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Unravelling vascular tumors : combining molecular and computational biology

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Chapter 2

Vascular tumors of bone: the evolvement of a classification based on molecular developments

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2.1 Abstract

The classification of vascular tumours of bone has been under debate over time. Vascular tumors in bone are rare, display highly overlapping morphology, and are therefore considered difficult by pathologists. Compared to their soft tissue counterparts, they are more often multifocal and sometimes behave more aggressively. Over the past decade, with the advent of next generation sequencing, recurrent molecular alterations have been found in some of the entities. The integration of morphology and molecular changes has led to a better characterization of these separate entities.

2.2 Introduction

The common denominator of vascular tumors consist of their endothelial differentiation, with a variable capability of forming mature or immature vessels. Literature on the cell of origin for vascular tumors (other than infantile hemangioma) is scarce, and point to an "endothelial precursor cell" or a haematopoietic precursor cell along its path of endothelial differentiation, for canine and murine hemangioma / angiosarcoma (1, 2). However, the definition of these cells in mice and human is controversial (3, 4).

The classification of vascular tumours of bone has been a matter of discussion over time (5–7). However, with the rapid elucidation of molecular changes in tumors using next generation sequencing, which also included vascular tumours of bone, the classification has evolved and morphology and molecular changes were integrated to better define the separate entities (8), that are sometimes extremely difficult to distinguish based on morphology alone. Like in soft tissue, the entity of "hemangiopericytoma of bone" is no longer recognized, as these lesions are rare presentations of synovial sarcoma, solitary fibrous tumour, and myofibroma primary of bone (9). Moreover, while in the past there has been ample discussion about "haemangioendothelioma of bone" being a separate entity (10), it is now more or less generally accepted that the previously reported cases represent epithelioid hemangioma of bone (8, 9, 11), and with the elucidation of specific genetic alterations in epithelioid hemangioma of bone (12, 13) this discussion may be definitively resolved in the future.

Now that the different vascular tumours have been better characterized, their distinct behaviour in bone as compared to when they are located in the soft tissues is becoming obvious. Vascular tumors of bone are more frequently multifocal, affecting multiple bones (6). Also, although histologically and genetically similar, epithelioid hemangioma in soft tissue is considered benign, while in bone it behaves as a locally aggressive, rarely metastasizing lesion and is therefore considered to be of the intermediate category (8). In addition, atypical epithelioid hemangioma has a preference for bone and penile lo-

Classification	Entity	Prognosis	Treatment
Benign	Hemangioma	100% survival, 0%	Treat symptoms
Intermediate	Epithelioid hemangioma	100% survival, 2% metastases, 9% local recurrence	Curettage or marginal excision
	Pseudomyogenic hemangioendothelioma	Limited follow-up, stable or progressive osseous disease	
Malignant	Epithelioid hemangioendothelioma	85% survival, 25% metastases	Wide resection
	Angiosarcoma	30% survival	Wide resection, consider systemic therapy
	Angiosarcoma	30% survival	Wide resection, consider systemic therapy

Table 2.1: Summary of prognosis and treatment for vascular bone tumors.

cation (14). Moreover, after the morphological and molecular characterization of pseudomyogenic (epithelioid sarcoma-like) haemangioendothelioma (15, 16), cases are reported that are exclusively located in bone, with unique histological findings (17).

Here we will discuss the most common vascular tumors of bone. These tumor entities range from the benign hemangioma of bone, with a good prognosis and no metastasis in all patients, to the intermediate epithelioid hemangioma (including the atypical variant) and the pseudomyogenic hemangioendothelioma whose survival is excellent but with some metastasis and recurrences. Epithelioid hemangioendothelioma is considered low grade malignant, with 85% survival and 25% metastases. Angiosarcoma is high grade malignant with a very poor survival of only 30% over 5 years (table 2.1). This review covers the classic presentations of these tumor entities including the diagnostic pitfalls and immunohistochemistry. We also discuss the recent developments regarding the genetics and tumorigenesis of these vascular tumors of bone (table 2.2).

Entity	Histologic and Molecular Findings	Immunohistochemistry
Hemangioma of bone	-Numerous smaller or larger blood -filled spaces, lined by flat endothelium -Reactive sclerosis of surrounding lamellar bone -No specific genetic alterations	CD31+ CD34+ ERG1+

Entity	Histologic and Molecular Findings	Immunohistochemistry
Epithelioid hemangioma of bone	-Lobular architecture (can be highlightedusing actin immunohistochemistry) -Well-formed vessels lined by epithelioidendothelial cells -Eosinophilic infiltrate -No prominent nuclear atypia or atypical mitoses -Hemorrhagic and spindle cell areas can be prominent, especially in acral lesions -Rearrangement of *FOS*	CD31+ CD34+ ERG+
Atypical epithelioid hemangioma of bone	-Similar to epithelioid hemangioma, with more solid growth, increased cellularity, nuclear pleomorphism, and necrosis -*ZFP36-FOSB* fusion	Similar to epithelioid hemangioma
Pseudomyogenic (epithelioid sarcoma-like) hemangioendothelioma	-Sheets of spindled or epithelioid cells with abundant eosinophilic cytoplasm -Infiltrative growth -Neutrophilic infiltrate -Reactive woven bone and osteoclast-like giant cells can be present -*SERPINE1-FOSB* fusion	ERG+ FLI1+ Keratin+ CD34- Desmin- Retention of INI1 FOSB+
Epithelioid hemangioendothelioma	-Epithelioid endothelial cells in strands and cords embedded in a hyaline or myxoid stroma -Intracytoplasmic vacuoles (blister cells) -No well-formed vessels-Infiltrative growth -Cytologic atypia and mitoses usually limited, but can be prominent -*WWTR1-CAMTA1* fusion	CD31 100% CD34 85% FLI1 100% Keratin 25%-38% D2-40 54% Prox1 54% ERG 98% Claudin-1 88% CAMTA1 86%-88%

Entity	**Histologic and Molecular Findings**	**Immunohistochemistry**
YAP1-TFE3 rearranged epithelioid hemangioendothelioma	-Focally well-formed vasoformative features in addition to solid areas -Voluminous deep eosinophilic or histiocytoid cytoplasm, sometimes feathery -Mild to moderate nuclear atypia -*YAP1-TFE3* fusion	Same as EHE TFE3+
Angiosarcoma	-Vasoformative, with multilayering, or solid -In bone often epithelioid (>90%) -Inflammatory infiltrate -Nuclear atypia (with large nucleoli) -Brisk mitotic atypia, including atypical mitoses -No specific genetic alterations	CD31 95%-100% ERG 96% VWF 60%-75% CD34 39%-40% Actin 61% Keratin 69%-80% D2-40 31%
Intravascular papillary endothelial hyperplasia (Masson tumor)	-Can occur in a blood vessel, a hematoma or in a preexisting vascular lesion -Papillary structures containing fibrin or collagen, covered by a single layer of endothelial cells -No or limited cytologic atypia, no or limited mitotic activity, no multilayering	CD31+ CD34+ ERG+

Table 2.2: Differential diagnosis of vascular tumors of bone.

2.3 Immunohistochemistry

In all vascular tumours endothelial differentiation can be highlighted using a panel of immunohistochemical markers including CD31, CD34 and ERG. ERG positivity can be highly specific for endothelial differentiation although this is dependent on the clone used: antibodies against the N-terminal part of the protein are more specific as compared to antibodies directed against the C-terminal part, which can also be positive in a variety of other mesenchymal tumors (18). Moreover, one should be aware that ∼50% of the prostate carcinomas harbour translocations involving ERG and thereby can be positive (19). FLI1

and Von Willebrand Factor (VWF, factor VIII) can also be used. Smooth muscle actin can highlight the pericytes, while D2-40 (podoplanin) and Prox1 can demonstrate lymphatic differentiation. A notorious pitfall that pathologists should be aware of, especially in bone where vascular tumors are often epithelioid (93-100% (20, 21)), is the expression of keratin in a significant percentage of vascular tumors (20, 22, 23).

2.4 Hemangioma

2.4.1 Definition, epidemiology and clinical features

Hemangiomas are common lesions that rarely ever reach a pathologist. Reported by Mirra et al these tumors are found in ∼10% of all autopsies and they are often seen by radiologists (24). They are usually asymptomatic. The vertebral bodies are most commonly affected (figure 2.1) (25). Kaleem et al analyzed all reported cases of hemangioma affecting the extremities in English literature till 2000 (n=104) and found a mean age of 32 years, and a slight preference for females (60%) (26). When affecting the long bones, the diaphysis or metadiaphysis are the most common location. Medullary origin is most frequent, but 45% of cases are either periosteal (33%) or intracortical (12%) (26). In literature 11 cases have been described with intracortical hemangioma of bone, seven of which were located in the distal tibia (27). Cavernous hemangioma is the most frequent type, although also (areas with) capillary hemangioma can be found. At imaging the lesions are relatively radiolucent due to the lack of bone and abundance of fat on radiological images. Reportedly the tumors give a high MRI signal in T1 and T2 owing to their high fat presence (5). No genetic aberrations have been described thus far.

2.4.2 Histological and immunohistochemistry features

Macroscopically (figure 2.2a, 2.2b and 2.2c) the lesions show trabeculated bone with a dark sponge-like appearance. Histologically, the lesions show numerous blood filled spaces, lined by a thin layer of flat endothelial cells, without atypia. The vascular spaces are surrounded by loose connective tissue, and grow in between the bone trabeculae, that are often thickened (figure 2.2d).

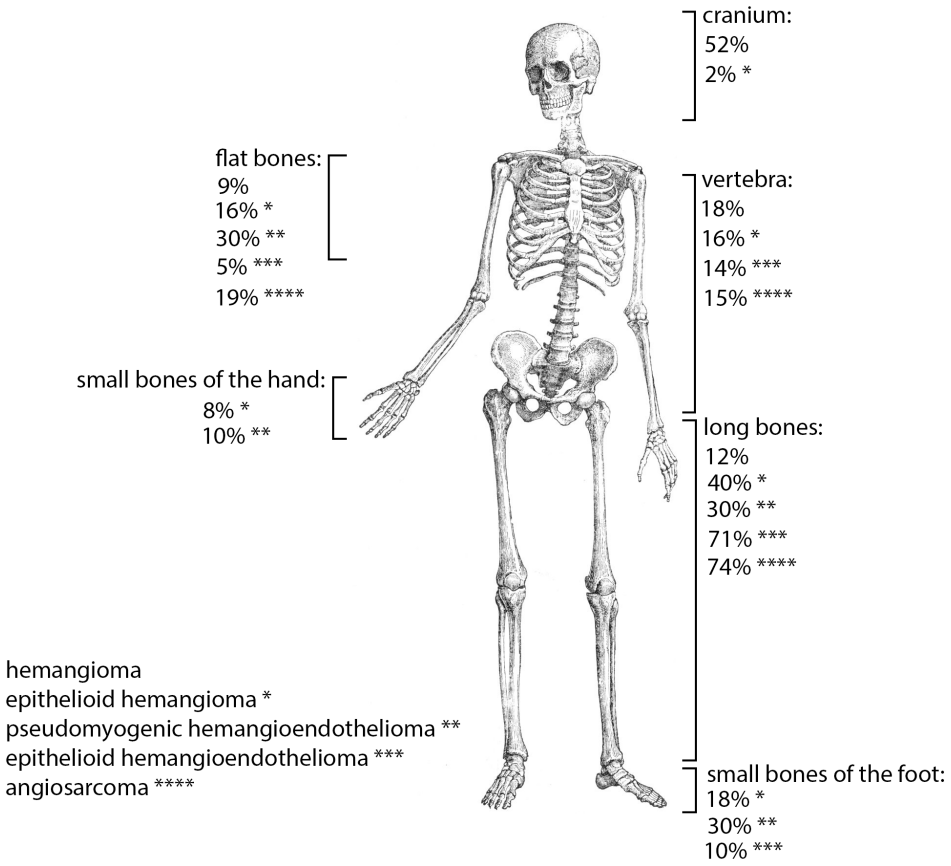

Figure 2.1: Distribution of vascular tumors.

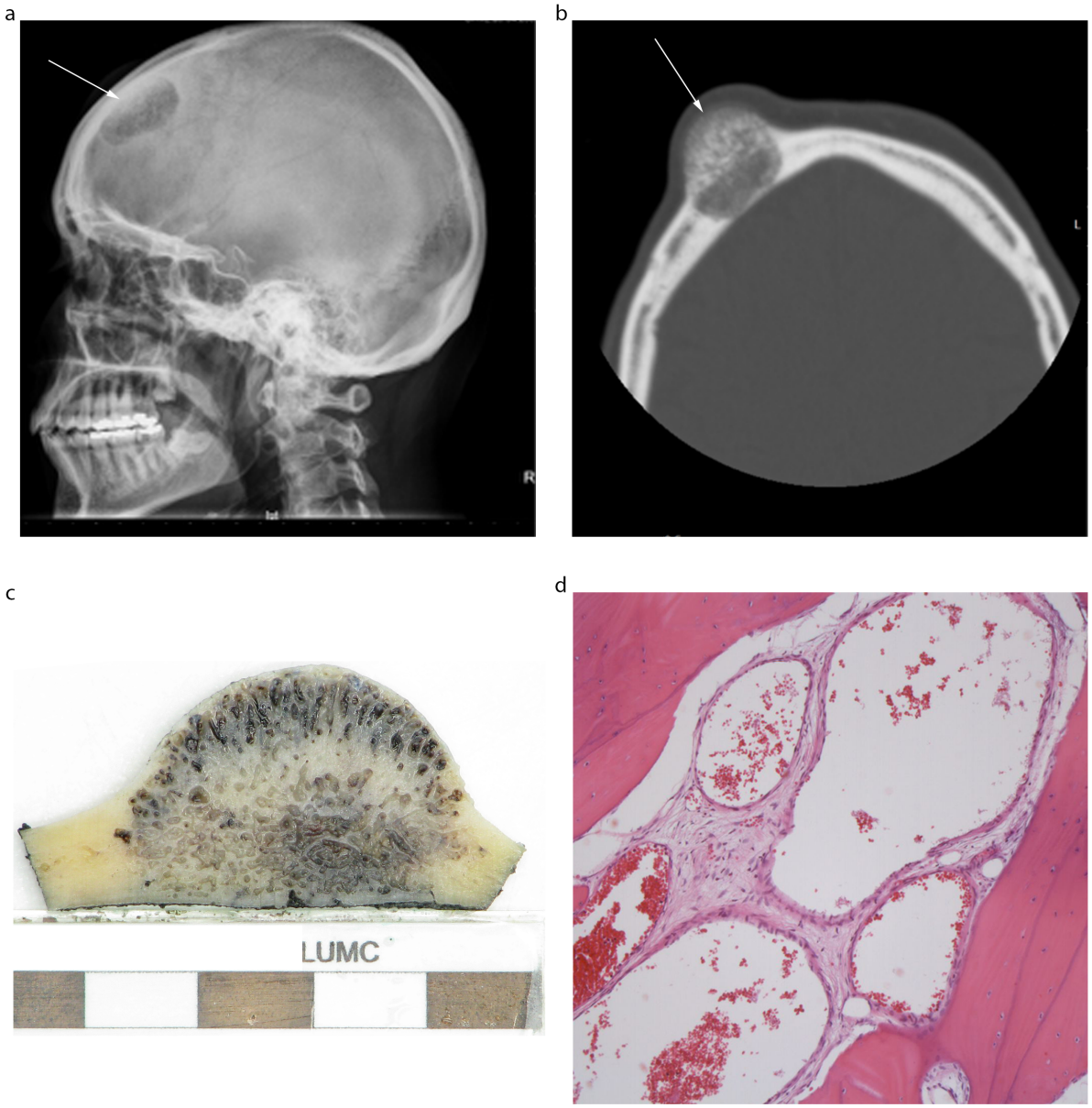

Figure 2.2: Hemangioma of bone. (a) X-ray of the skull with a sharply defined lytic lesion in the cranium. (b) Corresponding CT scan showing large protruding lytic lesion with calcifications. (c) Corresponding gross specimen shows trabeculations of the bone with sponge like appearance. (d) Microscopic image with large cavernous spaces lined by flat endothelium in between bony trabeculae.

2.5 Epithelioid hemangioma

2.5.1 Definition, epidemiology and clinical features

Epithelioid hemangioma of bone is classified as an intermediate, locally aggressive but rarely metastasizing vascular tumor of bone (8, 9). On CT scans a honeycomb pattern can be visible. Concise naming and classification of this tumor entity was only introduced recently by O'Connell et al (28, 29). Previously epithelioid hemangiomas of bone were reported as "haemangioendothelioma of bone" (11) or "haemorrhagic epithelioid and spindle cell hemangioma" (11, 30).

Nielsen et al revisited 50 epithelioid hemangiomas and described the age of occurrence to vary from 10 to 75 with a mean of 35 with a slight preference for males (11). As epithelioid hemangioma is a very rare entity exact prevalence is difficult to determine.

Epithelioid hemangioma has been described to occur in many different locations. Case reports and series of revisited cases seem to show there is a slight preference for the long tubular bones (31), but the spine is also often affected (32–35). Further reports include occurrences in the orbit (36–41). It is also frequently reported to occur in the small tubular bones of the extremities (42–45). Multifocal bone involvement occurs in ∼18% of the cases (11) with one case involving three different bones (46). Involvement of the draining lymph nodes has been described, but is not often confirmed as metastatic (47). Although as described by Nielsen et al the lymph node can contains cells resembling epithelioid hemangioma (11).

2.5.2 Histological and immunohistochemistry features

Epithelioid hemangioma is usually well-defined, with a lobular architecture (figure 2.3c), but can extend into the soft tissue. The vessels are usually well-formed, and lined by epithelioid endothelial cells (figure 2.3d, 2.3e). The cells have an enlarged nuclei with open chromatin, without prominent nuclear atypia. The often loose stroma surrounding the vessels can be infiltrated with eosinophils (figure 2.3e).

2.5.3 Tumorigenesis and genetics

Previously it was believed that epithelioid hemangioma could be a reactive lesion or a low grade variant of epithelioid hemangioendothelioma. This was refuted with the detection of fusion genes involving *FOS* (figure 2.3e) and *FOSB* with various fusion partners (12–14). Discovery of specific fusion genes also showed that tumors with multiple foci are monoclonal suggesting multifocal regional spread instead of multiple primaries (12). Overall, *FOS* rearrangements were found in 29% of epithelioid hemangiomas (13). The frequency

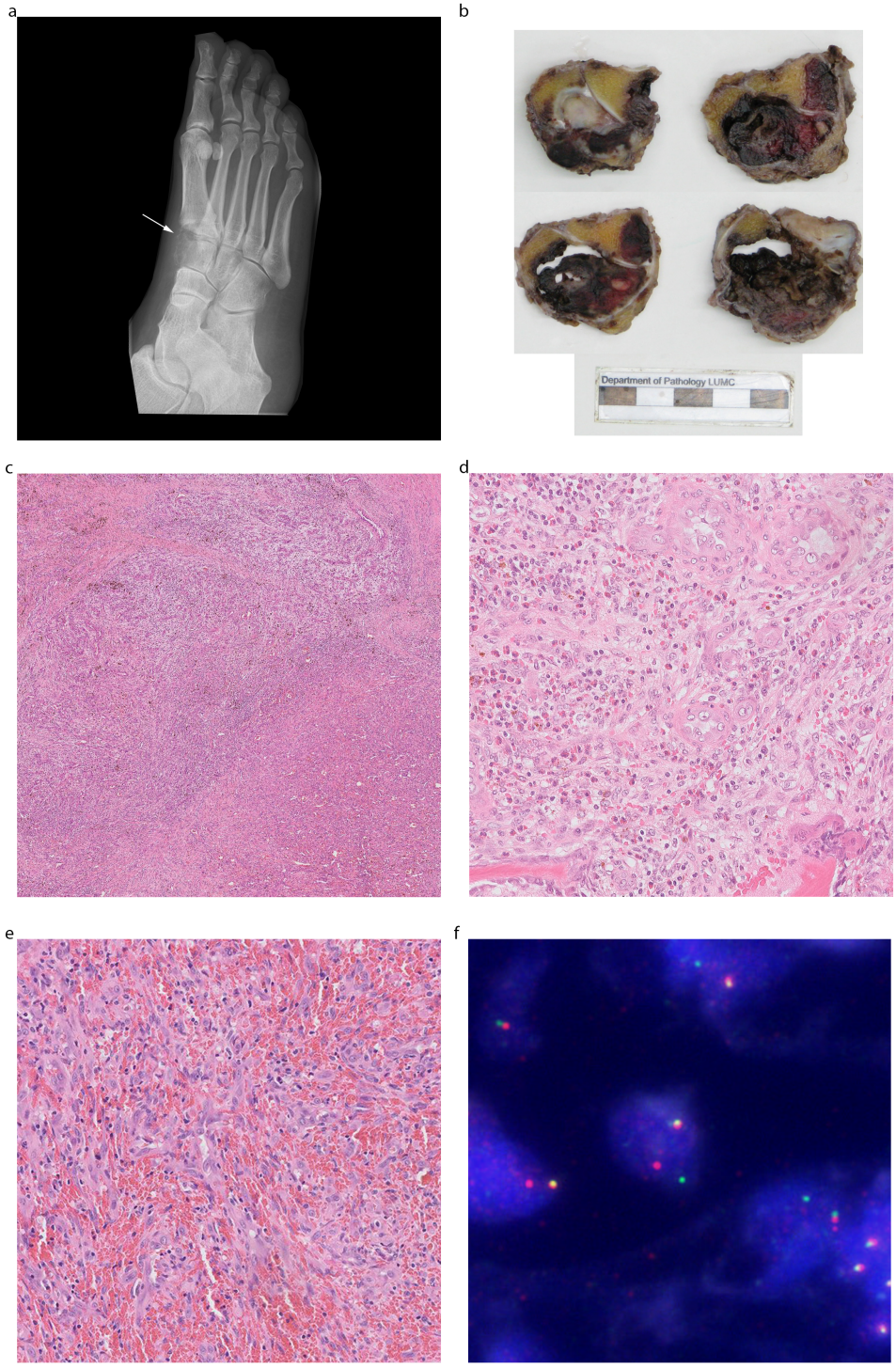

Figure 2.3: Epithelioid hemangioma of bone. (a) X-ray of the foot showing multiple sharply defined lesions. (b) Corresponding gross section. (c) Epithelioid hemangioma typically shows a lobular architecture. (d) Eosinophilic infiltrate surrounding the vessels lined by large epithelioid cells. (e) haemorrhagic and spindled cell appearance can be prominent especially in acral lesions. (f) FISH with break-apart probes surrounding *FOS* shows a split signal.

was higher in epithelioid hemangioma of bone as compared to soft tissue (59-71% versus 19%, respectively) (12, 13). In the *FOS* translocated epithelioid hemangiomas the fusion partners are at the C-terminal end of the protein and lead to a loss of the transactivating domain, regulating FOS turnover. It is speculated that this loss of the transactivation domain of FOS would lead to a reduced turnover, as FOS is normally rapidly degraded. Fusion genes involving *FOSB* are fused at the N-terminal end of the protein and are most likely activating promoter swap events. A specific subset of epithelioid hemangiomas was shown to harbour *ZFP36-FOSB* fusions (14). This subset has atypical histological features including more solid growth, increased cellularity, nuclear pleomorphism and necrosis (14). These cases are predominantly located at the penis and in bone. Both FOS and FOSB are part of the AP-1 transcription factor complex (48).

2.6 Pseudomyogenic (epithelioid-sarcoma like) hemangioendothelioma

2.6.1 Definition, epidemiology and clinical features

Most likely the first description in literature of pseudomyogenic hemangioendothelioma was published in 1992 by Mirra et al who reported a previously undescribed variant of epithelioid sarcoma. They reported five cases of epithelioid sarcoma displaying multicentric involvement of a single limb, osseous involvement, with bland diffuse fibrohistiocytic and rhabdoid cells (49). The first description as a distinct entity was in 2003 when Billings et al reported 7 histologically identical cases under the name epithelioid sarcoma-like hemangioendothelioma (50). In 2011 Hornick and Fletcher presented a large patient series including 29 cases. Twenty four percent of these had concurrent bone involvement. They proposed to designate the tumors as pseudomyogenic hemangioendothelioma. Most of the tumors arise in the extremities (see figure 2.1) with a male predominance (41 vs 9). Mean age was 31 years, ranging from 14-80. Strikingly, 33 of 50 patients presented with multifocal disease in which multiple discontiguous nodules were found in different tissue planes. In 2016 Inyang et al published the largest series of pseudomyogenic hemangioendotheliomas of bone to date, describing 10 cases with a male predominance (9 vs 1) and a mean age of 36 (range 12-74 years). They described the lesions to have intratumoral reactive woven bone and infiltration of osteoclast-like giant cells (17). The tumor is locally aggressive, and rarely metastasizing, and therefore of the intermediate category: one patient out of 50 developed distant metastasis (16). Reportedly the lesions are usually from 0.3 to 5.5 cm in size with ill-defined margins (figure 2.4a).

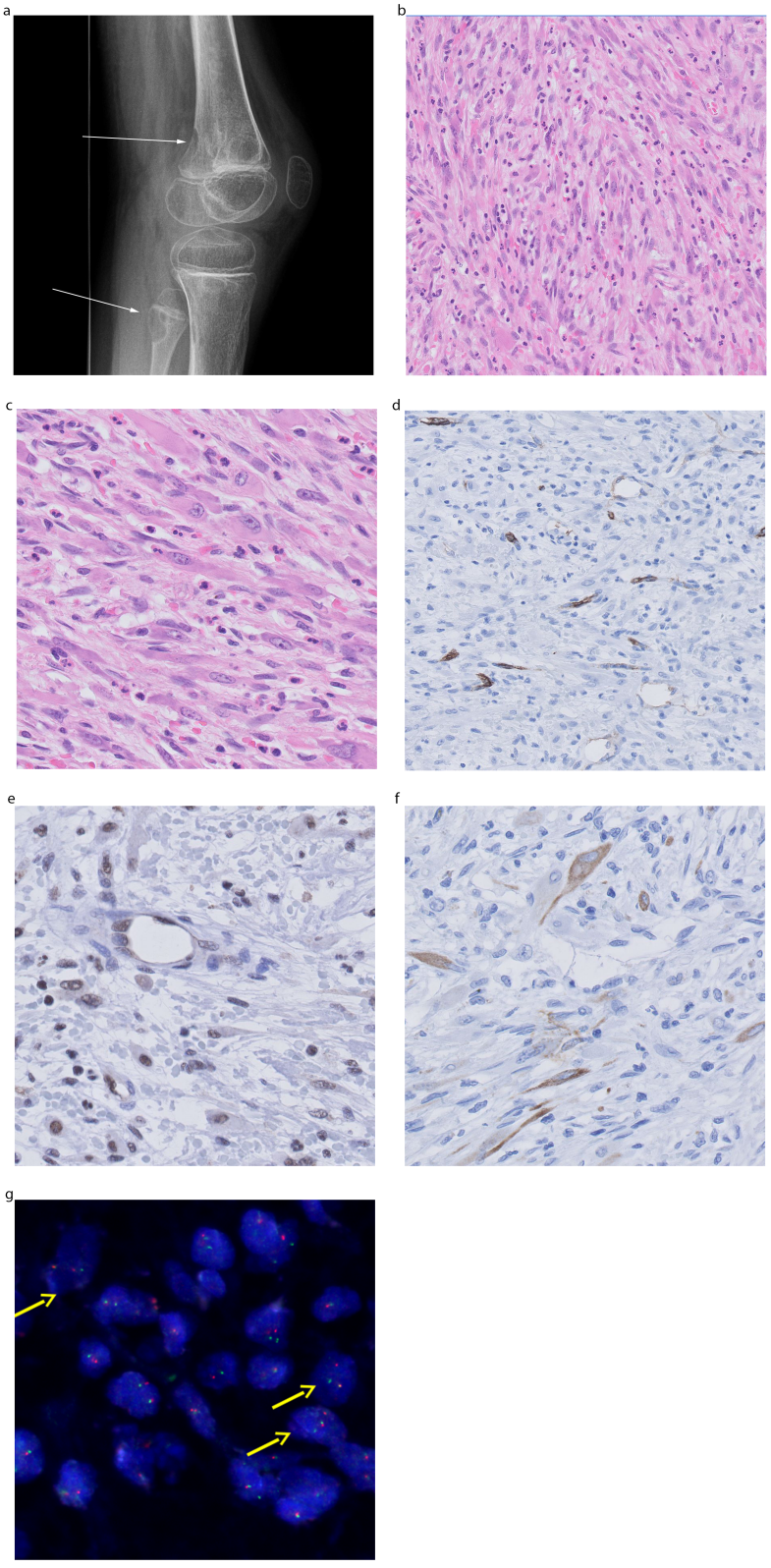

Figure 2.4: Pseudomyogenic hemangioendothelioma of bone. (a) X-ray shows a lesion the the left femur and fibula. (b) Spindle cells are seen admixed with neutrophils (c) the cells display a rhabdomyoblast-like appearance. (d) CD34 is consistently negative. (e) FLI1 shows nuclear staining in the endothelial cells. (f) Keratin staining is positive in the tumor cells. (g) FISH demonstrating split apart using a probe for *FOSB* indicative of *FOSB* rearrangement.

2.6.2 Histological and immunohistochemistry features

The tumor cells characteristically show an epithelioid sarcoma-like or rhabdomyoblast-like appearance, with abundant eosinophilic cytoplasm (figure 42.4b, 2.4c). The lesions can infiltrate in skeletal muscle. Infiltration with neutrophilic granulocytes can be prominent (figure 4b). The tumor cells characteristically express keratin AE1AE3 (figure 2.4f), ERG and FLI1 (figure 2.4e), while CD34 (figure 2.4d) and desmin are negative. CD31 is expressed in ∼50% of the cases and INI1 is retained. FOSB was shown to be an excellent immunohistochemical marker to detect the presence of the *SERPINE1-FOSB* fusion (see below), with 48 out of 50 pseudomyogenic hemangioendothelioma cases showing positive nuclear staining (51, 52).

2.6.3 Tumorigenesis and genetics

Trombetta et al published the first report of a balanced translocation between chromosomes 7 and 19 in pseudomyogenic hemangioendothelioma (53). The exact fusion partners were later on identified as *SERPINE1* and *FOSB* (54). FISH split probes for *FOSB* can be an excellent diagnostic marker (figure 2.4g). Most likely the *SERPINE1-FOSB* fusion leads to up-regulation of FOSB as *FOSB* is retained almost entirely (fused at exon 2) and gains the promoter of *SERPINE1* (fusion occurs in intron 1). Upregulation of FOSB could lead to activation of the AP-1 complex which is a potent transcription factor leading to the tumorigenesis, and thereby the underlying molecular mechanism is very similar to epithelioid hemangioma in which *ZFP36-FOSB*, as well as different types of *FOS* fusions, cause activation of the AP-1 complex.

2.7 Epithelioid hemangioendothelioma

2.7.1 Definition, epidemiology and clinical features

Epithelioid hemangioendothelioma of bone is classified by the WHO as a low grade malignant vascular sarcoma (8). Most are indolent, although 20-30% of the tumors metastasize and mortality is around 15% (55). The first distinction of epithelioid hemangioendothelioma from angiosarcoma was made by Thomas in 1942 who acknowledged that epithelioid hemangioendothelioma resembled epithelium in contrast to angiosarcoma. Moreover, he also described angiosarcoma to have a more malignant clinical course. The first comprehensive description of epithelioid hemangioendothelioma was formulated by Stout in 1943 who described two critical features. First the formation of atypical endothelial cells in greater numbers than are required for the lining of blood vessels. Second the formation of vascular tubes with a delicate framework of reticulin fibers (56). Later the characteristic

blister cells were added to the criteria for epithelioid hemangioendothelioma by Enzinger and Weiss (57). The tumor could easily be mistaken for a carcinoma due to its epithelioid appearance and lack of vasoformation.

The combined literature shows that the age of occurrence is regularly distributed between 10 and 60 years. There is a male predominance (68.9% of patients). The cases with reported metastasis showed that the prefered site for metastasis were the lungs followed by the skeleton, but it remains unclear whether these skeletal metastases should be considered as true metastases or multifocal regional spread. Overall, epithelioid hemangioendothelioma of bone is polyostotic in >50% of the cases (58). It predominantly affects the lower extremity (∼62%), and in up to 18% of the cases concurrent parenchymal tumors are found. Radiologically epithelioid hemangioendothelioma, like the other vascular tumors in bone, presents as a lytic lesion, without a sharp demarcation.

2.7.2 Histological and immunohistochemistry features

Histologically, epithelioid hemangioendothelioma typically consists of epithelioid cells, with abundant eosinophilic cytoplasm, sometimes with intracytoplasmic vacuolization (so-called "blister cells") (figure 2.5a, 2.5b). The cells are organized in short cords or strands and characteristically embedded in hyalinized or myxoid stroma. The tumor has an infiltrative growth pattern, and is lacking a lobular or vasoformative architecture. Marked nuclear atypia and / or necrosis is found in ∼33% of the cases. Inflammatory cells are usually absent. Immunohistochemically, the tumor cells are positive for CD31(100%) (figure 2.5c), CD34 (85%), FLI1 (100%), keratin (25-38%), D2-40 (54%), Prox1 (47%), ERG (98%) and Claudin-1 (88%) (23, 59, 60). Recently, nuclear staining for CAMTA1 was shown to be a highly specific marker for epithelioid hemangioendothelioma positive in 86-88% of the cases (figure 2.5d) (61, 62). TFE3 immunohistochemistry can be used to identify the very specific subset of epithelioid hemangioendotheliomas with *YAP1-TFE3* fusions, although not all TFE3 positive cases carry the translocation (see below) (63). The clinical behaviour of epithelioid hemangioendothelioma is highly variable and difficult to predict based on histological features. Deyrup et al proposed a risk stratification scheme in which tumors larger than 3cm, with >3 mitoses per 50 HPF have a 5 years survival rate of 59% and a metastatic rate of 32% as compared to 100% 5 year survival for patients with tumors smaller than 3 cm with less than 3 mitoses per 50 HPF (55). Whether this risk stratification scheme is also applicable to epithelioid haemangioendotheliomas with primary bone location remains to be established.

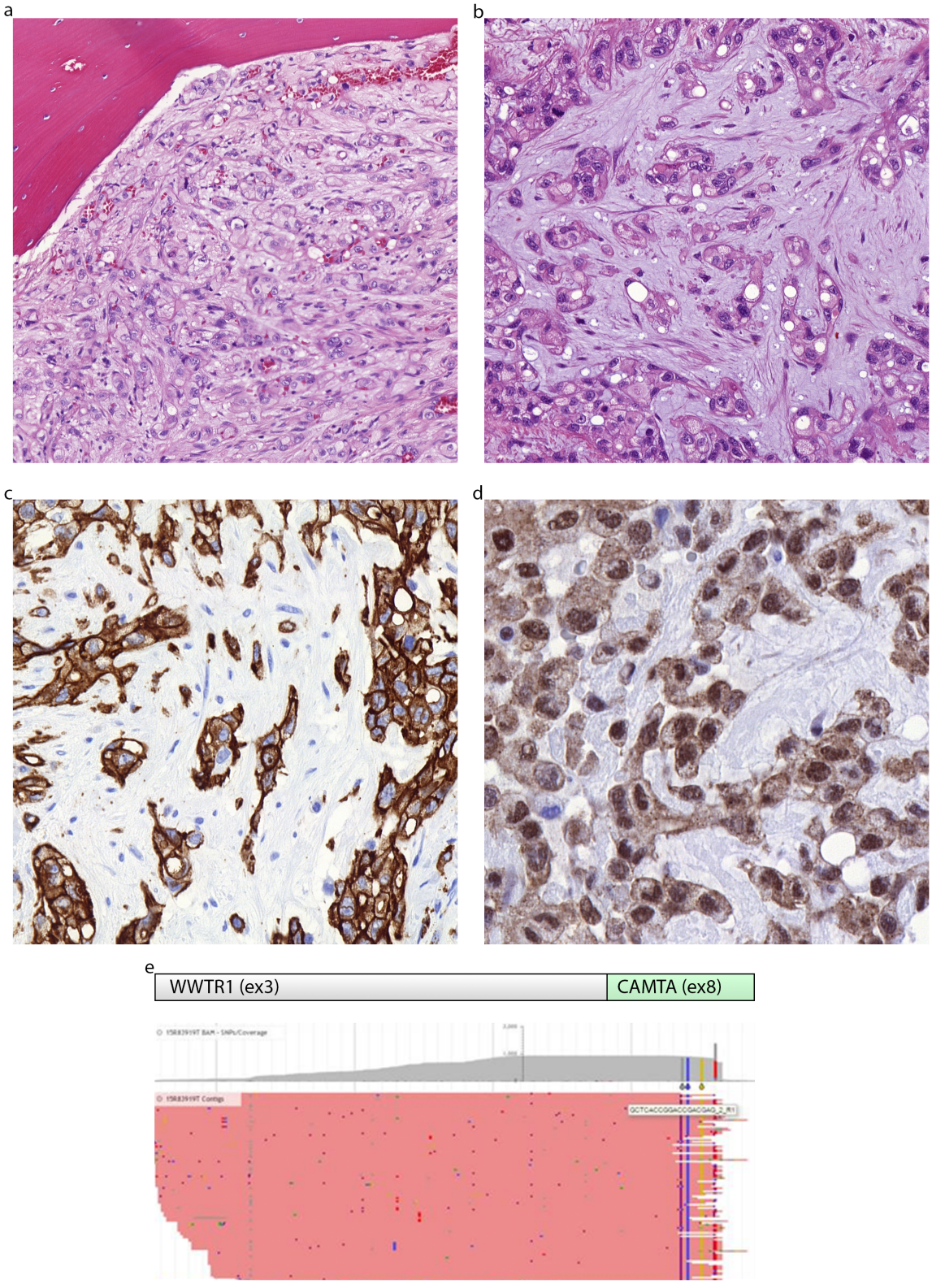

Figure 2.5: Epithelioid hemangioendothelioma of bone. (a) Epithelioid tumor cells in cords and strands embedded in stroma. (b) Intracytoplasmic vacuoles can be seen (Blister cells). (c) CD31 confirms endothelial differentiation. (d) CAMTA1 shows positive nuclear staining. (e) The *WWTR1-CAMTA1* fusion detected using next generation sequencing (Archer sarcoma fusion panel).

2.7.3 Tumorigenesis and genetics

Two fusions have been described for epithelioid hemangioendothelioma. The most common being the *WWTR1-CAMTA1* (figure 2.5e) fusion which was concurrently described (64, 65). Reportedly almost all (89-100%) epithelioid hemangioendothelioma with classic histological features harbor this fusion. Using genetic analysis the monoclonal origin of "multifocal" epithelioid hemangioendothelioma has been established using *WWTR1-CAMTA1* breakpoint analysis. This indicates that multiple lesions arise from local or metastatic spread from a single primary as opposed to multiple independent primaries (64).

In a distinct subset of epithelioid hemangioendotheliomas, which were negative for *WWTR1-CAMTA1*, a *YAP1-TFE3* fusion has been described (66). This specific subset affects predominantly young adults and has a distinct morphology, with vasoformative and vasoinvasive growth, combined with solid areas. The cytoplasm is voluminous, deeply eosinophilic or histiocytoid, sometimes feathery. The nuclei can be mild to moderately atypical. *TFE3* FISH can be used to confirm the diagnosis.

The *WWTR1-CAMTA1* fusion gene has been extensively studied. Interestingly, in contrast to what was speculated when the fusion was first described, the fusion is not a simple promoter swap where the *WWTR1* promoter drives CAMTA1. The *WWTR1-CAMTA1* fusion leads to activation of the Hippo signalling pathway, which is described to be an important regulator of organ size. As the chimeric protein contains the TEAD binding domain from WWTR1 it is able to activate the Hippo signaling pathway. The CAMTA1 part of the fusion protein leads to nuclear localization of the protein (67). Although less well studied, it would seem likely that the *YAP1-TFE3* chimeric protein would also lead to activation of the Hippo signaling pathway.

2.8 Angiosarcoma

2.8.1 Definition, epidemiology and clinical features

Angiosarcomas are highly aggressive sarcomas affecting the cutis, deep soft tissue, bone and viscera. Approximately 4% of all angiosarcomas arise primary in bone, and therefore angiosarcoma of bone is extremely rare. In bone, 30-40% of angiosarcomas are multifocal (21, 68, 69). Angiosarcomas are highly aggressive and predominantly occur in the seventh decade, with a male predominance. Angiosarcomas can be primary, or arise secondary to radiation (70). Angiosarcoma of bone should be treated with wide surgical resection, possibly with adjuvant radiation or chemotherapy. Virtually all patients die within a few years: the 1 year survival is 55% and the 5 year survival is 33% (21, 28). Radiologically angiosarcoma presents as a well-defined, osteolytic lesion, with a geographical

pattern of destruction (69). Cortical destruction is found in 65% of the cases. Because they are often multifocal, it is easily confused by radiologists with metastatic carcinoma. Macroscopically the tumors are hemorrhagic often with prominent necrosis.

2.8.2 Histological and immunohistochemistry features

Microscopically ill-defined blood vessels lined by enlarged endothelial cells with hyperchromatic, pleomorphic nuclei are seen (figure 2.6a, 2.6b). In bone, >90% of the angiosarcomas display epithelioid morphology (20, 21). In addition to the variable presence of vasoformative areas, often with multilayering, solid areas can be found. Mitoses are easily found, sometimes atypical forms (6, 21, 28, 71). Immunohistochemistry shows positivity for CD31 (95-100%) (figure 2.6d), ERG (96%), VWF (60-75%), CD34 (39-40%) and smooth muscle actin (61%) (20, 21, 23, 60). Keratin AE1AE3 is expressed in 69-80% of the angiosarcomas, and in combination with a radiological diagnosis of metastatic carcinoma and epithelioid morphology often causes misdiagnoses (21). D2-40 is expressed in 31% of the angiosarcomas of bone and is associated with a worse outcome (21). Likewise, loss of p16 is associated with a more aggressive clinical behaviour (72). In addition, the presence of a macronucleolus, three or more mitoses per 10 HPF, and less than 5 eosinophilic granulocytes are associated with poor outcome (21).

2.8.3 Tumorigenesis and genetics

Many different genetic alterations have been described for the angiosarcomas. As reported by Verbeke et al two groups of angiosarcomas could be identified; those with complex genetic profiles and a group with few gross genetic alterations (73). Inactivation of the p53 pathway is very common in angiosarcoma. A study on angiosarcoma of the liver reported frequent events leading to inactivation of p53 where p14, p15 and p53 were analyzed for mutations and for methylation. In almost all cases p53 was disabled due to promoter methylation or mutations in the aforementioned genes (74, 75). Antonescu et al described mutations in KDR in 7%, restricted to breast angiosarcomas but later also reported a case in the lumbar spine (76). This mutation could lead to auto-phosphorylation which would provide rationale for treatment with tyrosine kinase inhibitors. Furthermore, unsupervised clustering of gene expression profiles of angiosarcomas and other soft tissue sarcomas revealed that angiosarcomas cluster closely together, indicating they are indeed a highly similar entity in their gene expression pattern even though the specific mutations per case can be different (77). *MYC* amplifications are common events (55-100%) in secondary angiosarcomas (after irradiation or chronic lymphedema) (78). Although *MYC* amplifications were first reported exclusively for secondary angiosarcomas, more

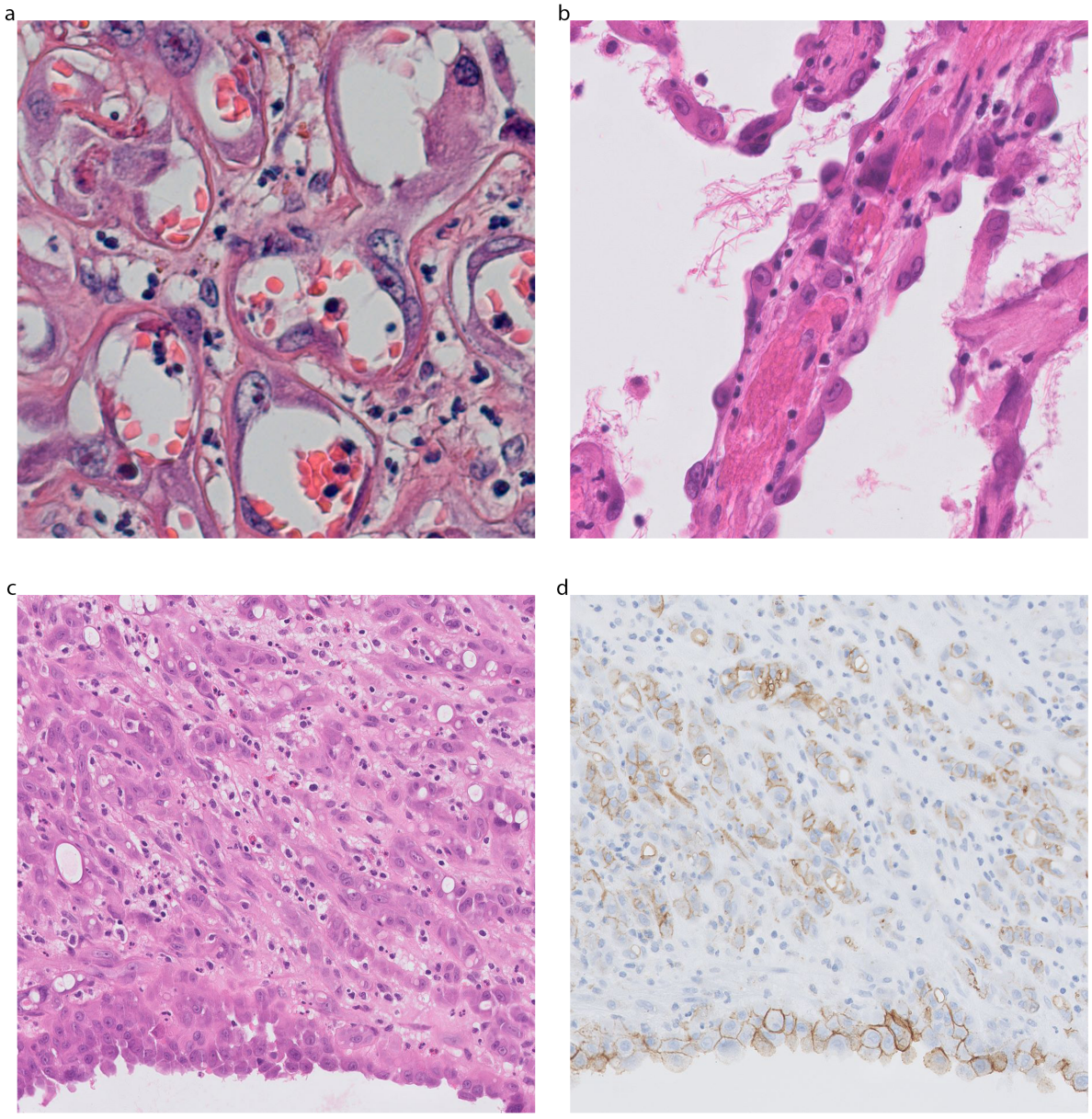

Figure 2.6: Angiosarcoma of bone. (a) Angiosarcoma of bone showing high degree of nuclear atypia, often with prominent nucleoli. (b) Vessels are lined by epithelioid endothelial cells. Cells show nuclear atypia. (c) Diffuse growth can be seen and tumor cells can have cytoplasmic vacuoles. (d) Corresponding CD31 immunohistochemistry.

recently they have been described in primary angiosarcoma cases as well including angiosarcoma of bone (73). Co-amplification of *FLT4*, and additional mutations in PLCG1 and PTPRB can be found in secondary angiosarcoma (70, 79). Furthermore, Huang et al recently reported *CIC* abnormalities occurring in 9% of cases, affecting younger patients with primary AS, with an inferior disease-free survival (80, 81). Although alterations in these genes have not been described in angiosarcoma of bone, it should be noted that genetic information for angiosarcoma of bone is limited.

2.9 Discussion

Vascular tumors consist of endothelial cells, often retaining the capacity to form vessels. Over time there has been much controversy surrounding the classification and naming of the various vascular tumors. But much of the confusion has been clarified with the discovery of a number of fusion genes and genetic alterations that point to distinct tumor entities.

Diagnosis of vascular tumors can be challenging as they are often very similar in appearance but required very different therapeutic approaches. Moreover, the epithelioid variants can show high keratin expression leading to confusion with metastatic carcinoma.

Bibliography

[1] Lamerato-Kozicki AR, Helm KM, Jubala CM, Cutter GC, Modiano JF. Canine hemangiosarcoma originates from hematopoietic precursors with potential for endothelial differentiation. Experimental Hematology. 2006;34(7):870–878. doi:10.1016/J.EXPHEM.2006.04.013.

[2] Kakiuchi-Kiyota S, Crabbs TA, Arnold LL, Pennington KL, Cook JC, Malarkey DE, et al. Evaluation of Expression Profiles of Hematopoietic Stem Cell, Endothelial Cell, and Myeloid Cell Antigens in Spontaneous and Chemically Induced Hemangiosarcomas and Hemangiomas in Mice. Toxicologic Pathology. 2013;41(5):709–721. doi:10.1177/0192623312464309.

[3] Yoder MC, Ingram DA. Endothelial progenitor cell: ongoing controversy for defining these cells and their role in neoangiogenesis in the murine system. Curr Opin Hematol. 2009;16(4):269–273. doi:10.1097/MOH.0b013e32832bbcab.

[4] Kovacic JC, Moore J, Herbert A, Ma D, Boehm M, Graham RM. Endothelial progenitor cells, angioblasts, and angiogenesis–old terms reconsidered from a current perspective. Trends Cardiovasc Med. 2008;18(2):45–51. doi:10.1016/j.tcm.2007.12.002.

[5] Wenger DE, Wold LE. Benign vascular lesions of bone: radiologic and pathologic features. Skeletal Radiology. 2000;29(2):63–74. doi:10.1007/s002560050012.

[6] Wenger DE, Wold LE. Malignant vascular lesions of bone: Radiologic and pathologic features. Skeletal Radiology. 2000;29(11):619–631. doi:10.1007/s002560000261.

[7] Bruder E, Perez-Atayde AR, Jundt G, Alomari AI, Rischewski J, Fishman SJ, et al. Vascular lesions of bone in children, adolescents, and young adults. A clinicopathologic reappraisal and application of the ISSVA classification. Virchows Archiv. 2009;454(2):161–179. doi:10.1007/s00428-008-0709-3.

[8] Fletcher CDM, Bridge JA, Hogendoorn P, Mertens F. Vascular tumours. Lyon: IARC; 2013.

[9] Verbeke SL, Bovee JV. Primary vascular tumors of bone: a spectrum of entities? Int J Clin Exp Pathol. 2011;4(6):541–551.

[10] Volpe R, Mazabraud A. Hemangioendothelioma (angiosarcoma) of bone: A distinct pathologic entity with an unpredictable course? Cancer. 1982;49(4):727–736. doi:10.1002/1097-0142(19820215)49:4<727::AID-CNCR2820490422>3.0.CO;2-V.

[11] Nielsen GP, Srivastava A, Kattapuram S, Deshpande V, O'Connell JX, Mangham CD, et al. Epithelioid hemangioma of bone revisited: A study of 50 cases. American Journal of Surgical Pathology. 2009;33(2):270–277. doi:10.1097/PAS.0b013e31817f6d51.

[12] van IJzendoorn DG, de Jong D, Romagosa C, Picci P, Benassi MS, Gambarotti M, et al. Fusion events lead to truncation of FOS in epithelioid hemangioma of bone. Genes Chromosomes Cancer. 2015;54(9):565–574. doi:10.1002/gcc.22269.

[13] Huang S, Chen H, Zhang L, Sung Y, Dickson B, Krausz T, et al. Frequent FOS Gene Rearrangements in Epithelioid Hemangioma. Lab Invest. 2015;95:18A–19A.

[14] Antonescu CR, Chen HW, Zhang L, Sung YS, Panicek D, Agaram NP, et al. ZFP36-FOSB fusion defines a subset of epithelioid hemangioma with atypical features. Genes Chromosomes and Cancer. 2014;53(11):951–959. doi:10.1002/gcc.22206.

[15] Walter JW, North PE, Waner M, Mizeracki A, Blei F, Walker JWT, et al. Somatic mutation of vascular endothelial growth factor receptors in juvenile hemangioma. Genes Chromosomes and Cancer. 2002;33(3):295–303. doi:10.1002/gcc.10028.

[16] Hornick JL, Fletcher CD. Pseudomyogenic hemangioendothelioma: a distinctive, often multicentric tumor with indolent behavior. Am J Surg Pathol. 2011;35(2):190–201. doi:10.1097/PAS.0b013e3181ff0901.

[17] Inyang A, Mertens F, Puls F, Sumathi V, Inwards C, Folpe A, et al. Primary pseudomyogenic hemangioendothelioma of bone. American Journal of Surgical Pathology. 2016;40(5):587–598. doi:10.1097/PAS.0000000000000613.

[18] Machado I, Mayordomo-Aranda E, Scotlandi K, Picci P, Llombart-Bosch A. Immunoreactivity using anti-ERG monoclonal antibodies in sarcomas is influenced by clone selection. Pathol Res Pract. 2014;210(8):508–513. doi:10.1016/j.prp.2014.04.005.

[19] Perner S, Mosquera JM, Demichelis F, Hofer MD, Paris PL, Simko J, et al. TMPRSS2-ERG fusion prostate cancer: An early molecular event associated with invasion. American Journal of Surgical Pathology. 2007;31(6):882–888. doi:10.1097/01.pas.0000213424.38503.aa.

[20] Deshpande V, Rosenberg AE, O'Connell JX, Nielsen GP. Epithelioid angiosarcoma of the bone: A series of 10 cases. American Journal of Surgical Pathology. 2003;27(6):709–716. doi:10.1097/00000478-200306000-00001.

[21] Verbeke SL, Bertoni F, Bacchini P, Sciot R, Fletcher CD, Kroon HM, et al. Distinct histological features characterize primary angiosarcoma of bone. Histopathology. 2011;58(2):254–264. doi:10.1111/j.1365-2559.2011.03750.x.

[22] Barak S, Wang Z, Miettinen M. Immunoreactivity for calretinin and keratins in desmoid fibromatosis and other myofibroblastic tumors: A diagnostic pitfall. American Journal of Surgical Pathology. 2012;36(9):1404–1409. doi:10.1097/PAS.0b013e3182556def.

[23] Miettinen M, Wang ZF, Paetau A, Tan SH, Dobi A, Srivastava S, et al. ERG transcription factor as an immunohistochemical marker for vascular endothelial tumors and prostatic carcinoma. American Journal of Surgical Pathology. 2011;35(3):432–441. doi:10.1097/PAS.0b013e318206b67b.

[24] Mirra JM, Gold RH, Marcove RC. Bone Tumors, Diagnosis and Treatment: Diagnosis and Treatment. Lippincott Williams & Wilkins; 1980.

[25] Picci P. Atlas of Musculoskeletal Tumors and Tumorlike Lesions. Springer Science & Business Media; 2014. Available from: `http://link.springer.com/10.1007/978-3-319-01748-8`.

[26] Kaleem Z, Kyriakos M, Totty WG. Solitary skeletal hemangioma of the extremities. Skeletal Radiol. 2000;29(9):502–513. doi:10.1007/s002560000290502.256.

[27] López-Barea F, Hardisson D, Rodríguez-Peralto JL, Sánchez-Herrera S, Lamas M. Intracortical hemangioma of bone. Report of two cases and review of the literature. The Journal of bone and joint surgery American volume. 1998;80(11):1673–8.

[28] O'Connell JX, Nielsen GP, Rosenberg aE. Epithelioid vascular tumors of bone: a review and proposal of a classification scheme. Advances in anatomic pathology. 2001;8(2):74–82.

[29] O'Connell JX, Kattapuram SV, Mankin HJ, Bhan AK, Rosenberg AE. Epithelioid hemangioma of bone: A tumor often mistaken for low-grade angiosarcoma or malignant hemangioendothelioma. American Journal of Surgical Pathology. 1993;17(6):610–617. doi:10.1097/00000478-199306000-00009.

[30] Keel SB, Rosenberg AE. Hemorrhagic epithelioid and spindle cell hemangioma: a newly recognized, unique vascular tumor of bone. Cancer. 1999;85(9):1966–1972.

[31] Mridha AR, Kinra P, Sable M, Sharma MC, Rastogi S, Khan SA, et al. Epithelioid hemangioma of distal femoral epiphysis in a patient with congenital talipes equinovarus. Malays J Pathol. 2014;36(1):63–66.

[32] Boyaci B, Hornicek FJ, Nielsen GP, Delaney TF, Pedlow FX, Mansfield FL, et al. Epithelioid hemangioma of the spine: A case series of six patients and review of the literature. Spine Journal. 2013;13(12):e7–13. doi:10.1016/j.spinee.2013.06.070.

[33] Calderaro J, Guedj N, Dauzac C, Wassef M, Guigui P, Bedossa P. Un cas d'hémangiome épithélioïde du rachis. Annales de Pathologie. 2011;31(4):312–315. doi:10.1016/j.annpat.2011.05.008.

[34] Weaver SM, Kumar AB. Epithelioid hemangioma of the spine: an uncommon cause of spinal cord compression. Acta Neurol Belg. 2015;115(4):843–845. doi:10.1007/s13760-015-0437-9.

[35] Akgun B, Ozturk S, Ucer O, Erol FS. Epithelioid hemangioma of the thoracic spine. Neurol India. 2015;63(4):610–611. doi:10.4103/0028-3886.162082.

[36] Cummings TJ, Proia AD, Woodward J. Epithelioid hemangioma of the orbit. Clinical and Surgical Ophthalmology. 2004;22(8):224–226. doi:10.1016/S0161-6420(00)00011-7.

[37] Budimir I, Demirovic A, Ivekovic R, Pazanin L. Epithelioid hemangioma of the orbit: case report. Acta Clin Croat. 2015;54(1):92–95.

[38] Sanchez-Orgaz M, Insausti-Garcia A, Gregorio LY, Duralde AA, Romero-Martin R. Epithelioid hemangioma of the orbit or angiolymphoid hyperplasia with eosinophilia. Ophthalmic Plastic and Reconstructive Surgery. 2014;30(3):e70–2. doi:10.1097/IOP.0b013e31829c4216.

[39] Alder B, Proia A, Liss J. Distinct, bilateral epithelioid hemangioma of the orbit. Orbit (Amsterdam, Netherlands). 2013;32(1):51–53. doi:10.3109/01676830.2012.739674.

[40] Fernandes BF, Al-Mujaini A, Petrogiannis-Haliotis T, Al-Kandari A, Arthurs B, Burnier MN. Epithelioid hemangioma (angiolymphoid hyperplasia with eosinophilia) of the orbit: a case report. Journal of Medical Case Reports. 2007;1(1):30. doi:10.1186/1752-1947-1-30.

[41] Baili L, Cheour M, Hachicha F, Aydi Z, Zoubeidi H, Ben Brahim E, et al. Hémangiome épithélioïde de l'orbite: à propos d'un cas. Journal Francais d'Ophtalmologie. 2014;37(9):e133–e136. doi:10.1016/j.jfo.2014.01.017.

[42] Svajdler M, Bohus P, Baumohlova H, Sokol L, Bielek J. [Epithelioid hemangioma of the foot]. Cesk Patol. 2006;42(2):86–90.

[43] Luna JTP, DeGroot H. Five-Year Follow-up of Structural Allograft Reconstruction for Epithelioid Hemangioma of the Talus and Navicular: A Case Report and Review of the Literature. Foot & Ankle International. 2007;28(3):379–384. doi:10.3113/FAI.2007.0379.

[44] Werhahn C, Lang M, Merkel KH, Pickartz H. [Epithelioid hemangioma–a rare tumor of the hand]. Handchir Mikrochir Plast Chir. 1990;22(4):214–218.

[45] El Harroudi T, Moumen M, Tijami F, El Otmany A, Jalil A. Hémangiome épithélioïde géant de la main. Chirurgie de la Main. 2008;27(5):240–242. doi:10.1016/j.main.2008.08.006.

[46] Sirikulchayanonta V, Arthit J, Suphaneewan J. Epithelioid hemangioma involving three contiguous bones: A case report with a review of the literature. Korean Journal of Radiology. 2010;11(6):692–696. doi:10.3348/kjr.2010.11.6.692.

[47] Zhou Q, Lu L, Fu Y, Xiang K, Xu L. Epithelioid hemangioma of bone: a report of two special cases and a literature review. Skeletal radiology. 2016;45(12):1723–1727. doi:10.1007/s00256-016-2482-8.

[48] Eferl R, Wagner EF. AP-1: A double-edged sword in tumorigenesis. Nature Reviews Cancer. 2003;3(11):859–868. doi:10.1038/nrc1209.

[49] Mirra JM, Kessler S, Bhuta S, Eckardt J. The fibroma-like variant of epithelioid sarcoma. A fibrohistiocytic/myoid cell lesion often confused with benign and malignant spindle cell tumors. Cancer. 1992;69(6):1382–1395. doi:10.1002/1097-0142(19920315)69:6<1382::AID-CNCR2820690614>3.0.CO;2-Y.

[50] Billings SD, Folpe AL, Weiss SW. Epithelioid Sarcoma-Like Hemangioendothelioma. The American journal of surgical pathology. 2003;27(1):48–57. doi:10.1097/PAS.0b013e31821caf1c.

[51] Hung YP, Fletcher CDM, Hornick JL. FOSB is a useful diagnostic marker for pseudomyogenic hemangioendothelioma. American Journal of Surgical Pathology. 2017;41(5):596–606. doi:10.1097/PAS.0000000000000795.

[52] Sugita S, Hirano H, Kikuchi N, Kubo T, Asanuma H, Aoyama T, et al. Diagnostic utility of FOSB immunohistochemistry in pseudomyogenic hemangioendothelioma and its histological mimics. Diagn Pathol. 2016;11(1):75. doi:10.1186/s13000-016-0530-2.

[53] Trombetta D, Magnusson L, von Steyern FV, Hornick JL, Fletcher CDM, Mertens F. Translocation t(7;19)(q22;q13)-a recurrent chromosome aberration in pseudomyogenic hemangioendothelioma? Cancer Genetics. 2011;204(4):211–215. doi:10.1016/j.cancergen.2011.01.002.

[54] Walther C, Tayebwa J, Lilljebjörn H, Magnusson L, Nilsson J, Von Steyern FV, et al. A novel SERPINE1-FOSB fusion gene results in transcriptional up-regulation of FOSB in pseudomyogenic haemangioendothelioma. Journal of Pathology. 2014;232(5):534–540. doi:10.1002/path.4322.

[55] Deyrup AT, Tighiouart M, Montag AG, Weiss SW. Epithelioid hemangioendothelioma of soft tissue: a proposal for risk stratification based on 49 cases. Am J Surg Pathol. 2008;32(6):924–927.

[56] Thomas A. Vascular tumors of bone: pathological and clinical study of 27 cases. Surg Gynecol Obstet. 1942;777–95.

[57] Stout AP. Hemangio-Endothelioma: a Tumor of Blood Vessels Featuring Vascular Endothelial Cells. Ann Surg. 1943;118(3):445–464.

[58] Weiss SW, Enzinger FM. Epithelioid Hemangioendothelioma - a Vascular Tumor Often Mistaken for a Carcinoma. Cancer. 1982;50(5):970–981. doi:Doi 10.1002/1097-0142(19820901)50:5<970::Aid-Cncr2820500527>3.0.Co;2-Z.

[59] Campanacci M, Boriani S, Giunti A. Hemangioendothelioma of bone: A study of 29 cases. Cancer. 1980;46(4):804–814. doi:10.1002/1097-0142(19800815)46:4<804::AID-CNCR2820460427>3.0.CO;2-1.

[60] Gill R, O'Donnell RJ, Horvai A. Utility of immunohistochemistry for endothelial markers in distinguishing epithelioid hemangioendothelioma from carcinoma metastatic to bone. Arch Pathol Lab Med. 2009;133(6):967–972. doi:10.1043/1543-2165-133.6.967.

[61] Miettinen M, Rikala MS, Rys J, Lasota J, Wang ZF. Vascular endothelial growth factor receptor 2 as a marker for malignant vascular tumors and mesothelioma: an immunohistochemical study of 262 vascular endothelial and 1640 nonvascular tumors. Am J Surg Pathol. 2012;36(4):629–639. doi:10.1097/PAS.0b013e318243555b.

[62] Shibuya R, Matsuyama A, Shiba E, Harada H, Yabuki K, Hisaoka M. CAMTA1 is a useful immunohistochemical marker for diagnosing epithelioid haemangioendothelioma. Histopathology. 2015;67(6):827–835. doi:10.1111/his.12713.

[63] Doyle LA, Fletcher CDM, Hornick JL. Nuclear expression of CAMTA1 distinguishes epithelioid hemangioendothelioma from histologic mimics. American Journal of Surgical Pathology. 2016;40(1):94–102. doi:10.1097/PAS.0000000000000511.

[64] Flucke U, Vogels RJ, de Saint Aubain Somerhausen N, Creytens DH, Riedl RG, van Gorp JM, et al. Epithelioid Hemangioendothelioma: clinicopathologic, immunhistochemical, and molecular genetic analysis of 39 cases. Diagn Pathol. 2014;9:131. doi:10.1186/1746-1596-9-131.

[65] Errani C, Zhang L, Sung YS, Hajdu M, Singer S, Maki RG, et al. A novel WWTR1-CAMTA1 gene fusion is a consistent abnormality in epithelioid hemangioendothelioma of different anatomic sites. Genes Chromosomes and Cancer. 2011;50(8):644–653. doi:10.1002/gcc.20886.

[66] Tanas MR, Sboner A, Oliveira AM, Erickson-Johnson MR, Hespelt J, Hanwright PJ, et al. Identification of a disease-defining gene fusion in epithelioid hemangioendothelioma. Sci Transl Med. 2011;3(98):98ra82. doi:10.1126/scitranslmed.3002409.

[67] Antonescu CR, Le Loarer F, Mosquera JM, Sboner A, Zhang L, Chen CL, et al. Novel YAP1-TFE3 fusion defines a distinct subset of epithelioid hemangioendothelioma. Genes Chromosomes Cancer. 2013;52(8):775–784. doi:10.1002/gcc.22073.

[68] Patel NR, Salim AA, Sayeed H, Sarabia SF, Hollingsworth F, Warren M, et al. Molecular characterization of epithelioid haemangioendotheliomas identifies novel WWTR1-CAMTA1 fusion variants. Histopathology. 2015;67(5):699–708. doi:10.1111/his.12697.

[69] Young RJ, Brown NJ, Reed MW, Hughes D, Woll PJ. Angiosarcoma. The Lancet Oncology. 2010;11(10):983–991. doi:10.1016/S1470-2045(10)70023-1.

[70] Vermaat M, Vanel D, Kroon HM, Verbeke SLJ, Alberghini M, Bovee JVMG, et al. Vascular tumors of bone: Imaging findings. European Journal of Radiology. 2011;77(1):13–18. doi:10.1016/j.ejrad.2010.06.052.

[71] Mentzel T, Schildhaus HU, Palmedo G, Buttner R, Kutzner H. Postradiation cutaneous angiosarcoma after treatment of breast carcinoma is characterized by MYC amplification in contrast to atypical vascular lesions after radiotherapy and control cases: clinicopathological, immunohistochemical and molecular analysis o. Mod Pathol. 2012;25(1):75–85. doi:10.1038/modpathol.2011.134.

[72] Evans HL, Raymond AK, Ayala AG. Vascular tumors of bone: A study of 17 cases other than ordinary hemangioma, with an evaluation of the relationship of hemangioendothelioma of bone to epithelioid hemangioma, epithelioid hemangioendothelioma, and high-grade angiosarcoma. Human Pathology. 2003;34(7):680–689. doi:10.1016/S0046-8177(03)00249-1.

[73] Verbeke SL, Bertoni F, Bacchini P, Oosting J, Sciot R, Krenacs T, et al. Active TGF-beta signaling and decreased expression of PTEN separates angiosarcoma of bone from its soft tissue counterpart. Mod Pathol. 2013;26(9):1211–1221. doi:10.1038/modpathol.2013.56.

[74] Verbeke SL, de Jong D, Bertoni F, Sciot R, Antonescu CR, Szuhai K, et al. Array CGH analysis identifies two distinct subgroups of primary angiosarcoma of bone. Genes Chromosomes Cancer. 2015;54(2):72–81. doi:10.1002/gcc.22219.

[75] Weihrauch M, Markwarth A, Lehnert G, Wittekind C, Wrbitzky R, Tannapfel A. Abnormalities of the ARF-p53 pathway in primary angiosarcomas of the liver. Human Pathology. 2002;33(9):884–892. doi:10.1053/hupa.2002.126880.

[76] Garcia JM, Gonzalez R, Silva JM, Dominguez G, Vegazo IS, Gamallo C, et al. Mutational status of K-ras and TP53 genes in primary sarcomas of the heart. Br J Cancer. 2000;82(6):1183–1185. doi:10.1054/bjoc.1999.1060.

[77] Antonescu CR, Yoshida A, Guo T, Chang NE, Zhang L, Agaram NP, et al. KDR activating mutations in human angiosarcomas are sensitive to specific kinase inhibitors. Cancer Res. 2009;69(18):7175–7179. doi:10.1158/0008-5472.CAN-09-2068.

[78] Segal NH, Pavlidis P, Antonescu CR, Maki RG, Noble WS, DeSantis D, et al. Classification and subtype prediction of adult soft tissue sarcoma by functional genomics. Am J Pathol. 2003;163(2):691–700. doi:10.1016/S0002-9440(10)63696-6.

[79] Manner J, Radlwimmer B, Hohenberger P, Mossinger K, Kuffer S, Sauer C, et al. MYC high level gene amplification is a distinctive feature of angiosarcomas after irradiation or chronic lymphedema. Am J Pathol. 2010;176(1):34–39. doi:10.2353/ajpath.2010.090637.

[80] Behjati S, Tarpey PS, Sheldon H, Martincorena I, Van Loo P, Gundem G, et al. Recurrent PTPRB and PLCG1 mutations in angiosarcoma. Nature Genetics. 2014;46(4):376–379. doi:10.1038/ng.2921.

[81] Huang SC, Zhang L, Sung YS, Chen CL, Kao YC, Agaram NP, et al. Recurrent CIC gene abnormalities in angiosarcomas: A molecular study of 120 cases with concurrent investigation of PLCG1, KDR, MYC, and FLT4 gene alterations. American Journal of Surgical Pathology. 2016;40(5):645–655. doi:10.1097/PAS.0000000000000582.