Virchows Arch (2010) 456:219–234
DOI 10.1007/s00428-009-0854-3

REVIEW AND PERSPECTIVE

Soft tissue tumors associated with *EWSR1* translocation

Salvatore Romeo · Angelo P. Dei Tos

Received: 20 August 2009 / Revised: 7 October 2009 / Accepted: 17 October 2009 / Published online: 20 November 2009

Abstract The Ewing sarcoma breakpoint region 1 (*EWSR1*; also known as *EWS*) represents one of the most commonly involved genes in sarcoma translocations. In fact, it is involved in a broad variety of mesenchymal lesions which includes Ewing's sarcoma/peripheral neuroectodermal tumor, desmoplastic small round cell tumor, clear cell sarcoma, angiomatoid fibrous histiocytoma, extraskeletal myxoid chondrosarcoma, and a subset of myxoid liposarcoma. The fusion products between *EWSR1* and partners usually results in fusion of the N-terminal transcription-activating domain of *EWSR1* and the C-terminal DNA-binding domain of the fusion partner, eventually generating novel transcription factors. *EWSR1* rearrangement can be visualized by the means of fluorescence in situ hybridization (FISH). As soft tissue sarcomas represent a diagnostically challenging group, FISH analysis is an extremely useful confirmatory diagnostic tool. However, as in most instances a split-apart approach is used, the results of molecular genetics must be evaluated in context with morphology.

Keywords EWSR1 gene · Soft tissue tumors · Sarcoma translocations

Introduction

The Ewing sarcoma breakpoint region 1 (*EWSR1*; also known as *EWS*) represents one of the most commonly involved genes in sarcoma translocations. These structural changes result in a fusion product between *EWSR1* and several distinct partners. *EWSR1*, as implied by its name, was initially identified in Ewing sarcoma [1, 2] but it is involved in a variety of distinct clinicopathological entities. This includes Ewing's sarcoma/peripheral neuroectodermal tumour (ES/PNET), desmoplastic small round cell tumour (DSRCT), clear cell sarcoma of soft tissue (ST-CCS), angiomatoid fibrous histiocytoma (AFH), extraskeletal myxoid chondrosarcoma (EMCS), and a subset of myxoid liposarcoma (MLPS). An up-to-date list of the known translocations involving the *EWSR1* gene and the corresponding soft tissue tumor types is given in Table 1. *EWSR1* rearrangements are also described in tumors other than soft tissue tumors: i.e., cutaneous hidradenoma and mucoepidermoid carcinoma of salivary glands [3].

EWSR1 maps on 22q12, and its coding sequence includes 17 exons [4]. Related pseudogenes have been identified on chromosomes 1(annotation NC_000001.10) and 14(annotation NC_000014.8) [5]. The encoded protein is made up of 656 amino acids [4]. The N-terminal domain, transcription activation domain, is encoded by exon 1–7 and contains a repeated degenerated polypeptide of 7 to 12 residues rich in tyrosine, serine, threonine, glycine, and glutamine with consensus SYGQQSX [4]. C-terminal contains three arginine- and glycine-rich tracts (respectively encoded by exons 8–9, 14, and 16) and an RNA-binding domain (encoded by exons 11–13) [4]. The sequence of this latter allows for RNA binding and/or single-strand DNA and is common to the novel TET protein family [6]. This family includes EWSR1, FUS, TAF15, the fruit fly protein CAZ, and the zebra fish proteins EWSR1A and EWSR1B [1, 7–10] TET family has a crucial role in sarcoma genetics as also FUS and TAF15 are involved in fusion protein formation upon translocation and remarkably with the same

S. Romeo · A. P. Dei Tos (✉)
Department of Pathology, General Hospital of Treviso,
Piazza Ospedale 1,
31100 Treviso, Italy
e-mail: apdeitos@ulss.tv.it

Table 1 Chromosomal translocations involving EWSR1 in sarcomas

Tumour	Translocation	Fusion product
Angiomatoid fibrous histiocytoma	t(12;22)(q13;q12)	EWSR1–ATF1
Clear cell sarcoma	t(12;22)(q13;q12)	EWSR1–ATF1
	t(2;22)(q34;q12)	EWSR1–CREB1
Desmoplastic round cell tumor	t(11;22)(p13;q12)	EWSR1–WT1
	t(21;22)(q22;q12)	EWSR1–ERG
Extraskeletal myxoid chondrosarcoma	t(9;22)(q22;q12)	EWSR1–NR4A3
Ewing sarcoma/PNET	t(11;22)(q24;q12)	EWSR1–FLI1
	t(21;22)(q22;q12)	EWSR1–ERG
	t(20;22)(q13;q12)	EWSR1–NFATC2
	t(2;22)(q33;q12)	EWSR1–FEV
	t(7;22)(p22;q12)	EWSR1–ETV1
	t(17;22)(q12;q12)	EWSR1–E1AF
	t(2;22)(q31;q12)	EWSR1–SP3
	t(1;22)(p36.1;q12)	EWSR1–ZNF278
	t(6;22)(p21;q12)	EWSR1–POU5FI
Myxoid–round cell liposarcoma	t(12;22)(q13;q12)	EWSR1–DDIT3

partners reported for EWSR1 in an exclusive way (respectively FUS in ES, AFH, MLPS, and low grade fibromyxoid sarcoma and TAF15 in EMCS) [11].

EWSR1 messenger RNA (mRNA) is ubiquitously expressed with little variation between different tissues, its expression is stable throughout cell cycle, and the half-life is long. These three features taken together suggest that *EWSR1* is a housekeeping gene [12]. The encoded protein is mainly located in the nucleus [13] and in the C-terminal, and is present a highly positively charged nuclear localization signal, again sharing strong similarity with the respective C-terminal sequences of the TET family [13]. The role of this protein is still a matter of investigation. The processes in which it is involved are several and includes interactions with TFIID subunits and RNA polymerase II complex [14], interaction with components of the spliceosome such as splicing factor1 and the small nuclear ribonucleoprotein polypeptide C [15–17], interaction with mitotic spindle and stabilization of microtubules [9, 18], meiosis, with proposed roles in DNA pairing and recombination/repair mechanisms, and cellular senescence via its interaction with lamin A/C [19].

The formation of fusion products between *EWSR1* and partners usually results in fusion of the N-terminal transcription activating domain of *EWSR1* with removal of its RNA-binding domain and substitution with the C-terminal DNA binding of the fusion gene partner. Among the genes that fuse with *EWSR1* are often members of the erythroblastosis virus-transforming sequence (avian ETS) transcription factor family: including *FLI1*, *ERG*, *ETV1*, *ETV4*, and *FEV*. The transformation effect of fusion protein was thought to be mediated by the abnormal activation of the target genes of the fusion partner contributing the DNA-binding domain, i.e., ETS family member. However, target genes have turned out to be distinct from the expected genes [20, 21].

The most important practical outcome of the above-mentioned knowledge is that ancillary techniques have been developed for recognizing *EWSR1* rearrangement in tumors. Recent availability of *ESWR1* split-apart fluorescence in situ hybridization (FISH) probes allow diagnostic confirmation for those tumors in which this gene is rearranged. Furthermore, it is possible to amplify the mRNA transcript of the fusion product, too. As mentioned above, the presence of pseudogenes demands some extra caution in the interpretation of the results of the molecular techniques [5]. For instance, some probes for FISH might recognize also the pseudogenes sequence with subsequent presence of extra spots. For the same reason, real-time polymerase chain reaction in the absence of a DNAse treatment might end up amplifying pseudogene DNA [5]. In this review, we will discuss the feature of the soft tissue tumors harboring a translocation involving *EWSR1*.

Ewing's sarcoma/PNET

Ewing's sarcoma/PNET (also recently referred to with the somewhat misleading term “Ewing's family of tumor”) refers to a group of sarcomas made up of small blue round cells showing variable extent of neuroectodermal differentiation [22], the vast majority of which shares rearrangements of the *EWSR1* gene [22]. Soft tissue ES/PNETs are rare neoplasms accounting for about 5% of soft tissue sarcomas in adults and 10–15% in childhood. They can present at any age, however, the peak incidence is between

the first and the third decades, with no sex predilection. Most cases occur in the deep soft tissues of the paravertebral region and proximal portions of the lower and upper extremities. Visceral location, such as kidney [23], pancreas [24], and meninges [25], has been documented. The involvement of a major nerve with subsequent neurologic symptoms is reported in up to one third of cases, as in the very first example of this entity, described by Arthur Purdy Stout in 1918 [26]. Examples of ES/PNET involving the thoracopulmonary region have been eponymically indicated as Askin's tumors [27] and are currently regarded as a distinct clinical presentation of the same tumor entity.

Grossly, ES/PNET is a large multilobulated soft tissue mass with extensive necrosis and/or hemorrhage [22]. In axial tumors, frequent osseous involvement makes difficult to determine whether site of origin was in bone or soft tissue.

Microscopically, the morphologic picture shows various degree of neuroectodermal differentiation along a spectrum ranging from ES to PNET. Growth pattern is mainly lobular (Fig. 1a). At the ES end of the spectrum, the neoplastic lobules are composed of small round cells exhibiting round or ovoid vesicular nuclei, distinct nuclear membrane, small nucleoli, and poorly defined, scanty cytoplasm. At the PNET end, the neoplastic cells may have more abundant, eosinophilic cytoplasm with discernible nucleoli. Importantly, at this better differentiated end of the spectrum, a variable number of rosettes (from scarce to numerous) can be detected (Fig. 1b). Most frequently, the rosettes are of the Homer–Wright type, similar to those seen in neuroblastoma, but occasionally Flexner–Wintersteiner rosettes, resembling those ones of ependymomas, are seen. Intracytoplasmic glycogen, highlighted by periodic acid Schiff stains, is present in the majority of undifferentiated cases but only in less than half of ES/PNET-containing rosettes. The mitotic activity tends to be quite variable. Necrosis is almost always present (Fig. 1c) and can be extensive, sometimes leaving collars of viable tumor cells around the richly ramified capillary network. Occasionally, ES/PNET shows focal atypical features: i.e., presence of spindled or large anaplastic tumor cells.

Immunohistochemistry plays a major role in the differential diagnosis of ES/PNET which includes alveolar rhabdomyosarcoma (ARMS), DSRCT, poorly differentiated synovial sarcoma (SS), and Merkel cell carcinoma (MCC). CD99 (the product of the MIC2 antigen) certainly represents the most useful marker [28]. Strong CD99 membrane immunopositivity is usually seen in most ES/PNET (Fig. 1d), independently from the degree of differentiation. However, as with most differentiation markers, CD99 tends to be very sensitive but not specific. When dealing with round cell neoplasms, it has to be kept in mind that CD99 is expressed in most lymphoblastic lymphomas, in poorly differentiated SS including its round cell variant [29], in MCC, and in a small percentage of DSRCT. CD99 immunopositivity evaluated in context with morphology and along with the results of other pertinent differentiation markers helps avoiding most of the diagnostic pitfalls. However, there are still cases that may represent a true challenge.

As it has been shown recently, ES/PNET can occur as primary cutaneous neoplasm, raising the problems of the distinction from MCC [30, 31]. It is important to remember that about 20% of ES/PNET does express cytokeratins and that, on the other hand, MCC can express CD99 in approximately 30% of cases [31]. Neuroendocrine markers can be detected in both lesions (of course much more consistently in MCC). CK20 immunopositivity favors a diagnosis of MCC, as it is consistently negative in all the cases of ES/PNET tested so far [32].

The presence of neuroectodermal differentiation in ES/PNET can be demonstrated by immunopositivity for Leu7, synaptophysin, S-100 protein, neurofilaments, and chromogranin. However, all these markers exhibit high variability eventually proving of scarce diagnostic utility.

ES/PNET has been increasingly reported to occur at visceral sites. When occurring in the kidney, the main differential diagnosis is with the so-called adult variant of Wilms' tumor, particularly when undifferentiated blastemal areas predominate [23]. It has to be stressed that CD99 is usually negative in Wilms' tumor, making immunohistochemistry extremely important in the differential diagnosis. As a consequence, most adult type Wilms' tumors have been now reclassified as ES/PNET, following either immunohistochemical or molecular genetics analysis [23].

Cytogenetics and molecular genetics findings

The central karyotypic anomaly is a t(11;22) with other variants found in about 15% of cases: a t(21;22), a t(7;22), a t(17;22) a t(2;22), and a t(6;22). Rare cases of ES/PNET harbor translocation involving *FUS* in place of *EWSR1*: i.e., t(16;21)(p11;q22) with a *FUS–ERG* fusion gene [33] or t(2;16)(q35;p11) with a *FUS–FEV* fusion gene [34].

The so-called Ewing's sarcoma translocation is the best known example of sarcoma translocation as it was the first one to be identified [35] as well as the first of which the involved genes were cloned [1, 2]. Molecularly, the result of the t(11;22) is a fusion of the *EWSR1* gene with the transcription factor *FLI1* (friend leukemia virus integration site 1) on 11q24 belonging to the ETS family. This molecular aberration leads to the oncogenic conversion of the *EWSR1* gene, and the normal function of which is still poorly understood. The replacement of the RNA-binding domain of the *EWSR1* gene with the DNA-binding domain

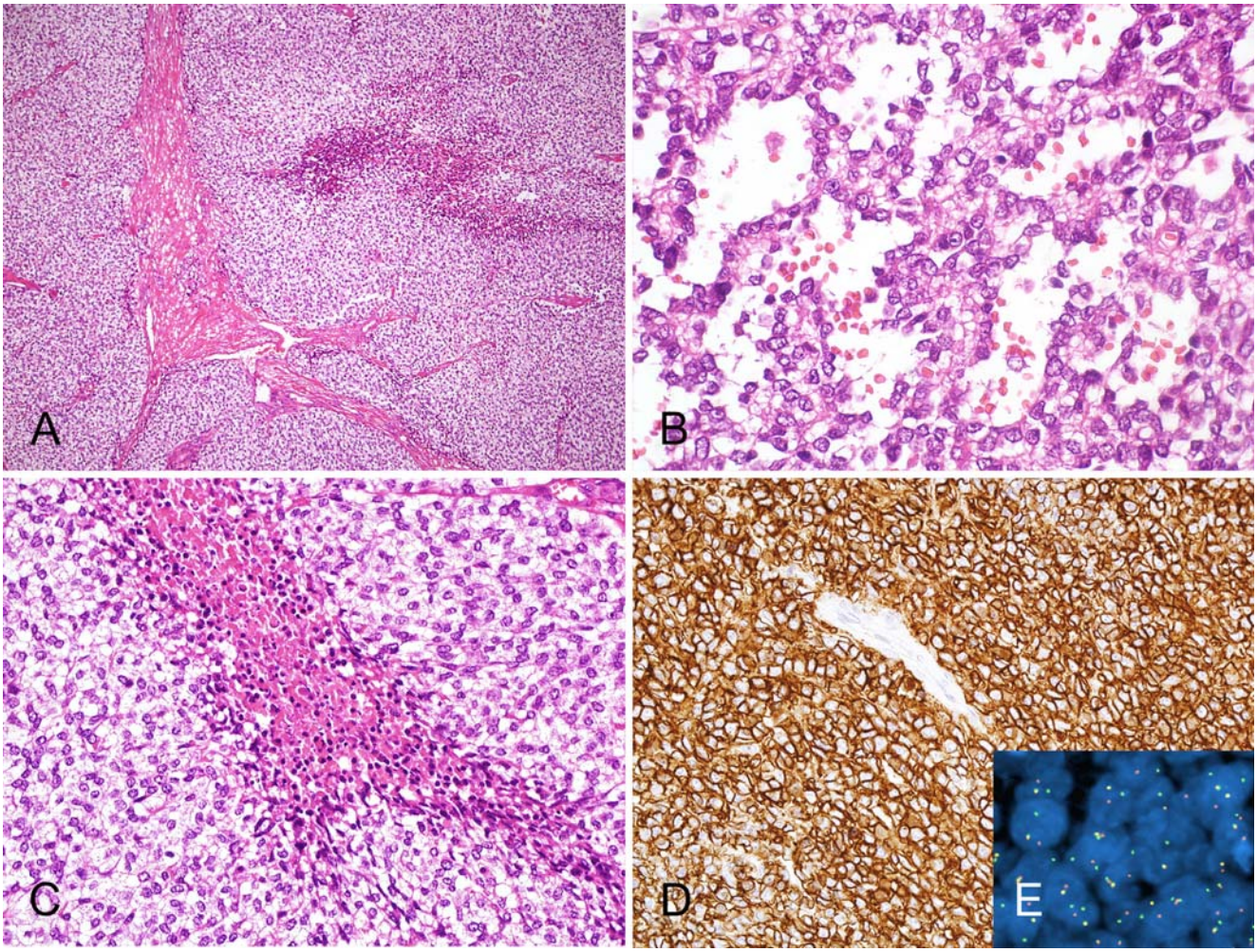

Fig. 1 ES/PNET's features: **a** Growth pattern is usually in solid sheets, **b** rosettes are often present, and **c** intratumoral necrosis is almost always observed. **d** A strong membranous positivity for CD99 immunohistochemistry is found. **e** Diagnosis is further corroborated by presence of rearrangement of *EWSR1* gene as shown by interphase FISH using split-apart probes

of the *FLI1* (or related genes) leads to the formation of a novel transcription factor whose target genes are now being elucidated. Recent in vitro and ex vivo experiments have been extremely beneficial for our understanding on the molecular effect of the fusion transcript expression. The possibility to induce expression of the fusion transcript via cell transfection has given some important clues. The main questions here were: Which is the originating cell of ESFT? Is the occurrence of the translocation and the subsequent fusion gene transcription necessary and sufficient for neoplastic transformation? Which are the key events in neoplastic transformation? Are there any secondary recurrent events? And what is their effect on the biological behavior?

In preliminary experiments, transfection of EWS–FLI1 construct in normal fibroblasts and TERT-immortalized fibroblasts was respectively inducing cell death and growth arrest (reviewed in Kovar H 2005) [36, 37]. The transfection in NIH3T3 not only augments tumorigenicity but also induces a phenotype closely resembling ESFT [37]. Taken together, these results allow concluding that the fusion transcript is indeed promoting tumorigenicity but only in the right cellular context, e.g., in NIH3T3 and not in normal fibroblasts [37]. Furthermore, transfection of fusion transcripts as for oncogenes transfection induces growth arrest unless there is already another operating mechanism, as it is happening for instance for *RAS* transfection [38]. NIH3T3 cell lines are known to be already tumorigenic [20]. In search of a cell of origin for ESFT, Riggi et al. [39] transfected murine mesenchymal stem cells with EWS–FLI1 transcript, remarkably the cells got tumorigenic, acquired a phenotype closely resembling ESFT, and more importantly, with neither apparent structural changes nor alteration in *ARF* and *TP53* pathway [39]. When the same group, Riggi et al. [40], transfected human mesenchymal stem cells with the EWSR1–FLI1 transcript, they did not get growth arrest, despite an expression profile similar to ESFT was triggered, no tumorigenicity was observed, implying that other events are necessary for transformation

[40]. These last two experiments show that mesenchymal stem cells are an attractive candidate of originating cells for ESFT: (1) they are permissive to translocation transfection with no growth arrest, (2) the expression of the fusion transcript induce a phenotype resembling ESFT, and (3) however, additional events are needed for malignant transformation. Established cell lines from ESFT allowed addressing other questions. Silencing of the fusion transcript results in a loss of the tumorigenicity [41] and a switch of phenotype toward mesenchymal stem cells [42]. These two results, on one hand, underline the importance of the fusion transcript expression for the tumorigenicity, and on the other hand, support the putative origin of ESFT from mesenchymal stem cells. Furthermore, culturing of ESFT cell lines acquired random alterations resulting in complex rearrangements [43]. Such rearrangements, although, may occur also in vivo and result in selective growth advantage of one clone with malignant progression and ultimately worse prognosis.

Recently, the fusion between *EWSR1* and a non-ETS family member has been reported. *NFATC2* is this new partner, and it functions as a T cell differentiation regulator [44]. Remarkably, *NFATC2* shares a sequence recognition with the ETS family with possible transcriptional control via activating protein complex 1 [44].

The possibility to detect these karyotypic abnormalities by the means of chromosome analysis as well as by molecular genetics on a routine basis has greatly increased diagnostic accuracy (Fig. 1e). However, even cytogenetics does not exhibit absolute specificity, as demonstrated by the existence of exceptional bonafide cases of ARMS, as well as of polyphenotipic sarcomas bearing a t(11;22) [45]. As a consequence, any result provided by molecular genetics should be evaluated in context with accurate morphological evaluation.

Several studies have claimed that molecular genetics may also prove useful in providing prognostic parameters. In particular, it seems that the presence of a type 1 EWS–FLI1 fusion (EWS exon 7 is linked in frame with exon 6 of FLI1) represents an independent positive prognostic factor [46]. However, large prospective EuroEwing trial is challenging such assumption (JCO in press).

The analysis of cell cycle regulators has also provided potentially valuable information regarding the prognosis of this important family of round cell sarcomas. Although p53 mutation and overexpression seem to delineate only a small subset of ES/PNET, they show remarkably poor clinical outcome [47, 48], and the same effect appears to be determined by deletion of the *CDKN2A* gene [49, 50]. Most likely, impairment of cell cycle/apoptosis regulation are late events in malignant progression affecting prognosis and response to therapy as shown also in other mesenchymal tumors such as gastrointestinal stromal tumors [51].

Prognosis and treatment

The prognosis of the ES/PNET family of neoplasms is poor; however, using multimodality therapy, which includes surgical resection and/or radiation therapy and chemotherapy, long-term survival has increased from less than 10% to approximately 30% to 40% [52]. It is a commonly shared opinion that lesions belonging to the less differentiated (ES) end of the spectrum are characterized by better response [53]. Unfortunately, such an assumption has not been entirely elucidated, with different studies leading to conflicting results [54, 55]. Moreover, most of cases tend to cluster in-between the extremes of the morphologic continuum making histopathologic distinction somewhat arbitrary.

The importance of the IGF1 receptor pathway in the development of ES/PNET has been recognized for some time [56, 57]. Targeting such pathway with the anti IGF1 receptor monoclonal antibody R1507 is the strategy for a single-agent clinical trial (www.clinicaltrials.gov) in patients with recurrent or refractory sarcomas including: ES/PNET, MLPS, EMCS, and DSRCT. Molecules targeting the fusion transcript are being searched, and promising results are coming from targeting the interaction of EWS–FLI1 and RNA helicase A [58]. Targeting tumor metabolism, in general, seems also an appealing alternative therapeutic strategy [59].

Desmoplastic small round cell tumor

Desmoplastic small round cell tumor is a highly malignant polyphenotipic mesenchymal neoplasm associated with a prominent fibrous stroma [60]. It mainly affects young adults with peak incidence in the second decade [60]. Male patients outnumber female patients 4 to 1 [60].

DSRCT most often arise in serous lined surfaces [60]. Originally, described as a predominantly intra-abdominal tumor, a broader distribution has emerged gradually that includes pleura [61] and tunica vaginalis [62]. Extraserous location has been occasionally reported, including parotid gland [63], posterior cranial fossa [64], central nervous system [65], bone and soft tissue [66–68], ovary [69], pancreas [70], and kidney [71, 72].

In its most frequent intra-abdominal presentation, DSRCT usually causes abdominal pain associated with ascites, abdominal distension, and/or intestinal obstruction. At surgery, the lesion usually presents as a large intra-abdominal mass associated with multiple peritoneal smaller implants and hematogenous metastases, especially to the liver [73].

Grossly, the neoplastic masses tend to be solid, firm, and multilobulated with a gray–white occasionally cystic cut

surface and most often featuring abundant coagulative necrosis. Microscopically, at low power, DSRCT is characterized by the presence of sharply demarcated clusters of small rounded cells, separated by a hypocellular desmoplastic stroma (Fig. 2a). However, desmoplasia in rare cases may be absent [74]. Cellular clusters may vary from centrally necrotic large islands to cord-like structures. In typical cases, tumor cells are usually uniform in size and shape and are characterized by small to medium size, round to oval shape, hyperchromatic nuclei, inconspicuous nucleoli, and cytoplasm varying from scanty to abundant (Fig. 2b). Mitotic figures are usually numerous, and prominent necrosis is frequently observed. More rarely, rhabdoid, spindle cell, or even signet-ring cell morphology has been reported [75, 76]. Rare cases of DSRCT exhibit a striking epithelial morphology that makes the differential diagnosis really challenging. It is also important to mention that pseudorosettes can occasionally be observed, making challenging the differential diagnosis with ES/PNET.

Immunohistochemistry plays a major role in the differential diagnosis with other small round cell tumors. The coexpression of epithelial (keratins, epithelial membrane antigen), myogenic (desmin), and neural/neuronal (neuron-specific enolase, S-100 protein, Leu 7, and synaptophysin) differentiation markers is a helpful diagnostic tool. Cytokeratin immunoreactivity is observed in the vast majority of cases (Fig. 2c); in particular when "cocktails" of different molecular weight keratins are used. It is worth noting that all DSRCTs tested so far appear to be negative for both cytokeratin 20 (usually expressed in Merkel cell carcinoma) and cytokeratin 5 (usually expressed in normal mesothelium and mesotheliomas), whereas both MOC31 and BerEP4, which are negative in mesotheliomas, are expressed in DSRCT [77]. One of the most characteristic immunohistochemical features of DSRCT is represented by the expression of desmin (Fig. 2d). A distinctive dot-like pattern of staining with tiny paranuclear globules is often observed. Of course, the expression of myogenic markers

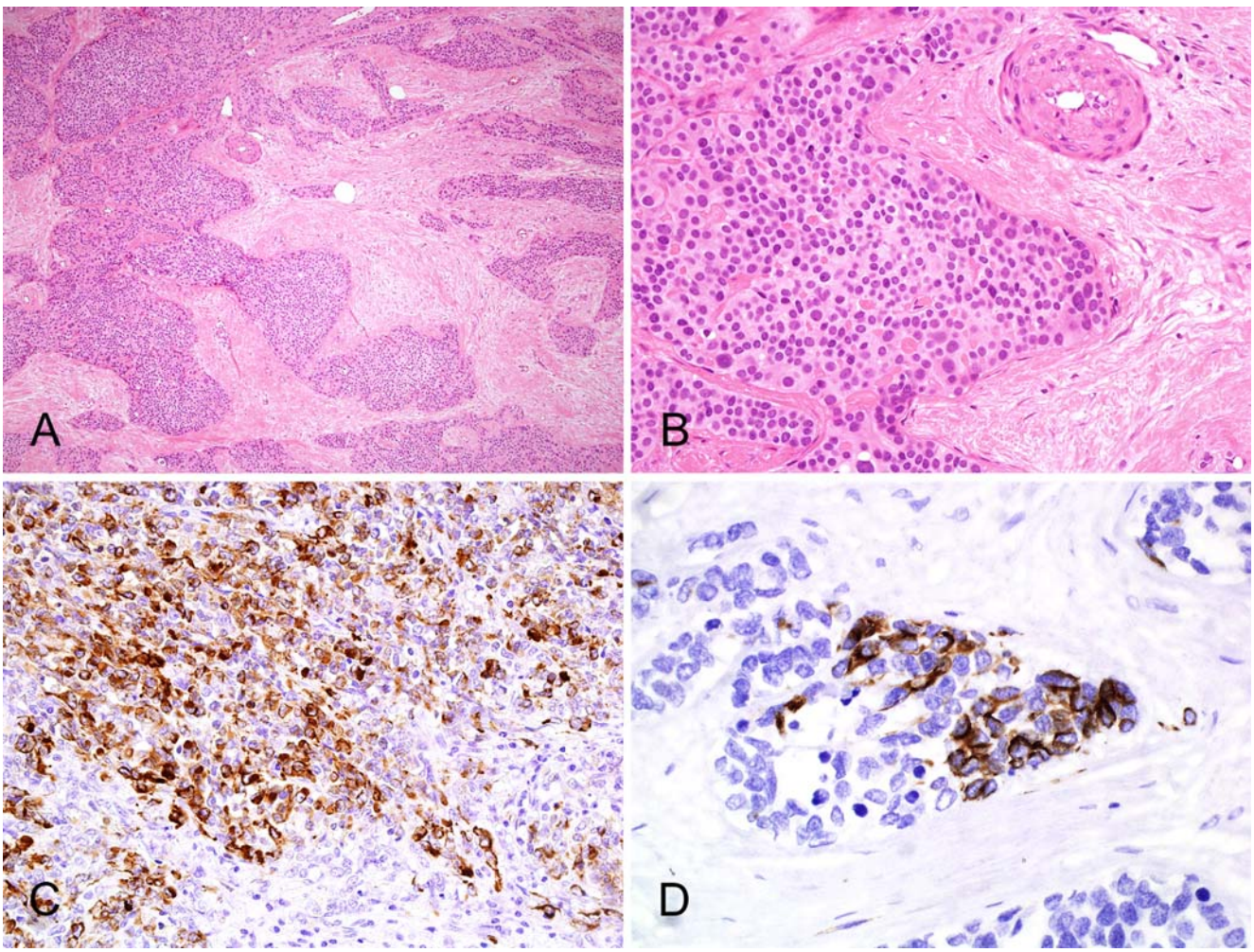

Fig. 2 DSRCT's features: **a** Solid nests of small round blue cells are surrounded by dense collagenous stroma. **b** Nuclei are hyperchromatic and cytoplasms of various size. Immunostains for cytrokeratins (**c**) and desmin (**d**) is usually positive and helpful for differential diagnosis

may mislead to a diagnosis of alveolar rhabdomyosarcoma. However, myogenin is positive in ARMS and negative in DSRCTs. Furthermore, DSRCT also tends to exhibit immunohistochemical as well as electron microscopic features of neural differentiation. The expression of neural differentiation markers is very variable [78], but both neuron-specific enolase (NSE) and CD57 have tested positive in about two thirds of cases. Unfortunately, considering the poor specificity of both markers, the presence of either NSE or CD57 immunopositivity cannot be regarded as unequivocal evidence of neural differentiation. Other more specific markers of neural/neuronal differentiation, such as synaptophysin and chromogranin, have been observed in about 20% of cases. Both MOC31 and BerEP4, which are negative in mesotheliomas, are expressed in DSRCT [77]. Interestingly, placental alkaline phosphatase, a marker for germ cell tumors, was found expressed with a paranuclear pattern of staining, similar to that of desmin expression, in 80% of the cases in a series of molecularly confirmed DSRCT [36], but its meaning has not yet been elucidated. Immunohistochemical analysis has also demonstrated overexpression of growth factors normally repressed by the wild-type WT1, as IGF-IR, PDGF-alpha, and its receptor, PDGFRbeta, latency-associated peptide of transforming growth factor beta, CCN2, a connective tissue growth factor, and IL2/15 receptor beta-chain in the neoplastic cells (the desmoplastic stroma produces IL2 and IL15). All of these molecules might contribute to the development of the typical desmoplastic stroma of this tumor [78–80].

The diagnostic use of anti-WT1 polyclonal antisera has been also suggested. However, WT1 appears to be expressed in a broad range of neoplasms including Wilms' tumors, renal cell carcinomas, mesotheliomas, and papillary serous carcinomas. As the differential diagnosis includes ES/PNET, it has to be stressed that CD99 immunopositivity is observed in less than 20% of DSRCTs. However, in contrast with the thick membrane pattern observed in ES/PNET, CD99 tends to decorate diffusely the cytoplasm of the DSRCT neoplastic cells.

Cytogenetics and molecular genetics

Cytogenetically, DSRCT is characterized by a reciprocal translocation, t(11;22)(p13;q12), that was first reported by Sawyer in 1992 [81]. At the molecular level, the Wilms' tumor gene (WT1) fuses with the EWS gene resulting in its oncogenic activation. The fusion protein functions as a novel, aberrant transcription factor that has the ability to transactivate genes that overlap with those normally regulated by WT1. One of the most interesting targets is represented by the platelet-derived growth factor alpha, a powerful fibroblast growth factor probably promoting the desmoplastic fibroblastic stroma that is typically seen in DSRCT [78]. Recently, two intra-abdominal pediatric tumors expressing EWSR1–WT1 fusion transcript and with a morphology and immunophenotype consistent with a diagnosis of leiomyosarcoma have been reported. Remarkably, both patients showed good prognosis which raises the possibility of a new entity showing the same molecular abnormality of DSRCT [82].

The existence of polyphenotypic neoplasms morphologically similar to DSRCT but containing chimeric transcripts associated with the ES/PNET category (EWS–FLI1 and EWS–ERG) underlines the importance of strict correlation between conventional morphology and genetic analysis [83].

Prognosis and treatment

DSRCT represents an extremely aggressive mesenchymal neoplasm with a dismal prognosis, despite all therapeutic efforts. Even if a modest increase of survival seems to be achieved by high-dose chemotherapy regimens, most patients die either of advanced uncontrolled local disease or of distant metastases, frequently within 24 months of diagnosis. Recent identification of ENT4, whose expression is related to clinical efficacy of a number of nucleoside analogs used in cancer chemotherapy, may represent an attractive pathway for targeting chemotherapeutic drugs into DSRCT [84].

Clear cell sarcoma of soft tissue

ST-CCS, also known as melanoma of soft parts, is a soft tissue sarcoma of young adults showing immunohistochemical as well ultrastructural features of melanocytic differentiation. It usually occurs in adolescents and young adults, with a peak incidence between the second and the fourth decades. ST-CCS is a slow-growing mass, associated with pain or tenderness in half of cases. The lower extremities are the commonest anatomic site (foot and ankle accounting for approximately 40% of cases). It is generally deep seated, most often in close proximity to tendons and aponeuroses. It has been shown that ST-CCS can involve the gastrointestinal tract wherein it tends to exhibit minimal features of melanocytic differentiation.

Grossly, ST-CCS is usually of small size, well circumscribed (Fig. 3a) but rarely encapsulated. Pigmented areas can occur. Necrosis (Fig. 3b), hemorrhage, and cystic degeneration are occasionally seen.

Microscopically, ST-CCS is composed of uniform nests and/or fascicles of polygonal to spindle cells with abundant clear or eosinophilic cytoplasm (more often, eosinophilic than clear; Fig. 3c). Nuclei are vesicular, round to ovoid,

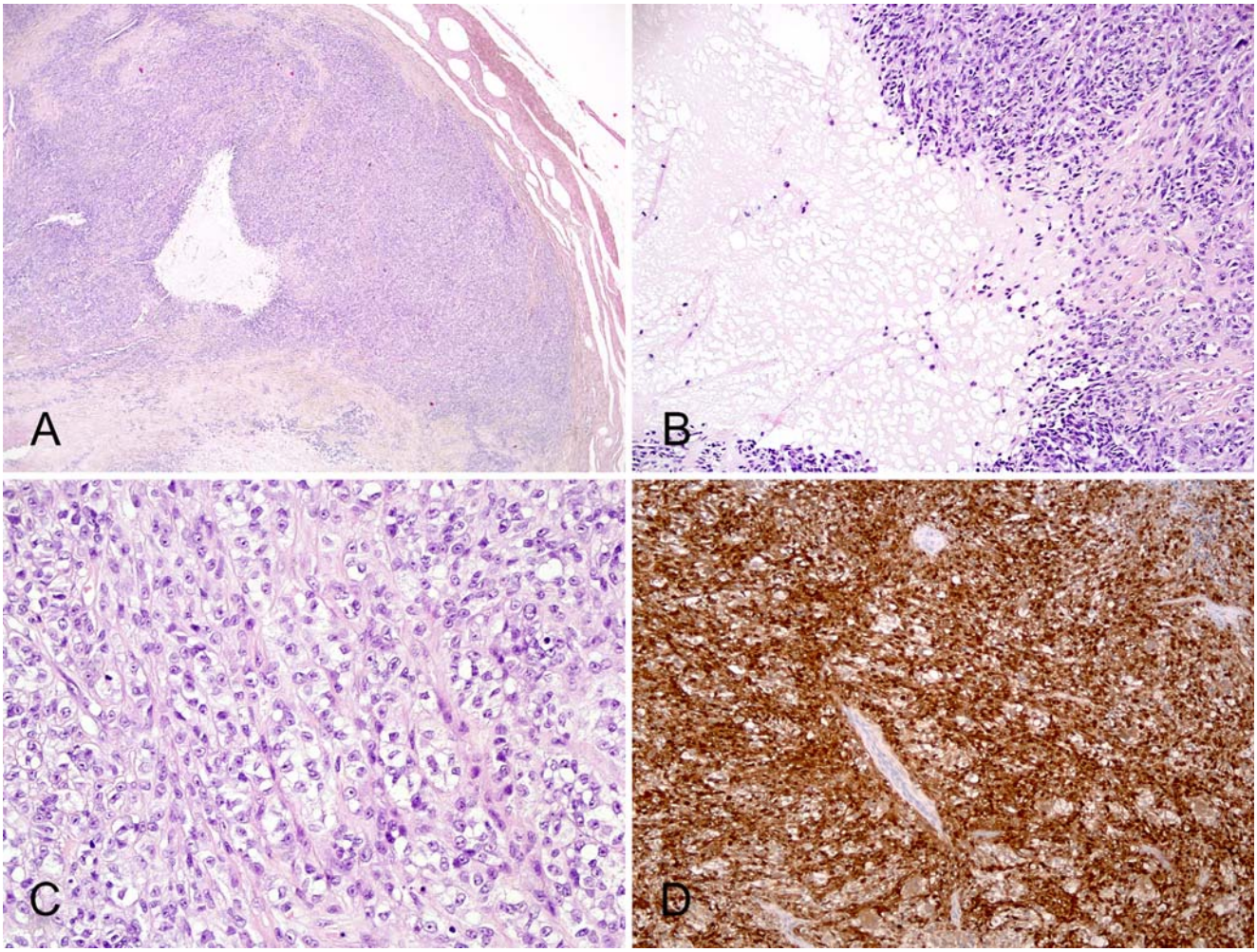

Fig. 3 ST-CCS's features: **a** Well demarcation is usually present, and **b** necrosis may occasionally be found. **c** Polygonal cells with clear cytoplasm are found, **d** almost always showing diffuse positivity for S100

with prominent nucleoli. Multinucleated giant cells are seen in half of the cases. Melanin pigment can be observed. Morphological variations are represented by presence of marked pleomorphism (associated with brisk mitotic activity) microcystic degeneration, and myxoid change of the stroma. Rare cases tend to assume a prominent spindled morphology. Pleomorphism seems to be more frequent in recurrent lesions as well as in metastases. ST-CCS shows immunoreactivity for S100 protein (Fig. 3d), HMB45, MART.1, and MITF-1 in almost all cases, with the notable exception of gastrointestinal (GI) tract cases in which only S-100 immunopositivity is most often detected [85, 86]. Expression profiling has shown consistent upregulation of avian erythroblastic leukemia viral oncogene homolog 3, and evaluation of its expression might be useful in clinical practice [87, 88].

Malignant melanoma needs to be ruled out on the basis of clinical history and absence of junctional activity [89, 90]. Metastatic melanoma can exhibits complete morphologic overlap with ST-CCS, making genetic analysis the only way to separate them [89, 90].

Cytogenetics and molecular genetics

ST-CCS is characterized by the presence of a specific translocation t(12;22)(q13;q12), fusing the *EWSR1* gene with the activating transcription factor-1 gene (*ATF1*) [91, 92]. A variant translocation t(2:22)(q34:q12) that seems to be associated with the GI tract location has been recently reported and involves the *EWSR1* gene on 22q12 and the *CREB1* gene on 2q34 [85]. Both ATF1 and cAMP-responsive element-binding protein (CREB1) belongs to the basic leucine zipper superfamily of transcription factors. Interestingly, CREB overexpression is associated with the acquisition of metastatic potential by melanoma cells [93]. Both EWSR1–ATF1 and EWSR1–CREB1 lead to the formation of chimeric transcripts in which the basic leucine zipper domain is retained. MITF upregulation is probably a

pre-existing characteristic of ST-CCS and not induced by fusion transcripts [91].

Prognosis and treatment

ST-CCS is an aggressive neoplasm, with an overall mortality ranging between 37% and 59%. Recurrences and metastases can occur even after 10 years. Nodal metastases are present in about 50% of cases. Also lung and bone metastases are frequent. Size above 5 cm, necrosis and local recurrence are unfavorable prognostic factors. The fact that multiple histone deacetylase inhibitors suppress MITF expression, it may potentially represent a novel therapeutic strategy in ST-CCS [94].

EMCS

EMCS is a rare malignant soft tissue tumor of uncertain differentiation, representing less than 3% of all soft tissue sarcomas [95]. It usually occurs in adults, with a peak incidence in the sixth decade [95]. A male predominance is reported. Despite the name, there is no convincing evidence of cartilaginous differentiation in most EMCS [96].

EMCS involves mainly the deep soft tissues of the proximal extremities and limb girdles. Thigh and popliteal fossa are the most commonly involved anatomic sites, followed by trunk, paraspinal region, foot, and head and neck regions. EMCS presents as a slow-growing soft tissue-enlarging mass, often associated with pain and tenderness. Skin ulceration and hemorrhage occur in some cases. The duration of symptoms spans from a few weeks to several years.

Grossly, EMCS is usually large and well demarcated by a fibrous pseudocapsule. It appears multinodular on cut section, with gelatinous nodules separated by fibrous septa. Hemorrhage, necrosis, and cystic degeneration can be present. Highly cellular tumors tend to be fleshy. Microscopically, EMCS can be subdivided into two types: a conventional "well differentiated" EMCS and cellular "high grade" EMCS. Low-grade forms are characterized by a multinodular architecture (Fig. 4a) with fibrous septa delimiting areas filled with strikingly hypovascular myxoid (Fig. 4b) or chondromyxoid stroma. Neoplastic cells present eosinophilic, granular to vacuolated cytoplasms, and uniform, round to oval nuclei and are organized in cords or clusters, and sometimes in a distinctive filigree or cribriform pattern. An accentuation of cellularity is typically observed at the periphery of the nodules. Ten percent of cases may contain at least focally neoplastic cells featuring an eosinophilic "rhabdoid" cytoplasm. Mitoses are usually scarce. Hemorrhage is common. True cartilage is uncommon. Cellular "high grade" EMCS shows increased cellularity associated with minimal myxoid stroma (Fig. 4c). Neoplastic cells are epithelioid and overtly atypical both in diffuse and cribriform areas (Fig. 4d). Mitotic count can be high in cellular EMCS. EMCS shows immunoreactivity for S100 protein in less than 20% of cases. Neuroendocrine differentiation, with chromogranin and synaptophysin immunoreactivity, has been reported and confirmed by gene-profiling data [97].

Mixed tumor of soft tissue/myoepithelioma/parachordoma represents the main differential diagnosis. Herein, S100 immunopositivity is generally observed as well as a relatively consistent expression of myoepithelial and epithelial differentiation markers, actin, and glial fibrillary acidic protein. Ductal differentiation can be seen focally. Chordoma exhibits a distinctive midline anatomic location, consistently coexpresses S100 and cytokeratins, and contains the characteristic physaliferous cells.

Cytogenetics and molecular genetics

The most frequent translocations observed in EMCS is t(9;22)(q22;q12) fusing *EWSR1* with *NR4A3* (also known as CHN) [98, 99]. Also, a t(9;17)(q22;q11) and a t(9;15)(q22;q21) are found, fusing the *NR4A3* gene with *RBP56* and *TAF15*, respectively. Recent data indicate that the EWSR1/NR4A3 fusion protein may activate the PPARG nuclear receptor gene that may not only play a key role in the molecular oncogenesis of EMCS but also represents a potential therapeutic target [100].

Prognosis and treatment

EMCS is associated with prolonged survival; however, local recurrences and metastases (usually to the lungs) occur in half of cases, generally more than 10 years after the diagnosis. Long survival even in the presence of metastases is reported. Large tumor size (in particular larger than 10 cm) and high-grade morphology (in particular with pleomorphic and rhabdoid cells) predict poor outcome. Response to chemotherapy is generally low, and the most effective therapeutic strategy is still represented by aggressive management of localized disease.

Angiomatoid fibrous histiocytoma

Angiomatoid fibrous histiocytoma is an intermediate, rarely metastasizing soft tissue neoplasm exhibiting a partial myoid phenotype. It should not to be confused with aneurysmal benign fibrous histiocytoma [101]. It occurs most frequently in children and young adults [101], males and females being equally affected [101]. Systemic signs such as pyrexia, anemia, or paraproteinemia are rarely

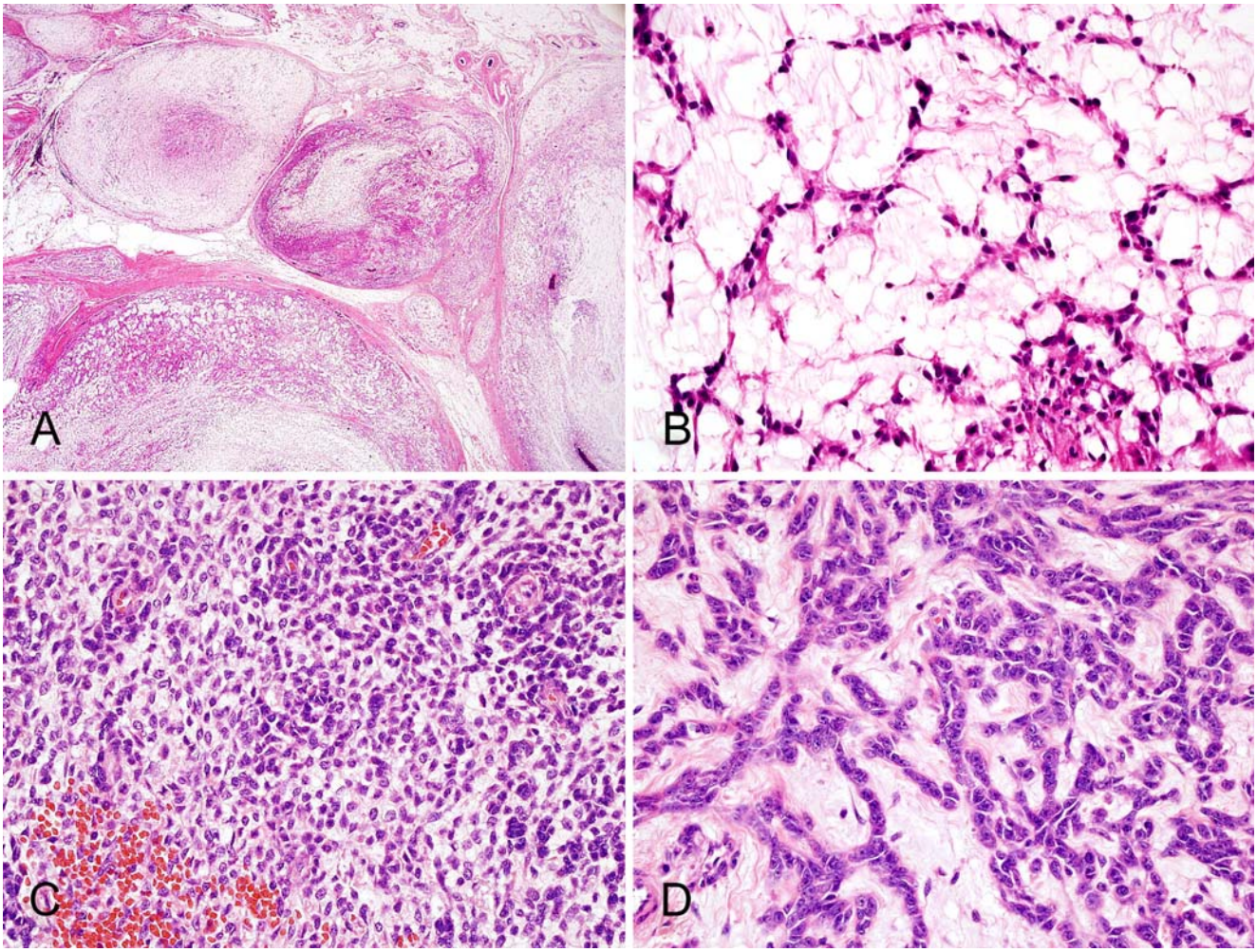

Fig. 4 EMCS's features: **a** Multinodular growth is evident, and **b** nodules are formed by hypovascular myxoid matrix mainly hypocellular. **c** More cellular, high-grade variant of EMCS with **d** epithelioid atypical cells

observed and tend to disappear following removal of the lesion.

The most frequently affected sites are represented by the superficial soft tissues of the extremities followed by the trunk and the head and neck regions [101]. Two thirds of cases occur in areas wherein lymph nodes are normally present.

Grossly, AFH is well circumscribed (Fig. 5a) and multinodular, showing cystic and hemorrhagic areas. A thick fibrous pseudocapsule is usually present at the periphery of the lesion wherein a lymphoplasmacytic infiltrate is also often observed. A multinodular proliferation of eosinophilic, "myoid" cells, featuring ovoid, uniform, plump nuclei is most often present (Fig. 5b). Hemorrhagic areas and pseudovascular spaces containing a proteinaceous material can be often observed. Stromal deposition of hemosiderin is also relatively common. Cell atypia is very rare but it can be detected occasionally (Fig. 5c). Mitotic activity is generally low. Immunohistochemically, AFH exhibits desmin positivity in approximately 40% of cases (Fig. 5d). Epithelial membrane antigen and muscle-specific actin are also positive in less than half of cases. Differential diagnosis include aneurysmal fibrous histiocytoma (more superficial, composed of a polymorphic cell population and usually desmin-negative), Intranodal Kaposi sarcoma may represent a diagnostic alternative which can be sorted out by HHSV8 immunodetection.

Cytogenetics and molecular genetics

AFH represents the first example of a mesenchymal lesion of intermediate malignancy to show a rearrangement of the *EWSR1* genes [102, 103]. Fascinatingly, AFH is characterized by the same chromosome translocation observed in ST-CCS leading to the formation of a *EWSR1–CREB1* fusion, and more rarely, of a *EWSR1–ATF1* fusion [102, 103]. Such distinctive phenotypes, in presence of the same gene rearrangement, may be explained hypothesizing that distinct differentiation programs pre-exist in two different mesenchymal progenitor cells. *EWSR1–ATF1*/*EWSR1–*

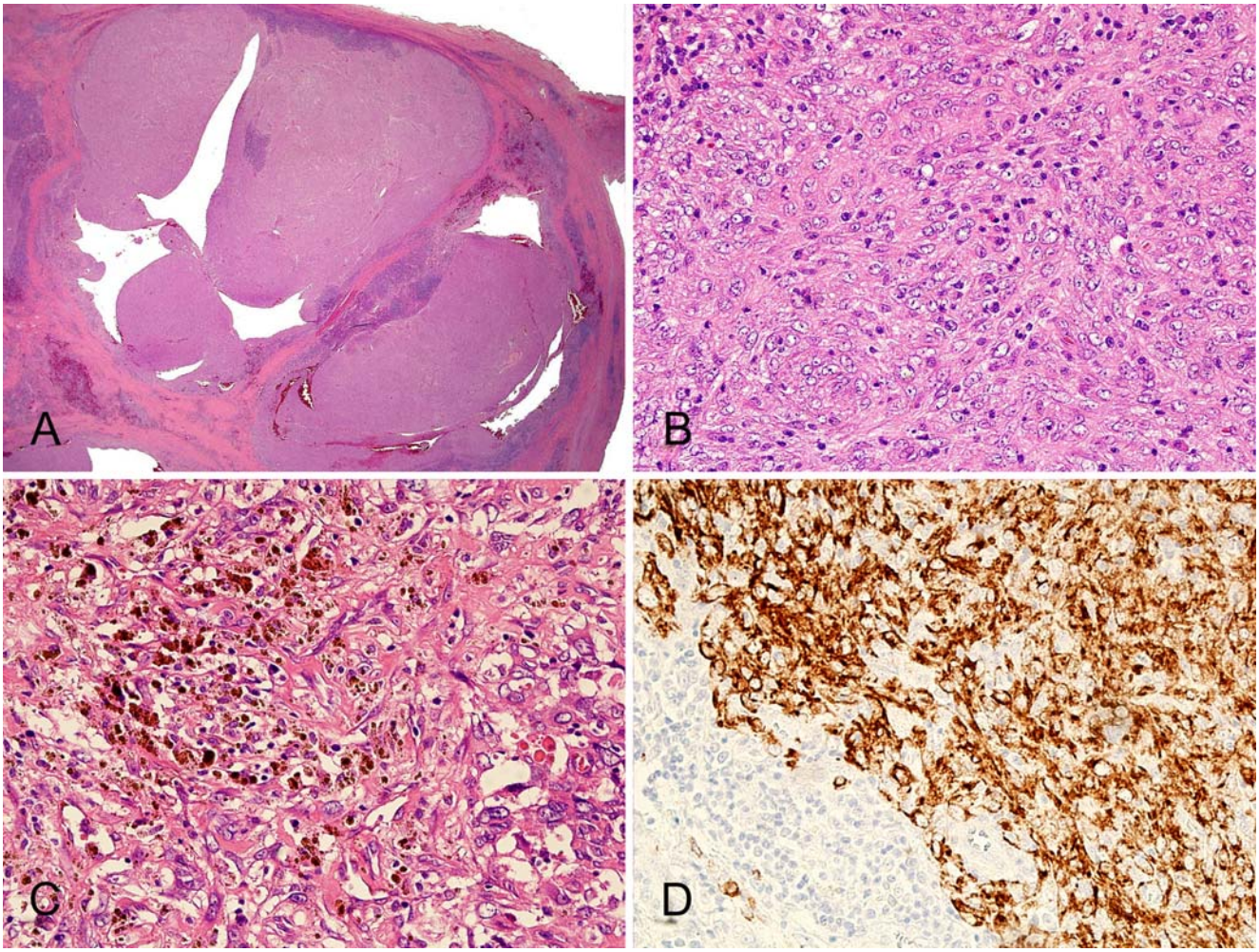

Fig. 5 AFH's features: **a** Multiple nodules with hemorrhagic changes are formed by **b** a proliferation of eosinophilic cells with plump nuclei. **c** Cell atypia may occasionally be found. **d** Neoplastic cells are often diffusely positive for desmin

CREB1 independent activation of MITF pathway in ST-CCS strongly supports this view.

Prognosis and treatment

As mentioned, current World Health Organization classification considers AFH as an intermediated malignancy soft tissue lesion, in which local recurrences are observed in less than 15% of cases, and metastatic spread to locoregional lymph nodes is being observed in less than 2% of cases. Complete local excision is considered curative.

Myxoid liposarcoma

Myxoid liposarcoma represents the second larger group of adipocytic malignancies accounting for about 30–35% of all liposarcomas [104]. Peak incidence is between the third and the fifth decade, and both sexes are equally affected [104]. Clinically, myxoid and round cell liposarcoma occur predominantly in the limbs whereas the retroperitoneal location is exceptional. Grossly, myxoid liposarcoma is most often a well-circumscribed, multinodular, gelatinous mass. The presence of hypercellular (round cell) areas may confer a fleshy appearance [104].

Microscopically, purely myxoid liposarcoma is composed by a hypocellular spindle cell proliferation set in a myxoid background, often featuring mucinous pools (Fig. 6a) [104]. Lipoblasts are most often monovacuolated, small, and tend to cluster around vessels or at the periphery of the lesion (Fig. 6b) [104]. The most distinctive morphologic clue is represented by the presence of a capillary network organized in a plexiform pattern (Fig. 6c) [104]. Myxoid/round cell liposarcoma is defined by the presence of hypercellular areas (that most frequently begins to form in a perivascular distribution) with an undifferentiated round cell component (Fig. 6d) ranging in extent between 5% and 80% [105, 106]. Pure round cell liposarcoma is a rare neoplasm in which hypercellularity or round cell differentiation account for more than 80% of tumor

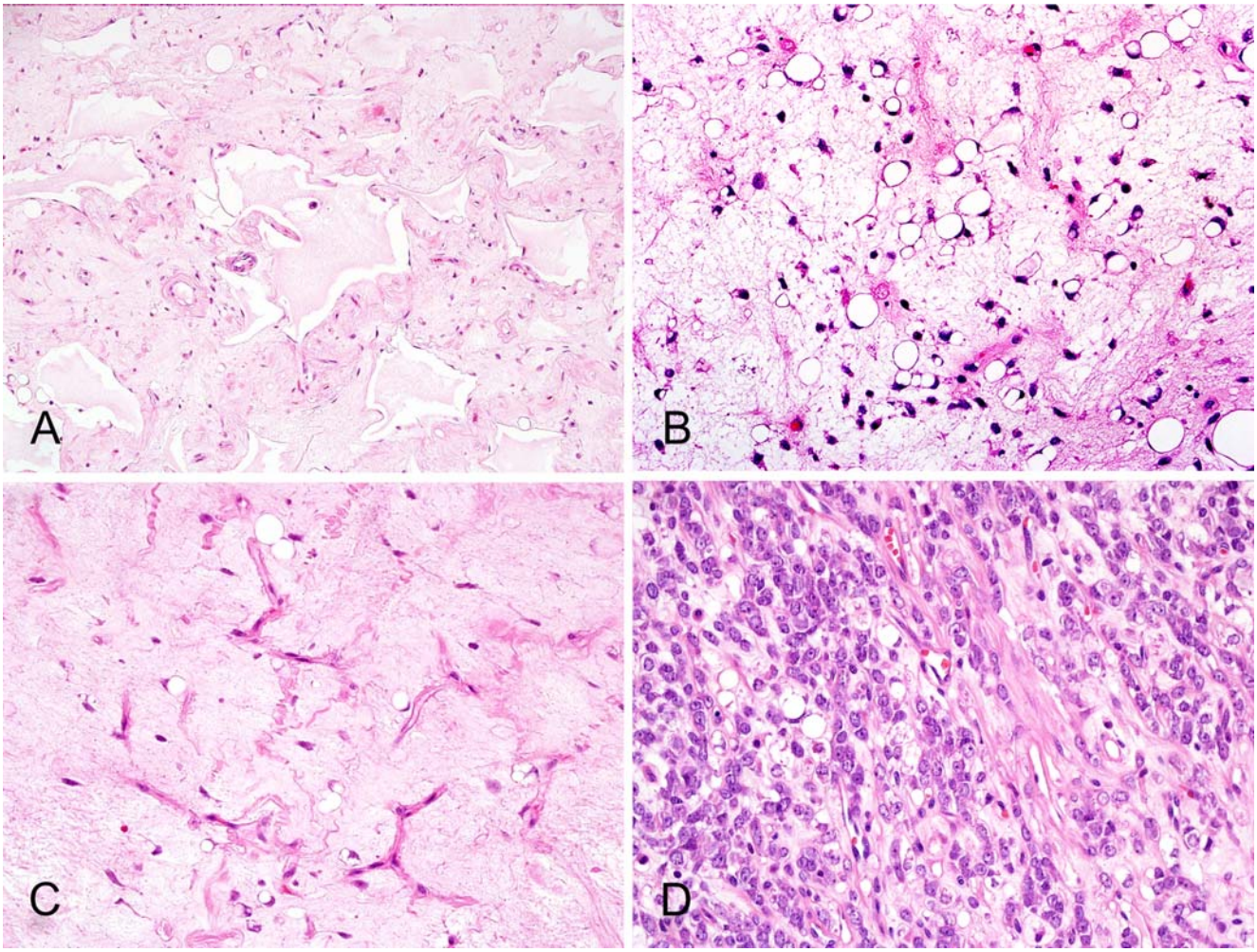

Fig. 6 MLPS's features: **a** Abundant myxoid matrix is found often deposited in mucinous pools, and **b** monovacuolated cells (lipoblasts) are encountered around vessel. **c** Typical plexiform vascular pattern resembling "crow feet" is present, and **d** a more hypercellular round cell component may be observed

tissue [104–106]. Adipocytic differentiation in pure round cell liposarcoma is hardly found, but the presence of S100 immunopositivity in the majority of the cases may be diagnostically helpful [107]. Transition to hypercellular/round cell areas is commonly observed in myxoid liposarcoma, therefore providing strong evidence in favor of the concept that myxoid and round cell liposarcoma represents a morphologic continuum of myxoid adipocytic neoplasia [108].

Well-differentiated myxoid liposarcoma must be differentiated from low-grade myxofibrosarcoma (a myxoid neoplasm composed of atypical stromal cells and pseudolipoblasts associated with distinctive archiform, capillary sized blood vessels) and EMCS (multinodular myxoid neoplasm showing oval round cells organized in strands and cords, clustering at the periphery of the nodules). Lipoblastoma can also mimic closely myxoid liposarcoma. Young age and lobular organization represent helpful clues.

Cytogenetics and molecular genetics

Myxoid liposarcoma is characterized by two main karyotypic aberrations: a t(12;16) that fuses the *DDIT3* gene on 12q13 (a member of the CCAAT/enhancer-binding protein family involved in adipocyte differentiation) [109, 110], with the *FUS* gene on 16p11. A subset is harboring a t(12;22) that fuses *DDIT3* with *EWSR1* on 22q12 [111]. The normal function of the *DDIT3* gene is to promote growth arrest, but as a consequence of the translocation, such an antiproliferative activity is lost [112]. Furthermore, both DDIT3 and FUS–DDIT3 expression in sarcoma cell lines induce adipogenesis [112].

Prognosis and treatment

The presence of hypercellularity or round cell differentiation is associated with worsening of prognosis [113]. Different

cutoff values, ranging between 5% and 25%, have been set by independent studies. A reliable assessment of the percentage of hypercellular areas is difficult to achieve as it may be hampered by inadequate sampling as well as by that degree of subjectivity that is intrinsic part of morphologic evaluation. For the time being, it appears safer to consider any amount of hypercellularity as prognostically relevant. Myxoid liposarcoma tends to recur repeatedly and tends to metastasize to both bone [114] and soft tissue locations including the retroperitoneum. Standard treatment is represented by wide surgical resection. In high-grade lesions, adjuvant therapy (radiotherapy and/or chemotherapy) may be associated. The drug trabectidin appears to be a highly promising treatment for metastatic myxoid liposarcoma [115, 116].

Conclusions

Mesenchymal tumors, in strong analogy with hematological malignancies, frequently harbor chromosome translocations. The *EWSR1* gene is one of the favorite partners as it is involved in several unrelated soft tissue lesions. Interestingly, *EWSR1* rearrangement has been recently observed in intermediate malignant tumors such as AFH. Even more, interestingly, the same alterations are observed in ST-CCS, a sarcoma phenotypically unrelated to AFH and characterized by full-blown malignant potential. This is a further example of the relative unspecificity of chromosome translocations, as also emphasized by the occurrence of ALK gene rearrangement in both inflammatory myofibroblastic tumor [117] and anaplastic large cell lymphoma [118] as well by the occurrence of the ETV6–NTRK3 fusion gene in infantile fibrosarcoma [119], mesoblastic nephroma [120], secretory carcinoma of the breast [121], and in an isolate report acute myeloid leukemia [122]. Another eloquent example is the occurrence of ASPSCR1–TEF3 fusion transcript in both alveolar soft part sarcomas as well as in pediatric forms of renal cell carcinoma [123, 124]. This does not hamper at all the diagnostic value of both cytogenetics and molecular genetics; however, it underscores the extreme importance of pairing genetic testing of tumors with proper diagnostic expertise. In particular, when using the split-apart FISH approach to *EWSR1* gene rearrangement, evaluation of the results in context with morphology becomes mandatory.

Conflict of interest statement We declare that we have no conflict of interest.

References

1. Delattre O, Zucman J, Plougastel B et al (1992) Gene fusion with an ETS DNA-binding domain caused by chromosome translocation in human tumours. Nature 359:162–165
2. Zucman J, Delattre O, Desmaze C et al (1992) Cloning and characterization of the Ewing's sarcoma and peripheral neuroepithelioma t(11;22) translocation breakpoints. Genes Chromosomes Cancer 5:271–277
3. Moller E, Stenman G, Mandahl N et al (2008) POU5F1, encoding a key regulator of stem cell pluripotency, is fused to EWSR1 in hidradenoma of the skin and mucoepidermoid carcinoma of the salivary glands. J Pathol 215:78–86
4. Plougastel B, Zucman J, Peter M et al (1993) Genomic structure of the EWS gene and its relationship to EWSR1, a site of tumor-associated chromosome translocation. Genomics 18:609–615
5. Bovée JVMG, Devilee P, Cornelisse CJ et al (1995) Identification of an EWS-pseudogene using translocation detection by RT-PCR in Ewing's sarcoma. Biochem Biophys Res Commun 213:1051–1060
6. Bertolotti A, Lutz Y, Heard DJ et al (1996) hTAF(II)68, a novel RNA/ssDNA-binding protein with homology to the pro-oncoproteins TLS/FUS and EWS is associated with both TFIID and RNA polymerase II. EMBO J 15:5022–5031
7. Crozat A, Aman P, Mandahl P et al (1993) Fusion of CHOP a novel RNA-binding protein in human myxoid liposarcoma. Nature 363:640–644
8. Morohoshi F, Arai K, Takahashi EI et al (1996) Cloning and mapping of a human RBP56 gene encoding a putative RNA binding protein similar to FUS/TLS and EWS proteins. Genomics 38:51–57
9. Azuma M, Embree LJ, Sabaawy H et al (2007) Ewing sarcoma protein ewsr1 maintains mitotic integrity and proneural cell survival in the zebrafish embryo. PLoS ONE 2:e979
10. Stolow DT, Haynes SR (1995) Cabeza, a Drosophila gene encoding a novel RNA binding protein, shares homology with EWS and TLS, two genes involved in human sarcoma formation. Nucleic Acids Res 23:835–843
11. Riggi N, Cironi L, Suva ML et al (2007) Sarcomas: genetics, signalling, and cellular origins. Part 1: the fellowship of TET. J Pathol 213:4–20
12. Aman P, Panagopoulos I, Lassen C et al (1996) Expression patterns of the human sarcoma-associated genes FUS and EWS and the genomic structure of FUS. Genomics 37:1–8
13. Zakaryan RP, Gehring H (2006) Identification and characterization of the nuclear localization/retention signal in the EWS proto-oncoprotein. J Mol Biol 363:27–38
14. Bertolotti A, Melot T, Acker J et al (1998) EWS, but not EWS-FLI-1, is associated with both TFIID and RNA polymerase II: interactions between two members of the TET family, EWS and hTAFII68, and subunits of TFIID and RNA polymerase II complexes. Mol Cell Biol 18:1489–1497
15. Zhang D, Paley AJ, Childs G (1998) The transcriptional repressor ZFM1 interacts with and modulates the ability of EWS to activate transcription. J Biol Chem 273:18086–18091
16. Knoop LL, Baker SJ (2001) EWS/FLI alters 5′-splice site selection. J Biol Chem 276:22317–22322
17. Knoop LL, Baker SJ (2000) The splicing factor U1C represses EWS/FLI-mediated transactivation. J Biol Chem 275:24865–24871
18. Leemann-Zakaryan RP, Pahlich S, Sedda MJ et al (2009) Dynamic subcellular localization of the Ewing sarcoma proto-oncoprotein and its association with and stabilization of microtubules. J Mol Biol 386:1–13
19. Li H, Watford W, Li C et al (2007) Ewing sarcoma gene EWS is essential for meiosis and B lymphocyte development. J Clin Invest 117:1314–1323
20. Thompson AD, Teitell MA, Arvand A et al (1999) Divergent Ewing's sarcoma EWS/ETS fusions confer a common tumorigenic phenotype on NIH3T3 cells. Oncogene 18:5506–5513

21. May WA, Arvand A, Thompson AD et al (1997) EWS/FLI1-induced manic fringe renders NIH 3T3 cells tumorigenic. Nat Genet 17:495–497
22. Ushigome S, Machinami R, Sorensen PH (2002) Ewing sarcoma/primitive neuroectodermal tumour (PNET). In: Fletcher CDM, Unni KK, Mertens F (eds) World Health Organization classification of tumours; pathology & genetics; tumours of soft tissue and bone. IARC Presss, Lyon, pp 298–300
23. Parham DM, Roloson GJ, Feely M et al (2001) Primary malignant neuroepithelial tumors of the kidney: a clinicopathologic analysis of 146 adult and pediatric cases from the National Wilms' Tumor Study Group Pathology Center. Am J Surg Pathol 25:133–146
24. Welsch T, Mechtersheimer G, Aulmann S et al (2006) Huge primitive neuroectodermal tumor of the pancreas: report of a case and review of the literature. World J Gastroenterol 12:6070–6073
25. Dedeurwaerdere F, Giannini C, Sciot R et al (2002) Primary peripheral PNET/Ewing's sarcoma of the dura: a clinicopathologic entity distinct from central PNET. Mod Pathol 15:673–678
26. Stout AP (1918) A tumor of the ulnar nerve. Proc N Y Pathol Soc 18:2–12
27. Askin FB, Rosai J, Sibley RK et al (1979) Malignant small cell tumor of the thoracopulmonary region in childhood: a distinctive clinicopathologic entity of uncertain histogenesis. Cancer 43:2438–2451
28. Ambros IM, Ambros PF, Strehl S et al (1991) MIC2 is a specific marker for Ewing's sarcoma and peripheral primitive neuroectodermal tumors: evidence for a common histogenesis of Ewing's sarcoma and peripheral primitive neuroectodermal tumors from MIC2 expression and specific chromosome aberration. Cancer 67:1886–1893
29. Dei Tos AP, Wadden C, Calonje E et al (1995) Immunohistochemical demonstration of glycoprotein p30/32^{mic2} (CD99) in synovial sarcoma. Appl Immunohistochem 3:168–173
30. Terrier-Lacombe MJ, Guillou L, Chibon F et al (2009) Superficial primitive Ewing's sarcoma: a clinicopathologic and molecular cytogenetic analysis of 14 cases. Mod Pathol 22:87–94
31. Hasegawa SL, Davison JM, Rutten A et al (1998) Primary cutaneous Ewing's sarcoma: immunophenotypic and molecular cytogenetic evaluation of five cases. Am J Surg Pathol 22:310–318
32. Nicholson SA, McDermott MB, Swanson PE et al (2000) CD99 and cytokeratin-20 in small-cell and basaloid tumors of the skin. Appl Immunohistochem Mol Morphol 8:37–41
33. Shing DC, McMullan DJ, Roberts P et al (2003) FUS/ERG gene fusions in Ewing's tumors. Cancer Res 63:4568–4576
34. Ng TL, O'Sullivan MJ, Pallen CJ et al (2007) Ewing sarcoma with novel translocation t(2;16) producing an in-frame fusion of FUS and FEV. J Mol Diagnostics 9:459–463
35. Aurias A, Rimbaut C, Buffe D et al (1983) Chromosomal translocations in Ewing's sarcoma. Letter to the editor. N Engl J Med 309:496–498
36. Lessnick SL, Dacwag CS, Golub TR (2002) The Ewing's sarcoma oncoprotein EWS/FLI induces a p53-dependent growth arrest in primary human fibroblasts. Cancer Cells 1:393–401
37. Kovar H (2005) Context matters: the hen or egg problem in Ewing's sarcoma. Semin Cancer Biol 15:189–196
38. Serrano M, Lin AW, McCurrach ME et al (1997) Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. Cell 88:593–602
39. Riggi N, Cironi L, Provero P et al (2005) Development of Ewing's sarcoma from primary bone marrow-derived mesenchymal progenitor cells. Cancer Res 65:11459–11468
40. Riggi N, Suva ML, Suva D et al (2008) EWS-FLI-1 expression triggers a Ewing's sarcoma initiation program in primary human mesenchymal stem cells. Cancer Res 68:2176–2185
41. Prieur A, Tirode F, Cohen P et al (2004) EWS/FLI-1 silencing and gene profiling of Ewing cells reveal downstream oncogenic pathways and a crucial role for repression of insulin-like growth factor binding protein 3. Mol Cell Biol 24:7275–7283
42. Tirode F, Laud-Duval K, Prieur A et al (2007) Mesenchymal stem cell features of Ewing tumors. Cancer Cells 11:421–429
43. Szuhai K, Ijszenga M, Tanke HJ et al (2006) Molecular cytogenetic characterization of four previously established and two newly established Ewing sarcoma cell lines. Cancer Genet Cytogenet 166:173–179
44. Szuhai K, Ijszenga M, De JD et al (2009) The NFATc2 gene is involved in a novel cloned translocation in a Ewing sarcoma variant that couples its function in immunology to oncology. Clin Cancer Res 15:2259–2268
45. Thorner P, Squire J, Chilton-MacNeill S et al (1996) Is the EWS/FLI-1 fusion transcript specific for Ewing sarcoma and peripheral primitive neuroectodermal tumor? A report of four cases showing this transcript in a wider range of tumor types. Am J Pathol 148:1125–1138
46. Ed A, Kawai A, Healy JH et al (1998) EWS-FL11 fusion transcript structure is an independent determinant of prognosis in Ewing's sarcoma. J Clin Oncol 16:1248–1255
47. Mangham DC, Cannon A, Li XQ et al (1995) p53 overexpression in Ewing's sarcoma/primitive neuroectodermal tumour is an uncommon event. J Clin Pathol 48M:M79–M82
48. Ueda Y, Dockhorn-Dworniczak B, Blasius S et al (1993) Analysis of mutant P53 protein in osteosarcomas and other malignant and benign lesions of bone. J Cancer Res Clin Oncol 119:172–178
49. Tsuchiya T, Sekine K, Hinohara S et al (2000) Analysis of the p16INK4, p14ARF, p15, TP53, and MDM2 genes and their prognostic implications in osteosarcoma and Ewing sarcoma. Cancer Genet Cytogenet 120:91–98
50. Wei G, Antonescu CR, de Alava E et al (2000) Prognostic impact of INK4A deletion in Ewing sarcoma. Cancer 89:793–799
51. Romeo S, Debiec-Rychter M, Van GM et al (2009) Cell cycle/apoptosis molecule expression correlates with imatinib response in patients with advanced gastrointestinal stromal tumors. Clin Cancer Res 15:4191–4198
52. Zogopoulos G, Teskey L, Sung L et al (2004) Ewing sarcoma: favourable results with combined modality therapy and conservative use of radiotherapy. Pediatr Blood Cancer 43:35–39
53. Schmidt D, Herrmann C, Jürgens H et al (1991) Malignant peripheral neuroectodermal tumor and its necessary distinction from Ewing's sarcoma. A report from the Kiel Pediatric Tumor Registry. Cancer 68:2251–2259
54. Parham DM, Hijazi Y, Steinberg SM et al (1999) Neuroectodermal differentiation in Ewing's sarcoma family of tumors does not predict tumor behavior. Hum Pathol 30:911–918
55. Terrier Ph, Henry-Amar M, Triche TJ et al (1995) Is neuroectodermal differentiation of Ewing's sarcoma of bone associated with an unfavourable prognosis? Eur J Cancer 31A:307–314
56. Toretsky JA, Kalebic T, Blakesley V et al (1997) The insulin-like growth factor-I receptor is required for EWS/FLI-1 transformation of fibroblasts. J Biol Chem 272:30822–30827
57. Toretsky JA, Steinberg SM, Thakar M et al (2001) Insulin-like growth factor type 1 (IGF-1) and IGF binding protein-3 in patients with Ewing sarcoma family of tumors. Cancer 92:2941–2947
58. Erkizan HV, Kong Y, Merchant M et al (2009) A small molecule blocking oncogenic protein EWS-FLI1 interaction with RNA helicase A inhibits growth of Ewing's sarcoma. Nat Med 15:750–756
59. Lessnick SL, Dei Tos AP, Sorensen PH et al (2009) Small round cell sarcomas. Semin Oncol 36:338–346

60. Antonescu CR, Gerald W (2002) Desmoplastic small round cell tumour. In: Fletcher CDM, Unni KK, Mertens F (eds) World Health Organisation classification of tumours, pathology and genetics, tumours of soft tissue and bone. pp 216–218
61. Parkash V, Gerald WL, Parma A et al (1995) Desmoplastic small round cell tumor of the pleura. Am J Surg Pathol 19:659–665
62. Cummings OW, Ulbright TM, Young RH et al (1997) Desmoplastic small round cell tumors of the paratesticular region. A report of six cases. Am J Surg Pathol 21:219–225
63. Wolf AN, Ladanyi M, Paull G et al (1999) The expanding clinical spectrum of desmoplastic small round-cell tumor: a report of two cases with molecular confirmation. Hum Pathol 30:430–435
64. Tison V, Cerasoli S, Morigi F et al (1996) Intracranial desmoplastic small-cell tumor. Report of a case. Am J Surg Pathol 20:112–117
65. Neder L, Scheithauer BW, Turel KE et al (2009) Desmoplastic small round cell tumor of the central nervous system: report of two cases and review of the literature. Virchows Arch 454:431–439
66. Adsay V, Cheng J, Athanasian E et al (1999) Primary desmoplastic small cell tumor of soft tissues and bone of the hand. Am J Surg Pathol 23:1408–1413
67. Hamazaki M, Okita H, Hata J et al (2006) Desmoplastic small cell tumor of soft tissue: molecular variant of EWS-WT1 chimeric fusion. Pathol Int 56:543–548
68. Murphy AJ, Bishop K, Pereira C et al (2008) A new molecular variant of desmoplastic small round cell tumor: significance of WT1 immunostaining in this entity. Hum Pathol 39:1763–1770
69. Slomovitz BM, Girotra M, Aledo A et al (2000) Desmoplastic small round cell tumor with primary ovarian involvement: case report and review. Gynecol Oncol 79:124–128
70. Bismar TA, Basturk O, Gerald WL et al (2004) Desmoplastic small cell tumor in the pancreas. Am J Surg Pathol 28:808–812
71. Su MC, Jeng YM, Chu YC (2004) Desmoplastic small round cell tumor of the kidney. Am J Surg Pathol 28:1379–1383
72. Wang LL, Perlman EJ, Vujanic GM et al (2007) Desmoplastic small round cell tumor of the kidney in childhood. Am J Surg Pathol 31:576–584
73. Stuart-Buttle CE, Smart CJ, Pritchard S et al (2008) Desmoplastic small round cell tumour: a review of literature and treatment options. Surg Oncol 17:107–112
74. Ordonez NG (1998) Desmoplastic small round cell tumor. I: a histopathologic study of 39 cases with emphasis on unusual histological patterns. Am J Surg Pathol 22:1303–1313
75. Gerald WL, Miller HK, Battifora H et al (1991) Intra-abdominal desmoplastic small round-cell tumor. Report of 19 cases of a distinctive type of high-grade polyphenotypic malignancy affecting young individuals. Am J Surg Pathol 15:499–513
76. Gerald WL, Ladanyi M, de Alava E et al (1998) Clinical, pathologic, and molecular spectrum of tumors associated with t(11;22)(p13;q12): desmoplastic small round-cell tumor and its variants. J Clin Oncol 16:3028–3036
77. Ordonez NG (1998) Desmoplastic small round cell tumor. II: an ultrastructural and immunohistochemical study with emphasis on new immunohistochemical markers. Am J Surg Pathol 22:1314–1327
78. Lee SB, Kolquist KA, Nichols K et al (1997) The EWS-WT1 translocation product induces PDGFA in desmoplastic small round-cell tumour. Na Genet 17:309–313
79. Werner H, Idelman G, Rubinstein M et al (2007) A novel EWS-WT1 gene fusion product in desmoplastic small round cell tumor is a potent transactivator of the insulin-like growth factor-I receptor (IGF-IR) gene. Cancer Lett 247:84–90
80. Wong JC, Lee SB, Bell MD et al (2002) Induction of the interleukin-2/15 receptor beta-chain by the EWS-WT1 translocation product. Oncogene 21:2009–2019
81. Sawyer JR, Tryka AF, Lewis JM (1992) A novel reciprocal chromosome translocation t(11;22)(p13;q12) in an intraabdominal desmoplastic small round-cell tumor. Am J Surg Pathol 16:411–416
82. Alaggio R, Rosolen A, Sartori F et al (2007) Spindle cell tumor with EWS-WT1 transcript and a favorable clinical course: a variant of DSCT, a variant of leiomyosarcoma, or a new entity? Report of 2 pediatric cases. Am J Surg Pathol 31:454–459
83. Katz RL, Quezado M, Senderowicz AM et al (1997) An intra-abdominal small round cell neoplasm with features of primitive neuroectodermal and desmoplastic round cell tumor and a EWS/FLI-1 fusion transcript. Hum Pathol 28:502–509
84. Li H, Smolen GA, Beers LF et al (2008) Adenosine transporter ENT4 is a direct target of EWS/WT1 translocation product and is highly expressed in desmoplastic small round cell tumor. PLoS ONE 3:e2353
85. Antonescu CR, Nafa K, Segal NH et al (2006) EWS-CREB1: a recurrent variant fusion in clear cell sarcoma association with gastrointestinal location and absence of melanocytic differentiation. Clin Cancer Res 12:5356–5362
86. Zambrano E, Reyes-Mugica M, Franchi A et al (2003) An osteoclast-rich tumor of the gastrointestinal tract with features resembling clear cell sarcoma of soft parts: reports of 6 cases of a GIST simulator. Int J Surg Pathol 11:75–81
87. Schaefer KL, Brachwitz K, Wai DH et al (2004) Expression profiling of t(12;22) positive clear cell sarcoma of soft tissue cell lines reveals characteristic up-regulation of potential new marker genes including ERBB3. Cancer Res 64:3395–3405
88. Segal NH, Pavlidis P, Noble WS et al (2003) Classification of clear-cell sarcoma as a subtype of melanoma by genomic profiling. J Clin Oncol 21:1775–1781
89. Langezaal SM, Graadt van Roggen JF, Cleton-Jansen AM et al (2001) Malignant melanoma is genetically distinct from clear cell sarcoma of tendons and aponeurosis (malignant melanoma of soft parts). Br J Cancer 84:535–538
90. Graadt van Roggen JF, Mooi WJ, Hogendoorn PCW (1998) Clear cell sarcoma of tendons and aponeuroses (malignant melanoma of soft parts) and cutaneous melanoma: exploring the histogenetic relationship between these two clinicopathological entities. J Pathol 186:3–7
91. Antonescu CR, Tschernyavsky SJ, Woodruff JM et al (2002) Molecular diagnosis of clear cell sarcoma: detection of EWS-ATF1 and MITF-M transcripts and histopathological and ultrastructural analysis of 12 cases. J Mol Diagnostics 4:44–52
92. Zucman J, Delattre O, Desmaze C et al (1993) EWS and ATF-1 gene fusion induced by t(12;22) translocation in malignant melanoma of soft parts. Nat Genet 4:341–345
93. Xie S, Price JE, Luca M et al (1997) Dominant-negative CREB inhibits tumor growth and metastasis of human melanoma cells. Oncogene 15:2069–2075
94. Yokoyama S, Feige E, Poling LL et al (2008) Pharmacologic suppression of MITF expression via HDAC inhibitors in the melanocyte lineage. Pigment Cell Melanoma Res 21:457–463
95. Lucas DR, Heim S (2002) Extraskeletal myxoid chondrosarcoma. In: Fletcher CDM, Unni KK, Mertens F (eds) Pathology & genetics. Tumours of soft tissue and bone. IARC Press, Lyon, pp 213–215
96. Aigner T, Oliveira AM, Nascimento AG (2004) Extraskeletal myxoid chondrosarcomas do not show a chondrocytic phenotype. Mod Pathol 17:214–221
97. Subramanian S, West RB, Marinelli RJ et al (2005) The gene expression profile of extraskeletal myxoid chondrosarcoma. J Pathol 206:433–444
98. Turc-Carel C, Dal Cin P, Rao U et al (1988) Recurrent breakpoints at 9q31 and 22q12.2 in extraskeletal myxoid chondrosarcoma. Cancer Genet Cytogenet 30:145–150

99. Panagopoulos I, Mertens F, Isaksson M et al (2002) Molecular genetic characterization of the EWS/CHN and RBP56/CHN fusion genes in extraskeletal myxoid chondrosarcoma. Genes Chromosomes Cancer 35:340–352
100. Filion C, Motoi T, Olshen AB et al (2009) The EWSR1/NR4A3 fusion protein of extraskeletal myxoid chondrosarcoma activates the PPARG nuclear receptor gene. J Pathol 217:83–93
101. Fanburg-Smith JC, Dal Cin P (2002) Angiomatoid fibrous histiocytoma. In: Fletcher CDM, Unni KK, Mertens F (eds) World Health Organisation classification of tumours. Pathology and genetics of tumours of soft tissue and bone. IARC Press, Lyon, pp 194–195
102. Rossi S, Szuhai K, Ijszenga M et al (2007) EWSR1-CREB1 and EWSR1-ATF1 fusion genes in angiomatoid fibrous histiocytoma. Clin Cancer Res 13:7322–7328
103. Antonescu CR, Dal CP, Nafa K et al (2007) EWSR1-CREB1 is the predominant gene fusion in angiomatoid fibrous histiocytoma. Genes Chromosomes Cancer 46:1051–1060
104. Antonescu CR, Ladanyi M (2002) Myxoid Liposarcoma. In: Fletcher CDM, Unni KK, Mertens F (eds) World Health Organisation classification of tumours. Pathology and genetics of tumours of soft tissue and bone. IARC Press, Lyon, pp 40–43
105. Kilpatrick SE, Doyon J, Choong PFM et al (1996) The clinicopathologic spectrum of myxoid and round cell liposarcoma. A study of 95 cases. Cancer 77:1450–1458
106. Smith TA, Easley KA, Goldblum JR (1996) Myxoid/round cell liposarcoma of the extremities. A clinicopathologic study of 29 cases with particular attention to extent of round cell liposarcoma. Am J Surg Pathol 20:171–180
107. Dei Tos AP, Wadden C, Fletcher CDM (1996) S-100 protein staining in liposarcoma. Its diagnostic utility in the high grade myxoid (round cell) variant. Appl Immunohistochem 4:95–101
108. Orvieto E, Furlanetto A, Laurino L et al (2001) Myxoid and round cell liposarcoma: a spectrum of myxoid adipocytic neoplasia. Semin Diagn Pathol 18:267–273
109. Tallini G, Rosai J, Fletcher CDM et al (1995) Myxoid liposarcoma represents a distinct type of liposarcoma characterised by the specific cytogenetic translocation t(12; 16)(q13; p11). Mod Pathol 8
110. Knight JC, Renwick PJ, Dal Cin P et al (1995) Translocation t (12;16)(q13;p11) in myxoid liposarcoma and round cell liposarcma: molecular and cytogenetic analysis. Cancer Res 55:24–27
111. Panagopoulos I, Hoglund M, Mertens F et al (1996) Fusion of the EWS and CHOP genes in myxoid liposarcoma. Oncogene 12:489–494
112. Engstrom K, Willen H, Kabjorn-Gustafsson C et al (2006) The myxoid/round cell liposarcoma fusion oncogene FUS-DDIT3 and the normal DDIT3 induce a liposarcoma phenotype in transfected human fibrosarcoma cells. Am J Pathol 168:1642–1653
113. Antonescu CR, Tschernyavsky SJ, Decuseara R et al (2001) Prognostic impact of P53 status, TLS-CHOP fusion transcript structure, and histological grade in myxoid liposarcoma: a molecular and clinicopathologic study of 82 cases. Clin Cancer Res 7:3977–3987
114. Schwab JH, Boland P, Guo T et al (2007) Skeletal metastases in myxoid liposarcoma: an unusual pattern of distant spread. Ann Surg Oncol 14:1507–1514
115. Grosso F, Jones RL, Demetri GD et al (2007) Efficacy of trabectedin (ecteinascidin-743) in advanced pretreated myxoid liposarcomas: a retrospective study. Lancet Oncol 8:595–602
116. Forni C, Minuzzo M, Virdis E et al (2009) Trabectedin (ET-743) promotes differentiation in myxoid liposarcoma tumors. Mol Cancer Ther
117. Lawrence B, Perez-Atayde A, Hibbard MK et al (2000) TPM3-ALK and TPM4-ALK oncogenes in inflammatory myofibroblastic tumors. Am J Pathol 157:377–384
118. Lamant L, Dastugue N, Pulford K et al (1999) A new fusion gene TPM3-ALK in anaplastic large cell lymphoma created by a (1;2)(q25;p23) translocation. Blood 93:3088–3095
119. Knezevich SR, McFadden DE, Tao W et al (1998) A novel ETV6-NTRK3 gene fusion in congenital fibrosarcoma. Nat Genet 18:184–187
120. Knezevich SR, Garnett MJ, Pysher TJ et al (1998) ETV6-NTRK3 gene fusions and trisomy 11 establish a histogenetic link between mesoblastic nephroma and congenital fibrosarcoma. Cancer Res 58:5046–5048
121. Tognon C, Knezevich SR, Huntsman D et al (2002) Expression of the ETV6-NTRK3 gene fusion as a primary event in human secretory breast carcinoma. Cancer Cells 2:367–376
122. Eguchi M, Eguchi-Ishimae M, Tojo A et al (1999) Fusion of ETV6 to neurotrophin-3 receptor TRKC in acute myeloid leukemia with t(12;15)(p13;q25). Blood 93:1355–1363
123. Argani P, Antonescu CR, Illei PB et al (2001) Primary renal neoplasms with the ASPL-TFE3 gene fusion of alveolar soft part sarcoma: a distinctive tumor entity previously included among renal cell carcinomas of children and adolescents. Am J Pathol 159:179–192
124. Ladanyi M, Lui MY, Antonescu CR et al (2001) The der(17)t (X;17)(p11;q25) of human alveolar soft part sarcoma fuses the TFE3 transcription factor gene to ASPL, a novel gene at 17q25. Oncogene 20:48–57