Introduction**

Vascular tumors represent one of the largest groups among soft tissue tumors. A classification scheme is shown in Table 1. They are histologically and clinically heterogeneous and, considering their ubiquity, are not all well understood. In general terms, the majority of vascular lesions are clinically benign and most such lesions occur in the skin. Angiosarcomas and the group of lesions which might be regarded as of low grade or intermediate malignancy are relatively rare, yet the capacity of benign lesions to mimic more aggressive tumors both clinically and morphologically is considerable and pathologists often find themselves having to rule out a malignant diagnosis. Frequently this may not be as straightforward as it sounds. As general rules (with occasional exceptions), the presence of a lobular growth pattern favors benignity, while the presence of endothelial multilayering, particularly if accompanied by nuclear hyperchromasia, pleomorphism and mitotic activity, favors malignancy. Endothelial atypia alone (without multilayering) or the presence of a dissecting growth pattern does not equate with angiosarcoma and may be seen in a variety of benign lesions.

This ambiguity in determining whether a vascular lesion is benign or malignant and the problem in predicting the behavior of some lesions has, in some ways, become embodied in the use of the term 'hemangioendothelioma', although in reality this is not how the term was originally used. In the early part of the last century, the term hemangioendothelioma was used principally for two lesions – *benign hemangioendothelioma*, which was used to describe what we recognize nowadays as juvenile capillary hemangioma (see below) and *malignant hemangioendothelioma* to connote the malignant subset of vascular tumors composed of

endothelial cells (in order to contrast them with so-called hemangiopericytoma). Juvenile capillary hemangiomas were designated hemangioendothelioma because they were notably more cellular than other types of benign hemangioma. In addition, in the 1930s, what we nowadays know as papillary endothelial hyperplasia (or Masson's change) was first described under the term *vegetant intravascular hemangioendothelioma*, because Masson believed this to be a truly neoplastic lesion. Soon thereafter, however, these lesions were recognized to be reactive in nature (see below). The term malignant hemangioendothelioma (which was popularized by Stout), rather than angiosarcoma, remained in quite common usage through the 1960s and even early 1970s and it is not difficult to imagine how often use of the term 'hemangioendothelioma' for both benign and malignant lesions may have given rise to confusion. Furthermore, in the pre-immunohistochemical era (mostly in the 1930s and 1940s) a variety of epithelial lesions with acinar or tubular architecture and with intraluminal red blood cells were quite often mislabelled hemangioendothelioma. In any event, by 1980 or so, the term malignant hemangioendothelioma had largely fallen into disuse. A further subset of low-grade malignant vascular tumors was subsequently introduced to the family of hemangioendotheliomas in the late 1960s – specifically Dabska's 'malignant endovascular papillary angioendothelioma', the definition of which has become more sharply defined over time (see below).

It was not until the early 1980s, with the first edition of the Enzinger and Weiss textbook, that a more refined and thoughtful approach to the use of the term 'hemangioendothelioma' was introduced. Specifically, Enzinger and Weiss utilized the term to describe the small subset of vascular tumors which did not fit neatly into either the benign or malignant categories at that time. The prototypical tumor type to be included under this heading was epithelioid hemangioendothelioma, which nowadays is more often regarded as frankly malignant (see below). By the time of the second edition of Enzinger and Weiss, published in 1988, the term 'hemangioendothelioma' had become more firmly established to describe this intermediate group of vascular lesions and, by then, the entities of epithelioid hemangioendothelioma, spindle cell hemangioendothelioma and malignant endovascular papillary angioendothelioma were included under this heading. Since that time, it has become evident that spindle cell hemangioendothelioma is in fact a benign lesion, better known as spindle cell hemangioma (see below) but this reappraisal did not become widely acknowledged until the mid 1990s and therefore, in the WHO Classification which was published in 1994, the grouping proposed by

Enzinger and Weiss was maintained. With the most recent (2002) revision of the WHO Classification, the term ‘hemangioendothelioma’ is no longer regarded as strictly defining a specific group of lesions with intermediate behavior but, instead, is now used to describe lesions which would fall into the locally aggressive, rarely metastasising and frankly malignant categories, as detailed below.

## **LESIONS FOR WHICH THE TERM ‘HEMANGIOENDOTHELOMA’ IS NO LONGER USED**

### **PAPILLARY ENDOTHELIAL HYPERPLASIA**

**(Syn: Intravascular papillary endothelial hyperplasia, Masson’s tumor, Masson’s vegetant intravascular hemangioendothelioma)**

#### **Clinical Features**

Papillary endothelial hyperplasia<sup>1-3</sup> is a distinctive form of organising thrombus which may occur in association with thrombosis either in a normal vessel, a pre-existing vascular lesion (e.g. hemorrhoidal vein or hemangioma) or, least often, in an extravascular hematoma.<sup>4</sup> The so-called primary type, occurring in normal vessels, is commonest in the fingers or neck, most usually in young adults of either sex, and it presents as a painless or occasionally tender nodule. Most examples measure less than 2 cm in diameter. The clinical features of those cases developing in the setting of a pre-existent vascular lesion (so-called secondary type) are determined by the nature of the prior pathology. Probably the two commonest lesions to be so affected are cavernous hemangiomas and hemorrhoidal veins. Only very rare examples of papillary endothelial hyperplasia are multiple<sup>5</sup> and this usually would reflect the multifocality of a pre-existing process with superimposed thrombosis. Examples of this process arising in extravascular thrombus are extremely uncommon.<sup>4</sup>

## **Pathologic Features**

Most examples of papillary endothelial hyperplasia are obviously intravascular, whether or not the vessel is normal or part of an angioma. Irrespective of the clinical setting in which papillary endothelial hyperplasia arises, its morphologic features are similar and consist primarily of innumerable small papillae with hyaline cores associated with adjacent thrombus. Often one can see transition from organizing fibrin thrombus into the papillae, which then seem to fall away from the thrombus. Papillae often show eosinophilic fibrin in their core. Each papilla appears to develop by a process of hyalinization and the simultaneous development of an attenuated endothelial covering. The papillary core generally is acellular but may contain rare thin-walled capillaries. The endothelial cells are always bland and monolayered and mitotic figures are rare. The characteristic papillae may appear to lie free within vascular lumina or may be attached to the vessel wall.

## **Differential Diagnosis**

The appearances and intravascular location are usually so distinctive that there is no real differential diagnosis. Angiosarcoma is almost never purely intravascular and rarely if ever shows the same degree of papillarity. The rare examples of angiosarcoma with an intravascular component show endothelial atypia, hyperchromasia and multilayering. Spindle cell hemangioma may have papillary structures but these are much more cellular and are associated with solid spindle cell areas.

## **Prognosis**

This is a benign non-recurrent process. Any evidence of recurrence, which is exceptional, would only reflect persistence or re-growth of a pre-existing lesion in which papillary endothelial hyperplasia had occurred.

## **JUVENILE CAPILLARY HEMANGIOMA**

**(Syn: Infantile hemangioendothelioma, Benign hemangioendothelioma,  
Cellular hemangioma of infancy, Strawberry naevus)**

The term capillary hemangioma encompasses a group of histologically similar but clinically distinct hemangiomas. Its best known and prototypical form is juvenile capillary hemangioma. Other variants of capillary hemangioma include pyogenic granuloma, tufted angioma, verrucous hemangioma and cherry angioma. All are clinically benign but, in their most cellular forms, are sometimes misdiagnosed as malignant.

### **Clinical Features**

The prototypical juvenile form of capillary hemangioma accounts for up to a third of vascular tumors in infants and children and has an incidence of 1-2 per 100 live births.<sup>6</sup> Although it may arise at almost any location, the skin and soft tissues of the head and neck region are by far the site of predilection. Females are affected more than males and these lesions typically are present at, or soon after, birth. They grow as a reddish-purple macule which then starts to involute in the second year of life. Most cases have involuted totally by the age of 6 or 7, with a minority persisting 2-3 years beyond that age.<sup>7,8</sup>

### **Pathologic Features**

The histologic appearances of juvenile capillary hemangioma depend upon the age of the lesion. In the earliest (growth) phase, when most biopsies are taken, this is a floridly cellular lesion which at low power has a well-formed lobular architecture and which may involve dermis, subcutis and deeper tissues. Individual lobules are composed of closely packed small capillaries showing little or no luminal canalization. At this stage the vascular nature of the tumor may not be immediately apparent. As time passes there is progressive vascular canalization and dilation. As regression begins there is a relative increase in fibrotic stroma which then separates ectatic vascular channels and eventually both components largely involute. In many cases, at any stage of their evolution, a larger feeding vessel (or vessels) can be identified. Especially in the active growth phase it is not unusual to find perineurial (or even endoneurial) invasion by tumor.<sup>9</sup>

## **Special Studies**

Although juvenile capillary hemangiomas appear to stain consistently for GLUT1, whereas most other benign vascular lesions do not,<sup>10</sup> in most cases additional stains are unnecessary. However, in the very cellular juvenile cases, reticulin staining helps to highlight the underlying vasoformative architecture. Interestingly, recent studies have suggested that juvenile capillary hemangiomas are composed, at least in part, of CD133-positive endothelial progenitor cells.<sup>11</sup> Additionally, by both X inactivation studies<sup>12</sup> and VEGFR mutational analysis,<sup>13</sup> these lesions have been shown to be clonal in nature, further blurring the definitions of a neoplasm or malformation/developmental anomaly.

## **Differential Diagnosis**

It is important to distinguish between juvenile capillary hemangioma and tufted angioma since they may present in a similar clinical setting but the latter does not regress spontaneously. Tufted angioma is composed of discontinuous/dispersed vascular lobules (so-called cannonball distribution) and some of the lobules typically have thin-walled, crescentic lymphatic channels at their periphery. A spindled pericytic component is often more prominent than in juvenile capillary hemangioma. The rapid growth, high cellularity and frequent mitoses in some lesions may raise concern to exclude angiosarcoma. The presence of a lobular architecture, often with feeder vessels, and the absence of endothelial multilayering or atypia are key distinguishing features. Angiosarcoma essentially never has a lobular architecture or feeder vessels. Highly cellular capillary hemangiomas with little or no luminal canalization may be hard to distinguish from other spindle cell neoplasms but the reticulin pattern and immunopositivity for CD31 and VWF are usually discriminatory.

## **Prognosis**

As a general rule, only the juvenile type of capillary hemangioma regresses spontaneously, while the other variants generally do not. Juvenile-type capillary hemangiomas are therefore only excised if they threaten vital structures or fail to regress. Each of the variants

is prone to non-destructive local recurrence unless completely excised.

## **SPINDLE CELL HEMANGIOMA**

**(Syn: Spindle cell hemangioendothelioma)**

Spindle cell hemangioma was first described under the rubric spindle cell hemangioendothelioma and, at that time, was thought to represent a distinctive low-grade form of angiosarcoma with potential for frequent recurrence and rare metastasis.<sup>14</sup> Time and the study of more cases has shown this not to be the case. Rather this lesion appears to be either a benign neoplasm or perhaps a reactive process engrafted on abnormal, often malformed vessels.<sup>15-19</sup> Its tendency for multicentricity likely reflects a field change effect.

### **Clinical Features**

Spindle cell hemangioma<sup>14-16,18</sup> may present over a very wide age range; many lesions are first noticed in childhood or early adulthood but may not come to medical attention for many years thereafter. Sex incidence is approximately equal. The majority of cases develop in the skin and subcutis of the distal extremities; more proximal or deep-seated examples are uncommon. Up to 50% of patients develop multiple lesions, usually in the same general anatomic region, and this process may occur over a period of decades. Individual lesions appears as small (< 2 cm), sometimes painful, nodules which produce bluish skin discoloration if located in the dermis. Up to 10% of patients have associated clinical anomalies which might contribute to an altered vasculature or blood flow in the affected limb; specifically these are lymphedema, enchondromatosis<sup>20</sup> (hence Maffucci's syndrome), Klippel-Trenaunay syndrome and early-onset varicose veins (presumed due to a venous valve defect).

### **Pathologic Features**

Grossly these lesions appear as small hemorrhagic nodules in deep dermal or subcutaneous tissue. Histologically they are unencapsulated but reasonably circumscribed and up to 50% show involvement of a large pre-existing vessel (usually a vein). Perhaps 20-30% are

entirely intravascular in location. At low power most lesions have two main components - solid spindle cell areas and dilated cavernous vascular spaces. The latter may contain thrombi, phleboliths or cellular papillary structures resembling strands of the more solid spindled tissue. An additional low power feature, evident in the majority of cases, is the presence of nearby abnormal thick-walled vessels which often show features of a localized arteriovenous shunt.

Closer examination of the spindle cell areas reveals slit-like spaces that represent either poorly formed or else collapsed vascular channels surrounded by pericytic and fibroblastic spindle cells. Thus there are typically admixed plump and spindled endothelial nuclei as well as spindled nuclei of supportive cells. Endothelial cells with plump more rounded nuclei often show striking cytoplasmic vacuolation (reminiscent of that seen in epithelioid hemangioendothelioma) and this is an important diagnostic clue. With very rare exceptions there is no nuclear atypia and mitoses are scarce. However occasional cases show nuclear atypia and hyperchromasia which most likely are degenerative or reactive in nature.

Reticulin stains demonstrate that the solid spindled areas are actually composed of vascular channels. Immunohistochemistry serves only to demonstrate that the cell population in the spindled areas is mixed, comprising some endothelial cells (CD31 or CD34 positive), some pericytes (actin positive) and some fibroblastic cells.

### **Differential Diagnosis**

The principal differential is Kaposi's sarcoma which rarely contains cavernous spaces, lacks vacuolated endothelial cells, shows hyaline globules (not seen in spindle cell hemangioma) and which shows much more uniform immunopositivity of the spindle cells for CD34 (as well as CD31). Furthermore, Kaposi's sarcoma stains positively for HHV-8, which spindle cell hemangioma does not. In addition, Kaposi's sarcoma is rarely, if ever intravascular. Kaposiform hemangioendothelioma lacks cavernous spaces or vacuolated endothelial cells and has a lobular growth pattern. Occasionally aneurysmal benign fibrous histiocytoma is mistaken for spindle cell hemangioma. However the former shows much greater cytologic polymorphism, a generally storiform growth pattern and overlying epidermal hyperplasia, and it lacks vacuolated endothelial cells or immunopositivity for endothelial antigens.

## **Prognosis**

Solitary lesions are cured by simple local excision. Patients with multiple lesions often continue to develop new lesions (usually in the same general area in the manner of a field defect) over many years but true recurrence in a previous excision site is rare. The originally reported metastasizing case is now generally accepted to have been a radiation-induced sarcoma and there is no evidence that spindle cell hemangioma affects life expectancy or has any significant morbidity whatever. These facts contributed significantly to the decision to drop the term 'hemangioendothelioma' for this entity.

## **TUMORS FOR WHICH THE TERM 'HEMANGIOENDOTHELIOMA' IS STILL USED**

### **INTERMEDIATE VASCULAR TUMORS**

Grouped under this heading are those lesions which some pathologists might refer to as of 'borderline' or 'intermediate' malignant in clinical terms. They may all pursue a clinically aggressive and even fatal course, some of them with metastasis. In the new WHO Classification,<sup>21</sup> these lesions are broken down into two separate biologic categories – 'intermediate (locally aggressive)' and 'intermediate (rarely metastasising)' – which helps to reflect the spectrum of behavior in this group of lesions. Many of the tumor types in this category are very uncommon.

### **INTERMEDIATE (LOCALLY AGGRESSIVE)**

#### **KAPOSIFORM HEMANGIOENDOTHELIOMA**

**(Syn: Kaposi-like infantile hemangioendothelioma)**

#### **Clinical Features**

Kaposiform hemangioendothelioma<sup>22-27</sup> occurs most often in infants and children (especially under the age of 2 years) but may also occur in adults<sup>26</sup> and, while it was originally thought to occur mainly in the retroperitoneum, it can also arise with equal and perhaps greater

frequency in soft tissue of the limbs and head and neck region.<sup>27</sup> Sex distribution is equal. It presents as a poorly marginated, multinodular infiltrative mass which may reach a considerable size, especially in the retroperitoneum. Clinical effects are due mainly to its infiltrative growth as well as a common association with consumption coagulopathy (Kasabach-Merritt syndrome), which seems to be an almost consistent feature of retroperitoneal cases,<sup>22-24</sup> but which also occurs in association with extra-abdominal lesions.<sup>27</sup> At least 20% of cases<sup>23</sup> (and possibly more<sup>27</sup>) may be associated also with lymphangiomatosis of the adjacent soft tissue.

### **Pathologic Features**

Macroscopically these lesions consist usually of multiple hemorrhagic nodules, each nodule measuring no more than 1-2 cm in most cases. The nodules and intervening fibrous tissue coalesce to form a firm mass.

Histologically the striking features at low power are high cellularity and a lobular architecture with poorly defined margins. Individual lobules closely resemble Kaposi's sarcoma in being composed of uniform spindle cells with pale eosinophilic cytoplasm and elongated nuclei. However these are associated, especially at the lobular periphery, with rounded, thin-walled capillaries and frequent fibrin microthrombi. Atypia is absent and mitoses are scarce. Many cases contain glomeruloid clusters of more epithelioid endothelial cells which may show cytoplasmic vacuolation. Hyaline globules are an occasional feature. Especially in deep-seated lesions the margins can be very infiltrative with destruction of adjacent normal tissue, such as pancreas.

Immunostains show that a significant proportion of the spindle cells stain positively with endothelial markers, while some are also actin positive in keeping with pericytes. Staining for HHV8 is negative and, in contrast to juvenile capillary hemangioma, these lesions are negative for GLUT1.

### **Differential Diagnosis**

On morphologic grounds, Kaposi's sarcoma (KS) is the main differential. In young children KS is exceptionally rare outside lymph nodes. In addition, KS generally lacks the

lobularity, dilated capillaries and fibrin microthrombi of kaposiform hemangioendothelioma. Spindle cell hemangioma differs by very rarely being deep-seated and the presence of cavernous spaces, frequent papillary structures and prominently vacuolated epithelioid endothelial cells. Tufted angioma is a dermal lesion in which the lobules are smaller, more widely dispersed, and usually less spindled in cytologic terms with a crescentic lymphatic at their periphery.

### **Prognosis**

Kaposiform hemangioendothelioma pursues a fatal course in perhaps 15-20% of cases but this seems only to occur in retroperitoneal lesions or those associated with consumption coagulopathy. At any location, recurrence is frequent and control of local disease is often difficult, sometimes necessitating radical surgery.

## **INTERMEDIATE (RARELY METASTASIZING)**

### **RETIFORM HEMANGIOENDOTHELIOMA**

#### **Clinical Features**

Retiform hemangioendothelioma<sup>28-30</sup> is a recently characterized tumor, formerly co-classified with angiosarcoma, which arises in the skin, mainly of the distal extremities, and usually affects young adults of either sex, although some cases occur in older patients. The lesion presents usually as a slowly growing plaque, sometimes with a reddish-purple hue. One patient with multiple lesions has been described.<sup>29</sup> Occasional cases occur in the setting of preceding lymphedema or irradiation.<sup>28</sup> Lesional size rarely exceeds 2-3 cm.

#### **Pathologic Features**

The tumor is centered mainly in the reticular dermis but often extends into both papillary dermis and subcutis. It is characterized, at low power, by arborizing, elongated and generally narrow vessels with hyperchromatic protuberant endothelial nuclei, hence the resemblance to rete testis which is implied in the name. Approximately 50% of cases show a prominent lymphoid infiltrate which may be both stromal and intraluminal. Closer examination of the neoplastic vessels, as they ramify through the dermis, reveals protuberant (hobnail) endothelial

nuclei with basal cytoplasm which merges imperceptibly with the vascular basement membrane. Some endothelial cells may form papillae reminiscent of Dabska's tumor (see below) and rare cells may be vacuolated. Most tumors have variably prominent, but usually small, more solid areas composed of plumper endothelial cells arranged in sheets. In exceptional cases these solid areas may be more spindled. There is no endothelial pleomorphism and mitoses are very scarce. In the single lymph node metastasis described so far the tumor cells were spindled.<sup>28</sup>

The neoplastic endothelial cells stain positively with usual vascular markers, generally marking more strongly with CD34 than with CD31 or VWF in these lesions. Keratin positivity is not usually a feature.

### **Differential Diagnosis**

Hobnail hemangioma is a smaller, more superficial and more localized lesion in which the vessels in the papillary dermis are more dilated and in which the vessels narrow and disappear in the reticular dermis. Angiosarcoma generally affects older patients, shows more irregular infiltration and collagen dissection and shows greater endothelial pleomorphism, multilayering and mitotic activity. Papillary intralymphatic angioendothelioma, often known as Dabska's tumor, (see below), while having similar cytology to retiform hemangioendothelioma, generally forms endovascular papillae within cavernous lymphatic-like spaces.

### **Prognosis**

Unless widely excised, these lesions recur locally and often do so repeatedly over a period of many years. The development of persistent uncontrolled disease in a distal location (where good margins are hard to obtain) sometimes leads to digital (or ray) amputation. To date only a single patient has developed metastatic disease and this was to a groin lymph node.<sup>28</sup> To date, no patient is known to have died of retiform hemangioendothelioma but this possibility cannot be excluded.

### **PAPILLARY INTRALYMPHATIC ANGIOENDOTHELIOMA**

**(Syn: Dabska's tumor, malignant endovascular papillary angioendothelioma)**

Since the first formal description of this tumor type in 1969,<sup>31</sup> less than thirty convincing additional case reports have been published and, as in the original description which also included lesions which would nowadays be classified as retiform hemangioendothelioma, not all were morphologically homogeneous. This is not a well-defined or well-characterized entity and some authors have expressed uncertainty as to its existence as a discrete pathological lesion, although this is being overcome through the recent publication of more uniform and better-defined data.<sup>32</sup>

### **Clinical Features**

Based on available data, papillary intralymphatic angioendothelioma affects mainly infants and children and shows a wide anatomical distribution with predilection for the skin.<sup>31-34</sup> It seems to present most often as a slowly growing indurated plaque.

### **Pathologic Features**

Again with the confines of limited data<sup>32-34</sup> that lesion most often seems to be characterized by dilated thin-walled intradermal vessels, reminiscent of lymphatics, within which there are cellular papillary structures composed of endothelial cells and admixed lymphocytes. Stromal lymphocytes are also a feature in some cases. The endothelial cells tend to be small and rounded with a small amount of eosinophilic cytoplasm and hobnail type nuclei lacking significant atypia. These papillary tufts of endothelial cells may have a hyaline core. Lymphocytes tend to cluster around these papillae. The differential diagnosis of papillary intralymphatic angioendothelioma is the same as for retiform hemangioendothelioma (see above).

### **Prognosis**

In the original series it was suggested that these lesions recur locally and may rarely spread to lymph nodes without systemic metastasis. However it seems that at least one of the originally reported patients later died with metastatic disease so the issue of how malignant these

lesions really are remains unresolved. In this circumstance it would seem advisable to widely excise any such lesion which shows a reasonable morphologic fit.

### **COMPOSITE HEMANGIOENDOTHELIOMA**

Composite hemangioendothelioma<sup>35</sup> is the term coined recently for a remarkable group of vascular lesions arising almost exclusively in the hands and feet of adult patients. These lesions behave similarly to retiform hemangioendothelioma, being characterized by frequent local recurrence while metastasis seems rare (although follow-up data so far are limited). Histologically, composite hemangioendothelioma generally consists of admixed components of both epithelioid and retiform hemangioendothelioma. Many cases also have areas indistinguishable from angiosarcoma (which, in other circumstances, might have heralded more aggressive behavior) and some examples additionally show features of spindle cell hemangioma or some other benign angiomatous process. Since the original publication describing this lesion, I have seen approximately 10 additional cases and these have shown similar distinctive clinicopathologic features (unpublished data).

### **POLYMORPHOUS HEMANGIOENDOTHELIOMA**

Polymorphous hemangioendothelioma has only been reported in very small case numbers and occurs mainly in lymph nodes,<sup>36</sup> usually in adults. Isolated cases also exist in soft tissue.<sup>37</sup> Although data are limited, it seems that, as well as recurring, these lesions have the potential to metastasize,<sup>37</sup> and hence this lesion may eventually prove to be an unusual morphologic variant of angiosarcoma. For this reason, this tumor type has not yet been formally accepted in the WHO Classification.<sup>21</sup> Polymorphous hemangioendothelioma comprises a complex admixture of solid, primitive vascular and more angiomatous endothelial proliferation. Tumor cells are consistently plump and even epithelioid with occasional vacuoles. Solid areas have a sheet-like, nested or trabecular growth pattern. The angiomatous component may have a retiform appearance (see retiform hemangioendothelioma above). Nuclear atypia is not a feature and mitoses are rare.

## **MALIGNANT VASCULAR TUMORS**

### **EPITHELIOID HEMANGIOENDOTHELIOMA**

#### **Clinical Features**

Although well known to occur also in lung, liver, bone and (less often) other organs,<sup>38</sup> this account is focussed on epithelioid hemangioendothelioma (EHE) arising primarily in soft tissue. In contrast to other locations, EHE arising in soft tissue is most often (at least initially) a solitary lesion and occurs mainly in mid-adult life, affecting patients of either sex equally.<sup>38-40</sup> Examples in children are distinctly rare. Almost any anatomic location may be affected and more cases are subfascial than subcutaneous; up to 10% are dermal in location. The usual presenting feature is an enlarging mass and a significant proportion of patients complain of pain, likely resulting from lesional angiocentricity and vascular occlusion. When excised, most tumors measure less than 5 cm and examples greater than 10 cm are uncommon. Importantly a subset of patients (perhaps 10% at most) are found to have had (or later develop) multi-organ EHE over a very prolonged period, often decades. This may include any combination (in any order) of soft tissue, lung, bone, liver and other involvement. Whether or not this truly represents indolent metastasis or whether it is indicative of field change disease is as yet unelucidated. No etiologic associations have been identified so far in EHE of soft tissue.

#### **Pathologic Features**

Macroscopically these lesions tend to be firm, fibrous or gritty with a pale cut surface and a variably delimited margin. Occasional cases may show obvious involvement of a large vessel. There is usually no gross evidence of hemorrhage or necrosis.

Histologically most examples are poorly marginated, infiltrative lesions while occasional cases are multinodular. Approximately a third of cases appear to originate from a vessel (usually a vein) and to spread centrifugally within a myxohyaline stroma. Other examples have an entirely diffuse growth pattern with no evidence of a specialized stroma. For the most part, tumor cells are arranged in cords, strands, small nests or singly with little or no evidence of overt vessel formation. Individual cells are plump, rounded or, less often, spindle-shaped with pale eosinophilic, often glassy cytoplasm and a vesicular nucleus with inconspicuous nucleoli. A

variable proportion of cells typically show striking cytoplasmic vacuolation (known as intracytoplasmic lumina), within which red blood cells are occasionally seen. Adjacent stroma may show recent or old hemorrhage, hyalinisation, focal chronic inflammation, and in up to 15% of cases, metaplastic ossification.<sup>40</sup> Some cases, particular those in the mediastinum, show an osteoclastic giant cell reaction in the stroma.<sup>41-43</sup>

Most cases show little or no cytologic atypia and few mitoses (generally < 2 per 10 HPF), but 10-15% of these tumors show worrisome features in the form of either nuclear atypia and pleomorphism, more spindle celled cytology, higher mitotic activity or focally more solid sheet-like growth reminiscent of epithelioid angiosarcoma. At the individual case level, these features do not correlate reliably with more aggressive behavior;<sup>40</sup> nevertheless there is a trend in this regard which suggests the existence of a morphologic and biologic continuum between EHE and epithelioid angiosarcoma. Rare cases may be very hard to subclassify in this context. It is my preference, when a lesion shows predominant characteristics of EHE but with obvious solid foci and increased atypia, to use the designation ‘malignant epithelioid hemangioendothelioma’ in order to convey greater concern about the likely clinical behavior.

Virtually all cases are immuno-positive for one or more endothelial antigens, amongst which CD31 and von Willebrand factor (VWP) are the most specific. In addition, up to a third of cases show keratin positivity, which is usually only focal but can be potentially misleading.<sup>40,44</sup> A significant proportion of cases also show actin positivity in the endothelial cells. By electron microscopy, tumor cells show usual endothelial features in the form of scattered Weibel-Palade bodies, prominent pinocytosis and usually a well-formed external lamina. Additional features seen in EHE are prominent sheets of cytoplasmic filaments (hence the epithelioid appearance) as well as intracytoplasmic lumina.

### **Differential Diagnosis**

Epithelioid hemangioma is distinguished by its well-formed, canalized vessels and often lobular architecture. Epithelioid angiosarcoma, by contrast, tends to grow in diffuse sheets and the tumor cells tend to be larger than those of EHE with a prominent amphophilic nucleolus. High grade myxoid (round cell) liposarcoma is sometimes mistaken for EHE but differs by the

presence of thin-walled arborising vessels, the usual presence of convincing lipoblasts and negativity for endothelial markers. Metastatic carcinoma or epithelioid sarcoma are separable by their usually strong and diffuse positivity for keratin and EMA while CD31 and VWF are negative. CD34 is less helpful in this context since up to 50% of epithelioid sarcomas are positive. Vacuolated cells in a carcinoma will commonly contain mucin; conversely vacuolated cells are usually sparse in epithelioid sarcoma. The distinctive necrosis or necrobiotic collagen of epithelioid sarcoma are not a feature of EHE.

### **Prognosis**

Aside from those rare patients who develop indolent multi-organ disease (mentioned above), up to a third of patients with EHE of soft tissue develop nodal or systemic metastases and overall mortality is of the order of 20%, hence the former designation as borderline seems inappropriate.<sup>40</sup> Predicting behavior reliably on histologic grounds is not possible since very bland lesions may metastasize. The local recurrence rate is only 10-15%, likely reflecting the comparative ease of obtaining adequate margins in relatively small tumors.

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## TABLE 1

### CLASSIFICATION OF VASCULAR TUMORS IN SOFT TISSUE

#### BLOOD VESSELS

##### BENIGN TUMORS AND TUMOR-LIKE CONDITIONS

##### Reactive vascular proliferations

- Papillary endothelial hyperplasia (Masson's tumor)
- Reactive angioendotheliomatosis
- Glomeruloid hemangioma
- Bacillary angiomatosis

##### Vascular ectasias

- Nevus flammeus (salmon patch, port-wine stain)
- Nevus araneus
- Venous lake
- Angioma serpiginosum
- Hereditary hemorrhagic telangiectasia (Osler-Weber-Rendu)
- Angiokeratoma

##### Capillary hemangioma

- Variants: Tufted angioma
- Verrucous hemangioma
- Cherry angioma
- Lobular hemangioma (pyogenic granuloma)

##### Cavernous hemangioma

- Variants: Sinusoidal hemangioma

##### Arteriovenous hemangioma

- Variants: Superficial (cirroid aneurysm)
- Deep

##### Epithelioid hemangioma (angiolymphoid hyperplasia with eosinophilia)

##### Microvenular hemangioma

##### Targetoid hemosiderotic hemangioma ("hobnail" hemangioma)

##### Venous hemangioma

##### Spindle cell hemangioma

##### Deep hemangiomas

- Intramuscular

- Synovial

##### Angiomatosis

**TABLE 1 (cont'd)**

INTERMEDIATE (LOCALLY AGGRESSIVE)

Kaposiform hemangioendothelioma

INTERMEDIATE (RARELY METASTASIZING)

Retiform hemangioendothelioma

Papillary intralymphatic angioendothelioma (Dabska tumor)

Composite hemangioendothelioma

Polymorphous hemangioendothelioma

Kaposi's sarcoma

MALIGNANT VASCULAR TUMORS

Epithelioid hemangioendothelioma

Angiosarcoma (includes lymphangiosarcoma)

Variants:      Associated with lymphedema ("lymphangiosarcoma")  
                    Epithelioid

**LYMPH VESSELS**

Lymphangioma

Variants:      Lymphangioma circumscriptum  
                    Cystic hygroma  
                    Progressive lymphangioma

Lymphangiomatosis

**Advantages and Pitfalls of Cytogenetic, Molecular  
Cytogenetic, and Molecular Diagnostic Testing in Bone  
and Soft Tissue Tumors**

**Julia A. Bridge, M.D.**

International Society of Bone and Soft Tissue Pathology  
2005

## **INTRODUCTION**

The pathogenesis of bone and soft tissue tumors is a multistep process stemming from somatic mutations that impair the regulation of normal cell development, cell proliferation, and other fundamental cellular activities. The elucidation of this process has been challenging because the genetic events are unique for different mesenchymal tumor subtypes. However, enormous progress has been achieved with the advancement of cytogenetic and molecular genetic techniques. As a result, relevant oncogenes and tumor suppressor genes have been identified and localized, and new gene constructs and their protein products that result from translocations during sarcomagenesis have been determined. The identification of tumor-specific genetic markers for bone and soft tissue tumors such as Ewing sarcoma has added a new dimension to the formulation of a diagnosis and the resolution of cellular origin. Many of the genetic markers appear to have prognostic value, and studies are under way to determine their potential applications as specific therapeutic targets.

## **GENETIC APPROACHES COMMONLY USED AS DIAGNOSTIC AIDS**

### ***CYTOGENETIC ANALYSIS***

#### ***Specimen Requirements***

Tissue submitted for cytogenetic analysis must be fresh (not frozen or fixed in formalin) because living, dividing cells are required. A mesenchymal tumor sample submitted for cytogenetic analysis should be representative of the neoplastic process and preferably be part of the specimen submitted for pathologic study. Ideally, a 1 to 2 cm<sup>3</sup> (approximately 0.5 to 1.0 g) fresh sample is provided for analysis. Also, small biopsy specimens or fine-needle aspirates (less than 500 mg) can be analyzed successfully, but prolonged culture may be needed to produce enough cells for examination. Necrotic tissue and nonneoplastic tissue should be dissected from the sample. The tumor tissue should be transported to the laboratory in sterile culture media or buffer solution (such as Hank's buffered salt solution) as soon as possible after surgical removal. Specimens sent over long distances (requiring 24 to 48 hours for delivery) to cytogenetic laboratories can be transported at room temperature or refrigerated (not frozen) in sterile isotonic saline or, preferably, culture media containing serum.

#### ***Cell Culture and Chromosome Banding***

The basic process of cell culturing is the same for all bone and soft tissue lesions. Briefly, sterile tumor tissue is minced mechanically with scissors or a scalpel and enzymatically disaggregated by incubation in collagenase. The resulting single cells and small cell clusters are incubated at 37°C and 5 percent CO<sub>2</sub> and are inspected daily under an inverted microscope for growth. When an optimal number of mitoses is observed, the proliferating cells are arrested in mid-division. The time that a bone or soft tissue tumor may be cultured to attain satisfactory karyotypic findings varies depending on the histopathologic type, grade of tumor, tumor cellularity, and size of specimen submitted for analysis. A short-term culture usually results in a sufficient number of mitoses in 10 days or less. Lengthy culture times should be avoided because undesired overgrowth by normal fibroblasts is more likely to occur.

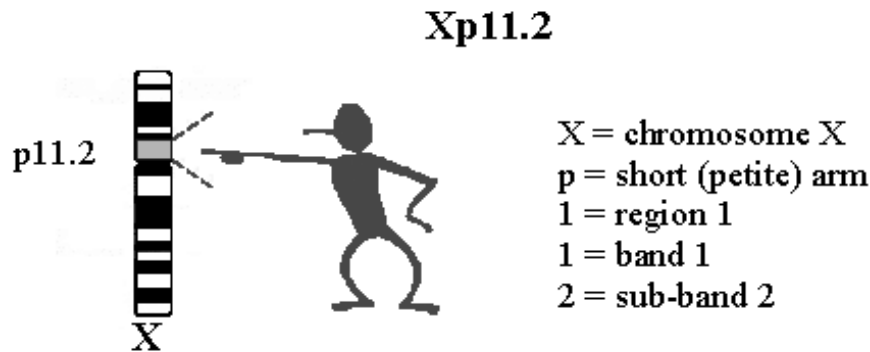
An alternative to tissue culture is direct harvest. With this technique, endemic dividing cells are arrested after a 1- to 12-hour incubation in colchicine and culture medium. This method is useful for obtaining fast or preliminary results but is limited by the in vivo mitotic index. Thus, direct harvest is most useful for high-grade tumors. Also, for best success, it is imperative that the laboratory receive the tissue sample within 1 hour after biopsy.

Chromosomes, as they appear in a metaphase spread, consist of tightly coiled DNA and protein. A karyotype is the somatic chromosomal complement of an individual or species. For humans, the normal karyotype consists of 46 chromosomes aligned in a standard sequence according to size, centromere location, and banding pattern. G-banding is the most common form of banding. This is attributable to the relative ease of performing the technique, the reliability of the results, and the permanence of the preparations. G-bands can be obtained with Giemsa or Wright stains pretreated with trypsin or phosphate

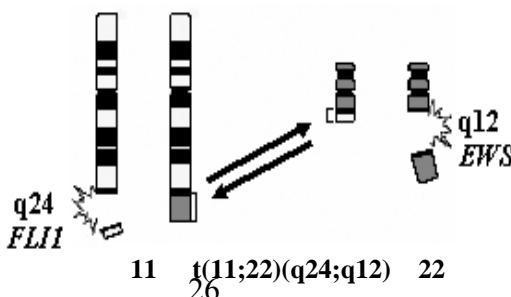
buffer, respectively. The number of alternating light and dark bands detectable with G-banding in the haploid genome varies with the level of chromosomal contraction in each metaphase cell, but it is in the range of 350 to 550 bands per haploid set. One band represents approximately  $5 \text{ to } 10 \times 10^6$  base pairs (bp) of DNA. A relationship exists between the different types of bands and gene density, base composition, and replication time; however, the functional basis for the interdependence of these features of chromosome structure and behavior is not known.

### Nomenclature

An international system for designating bands in human chromosomes was introduced at the 1971 conference in Paris. In this system, the short and long arms are divided into several regions, each defined as an area of chromosome lying between two adjacent landmarks. Landmarks are defined as consistent and distinct morphologic features important for identifying chromosomes. (Strictly, landmarks, like bands, are features of staining rather than morphologic features.) Regions are numbered consecutively from the centromere to the telomere (distal end of a chromosome) on each arm; within each region, the individual bands are numbered in the same direction. Thus, the complete designation of a band consists of the chromosome number, a letter to indicate the short or long arm, a number for the region, and a number for the band and subband; for example, Xp11.2 refers to the short arm of chromosome X, region 1, band 1, subband 2.



The two major types of chromosomal abnormalities are numerical and structural. Numerical abnormalities manifest as changes in complete sets of chromosomes (i.e., triploid [3N] or tetraploid [4N] complements) or in the number of individual chromosomes (i.e., loss of a single chromosome [monosomy] or gain of a single chromosome [trisomy]). Structural abnormalities of chromosomes result from chromosomal breakage and rejoining of the broken ends to form new combinations. A frequently observed structural abnormality is translocation. In a reciprocal translocation, chromosomal material is exchanged between two or more nonhomologous chromosomes. An example of the shorthand system used to describe numerical and structural aberrations is 47,XY,+8,t(11;22)(q24;q12), in which 47 indicates the total chromosome number, XY indicates the sex constitution, and +8 indicates an extra copy, trisomy, of chromosome 8. The “t” is an abbreviation for translocation and in this example specifies an exchange of chromosomal material between the long arms of chromosomes 11 and 22 at bands q24 and q12, respectively. The 11;22 translocation is a characteristic rearrangement in Ewing sarcoma, and trisomy 8 is a frequent secondary anomaly in this neoplasm.



## *Advantages and Limitations of Conventional Cytogenetic Analysis*

**Table 1: Conventional Cytogenetic Analysis**

Advantages	Limitations
Provides global information in a single assay. <ul style="list-style-type: none"><li>• includes primary &amp; secondary anomalies</li><li>• knowledge of anticipated anomaly or histologic diagnosis not necessary</li></ul>	Requires fresh tissue. <ul style="list-style-type: none"><li>• although direct preparations can be performed, cell culture is typically required (1-10 days).</li></ul>
Variants undetectable by interphase FISH or RT-PCR may be uncovered.	<ul style="list-style-type: none"><li>• may encounter complex karyotypes with suboptimal morphology.</li><li>• submicroscopic or cryptic rearrangements may result in a false negative result.</li></ul>
Diagnostically useful. <ul style="list-style-type: none"><li>• sensitive and specific</li><li>• can be performed on fine needle aspirates</li></ul>	Normal karyotypes may be observed following therapy-induced tumor necrosis or overgrowth of normal supporting stromal cells.
Provides direction for molecular studies of pathogenetically important genes.	Difficulties encountered with bone tumors include low cell density and the release of cells from the bone matrix.

## **MOLECULAR CYTOGENETICS**

A revolutionary tool in the analysis and characterization of chromosomes and chromosomal abnormalities has been the development of in situ hybridization (ISH) techniques. Hybridization refers to the binding or annealing of complementary DNA or RNA sequences that serve as probes. With this approach, specific nucleic acid sequences can be detected in morphologically preserved chromosomes, cells, or tissue sections.

Molecular cytogenetic assays typically are performed with chromosome-specific probes labeled with fluorescent dyes such as fluorescein and detected with fluorescence microscopy (fluorescence in situ hybridization [FISH]). Alternatively, hybridization signals can be detected with peroxidase or alkaline phosphatase, but these approaches are generally less sensitive.

### ***Specimen Requirements***

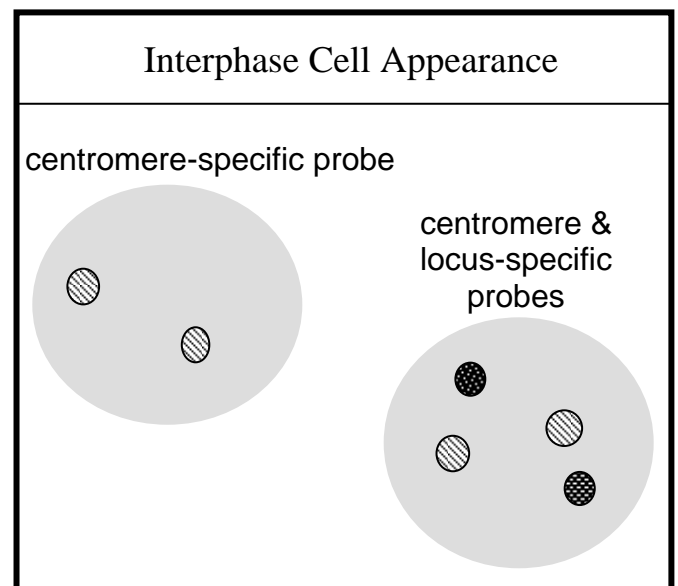
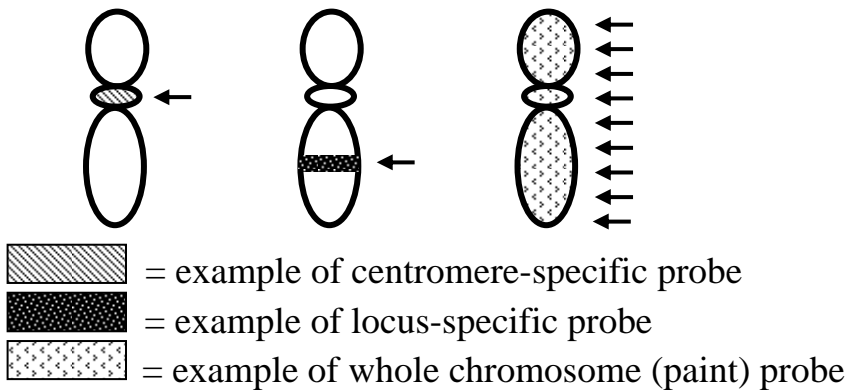
This technique can be performed on fresh or aged samples (such as blood smears, touch imprint cytologic preparations, or cytospin preparations), paraffin-embedded tissue sections, and disaggregated cells retrieved from fresh, frozen, or paraffin-embedded material. Blood smears, touch imprint cytologic preparations, and cytospin preparations are air-dried and subsequently fixed in methanol:glacial acetic acid (3:1) for 5 minutes. To visualize an anomaly within a specific region of a tumor or within a specific cell type, a 4- to 6- $\mu\text{m}$ -thick paraffin-embedded tissue section can be used. Analysis of thin sections, however, is limited because portions of most nuclei are lost during sectioning, and this may lead to false-positive results in the evaluation of chromosomal deletions or losses. For the most accurate assessment of subtle aneuploidy changes, the preferred approach is to obtain whole or intact nuclei by disaggregating and releasing cells from a much thicker (50 to 60  $\mu\text{m}$ ) section. FISH is a same day or overnight procedure, depending on the probes used or the type of specimen analyzed (or both).

## Probes

Chromosomal probes (complementary DNA sequences) frequently used to examine bone and soft tissue tumors can be divided into three categories:

- Centromere-specific  
tandemly repeated monomers or  $\alpha$ -satellite sequences (171 bp) that are unique for each chromosome. Useful for chromosome enumeration.
- Locus-specific  
single copy probes homologous to specific targets (15- >500 kb). Often used for assessing oncogenes or tumor suppressor genes.
- “Paint” or whole chromosome  
comprised of probe mixtures with homology at multiple sites along the target chromosomes. Useful for characterizing structural chromosomal anomalies.

### Chromosome Probe Examples



### Technical variations

Conventional karyotyping is limited by its inability to detect cryptic translocations or to identify marker chromosomes accurately. With recently developed universal chromosome painting techniques, all chromosomes can be analyzed simultaneously. Two similar approaches have been developed: spectral karyotype analysis (SKY) and multifluor fluorescence in situ hybridization (M-FISH). Both techniques are based on the principle that the differential display of colored fluorescent chromosome-specific paints provides a complete analysis of the human chromosomal complement. With the use of combinations of 23 different colored paints as a “cocktail probe,” subtle differences in fluorochrome labeling profiles after hybridization with the cocktail probe allow the computer to assign a unique color to each chromosome pair. Thus, abnormal chromosomes in the karyotype of a tumor can be identified by the pattern of color distribution along the axis of the chromosome so that rearrangements between different chromosomes lead to a distinct transition from one color to another at the position of the breakpoint, greatly facilitating the identification of subtle or cryptic rearrangements. This technique is suited particularly to solid tumors in which the complexity of the karyotypes may often mask the presence of recurrent chromosomal aberrations.

## *Advantages and Limitations of Molecular Cytogenetic Analysis*

**Table 2: Molecular Cytogenetic Analysis**

Advantages	Limitations
<p>Can be performed on metaphase or interphase cell preparations (fresh, frozen or paraffin-embedded material).</p> <ul style="list-style-type: none"> <li>• can localize anomaly within specific cells or tissue types</li> </ul>	<p>More targeted approach; not screening tool (generally requires prior knowledge of anomaly of interest).</p> <ul style="list-style-type: none"> <li>• exceptions would be CGH &amp; SKY</li> </ul>
<p>Can provide results when tissue is insufficient or unsatisfactory for cytogenetic analysis, when conventional cytogenetics has failed to yield results or when cryptic rearrangements are present.</p>	<p>Still a relatively gross approach when contrasting other molecular approaches capable of detecting single base mutations.</p>
<p>Diagnostically useful.</p> <ul style="list-style-type: none"> <li>• sensitive and specific</li> </ul>	<ul style="list-style-type: none"> <li>• requires fluorescence microscopy (signal fading).</li> <li>• interpretation may be challenging when analyzing suboptimal specimens (ie., background fluorescence or autofluorescence, particularly with paraffin-embedded material).</li> </ul>
<p>Rapid turn-around time.</p>	<p>FISH nomenclature not consistent among laboratories.</p>

## ***REVERSE TRANSCRIPTION – POLYMERASE CHAIN REACTION ANALYSIS (RT-PCR)***

Translocations, or exchange of chromosomal material between two or more nonhomologous chromosomes, are encountered frequently as tumor-specific anomalies in mesenchymal neoplasms. These tumor-specific translocations serve as important guides for molecular biologists conducting positional cloning studies of the genes at the translocation breakpoints. The most common genetic consequence of these translocation events is the fusion of two genes, one from each translocation partner, resulting in the formation of a chimeric gene. The fusion proteins encoded by these chimeric genes are not found in normal cells and are tumor-specific.

In sarcomas, the fusion genes most often code for aberrant transcription factors that result in inhibition of normal cellular differentiation, cell cycle activation, and loss of responsiveness to extracellular signals (Table 3). Note that new cytogenetic as well as molecular genetic variants continue to be defined. Cytogenetic variants are defined as differing chromosomal translocation partners [ie. t(1;13) and t(2;13) in alveolar rhabdomyosarcoma] and molecular variants are often the result of genomic breakpoint differences that lead to distinct fusion product exon combinations. For example, the two most frequent exon combinations in Ewing sarcoma-associated *EWS/FLI1* fusion transcripts include fusion of *EWS* exon 7 to *FLI1* exon 6 (type 1) and fusion of *EWS* exon 7 to *FLI1* exon 5 (type 2). These molecular variants can be detected by their unique RT-PCR product band size. The identity of less common or unexpected product band sizes should be confirmed utilizing additional approaches such as direct sequencing or digestion with specific restriction endonucleases.

**Table 3: Characteristic and Variant Chromosomal Translocations and Associated Fusion Genes in Bone and Soft Tissue Sarcomas**

Neoplasm	Translocation	Fusion gene(s)
Alveolar soft part sarcoma <sup>a</sup>	der(17)t(X;17)(p11.2;q25.3)	<i>ASPL/TFE3</i>
Alveolar rhabdomyosarcoma	t(2;13)(q35;q14) t(1;13)(p36;q14) t(X;2)(q13;q35) t(2;2)(q35;p23)	<i>PAX3/FKHR</i> <i>PAX7/FKHR</i> <i>PAX3/AFX</i> <i>PAX3/NCOA1</i>
Clear cell sarcoma	t(12;22)(q13;q12)	<i>EWS/ATF1</i>
Congenital fibrosarcoma <sup>b</sup>	t(12;15)(p13;q25)	<i>ETV6/NTRK3</i>
Dermatofibrosarcoma protuberans	t(17;22)(q22;q13)	<i>COL1A1/PDGFB</i>
Desmoplastic small round cell tumor	t(11;22)(p13;q12)	<i>EWS/WT1</i>
Epithelioid hemangioendothelioma	t(1;3)(p36;q25)	?
Ewing sarcoma/pPNET <sup>c</sup>	t(11;22)(q24;q12) t(21;22)(q22;q12) t(7;22)(q22;q12) t(17;22)(q21;q12) t(2;22)(q33;q12) inv(22)(q12q12) t(16;21)(p11;q22)	<i>EWS/FLI1</i> <i>EWS/ERG</i> <i>EWS/ETV1</i> <i>EWS/EIAF</i> <i>EWS/FEV</i> <i>EWS/ZSG</i> <i>FUS/ERG</i>
Extraskeletal myxoid chondrosarcoma	t(9;22)(q22;q12) t(9;17)(q22;q11) t(9;15)(q22;q21) t(3;9)(q11-12;q22)	<i>EWS/NR4A3<sup>d</sup></i> <i>RBP56<sup>e</sup>/NR4A3<sup>d</sup></i> <i>TCF12/NR4A3<sup>d</sup></i> <i>TFG/NR4A3<sup>d</sup></i>
Low Grade Fibromyxoid Sarcoma and Hyalinizing Spindle Cell Tumor with Giant Rosettes	t(7;16)(q33;p11)	<i>FUS/BBF2H7<sup>g</sup></i>
Myxoid/round cell liposarcoma	t(12;16)(q13;p11) t(12;22)(q13;q12)	<i>TL5<sup>f</sup>/CHOP<sup>h</sup></i> <i>EWS/CHOP<sup>h</sup></i>
Synovial sarcoma	t(X;18)(p11.2;q11.2)  t(X;20)(p11.2;q13.3)	<i>SYT/SSX1</i> <i>SYT/SSX2</i> <i>SYT/SSX4</i> <i>SSI8LI/SSX1</i>

<sup>a</sup> A balanced form of this translocation is seen also in a subset of pediatric renal neoplasms.

<sup>b</sup> This translocation is seen also in congenital mesoblastic nephromas.

<sup>c</sup> pPNET = peripheral primitive neuroectodermal tumor.

<sup>d</sup> Also referred to as *TEC*, *MINOR*, *CHN*, and *NOR-1*

<sup>e</sup> Also referred to as *TAF2N*

<sup>f</sup> Also referred to as *FUS*

<sup>g</sup> Also referred to as *CREB3L2*

<sup>h</sup> Also referred to as *DDIT3*

### ***Specimen Requirements***

The highly specific gene rearrangements that result from chromosomal translocations in bone and soft tissue tumors can be identified with reverse transcriptase polymerase chain reaction (RT-PCR) analysis. The PCR technique uses specific synthetic primers to amplify a section of a gene in vitro. With the additional step of reverse transcription (mRNA → cDNA), PCR can be carried out on RNA. Snap frozen tissue is preferred for RNA extraction and RT-PCR analysis, but this procedure can also be performed on archival (paraffin-embedded) material if the RNA is of sufficient quality.

RT-PCR analysis is remarkably sensitive. It may allow for the detection of abnormalities present in cells too few to be identified with traditional cytogenetic or FISH methods. It may be suitable for the detection or monitoring of minimal residual disease. Also, RT-PCR analysis is not dependent on successful cell culture and, similar to FISH, it is rapid, with a short turnaround time. Compared with cytogenetic analysis, the greatest disadvantage of RT-PCR analysis is the inability to detect chromosomal anomalies other than those for which the test was designed. With conventional cytogenetic analysis, all major chromosomal abnormalities, including those not initially anticipated by the clinician or laboratorian, may be uncovered.

### ***Advantages and Limitations of RT-PCR Analysis***

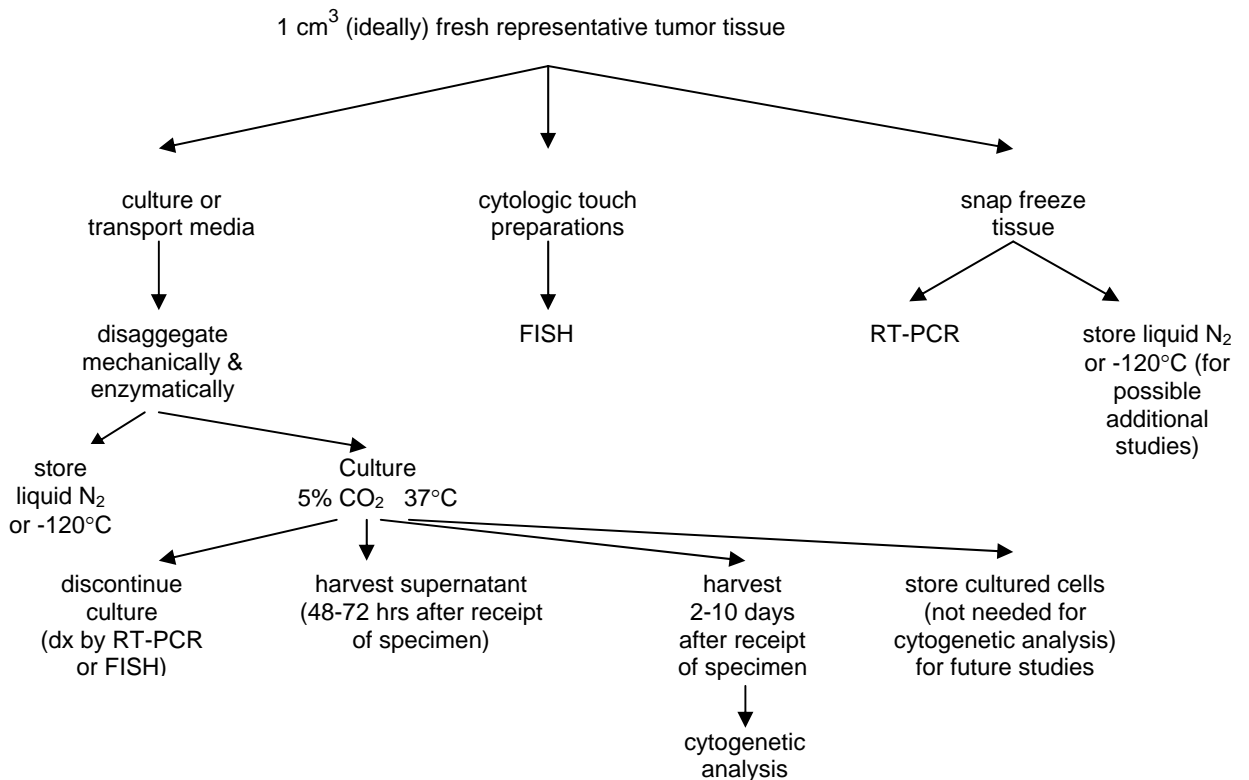
**Table 4: RT-PCR Analysis**

Advantages	Limitations
Can be performed on fresh, frozen or paraffin-embedded material. <ul style="list-style-type: none"> <li>• tissue quantity requirement is small</li> </ul>	Not all sarcomas exhibit characteristic fusion gene transcripts.
Can provide results when tissue is insufficient or unsatisfactory for cytogenetic analysis, when conventional cytogenetics has failed to yield results or when cryptic rearrangements are present.	Targeted approach; not screening tool. <ul style="list-style-type: none"> <li>• requires prior knowledge of fusion transcript.</li> </ul>
Diagnostically useful. <ul style="list-style-type: none"> <li>• sensitive and specific</li> <li>• rapid turn-around-time.</li> </ul>	RNA quality may be inadequate secondary to RNA degradation.
Because of its remarkable sensitivity, RT-PCR may be useful for the detection of minimal residual disease or early relapsed disease.	<ul style="list-style-type: none"> <li>• devised primer sets may not detect unusual molecular variants (false negative).</li> <li>• identification of some product bands may require validation by additional approaches such as direct sequencing, transfer and hybridization with internal oligonucleotide probes, digestion with specific restriction endonucleases, or re-amplification with internal primers (nested RT-PCR).</li> </ul>

## CONCLUSIONS

Dramatic advances in cytogenetic and molecular biologic techniques have furthered our understanding of sarcomagenesis. Cytogenetic and molecular genetic assays have a direct, potentially decisive role in the examination of bone and soft tissue tumors, and many such assays are used routinely for diagnostic and prognostic purposes in molecular pathology laboratories. However, genetic analysis is not a panacea for histopathologic study, but rather it is a powerful adjunct to complement conventional microscopy and radiographic assessment in the formulation of an accurate diagnosis. By virtue of their exquisite sensitivity, molecular techniques appear superior to standard methods in the assessment of minimal residual disease or early relapse of disease. Future advancements will include the development of a new class of antineoplastic agents based on the underlying biologic events in bone and soft tissue sarcomas for the clinical management of these malignancies.

### Summary: UNMC Processing of Tissue for Genetic Analyses (Algorithm Approach)



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## Soft tissue chondrosarcoma – A heterogeneous tumor or a dwindling entity?

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Chondrosarcomas arising in soft tissues are rare tumors accounting for less than 4% of soft tissue sarcomas. Currently four subtypes are recognized.

1. Conventional hyaline chondrosarcomas (similar to their intra-osseous counterparts). These are vanishingly uncommon.
2. Chondrosarcomas arising in synovial chondromatosis. Also extremely uncommon.
3. Extraskkeletal myxoid chondrosarcoma.
4. Mesenchymal chondrosarcoma.

The last two types comprise the bulk of soft tissue chondrosarcomas and will be the subject of this presentation. To start this discussion I will review some of the history surrounding the description of these tumor types and briefly summarize the current data pertaining to the clinical and pathological features of these tumors.

### Extraskkeletal Myxoid Chondrosarcoma

In 1953 Stout and Verner published an article entitled “Chondrosarcoma of the extraskkeletal soft tissues”. In this paper they described their experience with seven extraskkeletal chondrosarcomas. Two of these were composed of lobules of rounded cells, interpreted as “chondroblast”-like, that were embedded in abundant myxoid matrix. In 1972 Enzinger and Shiraki published a series of 34 cases similar to these two. They expanded the histologic description and emphasized that the extracellular matrix had similar histochemical staining properties to hyaline cartilage (colloidal iron and alcian blue positive staining of the matrix, resistant to hyaluronidase pre-treatment). Enzinger and Shiraki coined the term “Extraskkeletal myxoid chondrosarcoma” for this distinctive tumor. Interestingly in 1973 Martin et al. published a series of four histologically similar tumors and suggested the name “chordoid sarcoma” for these. The term “chordoid” had originally been used by Stewart in 1948 to describe these tumors because of their light microscopic similarity to chordoma. Since the publication of these two papers in the early 1970s there has been debate regarding which the most appropriate name for the tumor is, and whether or not cartilaginous differentiation is present within these lesions. At the time of this writing I think it is fair to say that the proponents of the extraskkeletal myxoid chondrosarcoma (EMC) nomenclature are in the ascendancy. Notwithstanding the controversy over nomenclature, the last 30 years has seen numerous solid clinicopathologic and basic research investigations of this tumor and its biology. EMCs occur predominantly in adults and affect males more commonly than females (2:1). They typically occur in the deep soft tissues, usually of the extremities. Like most soft tissue tumors they usually present as a painless mass. Grossly they appear well circumscribed and myxoid or gelatinous. Regions of hemorrhage are commonly present. Microscopically the tumors are hypovascular and distinctly lobulated. The lesional cells vary from spindled to epithelioid and usually do not exhibit high grade nuclear features or pleomorphism. Growth patterns include cords, and nests of cells that appear to touch adjacent cells. Most of the time the extracellular ground substance dominates the appearance although on occasion the cellularity of the tumor is considerably higher and

sheet-like growth of tumor cells occurs. An important feature to be aware of is that *bone fide* hyaline cartilage is only very rarely present in these tumors. Immunohistochemical stains are characteristically positive for vimentin and recently many tumors have been shown to label positively for “neuroendocrine antigens”. Of note, stains for S100 protein are commonly negative in the tumor cells. Recently it has become clear that EMCs harbor an apparently specific and diagnostic translocation, t(9;22) fusing the *EWS* and *CHN* genes in at least 50% of cases. In the original descriptions of this tumor it was considered to be a low grade sarcoma with a low metastatic rate. Numerous more recent studies that have included longer and more complete follow up data have shown that the long term rate of metastasis is high, with a corresponding increase in tumor related mortality.

### Mesenchymal Chondrosarcoma

Mesenchymal chondrosarcoma (MC) was first proposed as the name for two histologically distinct malignant cartilaginous tumors reported by Lichtenstein and Bernstein in 1959. Both of these were skeletal lesions. In 1962 Dahlin and Henderson further delineated this entity in a report of nine tumors, one of which arose in the soft tissues (meninges). Following these original reports the entity became more widely recognized and at this point numerous series of these tumors have been reported. Between one quarter and one third of MCs arise in the soft tissues. Males and females are affected equally. The tumors may occur at any age although most cases are diagnosed in adults within the second through fourth decades. The soft tissue tumors may occur at diverse locations although they most commonly arise adjacent to the cranio-spinal axis including the paraspinal musculature. The meninges are one of the commonest extra-skeletal sites for these tumors. As with most soft tissue sarcomas the patients usually present with a painless mass. Patients with meningeal tumors commonly present with focal neurologic signs depending on their intra-cranial location. MCs have a wide range in size from less than 5.0 to greater than 20.0 cm. The tumors appear grossly well defined and have a tan/gray cut surface. Focal hemorrhage or necrosis is commonly present. Gritty areas representing the calcified cartilage are often present. Typically the majority of the tumor consists of an undifferentiated “small blue cell” component. These cells are usually arranged as sheets and/or vague nests. A characteristic feature is the presence of a supporting capillary network that has acute angle branching resulting in a “hemangiopericytoma-like” growth pattern. The blue cells often are associated with bands of hyalinized eosinophilic collagen that may resemble the “rope-like” collagen of solitary fibrous tumor. The defining light microscopic feature is the presence of islands of hyaline cartilage. These typically form only a minority of the tumor and show an abrupt separation from the blue cell regions. Focal coarse purple deposits of calcification may occur within the hyaline matrix. Mitotic activity is variable but often high within the blue cell regions. Similarly necrosis usually involves this component of the tumor. Immunohistochemically the obvious cartilaginous components of the tumor label for S100 protein. The small cell apparently undifferentiated component usually labels for vimentin, CD99 and focally for desmin. MCs are high-grade clinically aggressive tumors that are treated by a combination of surgery and systemic chemotherapy. Up to 50% of patients have died of disease at 5 year follow up.

Given this background information, which essentially represents the state of our knowledge of these tumors, (at least up until the last few years), the question now needs to be asked “Are these tumors being appropriately classified as chondrosarcomas?” [Dictionary.com](http://Dictionary.com) defines classification as “the systemic grouping of organisms (entities) into categories on the basis of structural relationships between them” Certainly there are “structural/morphological” similarities between these two tumor types and normal cartilage as well as other cartilaginous neoplasms. However this area, like many others in surgical pathology has not been free of controversy and debate. The proponents of the “chordoid sarcoma” terminology have for many years rejected the concept of true cartilaginous differentiation in extraskeletal myxoid chondrosarcoma. Additionally, recently the identification of neuroendocrine differentiation in these tumors has reignited the concept that they are fundamentally different from conventional chondrosarcoma. Similarly other authors have suggested mesenchymal chondrosarcoma might be more appropriately classified as a variant of small cell osteosarcoma. Is there any way to resolve these issues?

In essence the debate revolves around exactly how we define cartilaginous differentiation. Initially this was done based on the appearance of cells stained with Hematoxylin and Eosin. Subsequently cartilaginous matrix was defined by its histochemical staining properties. Ultrastructural investigation and the immunohistochemical staining profile of constituent cells have also been used to define cartilaginous differentiation, but the problem with all of these methods is that the features that are used are not really specific, and hence the debate. Recently Aigner and several other investigators have adopted a different approach to address this issue of “what exactly defines cartilage?” Using immunohistochemistry they have examined the collagen subtypes within cartilage matrix of varying degrees of differentiation. This work has allowed a “profile” of collagen matrix components (as they relate to the level of differentiation of the cartilage cell) to be developed. This is summarized below

Cell type	Immunohistochemical staining profile (cells and matrix)
Mesenchymal precursor	Lacks all antigens
Chondroprogenitor	Coll 2A, vimentin
Mature Chondrocyte	Coll 2B, Coll 9, Coll 11, Vimentin, S100
Hypertrophic Chondrocyte	Coll 10, Coll 2, Vimentin, S100
Post hypertrophic osteoblast-like cell	Coll 1, Vimentin

Armed with this information Aigner et al. have re-examined various cartilaginous tumors including extraskeletal myxoid chondrosarcomas and mesenchymal chondrosarcomas for their collagen matrix staining profiles.

Investigated using this approach EMC shows no evidence of a cartilaginous matrix profile. The extracellular matrix shows a non-specific pattern of collagen deposition with positive staining for collagens 1, 3 and 6. The cartilage specific collagens 2 and 10 are

not identified except in morphologically characteristic foci of hyaline cartilage present in a minority of the tumors.

The results of investigating MCs using this approach are equally interesting. In contrast to the EMCs the tumor cells of MC clearly show a cartilaginous matrix staining profile. Interestingly this is true of the apparently undifferentiated small cells as well as the morphologically recognizable chondrocytes. The “blue cell” population of cells demonstrates a cellular and matrix immunophenotype of immature pre-chondrocytes (mesenchymal precursor (no antigen expression) and chondroprogenitor cell differentiation (Collagen 2a and vimentin expression)). The chondrocytes exhibit positivity for S100, vimentin and collagen 2 and 10 (matrix staining) supporting a level of differentiation of these tumor cells at the mature chondrocyte and post-hypertrophic chondrocyte levels.

Based on these immunohistochemical staining profiles in the two different tumor types Aigner et al. have concluded that “the basic cellular phenotype of EMC is not chondrocytic or prechondrocytic and that EMC is not a chondrosarcomatous entity”. On the other hand they conclude that their results “establish MC as the very neoplasm of differentiating premesenchymal chondroprogenitor cells.” They go on to suggest that “MC is the paradigmatic tumor entity of neoplastic chondrogenesis, which shows all steps of cell differentiation stages starting from the hardly mesenchymally differentiated cells.”

Additional support for the *bone fide* cartilaginous nature of MC has been presented by Wehrli et al. who have identified strong expression of the transcription factor Sox9 within the apparently undifferentiated blue cells of these tumors. Sox9 is a master transcription factor that is required for cartilage differentiation during embryogenesis. These investigators have found that Sox9 expression is limited to MC and is not present in other small blue cell tumors.

Given this “newer” data the question can now once more be asked is soft tissue chondrosarcoma a heterogenous tumor or a dwindling entity? In my opinion the answer is YES! It is both. The results suggest EMCs are not chondrosarcomas, at least in the traditional sense, and that MCs are. Whether our changing concepts of the biology of these neoplasms should affect the classification and nomenclature of these tumors is a question that is perhaps more philosophical than scientific? Personally I would favor preserving the current system because it is widely recognized by pathologists and clinicians and is clinically relevant, but ultimately these types of decisions are probably more appropriately addressed by our newly formed society of international bone and soft tissue pathologists.

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**CURRENT CONCEPTS OF DEDIFFERENTIATION IN SOFT TISSUE  
AND BONE SARCOMAS**

**Juan Rosai, M.D.**

The term *tumor dedifferentiation*, originally coined by cell biologists for the reversible changes in cell morphology they saw in tissue culture conditions<sup>1</sup>, was later adopted by surgical pathologists to designate the phenomenon by which certain low grade malignant/borderline neoplasms, characterized morphologically by a well-differentiated microscopic appearance, undergo a sudden drastic change in that appearance, resulting in the emergence of a highly undifferentiated component<sup>2</sup>. The implication of this terminologic choice was that this component had originated from the pre-existing well-differentiated elements through a reversal of the normal differentiation mechanism. Most familiar to surgical pathologists through the well-differentiated chondrosarcoma paradigm reported by Dahlin et al<sup>2</sup>, the phenomenon of dedifferentiation is generally characterized by the following attributes:

- 1) The emergence of the dedifferentiated component is accompanied by a marked acceleration of the clinical course of the disease;
- 2) There is a sharp segregation between the well-differentiated and the dedifferentiated components, resulting in a biphasic appearance;
- 3) The dedifferentiated component lacks all of the phenotypical attributes present in the well-differentiated component which had allowed for the recognition of the tissue type. For instance, microscopic examination of the dedifferentiated portion of a liposarcoma should not reveal any sign of “lipogenic activity” and therefore it would not be recognizable *per se* as a tumor of adipose tissue nature. Interestingly, the concept of dedifferentiation still allows for the presence within the dedifferentiated component of differentiated foci *as long as their line of differentiation is different from that of the pre-existent well-differentiated tumor*, a

phenomenon variously known as “divergent differentiation”, “phenotypical switch”, or simply as “tumor metaplasia”<sup>3,4</sup>.

Tumors to which the concept of dedifferentiation has been more often applied are those of mesenchymal nature, notably chondrosarcoma, liposarcoma, and chordoma. However, it is known that a transformation of an analogous type can occur in tumors of a wide variety of types, such as papillary thyroid carcinoma, renal cell carcinoma, grade I astrocytoma, and chronic lymphocytic leukemia. Actually, one could view *dedifferentiation* as simply the most overt version of the ubiquitous biologic phenomenon known as *tumor progression*, by which well-differentiated tumors acquire a progressively lesser differentiated appearance with the passage of time, particularly when recurring or metastasizing<sup>5</sup>. According to this expanded view, another family of tumors to which the concept of dedifferentiation could be applied is that of the epithelial (usually squamous) neoplasms generally arising in mucosal membranes (such as oral cavity, larynx, and esophagus) which are variously known as sarcomatoid, spindle cell, or metaplastic carcinomas<sup>6</sup>.

If this line of reasoning is accepted, one could make a point for doing away with the term *dedifferentiated* altogether because of its lack of biologic or morphologic specificity within the larger spectrum of *tumor progression*. Since this approach is likely to be resisted by the traditionalists, an alternative would be to attempt a more strict definition of the criteria for using the term. To recapitulate some of the points made above, these criteria could be the following:

- 1) The original tumor should be a low-grade, well-differentiated neoplasm;
- 2) The transition from the well-differentiated to the dedifferentiated component should be abrupt;
- 3) The dedifferentiated component should lack any evidence of differentiation along the lines of the original neoplasm, although it may show differentiation along other lines.

It may be appropriate at this point to remark on two peculiar and as yet unexplained aspects of this phenomenon, to wit:

- 1) The fact that it is much more common in some tumor types than in others, for example in well-differentiated liposarcoma than in myxoid liposarcoma, or in well-differentiated chondrosarcoma than in well-differentiated osteosarcoma;
- 2) The fact that it does not seem to occur in benign mesenchymal tumors, such as schwannoma, leiomyoma, lipoma, or chondroma (although it may occur in benign epithelial neoplasms, such as eccrine spiradenoma);

At the practical diagnostic level, some points worth making are the following:

- 1) One should think of dedifferentiation if faced with a high-grade sarcoma not easily labelled as one of the conventional types of sarcoma, particularly if the tumor is located in the retroperitoneum;
- 2) Since well-differentiated liposarcoma is one of the soft tissue tumors with greater tendency for dedifferentiation, sampling of the tumor (particularly in the retroperitoneum) should include the adipose tissue surrounding any high-grade sarcoma, even if this tissue appears grossly normal<sup>7</sup>;
- 3) Not all dedifferentiated tumors are high-grade tumors with poor prognosis. Some neoplasms fulfilling the criteria of dedifferentiation are actually low-grade lesions both on morphologic and behavioral grounds<sup>8-10</sup>;
- 4) As a corollary of the above, it is important to evaluate the microscopic type and grade of the dedifferentiated component, using similar criteria to those employed with de novo soft tissue sarcomas (an exercise which, for some peculiar reason, is often omitted)<sup>11</sup>.

From a microscopic standpoint, the dedifferentiated component of these tumors usually has a highly pleomorphic/anaplastic appearance, featuring multinucleated giant cell forms, high mitotic activity, and necrosis (in other words, it has the malignant fibrous histiocytoma “look”). However, many variations on the theme exist. These include a predominantly spindle cell form and a peculiar variety (seen in dedifferentiated liposarcoma) characterized by neural-like whorls and metaplastic bone<sup>12</sup>. It could also be argued that the rhabdoid cell is another manifestation of dedifferentiation, in

the sense that it can be seen in association with a variety of cell types, it is undifferentiated at a morphologic and often at an immunohistochemical level, and is invariably accompanied by an extremely aggressive clinical course<sup>13</sup>. As a matter of fact, one could regard the “undifferentiated malignant tumor with rhabdoid phenotype” as a *sui generis* variety of dedifferentiated tumor.

A final consideration worth making on the subject concerns the *sequence of events* in the differentiation process. The usual assumption – as implied by the term “dedifferentiated” – is that the sequence in a hypothetical dedifferentiated tumor is the following:

- Stem cell I → Well differentiated cell
- Stem cell II → Well differentiated cell
- Stem cell III → Well differentiated cell
- Stem cell IV → Well differentiated cell → Stem cell (dedifferentiated cell)

Other authors have proposed that a more likely sequence is the following:

- Stem cell I → Well-differentiated cell
- Stem cell II → Well-differentiated cell
- Stem cell III → Well-differentiated cell
- Stem cell IV → Remains as a stem cell (undifferentiated cell)

The basic difference between the two models should be evident: In the first, a stem cell that had differentiated reverts to its primitive undifferentiated state. In the second model, the tumor stem cell fails to differentiate (it becomes “arrested” or “blocked”) and therefore retains its stem cell (undifferentiated) character. As stated by the late Arkadi Rywlin “Cells, whether normal or neoplastic, do not dedifferentiate. Rather, they fail to differentiate ... It’s [therefore] unnecessary to resurrect the antiquated concept of dedifferentiation”<sup>14</sup>. James Robb, from the Scripps Clinic, agreed. He stated that he knew “of no proven case in which dedifferentiation occurred in any differentiated cell or neoplasm” and suggested that this “misleading and incorrect term no longer be

used”<sup>15</sup>. Many years before, Pierre Masson, in his usual peremptory style, commented that this phenomenon “does not in any way deserves the name *dedifferentiation*, a term often abused. In effect, dedifferentiation presupposes a pre-existing differentiation ... There is no turning backwards, but a more or less pronounced defect of progression”<sup>16</sup>.

Little is known regarding the molecular genetic correlates of the phenomenon<sup>17</sup>, although loss of E-cadherin and upregulation of N-cadherin have been found to be present in the most invasive and *dedifferentiated* cell lines, and that p53 mutations are the rule in undifferentiated (including *dedifferentiated*) tumors<sup>18, 19</sup>. The claims that the immunohistochemical profile and gene expression of the well-differentiated component of liposarcomas having a dedifferentiated component differ from those of well differentiated liposarcomas lacking that component<sup>20, 21</sup> are clearly in need of independent confirmation.

**TABLE I – Main tumor types in which the phenomenon of dedifferentiation has been described (the most common ones are highlighted)**

**BONE**

**Chondrosarcoma (central, peripheral, parosteal, clear cell)<sup>22-24</sup>**

Chordoma<sup>25</sup>

Osteosarcoma (parosteal, low-grade intraosseous)<sup>26, 27</sup>

Giant cell tumor<sup>28</sup>

Adamantinoma<sup>29</sup>

**SOFT TISSUES**

**Well-differentiated liposarcoma/atypical lipomatous tumor<sup>7</sup>**

Myxoid liposarcoma (very rare)<sup>30</sup>

Extraskeletal myxoid chondrosarcoma<sup>31</sup>

**EPITHELIAL**

Salivary gland tumors: Acinic cell carcinoma, adenoid cystic carcinoma, myoepithelioma<sup>32-34</sup>

Thyroid well differentiated carcinomas: Papillary carcinoma, follicular carcinoma<sup>35</sup>

Pulmonary adenocarcinoma<sup>36</sup>

Prostatic adenocarcinoma<sup>37</sup>

Sweat gland tumors: Eccrine spiradenoma<sup>38</sup>

**NEURAL**

Grade I astrocytoma<sup>39</sup>

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