

Fig. 2 (A) The lesion shows maturation, as expected in classical skin naevi [hematoxylin, eosin, safran (HES)]. (B) P16 expression is retained [3,3'-diaminobenzidine (DAB)]. (C) No invasion of the overlying epidermis is seen (HES). (D) MiB1 expression is seen in a very few naevocytes (DAB).

tumours and even in teratomas experimentally formed after transplantation of undifferentiated embryonic stem cells in subcutaneous tissues,¹ melanocytic lesions in mature teratomas are extremely rare. This could represent the actual rarity of the lesion or it could be due to underdiagnosis, inadequate sampling or not reporting of these lesions. Rare cases of dermal,⁵ compound^{6–8} or dysplastic⁹ naevi, as well as melanomas¹⁰ have been reported, representing a rare phenomenon of a 'neoplasm inside a neoplasm'.⁵ These naevi were reported with teratomas that were large (range 4 cm⁷ to 10.5 cm⁶), while in our case a small 3 mm naevus inside a small teratoma was found. Molecular characterisation of these melanocytic lesions has never been performed. We show here that melanocytic naevus arising inside mature teratoma harbours *BRAF* mutation, the most common alteration of acquired naevi.

Thus, melanocytes existing inside the skin tissues of ovarian teratomas, away from sun exposure, can undergo the same mutations and give the same clonal proliferations as melanocytes of normal epidermis.

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Myoepithelioma-like tumour of the vulvar region



Sir,
Myoepithelioma-like tumour of the vulvar region (MELTVR) is a rare mesenchymal neoplasm of the vulvar area. Histologically, MELTVRs are usually similar to soft

tissue myoepitheliomas and extraskeletal myxoid chondrosarcomas; however, they have a characteristic immunohistochemical profile, including positivity for oestrogen receptor, negativity for S100 protein and glial fibrillary acidic protein, and loss of INI1/SMARCB1 expression. EWSR1 and NR4A3 rearrangements are absent. In this report, we present a case of MELTVR harbouring only oestrogen receptor positive cells in the myxoid area.

The patient was a 34-year-old woman who presented with a 6-month history of a slowly enlarging subcutaneous nodule in the right area of the mons pubis. The patient reported pain beginning one month previously. Ultrasound examination revealed a mixed echo subcutaneous mass with heterogeneous texture, measuring 35 mm in diameter. She had a history of pregnancy. The clinicians made a diagnosis of 'angioliipoma'. The lesion had been enucleated. No adjunctive therapy was administered. The patient was free from local recurrence and distant metastasis at the last follow-up, 6 months after the initial excision.

Grossly, a 30×30×20 mm circumscribed and lobulated nodule was seen. The tumour was described as soft and partially glistening. The cut surface was white to yellow in colour, without necrosis. Microscopically, at low magnification the tumour was lobulated, being septated by fibrous tissue and focally encased by a thin fibrous pseudocapsule. The surgical margin was positive. The ratio of myxoid area was approximately 60% of the tumour volume. At the peripheral area, tumour cells were mainly arranged in a reticular manner with cords in a myxoid matrix. In the myxoid area, the epithelioid or spindle-shaped tumour cells proliferated singly or in a loosely cohesive reticular manner with cords, chains, or clusters (Fig. 1). In the non-myxoid areas, the epithelioid or short-spindle shape tumour cells grew in diffuse sheets. A characteristic hypervascular stroma, showing a haemangiopericytomatous pattern with elongated and branching vessels, was present throughout the tumour.

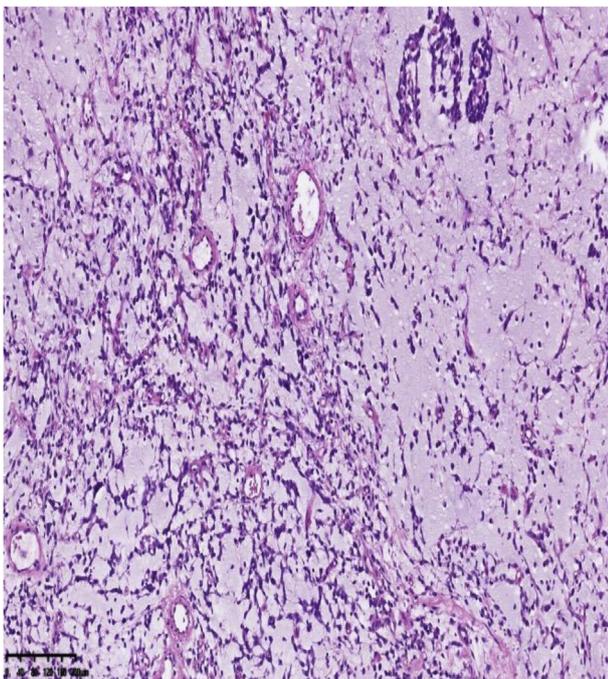


Fig. 1 Tumour cells proliferated singly or in a loosely cohesive reticular manner with cords, chains, or clusters in the myxoid area.

These tumour cells formed alternating hypocellular and hypercellular areas. The epithelioid tumour cells had amphophilic cytoplasm and round nuclei with vesicular chromatin and conspicuous nucleoli. Tumour cells showed moderate or severe nuclear atypia. Mitotic figures were readily observed in the tumour, with a median rate of 6/10 high-power fields (×40 objective and an eyepiece of field number 22). No necrosis was observed.

Immunohistochemically, the tumour cells showed strong focal oestrogen receptor (ER) expression. The ER positive cells were only seen in the myxoid area. Progesterone receptor positive cells were focally distributed in the peripheral area of the tumour. The tumour cells reacted focally for epithelial membrane antigen (EMA). The tumour cells were completely negative for pan-cytokeratin (clone AE1/AE3), desmin, S100 protein, glial fibrillary acidic protein (GFAP), and CD34. Loss of expression of INI1/SMARCB1 was observed in all tumour cells (Fig. 2). The Ki-67 index was approximately 20%.

The tumour was tested for EWSR1 rearrangement by fluorescence *in situ* hybridisation (FISH). No split signals were observed.

In this report, we discuss the clinical, histological, morphological, immunophenotypical and cytogenetic features of MELTVR. To date, about 11 cases have been reported in the literature.^{1–3} The clinical findings and pathological findings of this case and 11 cases in the literature are summarised in Table 1.

All of the tumours grossly seemed well circumscribed; however, four cases showed minimal micronodular extracapsular infiltration, one case showed extensive infiltration into the adipose tissue. MELTVRs were characterised by histological heterogeneity, and each tumour consisted of a combination of myxoid epithelioid, myxoid spindle, non-myxoid epithelioid and non-myxoid spindle patterns in varying proportions. Myxoid areas were at least focally

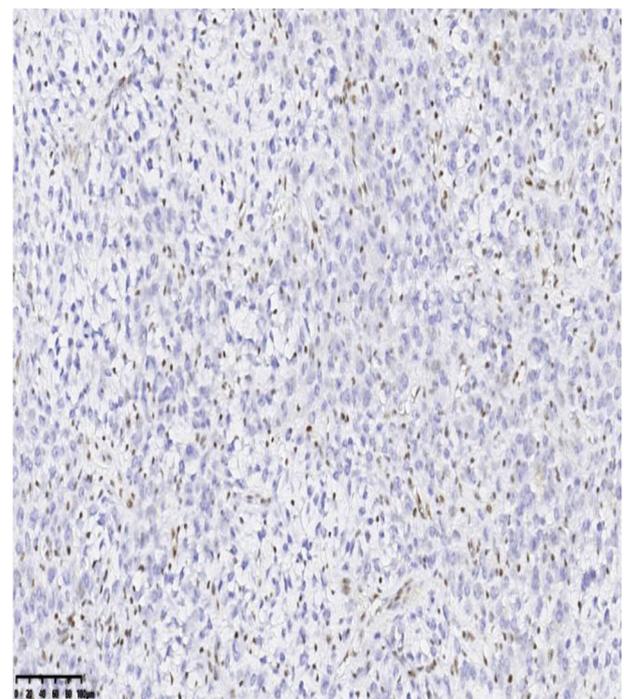


Fig. 2 All tumour cells exhibited loss of INI1/SMARCB1 expression.

Table 1 Clinical summary and histological findings of 12 MELTVRs

No	Age	Site	Size, cm	Myxoid area, %	Nuclear grade	Mitosis (/10HPF)	Necrosis	Outcome
1	49	Labium majus	4.3	5	Low	6	Yes	No
2	42	Labium majus	7	60	Low	4	Yes	Regrowth
3	24	Labium majus	3.3	75	High	8	No	No
4	41	Labium majus	2	95	High	2	No	Regrowth
5	52	Mons pubis	7.7	20	High	12	Yes	No
6	28	Labium majus	3	80	High	2	Yes	No
7	35	Groin	2.4	90	High	7	No	No
8	65	Groin	3.2	95	High	11	No	No
9	35	Mons pubis	^a	<5	Low	<1	No	Recurrence
10	31	Mons pubis	2	10	Low	3	No	No
11	70	Groin	3	40	High	2–3	Yes	No
12	34	Mons pubis	3	60	High	6	No	No

^a The size of a ping-pong ball; outcome, recurrence or distant metastasis.

present in all tumours and ranged from <5% to 95% of the tumour volume. In the myxoid area, the epithelioid or spindle-shaped tumour cells proliferated singly or in a loosely cohesive reticular manner with cords, chains, or clusters. In non-myxoid areas, the epithelioid tumour cells grew in diffuse sheets, or the spindle-shaped tumour cells formed storiform arrangements.

MELTVRs should be differentiated from soft tissue myoepitheliomas, extraskeletal myxoid chondrosarcomas (EMCs), epithelioid sarcomas (ESs), and benign genital stromal tumours (such as cellular angiofibromas, aggressive angioyxomas, and angiofibroblastomas). Soft tissue myoepitheliomas are the most important differential diagnosis for MELTVRs. Histologically, MELTVRs are quite similar to soft tissue myoepitheliomas; however, the immunophenotypes of MELTVRs deviate from those of classic myoepitheliomas. First, cytokeratin expression was negative in most MELTVRs and all MELTVRs lacked reactivity to S100 and GFAP. In a large study by Hornick and Fletcher,⁴ 93% of their myoepithelioma cases expressed keratin, and 87% expressed S100 protein. Secondly, all cases of MELTVRs were deficient for INI1 expression, although myoepitheliomas occasionally demonstrate the loss of SMARCB1 immunoreactivity (approximately 10%).⁵ Finally, about half of soft tissue myoepitheliomas reportedly harbour *EWSR1* gene rearrangements⁶ but MELTVRs lacked this rearrangement.

EMCs resemble MELTVRs because they comprise uniform, loosely cohesive tumour cells in a myxoid matrix. However, EMCs usually arise in the deep soft tissue of the extremities in middle-aged and elderly patients, and superficial vulvar/inguinal involvement is rare. EMCs are characterised by deeply eosinophilic, finely granular to vacuolated cytoplasm and strikingly hypovascular stroma. EMA expression is uncommon, and ER expression was rare in EMCs.^{7,8} NR4A3 gene rearrangements are present in >90% of EMCs.^{7,9} Six cases of MELTVRs were tested for NR4A3 and none showed these gene rearrangements.

Another differential diagnosis of MELTVR is ES. ESs share characteristics with MELTVRs such as cohesive epithelioid tumour cells with vesicular nuclei and the loss of SMARCB1 immunoreactivity, particularly proximal variants which affect the vulva and inguinal region of adults. Myxoid epithelioid sarcoma has been reported as a rare variant of ES.¹⁰ However, ESs typically show more pleomorphic epithelioid (carcinoma-like) cells with enlarged vesicular

nuclei and prominent nucleoli; they also show infiltrative growth. Immunohistochemically, ESs virtually always express cytokeratin, and up to 80% label for CD34.⁹

Benign genital stromal tumours should be differentiated from MELTVRs because of their shared location and ER expression. Cellular angiofibromas are usually composed of mild spindle cells, and lack the epithelioid cytology and myxoid reticular architecture. The tumour cells are typically immunopositive for CD34. Angiomyofibroblastomas typically show consistent perivascular aggregation of tumour cells and the majority express desmin. Aggressive angioyxomas are often deep-seated, infiltrative lesions, characterised by hypocellular myxoid tissue. The spindle cells are desmin positive, and sometimes CD34 positive.

In summary, we have reported a case of MELTVR harbouring only ER positive cells in the myxoid area. We have also elucidated the clinical, histological, morphological, immunophenotypic and cytogenetic features of MELTVR, and discussed the various differential diagnoses. There is limited information regarding MELTVRs, and a larger series is necessary to determine their histological diversity and test clinical course.

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Comparison of Vitek 2 YS08 with Sensititre YeastOne for *Candida* susceptibility testing



Sir,

An early and accurate antifungal susceptibility result is important for the treatment of invasive candidiasis. Sensititre YeastOne (Thermo Scientific, USA) has good concordance with the standard Clinical and Laboratory Standards Institute (CLSI) reference method for *Candida* susceptibility testing,¹ and therefore is a widely utilised commercial method of determining *Candida* susceptibility. The automated Vitek 2 AST YS08 (bioMérieux, France) has advantages in decreased turnaround time, reduced costs and ease of use; however, there are limited data regarding its performance for resistant isolates.

A total of 68 clinical isolates of *Candida* species, many known to have antifungal resistance, were tested by Vitek 2 AST YS08 and the Sensititre YeastOne method according to the manufacturer's instructions. They were comprised of *Candida albicans* ($n=20$), *Candida glabrata* ($n=21$), *Candida tropicalis* ($n=9$), *Candida parapsilosis* ($n=10$) and *Candida krusei* ($n=8$). *Candida* species were identified with a log score >2.0 using the MALDI Biotyper (Bruker, USA).

Essential agreement was defined as ≤ 2 minimum inhibitory concentration (MIC) dilution difference and categorical agreement was obtained when the MIC result fell within the same interpretive categories according to CLSI breakpoints for azoles and echinocandins,² and according to the epidemiological cutoff values for amphotericin B.³ Very major errors, major errors and minor errors were defined according to a prior study, with Sensititre YeastOne as the reference method.⁴ Very major errors occurred where the reference method categorised the isolate as resistant and Vitek 2 categorised it as susceptible. Major errors occurred where the reference method categorised the isolate as susceptible and Vitek 2 categorised it as resistant. Minor errors occurred where one of the methods categorised the isolate as susceptible or resistant and the other method categorised the isolate as intermediate or susceptible dose dependent.

Table 1 demonstrates that essential agreement and categorical agreement were suboptimal for fluconazole and voriconazole with some very major errors, while there was good agreement for the other antifungals tested, acknowledging the lack of isolates which were non-susceptible to micafungin and amphotericin. Poor agreement for fluconazole was largely found in *C. albicans* where essential agreement occurred in 14/20 isolates and categorical agreement in 17/20 isolates. Poor agreement for voriconazole was predominantly found in *C. krusei* where essential agreement occurred in 2/8 isolates and categorical agreement occurred in only 2/8 isolates, mostly classified as minor errors. Caspofungin non-susceptibility occurred predominantly in *C. glabrata* complex and it was in this species complex that poor categorical agreement for caspofungin occurred with essential agreement in 15/15 isolates and categorical agreement in 5/15 isolates, mostly classified as minor errors.

Many previous studies of Vitek 2 *Candida* susceptibility testing had a low percentage of resistant isolates,^{5–7} hampering the comparison to our study; however, some studies which were enriched for resistant isolates like ours have shown better performance. Cuenca-Estrella *et al.* tested a set of 154 *Candida* isolates, approximately half with elevated azole MICs.⁸ They found essential agreement between Vitek 2 and the formal CLSI method of $>96\%$ for fluconazole and voriconazole. Using old CLSI breakpoints for fluconazole, minor errors occurred for 25 isolates, and major or very major errors in three isolates. For voriconazole, minor errors occurred for two isolates, and major or very major errors for four isolates. Posteraro *et al.* found good essential agreement and categorical agreement for their set which included 11 azole resistant *C. albicans* and 48 azole resistant *C. glabrata*.⁴ The better essential agreement seen in these two studies compared to our study may have been influenced by the *Candida* species which were tested. Both studies included an assessment of fluconazole and

Table 1 Comparison of Vitek 2 AST YS08 to reference method Sensititre YeastOne *Candida* susceptibility testing

Antifungal agents	Isolates ^a (n)	Resistant ^b (n)	I/SDD ^b (n)	EA (%)	CA (%)	Misclassified isolates (n)		
						VME	ME	mE
Fluconazole	39	18	1	77	90	2	0	2
Voriconazole	47	14	11	79	66	2	1	13
Caspofungin	62	3	7	100	82	0	2	9
Micafungin	54	1	0	100	100	0	0	0
Amphotericin	67	0	0	100	100	0	0	0
Flucytosine ^c	68	NA	NA	99	NA	NA	NA	NA

CA, categorical agreement; EA, essential agreement; mE, minor errors; ME, major errors; VME, very major errors.

^a Vitek 2 AST YS08 does not provide results for *Candida glabrata* against fluconazole and voriconazole, nor for *Candida krusei* against fluconazole. Six isolates for caspofungin and one isolate for amphotericin terminated by Vitek AST YS08 so did not allow comparison. Fourteen isolates were not tested for micafungin by YeastOne so did not allow comparison.

^b Resistant or intermediate/susceptible dose dependent according to Sensititre YeastOne results with CLSI interpretative criteria.

^c There is no CLSI breakpoint or epidemiological cutoff value for flucytosine, therefore no CA was calculated.