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Orbital Pathology Update

An update on mesenchymal tumours of the orbit with an emphasis on the value of molecular/cytogenetic testing

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Abstract

Mesenchymal tumours of the orbit are uncommon. Beyond childhood primary sarcomas are extremely rare and the literature is limited to case reports and short case series. However there is a diverse assortment of benign and malignant soft tissue tumours that may involve the orbit. Techniques to identify tumour specific cytogenetic or molecular genetic abnormalities often resulting in over- expressed proteins are becoming an increasingly important ancillary technique for these tumours. This review focuses on 3 specific areas: 1. Orbital mesenchymal tumours where cytogenetics are important to reach the correct diagnosis. The majority of these are chromosomal translocations that often result in a fusion gene and protein product; 2. Orbital mesenchymal tumours where cytogenetics are important to identify patients who will do well versus those with a poorer prognosis. This is turn helps with therapeutic options. In some tumours e.g. synovial sarcoma the chromosomal translocations can occur with 2 different regions resulting in different fusion products that carry a different prognosis. Alternatively whilst the majority of alveolar rhadomyosarcomas are fusion positive a minority are fusion negative with a better prognosis; 3. Orbital mesenchymal tumours where the identification of specific cytogenetic abnormalities has resulted in overexpression of specfic proteins which are diagnostically useful biomarkers for immunohistochemistry.

Keywords: Cytogenetics, Molecular genetics, Mesenchymal tumour, Soft tissue tumour, Orbit

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Introduction

There is a predominance of mesenchymal tissues in the orbit however despite this primary mesenchymal tumours are relatively rare. In adults the most common mesenchymal tumour (excluding cavernous haemangioma) is solitary fibrous tumour. In children embryonal rhabdomyosarcoma is the most common mesenchymal tumour (excluding capillary haemangioma). Beyond childhood primary sarcomas are rare with only individual reports and small case series in the literature.

Cytogenetic and molecular genetic assays are used routinely for diagnostic purposes in soft tissue pathology and represent a powerful adjunct to complement conventional microscopy. Many soft-tissue tumours are characterised by recurrent chromosomal rearrangements commonly translocations that produce specific gene fusions which allow precise classification of tumours.[1] This has been particularly useful for separating small round blue cell tumours of childhood. Furthermore overexpressed genes in mesenchymal tumours may result in overexpression of protein products that have provided novel and specific immunohistochemical markers.[2] In addition the identification of morphologically similar

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tumour groups with different behaviour and different cytogenetic findings has allowed better prognostication of some mesenchymal tumours.[3]

Molecular techniques have transformed the diagnosis of mesenchymal tumours in soft tissue. The diagnosis of orbital mesenchymal tumours should follow the same algorithm as soft tissue diagnosis elsewhere and include morphological, immunohistochemical and where appropriate molecular diagnostic techniques. The aim of this review is to consider the appropriate uses of these molecular techniques as applied to mesenchymal tumours occurring in the orbit (see Table 1).

Orbital mesenchymal tumours where cytogenetics is useful for diagnosis

Small round blue cell tumours

A number of small round blue cell tumours can rarely present within the orbit. Those encountered include Ewing's sarcoma, poorly differentiated synovial sarcoma, alveolar rhabdomyosarcoma and mesenchymal chondrosarcoma.

Ewing's sarcoma

Ewing's sarcoma is a high grade malignant tumour which typically presents in the long bones of children and young adults. It is only rarely seen in the head and neck and then generally involves the jaw bones. Most cases are metastatic from distant sites and primary orbital Ewing's sarcoma is extremely rare.[4,5] Histologically the tumour is composed of sheets of small round blue undifferentiated cells. They have a very small amount of cytoplasm. Nucleoli are usually not discerned. Immunohistochemical staining reveals strong membranous CD99 positivity. FLI1 shows nuclear staining. It is important to ensure that lymphoma is excluded by immunohistochemistry. Molecular studies are extremely helpful in the diagnosis of Ewing's sarcoma and should be carried out on all cases. A translocation involving chromosomes 11 and 22 is the commonest abnormality seen and is present in 85% of cases.[6] A rearrangement fuses the *EWSR1* gene with part of the *FLI1* gene forming the *EWSR1/FLI1* fusion gene in the vast majority of cases. This abnormality can be detected on fluorescent in situ hybridisation (FISH) analysis and by Reverse transcriptase polymerase chain reaction (RTPCR). There are alternative fusion partners for EWSR1 in a smaller number of cases. These include the *ERG* gene

Table 1. Summary of the key role of molecular genetics in the more common mesenchymal tumours of the orbit.

Mesenchymal origin	Chromosomal abnormality	Gene involved or fusion gene	Prevalence	Role of cytogenetics	References
Small blue cell tumours					
Ewing Sarcoma	t(11;22)(q24;q12) t(21;22)(q21′p12)	*EWSR1-FLI1* *EWSR1-ERG*	85% 5–10%	Diagnostic	Chen et al. 7
Synovial Sarcoma	t(X;18)(p11;q11)	*SS18-SSX1/SSX2 or SSX4*	66% SS18-SSX1 33% SS18-SSX2 Few SS18-SSX4	Diagnostic and Prognostic (SSX1 less favourable)	Stagner et al. 9
Mesenchymal Chondrosarcoma	t(8;8)(q21.1:q13.3)	*HEY1-NCO2*	Most	Diagnostic	Moriya et al. 14
Rhadomyosarcoma					
Embryonal RMS	Multiple events – no specific gene	–	–	–	Parham et al. 16
Alveolar RMS	t(2;13)(q35;q14)	*PAX3-FOXO1*	60%	Diagnostic & Prognostic (Less favourable for those without translocation)	Parham et al. 16
	t(1;13)(p36:q14)	*PAX7-FOXO1*	20%		Kubo et al. 57
Liposarcoma					
Well differentiated LS/ ALT	Amplication of 12q14-15	Overexpression of *MDM2*	100%	Diagnostic	Jakobiec et al. 18
Myxoid LS	t(12;16)(q13;p11)	*FUS-DDIT3* *EWSR1-DDIT3*	95% 5%	Diagnostic	Rao et al. 27
Spindle cell proliferations					
Low grade Fibromyxoid Sarcoma	t(7;16)(q33;p11)	*FUS-CREBL2*	76–96%	Diagnostic	Mohamed et al. 30
	Ring chromosome t(11;16)(p11;p11)	*FUS-CREBL1*	4–6%		
	3q29	Over expression *MUC4*	100%	Biomarker – MUC4 IHC	Doyle et al. 55
Nodular Fasciitis	t(17;22)(p13;q13)	*USP6-MYH9*	100%	Diagnostic	Compton et al. 31
Solitary Fibrous Tumour	Inv(12)(q13;q13)	*NAB2-STAT6*	100%	Diagnostic Biomarker – STAT6 IHC	Thway et al. 45
Miscellaneous mesenchymal tumours					
Alveolar Soft Part Sarcoma	t(X;17)(p11;q25)	*ASPOL-TFE3*	NK – majority	Diagnostic Biomarker – TFE3 IHC	Folpe et al. 47
Chordoma	6p27	Brachyury	NK – majority	Biomarker – Brachyury	Miettinen et al. 58

LS-Liposarcoma; ALT – atypical lipomatous tumour; t – translocation; NK – not known; IHC – immunohistochemistry.

from chromosome 21 which forms the *EWS/ERG* fusion gene and a smaller group of rarer variants.[7]

Synovial sarcoma

Synovial sarcoma is a malignant tumour which usually presents in young patients as a mass in the deep musculature of the limbs often close to large joints. It has however been described in many varied anatomic locations, including the orbit.[8,9] It is a spindle cell tumour which has both biphasic and monophasic forms. In its classic biphasic form it is composed of cellular fascicles of short spindle cells with an admixed epithelial component in varying proportions. Monophasic forms consist of spindle cell tumour alone (Fig. 1A and B) or exceedingly rarely, an epithelial component alone. Occasionally a poorly differentiated variant is observed. This tumour is composed of small round blue cells and can be difficult to diagnose, requiring a high index of suspicion coupled with confirmatory ancillary immunohistochemical and molecular investigations. On immunohistochemistry, both the spindle cell and epithelial components show some staining with EMA and broad spectrum cytokeratin. The intensity of staining is often higher in the epithelial component. Around 90% of all synovial sarcomas express cytokeratin. Gene expression profiling has more recently identified TLE1 as a sensitive and specific marker of synovial sarcoma. It is a regulator of the Wnt signalling pathway and appears to be expressed in over 90% of synovial sarcomas. Molecular analysis is directed towards identifying an X:18 translocation t(X;18)(p11.2; q11.2). These are present in around 90% of cases. There is translocation of the SYT18 gene on chromsome 18 with one of the three SSX genes (SSX1, SSX2 and SSX4) to form an SYT18-SSX fusion gene. There appear to be some prognostic significance associated with fusion type *vide infra*. This molecular abnormality can be detected by FISH and RTPCR techniques using frozen or paraffin embedded material. A positive result can be particularly helpful in cases presenting as small round blue cell tumours and indeed in the very rare instance of the monophasic epithelial variant in its distinction from adenocarcinoma.

Mesenchymal chondrosarcoma

Mesenchymal chondrosarcoma is a rare form of chondrosarcoma characterised by its biphasic morphology which includes a poorly differentiated small round blue cell component. These cells are admixed with islands of well differentiated hyaline cartilage. Initial biopsy may however not contain both parts making correct diagnosis particularly challenging. These tumours show widespread anatomic distribution with two thirds arising in the skeleton and one third affecting soft tissue sites. Occasionally these occur in the orbit either primary in the bone or more rarely soft tissue.[9–11] Histologically the small round blue cell component typically resembles Ewing's sarcoma. There is often a haemangiopericytomatous vascular pattern (Fig. 1C). Scattered osteoclast type giant cells may be seen as may osteoid-like matrix. The presence of adjacent hyaline cartilage is helpful in establishing the correct diagnosis (Fig. 1D). In its absence however immunocytochemistry may be needed. The small round blue cells are positive for SOX-9.[12] It should be noted that CD99 and desmin may be

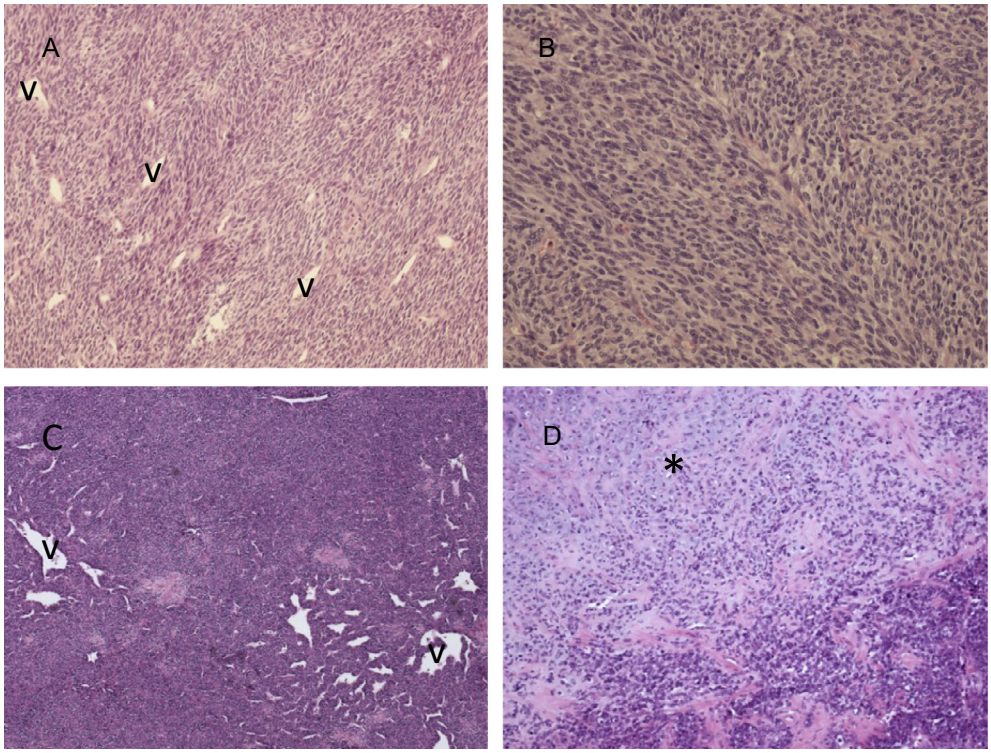

Fig. 1. Synovial sarcoma and mesenchymal chondrosarcoma. A. Orbital tumour in a 26 year old male showing a monophasic synovial sarcoma displaying areas with a haemangiopericytomatous vascular pattern (v) (H&E, ×25). B. On higher power synovial sarcoma is composed of sheets of short, uniform spindle cells (H&E, ×200). C. Tumour involving orbital bone in a 25 year old male. In many areas the tumour is composed of sheets of primitive spindle shaped cells often with a haemangiopericytomatous vascular pattern (v) similar to synovial sarcoma. D. The diagnosis is more straightforward when areas of cartilagenenous differentiation (*) are identified juxtaposed to the primitive spindle cell area.

expressed by the blue cell component of mesenchymal chondrosarcoma making differentiation from Ewing's sarcoma or alveolar rhabdomyosarcoma difficult.[13]

A consistent genetic abnormality has been described in mesenchymal chondrosarcoma. This is the *HEY-NCOA2* fusion which is present on chromosome 8 and may offer a potential therapeutic target.[14]

Rhabdomyosarcoma

Rhabdomyosarcoma is a highly malignant tumour which is the most common soft tissue sarcoma of the head and neck in childhood. 10% of all cases occur in the orbit.[15] There are three principle variants of rhabdomyosarcoma–embryonal, alveolar and pleomorphic. Between 50 and 70% of orbital RMS are of the embryonal subtype. Histologically these are composed of primitive mesenchymal cells resembling embryonic skeletal muscle (Fig. 2A and B). Around 20–30% are alveolar RMS which is a highly malignant and highly cellular tumour composed of a monotonous population of small round blue primitive cells (Fig. 2C). They can form solid sheets or they may form nests separated by thin fibrous septa. The nests classically show loss of cohesion centrally. The blue cells stain express the muscle markers desmin, MyoD1 and myogenin on immunostaining. Cytogenetic analysis has shown consistent and specific translocations associated with alveolar RMS. A t(2:13)(q35;q14) occurs in most cases (Fig. 2D). A small group contain the t(1;13)(p36;q14). These translocations involve the *PAX3* and *PAX7*genes which become translocated to the *FOXO1*gene locus on chromosome 13.[16] The fusion products encoded have oncogenic function as transcription activators. FISH analysis should be carried out looking for these fusion genes in any case of suspected alveolar rhabdomyosarcoma.

In terms of prognosis, there is some data to suggest that metastatic tumours with a *PAX7-FOXO1* fusion behave less aggressively than those with a *PAX3-FOXO1* fusion as discussed below.

In the majority of embryonal rhabdomyosarcomas there is loss of genomic material from chromosome 11 at the 11p15 locus, but in contrast to the alveolar subtype, it is not yet known which gene is involved.[16] The molecular genetics of pleomorphic rhabdomyosarcoma are even less well defined at present.

Adipocytic tumours

Liposarcoma, a malignant tumour of adipocytic tissues, is the most common soft tissue sarcoma in adulthood, comprising approximately 20% of all sarcomas; most present in the extremities and retroperitoneum. Despite the relatively large amount of adipose tissue in the orbit, orbital liposarcomas are rare. Approximately 60 cases of liposarcoma have been reported in the literature.[17–19]

The majority of cases of liposarcoma are intraconal or in the superior orbit with fewer cases in medial, lateral and inferior orbit.[17] There are 4 recognised subtypes of liposarcoma: myxoid, pleomorphic, atypical lipomatoustumour/well differentiated and dedifferentiated and these have all been

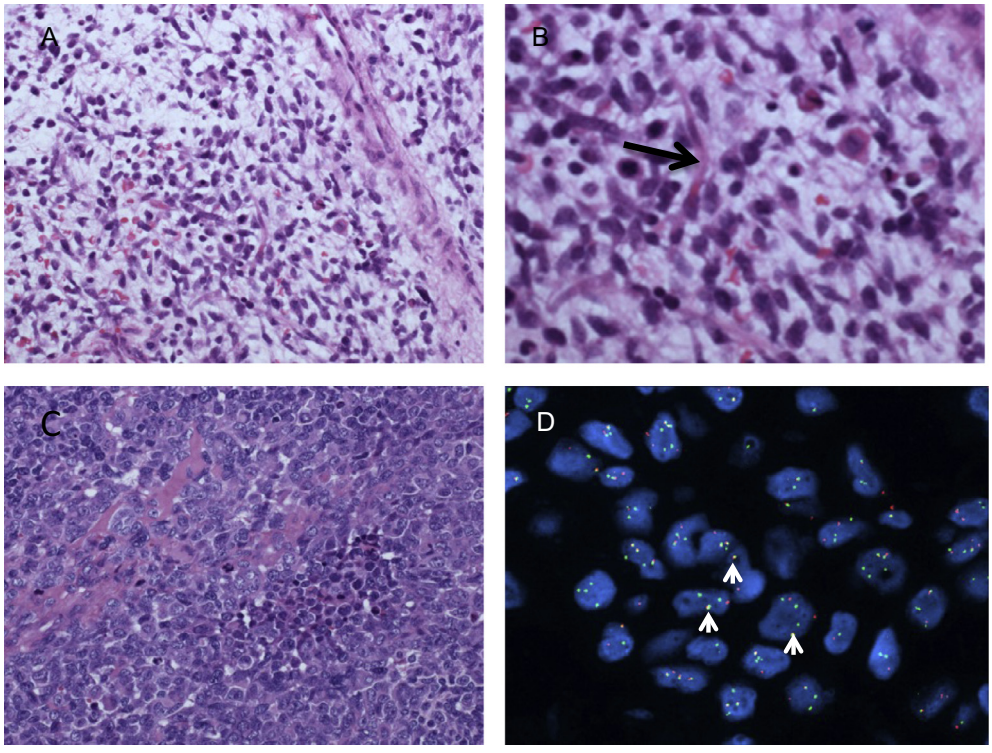

Fig. 2. Rhabdomyosarcoma. A. Orbital mass in a 6 year old child. Embryonal rhabdomyosarcoma composed of primitive spindle cells (H&E, ×100). B. Embryonal rhabdomyosarcoma with occasional strap like cells (arrow; H&E, ×200). C. Orbital mass in 13 year old child. Alveolar rhadomyosarcoma composed of sheets of round blue cells with discohesion (H&E; ×100). D. FISH demonstrating a t(2;13)(q34;q14) in alveolar rhabdomyosarcoma. The *FOXO1* chromosome probe is orange and the = chromosome probe is green. Fusion sites are indicated as a yellow signal (arrowheads).

described in the orbit.[17–20] Most case reports in the orbit pertain to myxoid and well-differentiated subtypes.

Atypical lipomatous tumour/well differentiated liposarcoma

Of all the subtypes atypical lipomatous tumour/well-differentiated liposarcoma (ALT/WDLS) presents the biggest diagnostic challenge (Fig. 3A and B). These can show a resemblance to lipoma variants such as spindle cell and pleomorphic lipoma, both of which have been described in the orbit.[21,22] Spindle cell lipomas are benign with fibrous septa containing bland spindle cells and ropey collagen. They are CD34 positive and lack infiltration. Lipoblasts are absent. However intramuscular spindle cell lipoma does occur and can be mistaken for infiltration. In pleomorphic lipoma there may be atypical pseudolipoblasts which can cause confusion. Furthermore CD34 staining has been described in ALT/WDLS. Other potential differential diagnoses that should be considered include a reaction to silicone where pseudolipoblasts may be plentiful, fat containing solitary fibrous tumour and prolapsed orbital fat which may contain floret like cells.

Cytogenetics studies have shown that ALT/WDLS is characterised by amplification of chromosomal region 12q14-15.[18,23] This region contains the murine double minute-2 (*MDM2*) and cyclin dependent kinase-4(*CDK4*) genes. *MDM2* is an oncogene and expression of this promotes degradation of the tumour suppressor protein p53. *MDM2* is consistently amplified in cases of ALT/WDLS. The adjacent gene *CDK4* on 12q14.1 is frequently amplified when *MDM2* is amplified and its protein product phosphorylates the retinoblastoma 1 protein (RB) which disrupts its interactions with E2F transcription factors allowing progression of the cell cycle form G1 to S phase.

Amplification of the MDM2 can be detected by FISH or by immunohistochemistry for the MDM2 and CDK4 proteins. Immunohistochemical staining has the advantage of being a routine technique available in almost all laboratories. However, the disadvantage is that whilst the sensitivity is high, specificity as relatively low as 59%.[23,24] Sensitivity is lower in needle biopsies which can have a false positive rate of up to 11%.[24] Using FISH for the *MDM2* gene has been shown to have a much higher sensitivity and specificity (even in small biopsies) provided it is applied in the correct clinical and histological context.[24]

MDM2 is also amplified in dedifferentiated liposarcoma and FISH studies can be useful in distinguishing this from other high grade sarcomas.[25]

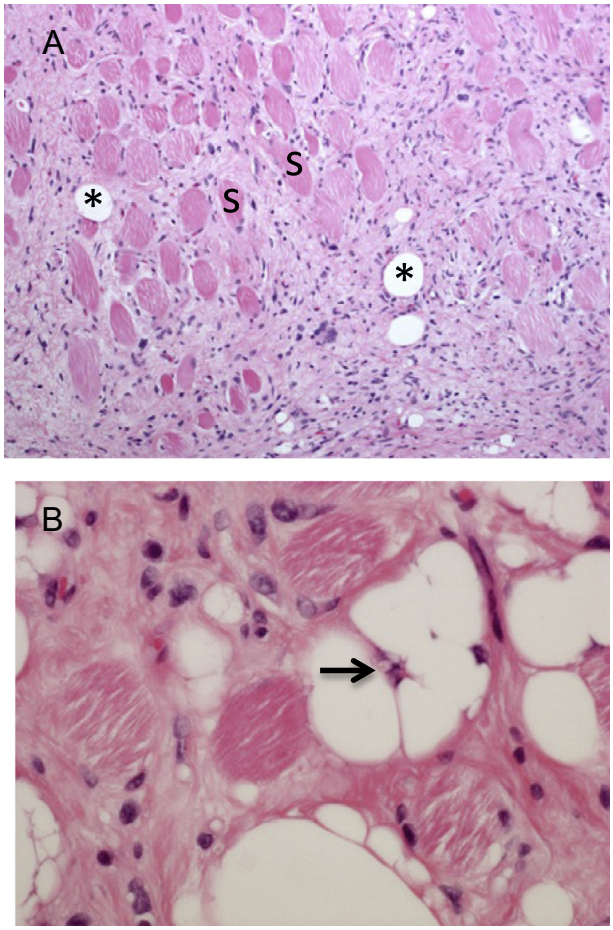

Fig. 3. Well differentiated Liposarcoma/Atypical lipomatous tumour. A. Orbital mass in a 55 year old female. A bland spindle cell tumour with occasional fat spaces (*) infiltrates between skeletal muscle bundles (arrowhead); (H&E; ×100). B. On closer inspection there are occasional lipoblasts (arrow); (H&E; ×400).

Myxoid liposarcoma

Myxoid liposarcoma is a low grade, paucicelluar tumour with a prominent chicken wire vascular pattern within a mucoid matrix often with large mucoid pools. Within the background there may be scattered signet ring lipoblasts. Higher grade tumours may contain larger numbers of small round monomorphic cells particularly towards the edge of tumour lobules (previously known as round cell liposarcoma). On histology the differential diagnosis includes other lipomatous tumours such as chondroid lipoma or myxolipoma, extraskeletal myxoid chondrosarcoma, myxofibrosarcoma and myxoma

Cytogenetic studies have shown that myxoid liposarcoma (MXLS) is characterised by a specific translocation, t(12;16) resulting in the juxtaposition of *FUS* (fused in sarcoma) and *DDIT3* (DNA-damage-inducible transcript 3) or the *EWS* (Ewing sarcoma breakpoint region 1).[26] The resulting protein functions as a transcriptional activator of downstream genes. This translocation can be detected by FISH and RT-PCR with high specificity and sensitivity.[27,28]

Spindle cell proliferations

Low grade fibromyxoid sarcoma

This is a low grade sarcoma that typically occurs in the proximal extremities or trunk. Occurrence in the head is rare and orbital tumours are exceptional. However two cases of hyalinising spindle cell tumour with giant rosettes a subtype of LGFMS have been described in the orbit.[29] Interestingly both cases occurred at a younger age group than is usual elsewhere in the body. These tumours are liable to local recurrence and late metastasis.

Histologically these tumours are challenging. They have low grade morphology and often have myxoid areas, which can occur in several other tumours including peripheral

nerve sheath tumours and solitary fibrous tumour (Fig. 6A and B).

LGFMS is characterised by a recurrent a t(7;16)(q34;p11).[30] This results in *FUS-CREB3L2* fusion gene in around 95% of cases that generates a protein with transcriptional regulatory activity. This is present in about 95% of cases with a smaller number of cases harboring a *FUS-CREB3L1* fusion resulting from t(11;16)(p11;p11).[30] Occasional rare cases harbor an *EWSR1-CREB3L1* fusion. Hyalinizing spindle cell tumour with giant rosettes shares the same cytogenetic findings.[30]

Nodular fasciitis

This is a benign reactive fibroblastic proliferation characterised by rapid growth. It usually occurs in subcutaneous or superficial fascia of the extremities. Orbital lesions are relatively rare and typically occur in the anterior orbit or periorbital region.[31,32] These proliferations are unencapsulated and usually less than 3 cm. These consist of a proliferation of spindle shaped cells often with a tissue culture type appearance. There may be myxoid areas and extravasation of red cells (Fig. 4A and B). Mitotic figures are frequent although these are never atypical. Morphologically these may be mistaken for spindle cell neoplasms including smooth muscle tumours as they express smooth muscle actin. However these proliferations consistently show a t(17;22)(p13;q13) representing a *USP6-MYH9* fusion.[32,33] This rearrangement of *USP6* can be detected by FISH in 90% of cases of nodular fasciitis and is not present in lesions that mimic nodular fasciitis.[33] In one study it had a sensitivity of 86% and a specificity of 100%.[33]

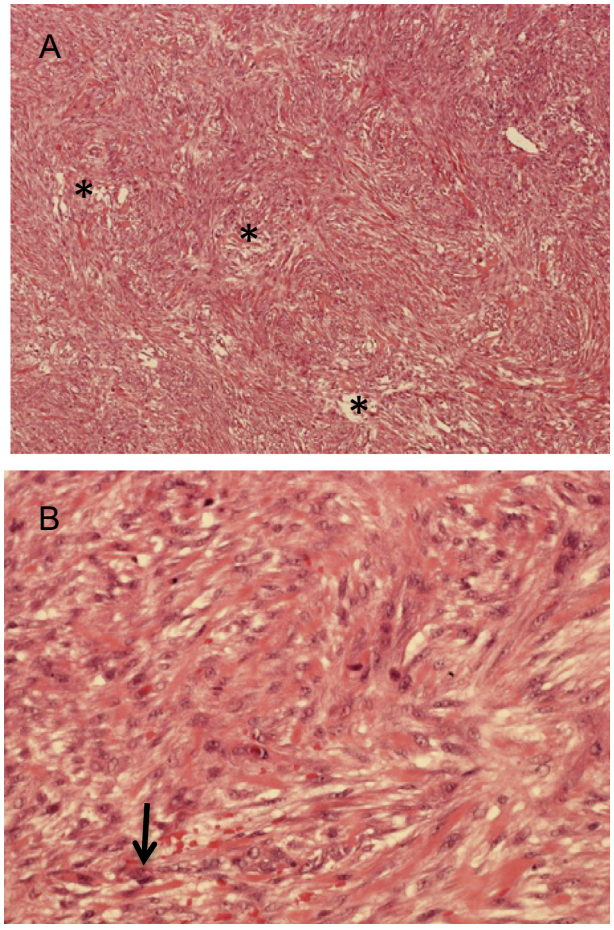

Fig. 4. Nodular Fasciitis. A. Orbital mass in a 28 year old male showing a spindle cell tumour with paler myxoid areas (*); (H&E; ×100). B. On higher power there is also extravasation of red cells (arrow); (H&E; ×400).

USP6 is part of a subfamily of deubiquitinating enzymes. MYH9 is a member of the non-muscle myosin class II family important in cell motility. Fusion leads to transcriptional upregulation of *USP6*. USP6 is involved in intracellular trafficking, protein turnover and inflammatory signaling and cell transformation. This is the first example of self-limited human lesion that is characterised by a recurrent somatic gene fusion event.[34]

Orbital mesenchymal tumours where cytogenetics is useful for risk stratification

Rhabdomyosarcoma

Of the two main subtypes of rhabdomyosarcoma seen in the orbit (embryonal and alveolar), the embryonal variant typically has a better prognosis. Many studies seem to indicate that in alveolar rhabdomyosacroma the type of fusion gene present has prognostic value. It has been reported that the presence of a *PAX3-FOXO1* fusion carries a worse prognosis than a *PAX7-FOXO1* fusion.[35,36] Other studies have shown that the very presence of a fusion gene may be prognostic in itself. The 70–80% of alveolar rhabdomyosarcomas with a fusion (either *PAX3* or *PAX7* to *FOXO1*) have been demonstrated to have a poorer prognosis than those without.[35,37,38] Those non-fusion cases are molecularly and clinically indistinguishable from embryonal rhabdomyosarcoma despite their histology. This means that patients with a fusion negative alveolar rhabdomyosarcoma can receive less intensive therapies and expect a better outcome. Debate still remains around this evolving subject although it would seem reasonable to assume that molecular status will inform risk stratification for rhabdomyosarcoma in the long term. Emerging biomarkers at the forefront of active research in this area include *MYOD1* mutations, *RAS* pathway mutations and *NCOA2* gene fusions.[36,39]

Synovial sarcoma

As discussed above, the chromosomal translocation t (X;18) fuses the SS18 (SYT) gene to the SSX gene (predominantly SSX1 or SSX2) and is the precipitating event in the oncogenic development of synovial sarcoma. It is present in almost all cases of synovial sarcoma. There is an association with histological subtype, SSX2 fusions rarely being encountered in biphasic tumours. A number of studies have shown that the particular fusion variant present affects prognosis. It has been reported that those patients with an SYT-SSX1 fusion have poorer median survival than those possessing the SYT-SSX2 fusion gene. Indeed in one large study, the median survival was twice that for those with the SSX2 variant.[40] Additionally the presence of the SYT-SSX1 has been reported to double the risk of developing metastatic disease. Not all studies have been able to show this association however suggesting that there are other as yet unspecified factors which play a role in determining outcome. Other biomarkers which have been proposed as markers of poor prognosis include aberrant expression of p53 as well as expression of insulin-like growth factor receptors 1 and 2. These are active areas of research.

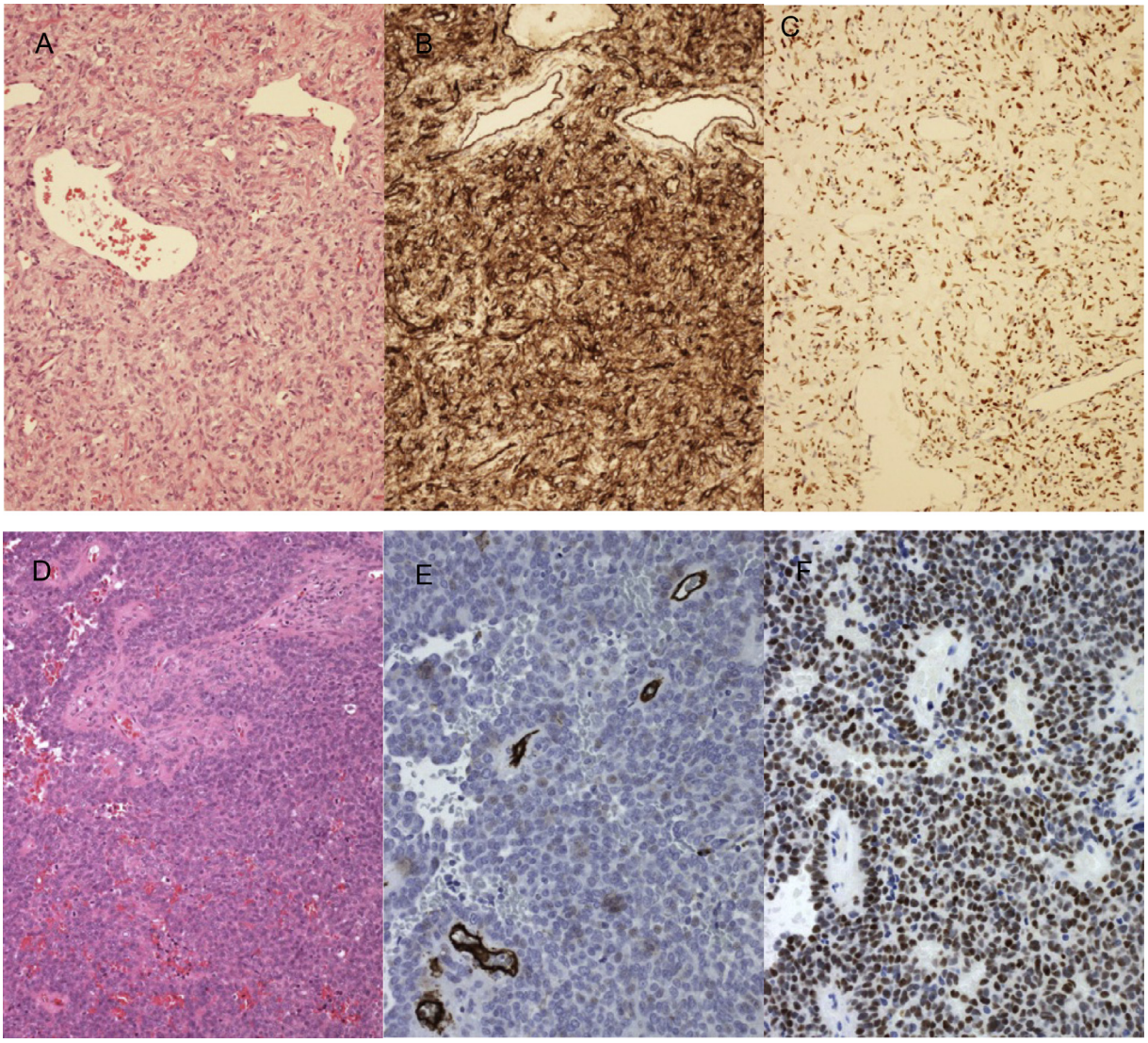

Fig. 5. Solitary Fibrous Tumour. A. Orbital mass from a 55 year old female showing conventional SFT with a patternless pattern of spindle cells in a dense collagenous background (H&E, ×40). B. Immunohistochemical staining for CD34 is strongly positive (CD34, ×40). C. Immunohistochemical staining shows widespread nuclear staining for STAT 6 (STAT 6; ×40). D. Recurrent tumour in the orbit of a 61 year old male. Malignant SFT showing a more densely cellular tumour composed of plumper spindle cells. E. Immunohistochemical staining for CD34 is negative (CD34; ×100) which commonly occurs in malignant SFT. F. Immunohistochemical staining for STAT6 is however maintained (STAT6, ×100).

Orbital mesenchymal tumours where cytogenetics has resulted in the identification of diagnostically useful biomarkers

Solitary fibrous tumour

Solitary fibrous tumour (SFT) was first described in the orbit in 1994 is probably the most common primary soft tissue neoplasm of the orbit.[41] It is characterised by proliferation of spindle shaped cells in a so-called patternless pattern with a staghorn vascular pattern (Fig. 5A).[41–43] These tumours can be variably cellular and myxoid and contain fat or multinucleated giant cells. Until recently positive immunohistochemical staining for CD34, bcl2 and CD99 (Fig. 5B) was used in confirming the diagnosis although some tumours (particularly more aggressive tumours) can be negative for some or all of these markers (Fig. 5D and E).[41–43] However, in 2015 a NAB2-STAT6 gene fusion product derived from inversion of chromosome 12 (q13q13) was identified as the genetic hall mark of SFT.[44] This results in nuclear overexpression of STAT6 which can be detected immunohistochemically in 97% of SFT (Fig. 5C and F). STAT6 is present in all variants of SFT and is maintained in more aggressive tumours unlike CD34.[45] STAT6 is also expressed in dedifferentiated liposarcomas. However the different morphology means this is rarely a diagnostic dilemma.[46] It is therefore a sensitive and specific marker of SFT.

Alveolar soft part sarcoma

Alveolar soft part sarcoma (ASPS) may arise in the orbit of children and young adults[47]. The tumours are composed of cells with granular eosinophilic cytoplasm separated by connective tissue network but in the orbit there may also be a more solid growth pattern[48,49] In the orbit the differential diagnosis includes metastasis from renal cell carcinoma, hep-

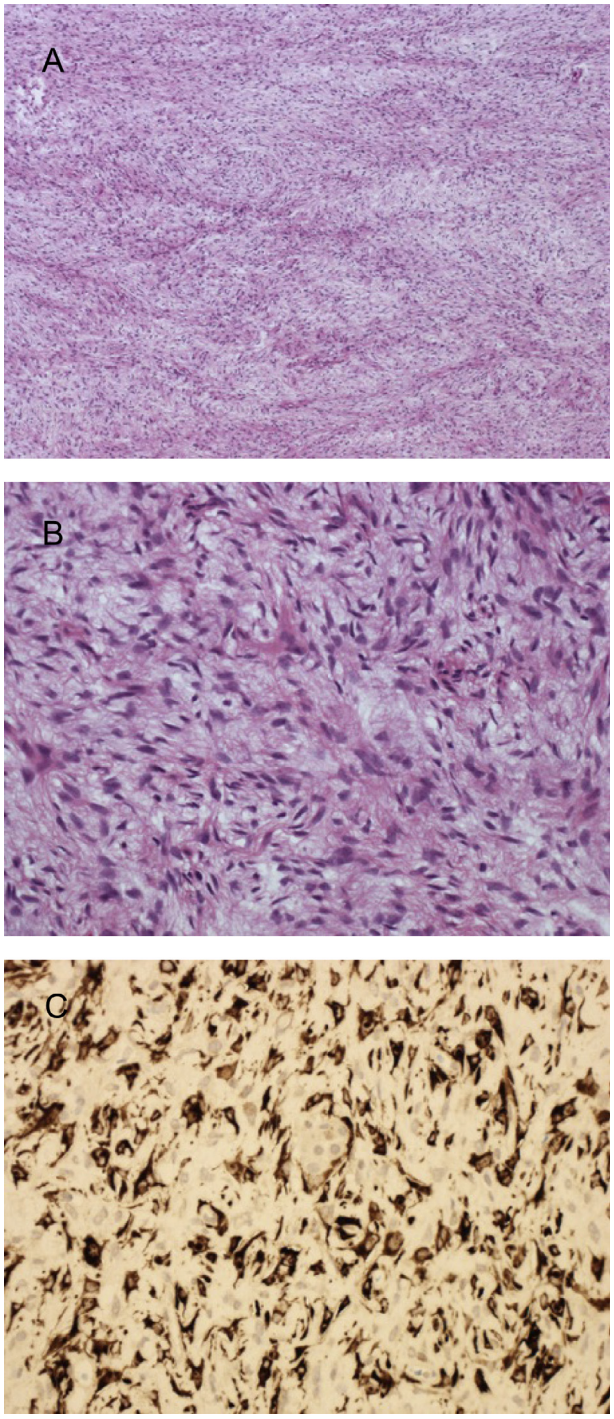

Fig. 6. Low Grade Fibromyxoid Sarcoma. A. Tumour extending along the lateral wall of the orbit in a 33 year old male. This shows a poorly cellular tumour with a myxoid stroma (H&E, ×25). The differential diagnosis includes neural tumours and SFT which can both have myxoid areas. B. Higher power shows this is composed of bland spindle shaped cells (H&E; ×200). C. Immunohistochemical staining for MUC4 shows widespread cytoplasmic staining confirming the diagnosis of LGFMS (MUC4; ×200).

atocellular carcinoma and melanoma. Granular cell tumour may also occur in the orbit and is included in the differential diagnosis.[50,51] Cytogenetics studies have shown a t(X;17)(p11.2;q25) in the majority of cases.[52] This results in an ASPOL-TFE3 fustion gene and nuclear staining with antibodies to TFE3 in ASPS.[52] Only nuclear expression of TFE3 is of diagnostic value, as cytoplasmic staining (possibly non-specific) is seen in various tumours. However some renal cell carcinomas and granular cell tumours show strong nuclear staining for TFE3.[53] Therefore whilst this is a sensitive marker for ASPS it is important to use an antibody panel to avoid these diagnostic pitfalls.

Low grade fibromyxoid sarcoma

The histology and cytogenetic findings in LGFMS has been discussed previously (Fig. 6A and B). In addition studies have shown that the *MUC4* gene is one of the top upregulated in LGFMS.[54,55] MUC4 is an epithelial glycoprotein which can be detected with immunohistochemistry and is a sensitive and specific marker of LGFMS (Fig. 6C).[55]

Chordoma

Secondary involvement of the orbit by chordoma originating from midline locations in the base of the skull has been described.[56] This usually occurs in late in disease such that diagnosis from orbital biopsy is unlikely to be challenging. However, rare cases originate in the head away from the cranial base and these ''ectopic'' chordomas may occur in the bony orbit.[57] It is thought that ectopic rests of cells may migrate through the superior orbital fissure during embryogenesis. Chordomas are composed of large cells with vacuolated cytoplasm (so-called physaliphorous cells). The cells are arranged in cords and ribbons embedded in extracellular matrix. On immunohistochemical staining these coexpress S100 and cytokeratins or EMA. Brachyury is a T-box family transcription factor which is overexpressed in chordoma (and notochord). Nuclear staining on immunohistochemistry is a sensitive and fairly specific marker for chordoma in distinguishing this from similar neoplasms such as carcinomas, chondrosarcoma and chondroid meningioma.[58] Whilst several other tumours, such as small cell carcinoma, may be positive, this rarely poses diagnostic difficulty.

Discussion/conclusions

Mesenchymal tumours of the orbit are a diverse range of tumours with wide ranging morphology. Primary mesenchymal tumours of the orbit are rare and the more common entities include rhabdomyosarcoma in childhood and solitary fibrous tumours in adults. The pathological work up of these tumours should be identical to other sites and immunohistochemistry and cytogenetics and molecular genetics are important ancillary techniques. In particular many soft tissue tumours have distinctive fusion gene products resulting from chromosomal translocations that are most efficiently identified by FISH or RTPCR. In some tumours, for example, alveolar rhabdomyosarcoma different gene fusions or absence of these gene fusions has a more significant effect on prognosis than histology such that cytogenetics is now mandatory. In addition, overexpression of certain proteins due to the action of these fusion products has resulted in new immunohistochemical antibodies that are highly sensitive and specific and thus useful for diagnosis. This review has highlighted the role of cytogenetics and molecular genetics in the more common mesenchymal tumours of the orbit.

Conflict of interest

The authors declared that there is no conflict of interest.

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