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Case Report

Phyllodes tumor with myoepithelial phenotype: a case report

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Abstract: Malignant phyllodes tumors of the breast are aggressive, occurring less frequently than fibroadenomas. The diagnosis is established after excluding metaplastic carcinoma and sarcomas. A 47 year old woman presented a primary tumor composed of hyperplastic epithelial and mucoid component, typically seen in fibroadenomas. Further investigations revealed PanCK, EMA and CK8/18 stained epithelial cells, stromal cells were positive for CD34, weak expression of SMA and CD10;calponin, S100, and p63 negative. Two years after resection, a new mass reappeared in the same breast mainly composed by spindle cells expressed strongly for CD10, SMA, Calponin, Vimentin, CD117, and EGFR, conversely, CD34 and epithelial markers were negative. These features bring into account of mammary not-otherwise specified-type sarcoma with CD10 expression along with myoepithelial features. After extensive sampling, a focal epithelial component was observed. Further comparative genome hybridization revealed similar LOH between both neoplasms, which prompts the possible transformation into amalignant tumor. Among chromosomal alterations, 1q is associated with the malignancy and recurrence.We illustrate the first malignant phyllodes tumor with myoepithelial phenotype that progressed from fibroadenoma. It possibly sheds light on a variant of this disease. Extensive sampling and molecular analysis might assist in the differential diagnosis with NOSCD10 sarcomas with myoepithelial phenotype expression.

Keywords: Fibroadenoma, malignant phyllodes tumor, breast, myoepithelial phenotype, CGH

Background

Phyllodes tumors (PTs) are fibroepithelial neoplasms of the breast that have the potential of recurrence. They are uncommon tumors that account for 0.5% to 0.9% of all breast malignancies in women [1, 2] occurring much less frequently than fibroadenomas (FAs). FAs are well circumscribed neoplasm, composed of stromal and epithelial cells that give raise to two distinct patterns; pericanalicular is the proliferation of stromal cells around ducts and intracanalicular is caused by compression of ducts intro clefts by proliferating stromal cells. Generally speaking, PTs are unilateral, great in size and are presented as a painless, mobile mass. Malignant PTs are the most aggressive subtype in this spectrum corresponding to about 10 to 20% of all PTs. They develop rapidly and reach up to 10 cm in diameter. Histologically, they are characterized by a combination of stroma and one or more layers of epithelial components, luminal epithelial and myoepithelial cells to forms leaf-like projections of stroma into the cystic cavities and infiltrative borders, the areas of highest stromal cellular activity usually diffuse to show various degrees of nuclear atypism and increased mitosis (≥10 per 10 high power fields), stromal overgrowth defined as absence of epithelial elements in one low power microscopic field containing only stroma [2-5]. The World Health Organization proposes that the pronounced cellular stroma along with the formation of leaf-like processes is the diagnostic criteria of PTs. It is exhibited in all forms of PTs, however, in certain circumstances; such as malignant cases, the stromal overgrowth proliferates to the point where epithelial elements are absent [5].

MPT are oftenmisperceived as pure sarcomas of the breast since the overgrowth of sarcomatous components in MPT may lead in absence or very focal identification of epithelial compo-

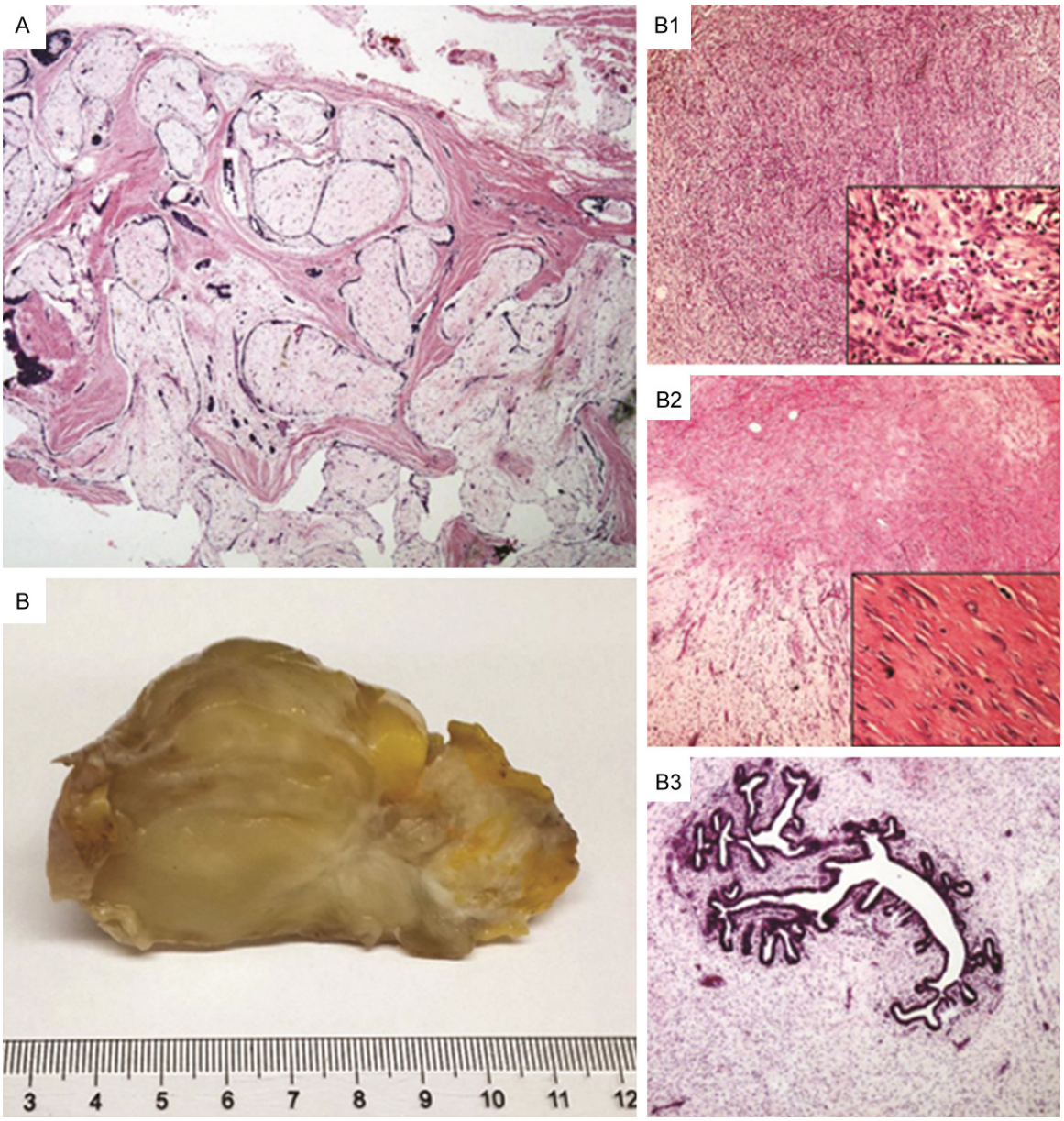

Figure 1. A. Fibroadenoma and second malignant tumor. Fibroadenoma, composed by capsule and epithelium and stroma, (H&E x 100). B. Gross specimen of malignant phyllodes tumor. B1. Spindle cells (H&E x 40). B2. Scar sequel from the previous surgery, infiltration of spindle cells. (H&E x 40). B3. Focal area of malignant tumor with epithelial and spindle component. (H&E x 40). The inserted pictures shows H&E staining in x 400 magnification of spindle cell (B1) and bundles of collagen mixed with scarce spindle cells (B3).

nent [5]. Metaplastic Carcinomas (MCs) are included in the differential diagnosis due to substantial arrangement of spindle cells; however, immunohistochemical demonstration of epithelial differentiation assists indetermining the diagnosis [6, 7]. In breast sarcoma, not otherwise specified-type sarcoma with CD10 expression (NSCD10s) with myoepithelial features has been proposed as a new entity. This assumption is supported by the fact that aside from CD10 expression, this tumor shows positivity for at least one marker of the following combination CD29/SMA/p63/calponin [8, 9].

Herein, we present a case of a malignant tumor that has progressed from fibroadenoma. Although, morphological and immunohistochemically investigations demonstrated myo-

Table 1. Primary antibodies for immunohistochemistry, antigen retrieval, and detection

Antibody	Source	Clone	Dilution	Retrieval Method	Detection System
CD10	Novocastra	56C6	1:50	CC1	BM, DAB
CD117	DAKO	c-kit	1:100	citrate 6.0	DAKO K5007
Ki67	DAKO	MIB-1	1:50	citrate 6.0	DAKO K5007
P63	MAIXIN	4A4	1:200	citrate 6.0	DAKO K5007
SMA	Sigma	1A4	1:5000	CC1	BM, DAB
MSA	Monoclonal	HHF35	1:50	citrate 6.0	DAKO K5007
Calponin	Novocastra	26A11	1:25	MW trs	DakoCHemMate, DAB
H-caldesmon	Biogenex	h-CD	Rtu	citrate 6.0	DAKO K5007
Vimentin	Linaris	V9	Rtu	None	DakoCHemMate, DAB
CD34	Neomarkers	QBEnd/10	1:800	CC1	BM, DAB
CK 8/18	INVITROGEN	ZyM5.2	Rtu	citrate 6.0	DAKO K5007
EMA	DAKO	E29	1:100	citrate 6.0	DAKO K5007
EGFR	Biogenex	POLYCLONE	Rtu	citrate 6.0	DAKO K5007
S-100	DAKO	POLYCLONE	1:1000	citrate 6.0	DAB
PanCK	DAKO	AE1/AE3	1:100	citrate 6.0	DAKO K5007

SMA, smooth muscle actin; EGFR, epidermal growth factor receptor (Her-1); rtu, ready to use; MSA, Muscle-specific actin, citrate; MW, microwave 30 min, CC1, CC1M solution, 32 min; BM, Ventana benchmark; trs, Dako retrieval solution pH 9.0; DAB, diaminobenzidine.

epithelial features which are similar to NSCD10s, a focal presence of epithelial elements and genetic findings confirm a variant of phyllodes tumor withmyoepithelial immunophenotype. This should promptfuture caution with the differential diagnosis of these two tumors.

Case presentation

Two years ago, a 47-year-old woman presented a palpable, painless and mobile discrete lump in her left breast. After excisional biopsy the gross tumor measured 0.8 cm in diameter, well circumscribed, the cut surface was white, smooth and rubbery. Under microscopic inspection, it was a noncancerous, encapsulated tumor composed of fibrous and glandular tissue; following an intracanalicular pattern that the stromal proliferation predominates and compresses the ducts, which are irregular and reduced to slits (**Figure 1A**).

After two years, Color Doppler Ultrasound revealed the reappearance of a mass in the left breast. The patient stated a rapid development over the course of a month. Unilateral radical mastectomy was performed as an invasive treatment. The gross specimen measuring 7.5 × 5.0 × 6.0 cm appeared to be a circumscribed neoplasm. The cut surface appearance was yellow-grey, glistening and fibrous (**Figure 1B**).

Pathology review confirmed a tumor mainly characterized by spindle cells and scarce to almost unperceivable amounts of collagen, presence of increased and atypical mitotic activity accompanied with exaggerated stromal overgrowth and infiltrative borders, confirming a high-grade tumor (**Figure 1C**). An area of well-organized collagen fibers infiltrated by spindle cells seemed to be consistent with the scar produced by the previous surgery (**Figure 1D**). The first samples of the structure appeared to be sarcomatoid. Among mammary sarcomas of the breast, few of them lack features of a specific type of sarcoma. These tumors require extensive immunohistochemical reevaluation for best differentiation. In this regard, both tumors underwent immunohistochemical analysis for further investigation (**Table 1**).

The immunostaining performed in the first tumor (**Figure 2**), revealed moderate membranous expression of CD34 (2+) and weak cytoplasmic expression of SMA (1+). PanCK, EMA, CK 8/18 were all negative, as well as CD10, S-100, Calponin, p63 and EGFR. Ki67 index proliferation was as low as 0.04%.

The immunostaining profile for the second tumor (**Figure 2**) showed strong cytoplasmic and membranous CD10 expression (3+) in the spindle cells (**Figure 2C2**). Two other myoepi-

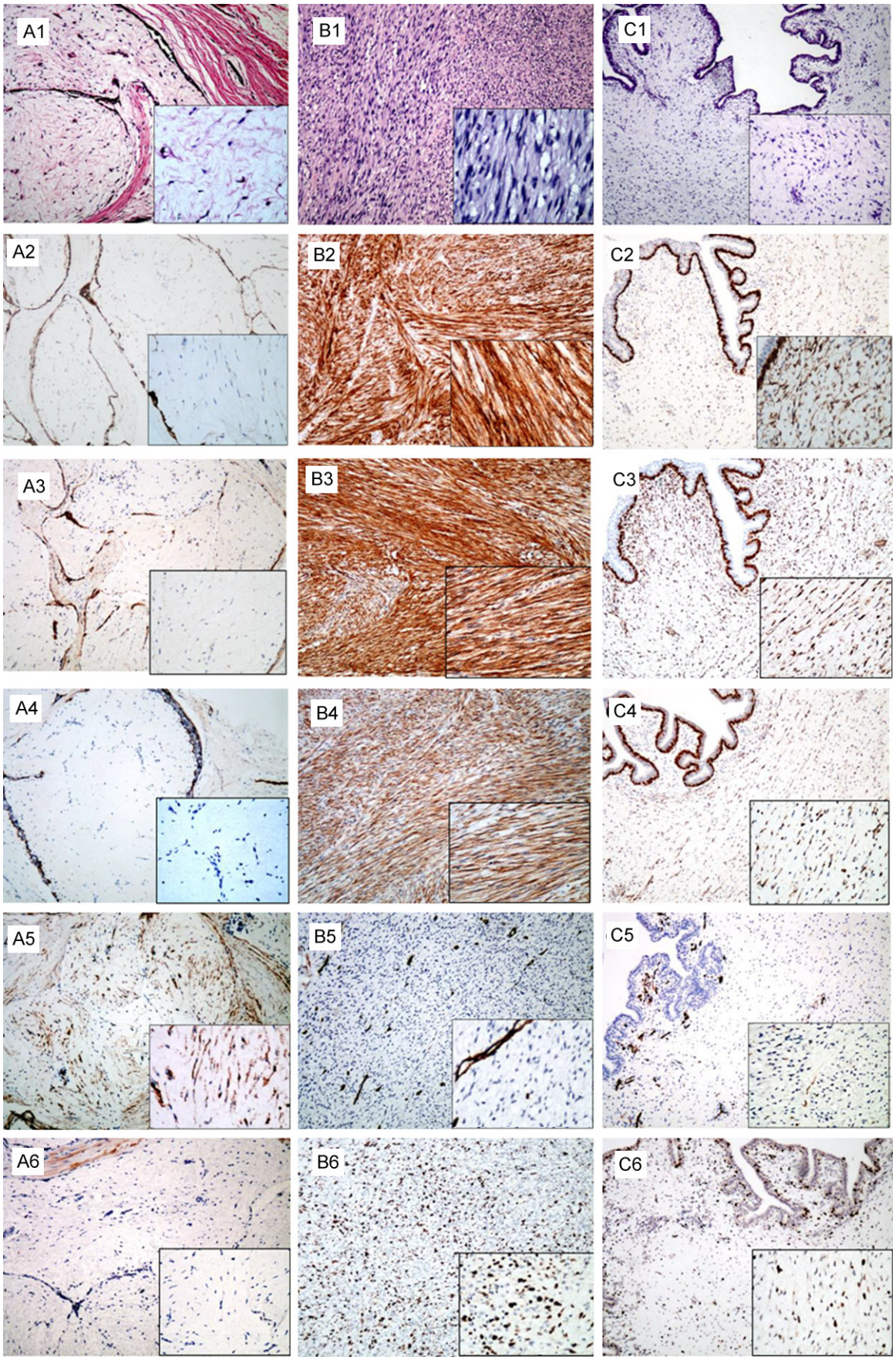
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Figure 2. Immunohistochemistry. A: Fibroadenoma. B: Spindle cells of the malignant tumor. C: Atypical leaf-like area of the malignant tumor. 1. H&E staining. 2. Cytoplasmic and membranous staining for CD10. 3. Cytoplasmic staining for SMA. 4. Cytoplasmic staining for Calponin. 5. Membranous staining for CD34. 6. Nuclear staining for Ki67. All pictures in x 100; inserted pictures in x 400 show the intensity of the respective staining in stromal cells.

Table 2. Immunohistochemical reactivity of positive markers

Marker	Malignant Phyllodes Tumor				Fibroadenoma			
	P	I	Q	IR	P	I	Q	IR
CD10	86	3	258	3+	—	—	—	—
Calponin	85	3	255	3+	—	—	—	—
p63	8	1	8	0	—	—	—	—
SMA	80	3	240	3+	28	1	40	1+
Vimentin	98	3	294	3+	—	—	—	—
S-100	—	—	—	—	—	—	—	—
MSA	—	—	—	—	—	—	—	—
CD34	—	—	—	—	65	2	130	2+
H-Caldesmon	—	—	—	—	—	—	—	—
CK14	—	—	—	—	—	—	—	—
panCK	—	—	—	—	—	—	—	—
EMA	—	—	—	—	—	—	—	—
CK8/18	—	—	—	—	—	—	—	—

SMA, smooth muscle actin; EGFR, epidermal growth factor receptor (Her-1); MSA, Muscle-specific actin; CK, citokeratin; EMA, Epithelial Membrane Antigen; P, % positive cells; I, intensity of staining; Q, Quick score. Intensity of staining: 1 = weak, 2 = moderate, 3 = strong. Quick score: Q = P*I, maximun 300. IR, Immunohistochemical reactivity: 0= ≤ 10; 1 + = 10-40; 2 + = 41-140; 3 + = 141-300.

thelial markers; calponin and SMA were strongly cytoplasmic positive in the spindle cells (3+), as well as Vimentin. EGFR and CD117 showed moderate cytoplasmic expression (2+). CD34 membranous expression was negative for the spindle cells. H-Caldesmon, MSA, S-100, p63, EMA, PCK, CK14, and CK8/18 were all negative. Ki67 index proliferation was more than 60%.

Striking differences of immunophenotype profile of both tumors were listed in **Table 2**. Some of the myoepithelial markers such as CD10, calponin, SMA and p63 were significantly upregulated and CD34 was downregulated. The strong expression of CD10 brings into account the possibility of NSCD10s and MCs with myoepithelial features. Liebl and Moinfar [8] reported 7 cases of NSCD10s where all tumors were positive for CD10 in addition to at least one myoepithelial marker of the combination CD29/SMA/P63/calponin, which they proposed as myoepithelial differentiation. It should be noted that there is no myoepithelial marker specific for myoepithelial cells. A study performed by Liebl et al [7] showed myoepithelial immunophenotype in 20 MCs, demonstrated by the presence of basal cell type CKs and the combination of myoepithelial markers CD10/p63/SMA/S100 that grant myoepithelial differentiation [7]. NSCD10s share the myoepithelial differentiation inherent to MCs, according to Liebl and Moinfar [8] and may represent the end of the MCs' spectrum. In our study, some of the most common markers (CD10, SMA, P63 and Calponin) used for myoepithelial immunophenotype were positive and considered to be effective inmerging sensitivity, specificity and ease of interpretation [10, 11]. The fact that CKs were negative clearly excluded the possibility of MCs with myoepithelial differentiation and considers the possible diagnosis of NSCD10s.

Further extensive sampling of the tumor revealed a focal presence of epithelial components (**Figure 1F**). At this point, we wonder whether it is an entrapped duct or a tumor component that suggests MPT; one of the most common malignant mammary fibroepithelial tumors [5]. As previously established, the epithelial component characterized by the leaf like biphasic structure is the more important diagnostic criteria for PTs [5]. Occasionally, some MPTs, especially the recurrent cases, may lack this structure due to an obvious stromal overgrowth [5, 7, 8]. In this case, the stromal overgrowth shows what could be an atypical leaf-like structure. FAs and PTs arise from the proliferation of mammary CD34 positive stromal and epithelial components [5], CD34 might help with the diagnosis [8], while as compared with FAs and benign PTs, MPTs displayed lower percentage of CD34 expression [8, 12-14]; this

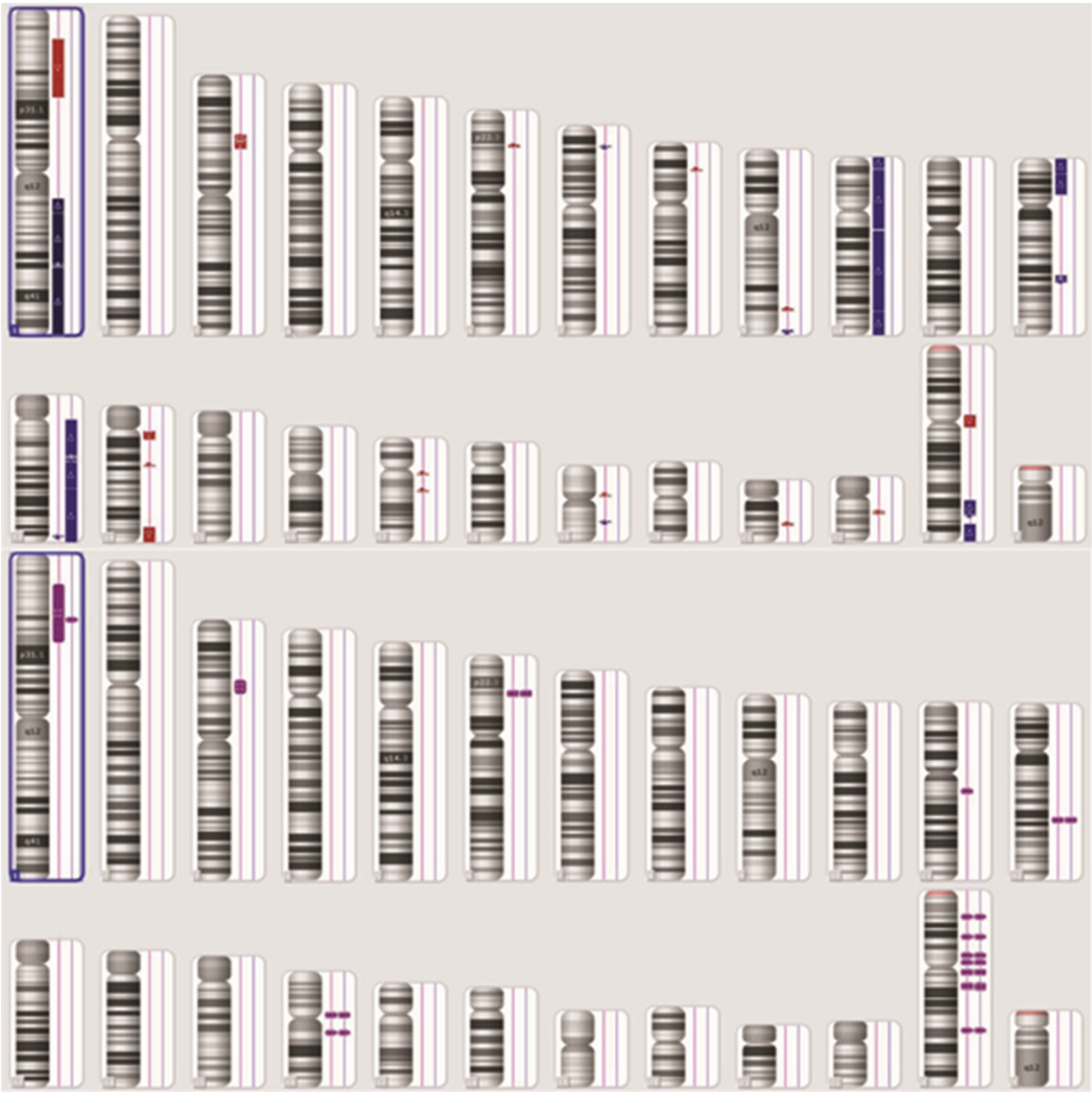

Figure 3. Comparative Genome Hybridization. The first sequence of chromosomes shows the distribution of the copy number variation in each chromosome, the breakpoints are indicated by arrows: blue = gain, red = loss. The Loss of heterozygosity breakpoints in the sequence of chromosomes above, is indicated by a purple star. For both pictures, the pink line next to the chromosome is expressing data from fibroadenoma and the line blue is from MPT. All chromosomes are identified in the right corner at the bottom.

observation of loss of CD34 expression in high grade PTs is a feature underlined in the present study. On the other hand, expression of CD10 and CD117 as well as EGFR was upregulated in MPT as compared with those benign fibroepithelial tumors [8]. Some researchers speculate that CD10 might even grant specificity for predicting malignancy,although the second tumor of our case demonstrates strong expression of C10, CD117, EGFR and some other myoepithelial markers, which have never been previously identified in MPTs [15-17].

For differential diagnosis of MPT and NSCD10s, we further performed CGH in both tumors. FA has shown only 13q gain; whereas the second tumor has several structural chromosomal abnormalities: gain in 1q, 7p, 10, 12, 13q, 19q, Xq; and loss in 1p, 3p, 6p, 8p, 9q, 14q, 17q, 19p, 21q, 22q and Xp. The copy-number analysis detected Loss of Heterozygosity (LOH) that have been found in similar locations in both tumors (6p, 12q, 16 and X). Second tumor shows an extra LOH in 3p and 11q. Moreover, the mutation table disclosed activity in the

oncogenes KRASG12D and KRASG12V in the malignant tumor.

Some studies had found a variety of chromosomal alterations in FA, Ojopi et al [18] studied 24 cases and found aberrations in 5p, 5q, 7q, 10q, 13q and 18; Amiel et al [19] discovered alterations in 13q, 6q, 11p, 18p, 22q in just one case; while another did not discover any alteration in DNA copy numbers [5, 20, 21]; because of these variability, the data related to CGH in FA is still uncertain. Nikita et al [22], Jardim et al [23] and Jones et al [24] demonstrated different alterations in PTs independently among these chromosomal instabilities; 1q alteration is seen in all of them and it is characteristically found in borderline and malignant categories. This finding further supports the diagnosis of MPT as well as several correlations of loss and gains (**Figure 3**). 1q has been reported to be present in a variety of malignancies as well as in breast carcinomas and is a constant finding in this entity [5, 25]. No specific chromosomal aberrations to PTs have been identified so far, gains of 1q have emerged as the hallmark alterations in the previous studies including the current one.

Conclusion

In summary, we reported the first case of a malignant phyllodes tumor with unique morphology of myoepithelial phenotype. The presence of atypical epithelial structure is noted to distinguish it from the so called NSCD10s with myoepithelial differentiation. The presence of the patient's clinical history, which initially suggested a benign or borderline lesion that morphed into asarcomatous overgrowth, is one of common phenomena in MPTs. In our opinion, the presence of the epithelial component, rule out NSCD10s and MCs, and promote the diagnosis of MPTs that exhibit myoepithelial immunophenotype irrespective of its heterogeneous morphology.

Molecular properties are crucial in the diagnosis. The use of CGH in this case has identified new regions of chromosomal gain and deletion; 1q that has been proved to be an important characteristic of PTs. These results may help subsequent studies to generate new insights in the pattern of genetic alterations that perhaps are associated in the progression from FAs to MPTs with the presence of myoepithelial differentiation. Our research only involved one patient, in the future; more cases shall be obtained to furtherthe investigation.

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Written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of the written consent is available for review.

Disclosure of conflict of interest

None.

Abbreviations

PTs, Phyllodestumors; FA, Fibroadenoma; MPTs, Malignant Phyllodes tumors; CD10, Cluster of differentiation 10; NSCD10s, Not-Otherwise specified type sarcoma with CD10 expression; CD29, Cluster of Differentiation 29; SMA, Smooth muscle actin; MCs, Metaplastic carcinomas; CD34, Cluster of differentiation 34; PanCK, Pan cytokeratin; EMA, Epithelial membrane antigen; CK, Cytokeratin; EGFR, Epidermal growth factor receptor; CD117, Cluster of differentiation 117; MSA, Muscle-specific actin; CGH, Comparative Genomic hybridization; LOH, Loss of heterozygosity.

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