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Summary

Myoepithelial cells (MECs) constitute a continuous layer of cells surrounding the breast glands, localized between the epithelial cells (ECs) and the basal membrane. MECs play important roles in normal mammary gland as they produce basal membrane and stimulate secretion. During neoplastic transformation, MECs act as a barrier preventing stromal invasion.

MECs themselves can undergo a great variety of changes, ranging from hyperplastic to metaplastic, to neoplastic, and giving rise to a wide spectrum of morphological pictures sometimes difficult to interpret on routine diagnoses.

Several benign and malignant breast tumours can present features of MECs differentiation.

As these latter tumours are quite infrequent, the purpose of the present paper is to offer a review of the morphological spectrum of MECs lesions, with correlations to prognosis.

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Introduction

The breast glandular tree is entirely lined by a double cell layer composed of epithelial cells (ECs) and myoepithelial cells (MECs) these latter located between the basal membrane and the epithelial cells. Topographically speaking, the ECs are luminal in orientation, and the MECs are the basal cell layer.

The MECs present as elongated or oval cells, that can be deeply eosinophilic (a sign of myoid differentiation), clear, or dark with minimal cytoplasm; they contain contractile filaments and cytokeratins (1,2).

MECs can be easily seen on haematoxylin and eosin (H&E) stained slides, but they are better identified on immunohistochemistry using more or less specific makers. A great variety of immunohistochemical MEC markers are now available, among which smooth muscle actin (SMA), calponin, smooth muscle myosin heavy chain (SMMHC) and CD10 are the most reliable (3). Among others, p63/p40, S100, maspin and podoplanin (D2-40) can also be used. Antibodies to high molecular weight cytokeratins (HMW CKs) can also be applied to evidence MECs. Antibodies

staining contractile filaments have the advantage to be more specific MEC markers, but they stain also stromal myofibroblast. Anti-HMW CKs are similarly rather specific but they show some variations in staining distribution (3), like the low expression of CK14 in MEC surrounding the normal acinar structures (4) or their expression in epithelial cells (5,6). In addition, a number of other immunostains used for other purposes may emerge as preferentially labelling the MECs. For example, SOX10, used as a marker of breast origin in triple negative breast carcinomas, labels the MECs (7); EGFR or CD117(c-kit) also stain these cells, and have been implicated to highlight the myoepithelial differentiation (8); glial fibrillary acidic protein (GFAP) may also stain MECs (9); and the list is much longer.

MEC normally produce basal membrane and favour secretion in lactating breast. In addition, several studies demonstrated that MECs constitute a dynamic barrier preventing the stromal invasion of neoplastic cells (Reviewed in references 10 and 11.) and playing a crucial role in the transition between in situ and invasive breast carcinoma (12). The MEC phenotype is modified in the BRCA1 and BRCA2 germline mutation carriers, they lose the co-expression of p63 and TCF7 transcription factors, which further supports the role of MECs in the malignant transformation (13).

In daily practice, MEC presence is a useful marker to differentiate in situ from invasive carcinoma (14). Nevertheless, care should be paid as benign and non-invasive apocrine lesions can show MEC reduction and occasional complete loss. Therefore, at least 2 MEC markers should be used especially when evaluating apocrine lesions without obvious MEC layer (15). Furthermore, in spite of the use or more MEC markers, some benign looking apocrine lesions (16,17) can completely lose the MEC cell layer (Fig. 1 and Fig. 1S).

As the title of our review refers to myoepithelial phenotype, it is worth defining what characteristics of MECs we mean by this. In the current oncology and pathology literature, the gene-expression profile based basal-like breast carcinoma term refers to MECs (myoepithelial features) in contrast to luminal carcinomas, these latter displaying characteristics of ECs. Therefore, a relatively high proportion of triple-negative breast carcinomas (defined as such on the basis of absence of oestrogen receptors, ER; progesterone receptors, PR; and human epidermal growth factor receptor-2, HER2) also display MEC markers, and correspond to basal-like carcinomas (18). This paper will omit such tumours and will concentrate on lesions where myoepithelial differentiation is manifested by the presence of hyperplastic or neoplastic MECs both by morphology and immunophenotype (i.e. the display of MEC markers). It is noteworthy that MECs are generally lacking ER, PR and HER2, but MECs may show a variety of phenotypic changes in pathologic conditions including the expression of steroid hormone receptors or the loss of some MECs markers (19,20).

MEC lesions discussed here, therefore, show histologic evidence of MECs and display either a genuine or an aberrant MEC phenotype by immunihistochemistry.

To conclude, the purpose of the present paper is to review the spectrum of benign and malignant breast lesions, showing the presence of MECs.

Myoepitheliosis / Myoepithelial hyperplasia

MECs can undergo a great variety of changes ranging from hyperplastic to metaplastic or neoplastic (21), sometimes being difficult to correctly identify on pure morphological basis.

Tavassoli (21) coined the term “myoepitheliosis” to indicate a MEC proliferation in the peripheral duct system. Since then, the term myoepitheliosis has been rarely applied in the literature (22,23), nevertheless, it is not uncommon to see MEC hyperplasia (a synonymous, and currently

preferred term) in common breast benign lesions, as intraductal papillomas (Fig. 2S) and sclerosing adenosis.

We recently observed a case of myoepitheliosis associated with mammographically detected microcalcification (Fig. 2).

When MEC hyperplasia is prominent, the differential diagnosis with tumours, as adenomyoepitheliomas (AMEs) can be difficult. Based on the fact that some AMEs carry mutations in *HRAS* family gene (24), Pereja et al. (25) demonstrated that the mutation specific antibody anti-RAS Q61R is helpful in the differential diagnosis between neoplastic and non-neoplastic myoepithelial proliferations.

Another non-neoplastic condition, correlated with MEC proliferation is collagenous spherulosis (CS).

CS is a benign condition originally described by Clement et al. (26) as a basal membrane deposition produced by MECs present in usual duct hyperplasia/epitheliosis (UDH/EP)(27). CS has been rarely reported in the literature and knowledge is mainly based on single case reports, with only one paper reporting on a large series (28). In daily practice CS maybe accidentally encountered in specimens resected for other reasons or in biopsies obtained from screen detected lesions. Therefore, it can be challenging to make a diagnosis of CS, especially in biopsy specimens. CS can affect women of all ages, being localized in any breast quadrants. It can present alone or in association with other breast lesions. CS alone can present as a clinically detectable mass (28) or as mammographically detected parenchymal distortions and/or microcalcification. Most often CS can be seen in association with benign lesions or at the periphery of breast carcinoma. On very rare occasions, it can be intermingled with in situ lobular carcinoma (29) or, even rarer, with in situ duct carcinoma (30).

CS is characterized by spherical inclusions in ducts or lobules with UDH/EP (Fig. 3). The inclusions are fibrillary, with delicately arranged fibrils and a more eosinophilic peripheral rhyme. Immunohistochemistry demonstrates the presence of epithelial and myoepithelial markers, the latter lining the spherical inclusions. The spherical inclusions stain positively for basal membrane markers as laminin and collagen IV.

Differential diagnoses comprise cribriform ductal carcinoma in situ (DCIS) and adenoid-cystic carcinoma (AdCC). Cribriform DCIS is easily differentiated as the myoepithelial cells are not part of the lesion, but present at the periphery of the involved ducts or lobules. In addition, ER is diffusely positive in cribriform DCIS, while ER is present in scattered cells only in CS.

Differential diagnosis between AdCC and CS can be quite difficult, as both lesions show a similar cell composition, to the point that Wells et al. (27) hypothesized that CS is a precursor of AdCC. Nevertheless, in daily practice, it is very important to differentiate the two lesions (31), as CS is benign and no proofs of malignant transformation have been published to date. CS is an intraductal lesions, associated with features of UDH/EP.

Tumours composed of myoepithelial cell

Benign Myoepithelioma (BM)

Benign tumours with pure MEC differentiation are extremely rare (32), and only a few examples are on record. BM affects adult female patients, presenting with nodular or cystic lesions.

On histology (Fig. 4) BMs are composed of elongated or epithelioid cells, sometimes with clear cytoplasm, with no atypia. Margins are usually well defined, even if they were reported to be infiltrative in one case (33).

Ultrastructural examination (33) and, more recently, immunohistochemistry is necessary to demonstrate pure MEC differentiation. MECs markers are diffusely positive.

Prognosis is difficult to predict as a few cases only have been reported (32). Most of the cases followed a benign course, but one case recurred three times (34), and one case diagnosed as BM gave rise to metastases in spite of the benign appearance (35).

Malignant myoepithelioma (MME) (synonim Myoepithelial cell carcinoma)

Pure MEC differentiation has been rarely reported in breast carcinomas (reviewed in reference 32). MMEs reorted to date, affected adult female patients, presenting as breast nodules (36). MME can be multifocal (37). MME can present as malignant transformation in AME, as discussed above.

On histology, MMEs are characterized by proliferation of markedly atypical spindle or round cells (Fig. 5). Atypical mitotic figures and necrosis are frequent.
Positive immunohistochemical findings of MEC markers is needed to reach a final diagnosis. Differential diagnosis between MME and metaplastic carcinoma, with spindle cell appearance can be difficult to objectify, as they share similar immunohistochemical profile. Indeed, MME can be one type of spindle cell metaplastic carcinomas according to the WHO 2019 (38).

The rare MME reported behaved in an aggressive fashion, with a high rate of systemic metastases and tumour-related deaths (32,39).

Tumours with mixed epithelial and myoepithelial phenotype

Rare cases of lobular in situ and invasive carcinoma of the breast, composed of cells showing dual epithelial and myoepithelial ("myosecretory") differentiation have been described (40).

Three cases of in situ lobular carcinoma composed of clear to eosinophilic cells, showing double immunoshitochemical positivity for epithelial membrane antigen (EMA) and SMA were reported. The "myosecretory" cells were located at the periphery of the neoplastic glands and showed fragmented E-Cadherin positivity.

Since the original publication no other cases have been described, therefore it is difficult to assess the possible prognostic meaning of myosecretory differentiation.

MECs changes, similar to those described as "myosecretory" have been described in association with lobular in situ carcinoma (41).

Tumours with dual epithelial and myoepithelial cell differentiation

Pleomorphic adenoma (PA).

PA is the most common benign tumour of the salivary glands, while it's very rare in the breast, with less than 100 cases reported.

Breast PA shows the same histological features observed in salivary glands, being characterized by epithelial and myoepithelial cells with variable cytological and architectural features laying in a chondromyxoid stroma (38).

Breast PA usually affects adult female patients, but, on rare occasions, it can also arise in males (42).

It is usually located in the retro-areolar area, not excluding other breast sites (43)

Pre- and intra-operative diagnosis can be difficult, both on imaging (43,44) and fine needle aspiration cytology (FNAC) or frozen sections (45).

On histology, PA shows the same morphological features observed in salivary glands. It presents as a nodule, with lobulated but well defined margins. ECs and MECs, devoid of any atypia are arranged in glandular structures, immersed in a chondromyxoid stroma. Sometimes PA presents an intraductal growth, thus expanding through the breast lobe.

Immunohistochemical demonstration of the double cell layer (EC and MEC) lining the glandular structures is of outmost importance to differentiate PA from metaplastic carcinoma (38).

Similarly, to the salivary gland counterpart, breast PA can show PLGA1 rearrangements (46).

Prognosis is usually good. Diaz et al (47) reported 10 cases, treated with surgical excision alone, that did not show recurrences or metastases, 4.9 years after diagnosis. However, recurrences have been described by John et al. (48), probably as a consequence of the multifocal intraductal growth. Therefore, surgical excision with a rim of normal breast tissue is suggested.

Malignant transformation of breast PA has been described on rare occasions only (49,50).

Adenoid-cystic carcinoma (AdCC)

Breast AdCC is an invasive carcinoma composed of ECs and MECs arranged in different architectural patterns (tubular, cribriform and/or solid) associated with basophilic matrix and reduplicated basement membrane material, frequently associated with *MYB-NFIB* fusion (38).

Breast AdCC usually arises in adult or elderly women (51,52,53) but rare cases have been reported in young women (54) and men (55). It can affect all breast quadrants, with a slight predilection for the retro-areolar region, sometimes associated with nipple discharge (51,52,53). On rare occasions it can be multifocal (56).

Preoperative diagnosis can be difficult. On conventional mammograms it presents as a solid neoplasm with well-defined margins, thus simulating a fibroadenoma (57,58). FNAC can be difficult to interpret, while a correct pre-operative diagnosis is better obtained on needle core biopsies.

AdCC can present three different histological patterns: classical AdCC (C-AdCC), solid-basaloid AdCC (SB-AdCC) and AdCC with high grade transformation AdCC (HG-AdCC).

C-AdCC (Fig. 6) is characterized by tubular, cribriform and solid architectural growth patterns. The morphological aspect common to all patterns is the presence of lumina and pseudolumina. Lumina are lined by ECs and filled with epithelial-type of mucins. On the contrary pseudolumina are lined by MECs and filled with basal-membrane material. Usually the three different architectural patterns are intermingled with each other, with the cribriform and the solid patterns constituting the bulk of the tumour and the tubular pattern present at the periphery.

Immunohistochemistry helps evidencing the presence of ECs and MECs. ECs are stained with typical EC markers, as low molecular weight CKs (CKs 7/8) and EMA. MECs are positive with the classical MEC markers mentioned above. Attention should be paid to expression of CK 5/6 in epithelial cells (59).

Additional markers are of help, if correctly applied (60,61,62). CD117 is considered a marker of AdCC, as it shows positive membranous reactivity in the ECs, thus highlighting the lumina. However, it should be remembered that CD117 can be positive in other triple negative breast carcinomas, too (63). MYB labelling is very important, especially in the differential diagnosis with AME and CSs. Positivity for additional markers, such as SOX10 has been recently reported. Collagen IV and laminin can evidence the basal membrane filling the pseudolumina (review in reference 52). Traditionally, periodic acid Schiff (PAS) – alcian blue (AB) combined staining has also been used to highlight the difference between lumina and pseudolumina, as the true secretory material is PAS-positive, whereas the basement membrane material stains with AB.

SB-AdCC (64) (Fig. 7) is characterized by solid nests of markedly atypical cells, with numerous mitotic figures and necrosis. Areas of C-AdCC are usually present, even if focally and sometimes difficult to detect.

Both C-AdCC and SB-AdCC can present a multifocal type of growth.

HG-AdCC (Fig. 8) is a very rare condition, characterized by the appearance of a high grade type of carcinoma arising in AdCC (51).

Most AdCC cases fall in the "triple negative breast cancer" (TNBC) category, as they lack ER, PR and HER2 expression. Nevertheless, a minority of neoplastic cells, usually ECs can present ER positivity (65). ER protein expressed in AdCC can be the alpha-36 variant, a splice variant of the full length ER-alpha molecule playing a role in membrane-initiated ER signalling (66). Rare cases of androgen receptor (AR) positive C-AdCC are on record (67).

AdCC is characterized by molecular alterations on the MYB/MYBL1 pathway, the most common being the MYB-NFIB fusion gene, resulting from the t(6;9)(q22-23;p23-24) translocation (68,69).

These alterations have been detected mainly in C-AdCC and, on rare cases of SB-AdCC (70).

SB-AdCC cases presented, in addition to the classical MYB/MYBL1 pathway alterations, other differentially expressed genes, as the NOTCH and CREBB mutations that can be of therapeutic importance (71),

AdCC prognosis greatly depends on the AdCC subtypes and stage at presentation.

C-AdCC is usually detected at early stage as pT1/T2, with axillary lymph-node involvement rate ranging from 0 to 8% (reviewed in ref. 66), and has a very low distant metastases risk (66). Consequently, the 10-year survival rate is higher than 90% (53, 66, 72,73).

Nevertheless, care should be taken as the multifocal growth and the presence of a peripheral rim of tubular growth pattern, can lead to real size under-evaluation on pre-operative radiological imaging resulting in inadequate surgery (74). All these features can lead to higher rate of local recurrences. Furthermore, C-AdCC local recurrences can show transformation into more aggressive AdCC variants (51).

SB-AdCC carries a higher risk of tumour progression. Lymph-node metastases can be detected at presentation in up to 30% of the cases (75). Moreover, SB-AdCC has a faster progression time than the C-AdCC. A large study, based on a multicenter series, demonstrated that the mean progression time of SB-AdCC is 22 months while it reached 84 months in C-AdCC (75).

At the moment, a few data only are available on HG-AdCC prognosis. The morphological features of the carcinomatous components associated with AdCC features (metaplastic carcinoma with melanomatous differentiation and small cell carcinoma, TNBC), suggest an aggressive clinical behavior (51).

The value of ER positivity in AdCC is still poorly known; one paper only (76) described a higher local recurrence rate in ER negative AdCC cases.

Adenomyoepithelioma (AME)

AME is a biphasic tumour characterized by small epithelium-lined spaces with inner luminal ductal ECs and a proliferation of variably enlarged and clearly noticeable abluminal MECs. Malignant transformation may occur from either the luminal or myoepithelial component (38).

AME can affect individuals of a wide age range and both sexes with a predilection for elderly women (51). The classic presentation is of a palpable retro-areolar nodule, however with the increasing diffusion of mammography screening cases are starting to be seen screen detected.
Pre-operative diagnosis can be very difficult, as on radiological imaging AME simulates fibroadenoma and on FNAC is highly cellular simulating an aggressive tumour. According to the literature review by Chang et al (77), AME frequently mimics the FNAC features of a number of benign and malignant breast lesions, thus representing not only an important potential pitfall in the diagnosis of carcinoma but also a differential diagnosis to consider in a variety of breast lesions.
Core needle biopsy (CNB) is advised to reach a reliable diagnosis, though often it might only result in a differential diagnosis (e.g. papillary AME vs papilloma with MEC hyperplasia, see below).

Histological features of AME are quite variable and constitute a wide spectrum ranging from benign to malignant tumours, with intermediate aspects of difficult interpretation.
The common denominator and basis for the diagnosis is the finding of neoplastic glands and nests composed of ECs and MECs.
Previous papers (51,78) have tried to better define all the different AME features, as follows:
Classical AME (C-AME) (Fig. 9) is the more typical and better recognizable form. It is characterized by small glandular structures lined by an outer layer of prominent MECs and by and inner layer of EC often presenting apocrine differentiation. The neoplastic glands are lined by a thick basal membrane.
The MECs can have different features, ranging from elongated cells with clear cytoplasm to spindle eosinophilic cells. ECs can show sebaceous or squamous differentiation.
C-AME can have different architectural patterns, as lobulated, tubular and papillary. Lobulated C-AME is characterized by nodules with lobulated margins (Fig. 10). Tubular AME is composed of neoplastic glands haphazardly infiltrating the breast parenchyma. Tubular AME is differentiated from microglandular adenosis (MGA)(79) as it shows the double cell population (EC/MEC) lacking in MGA. (For historical completeness, tubular AME has been named apocrine adenosis in early papers (80,81,82), but the same name was also applied for sclerosing adenosis with diffuse apocrine metaplasia). Papillary AME is characterized by a polypoid intraductal growth along the breast lobe, thus ending in an apparently multifocal pattern. Papillary intraductal AME should be differentiated from intraductal papilloma with prominent myoepithelial hyperplasia. In daily practice AME shows a diffuse and prominent MEC proliferation along the entire lesion. When available, RAS Q61R mutation specific antibody or molecular characterization can help (24,25).

Atypical AME (A-AME) (Fig. 11) are considered those cases showing typical AME architectural patterns, but cytological atypical features affecting EC and/or MEC. Exact criteria to define cytological atypia in breast AME have not been well defined, therefore it is suggested to apply those proposed by Seethala et al. in epithelial-myoepithelial cell carcinoma of salivary glands (83). Accordingly, atypical cells are 3 times larger than the normal epithelial cells, have a large nucleus, granular chromatin and prominent nucleolus. Features of atypia are more frequently encountered in MECs.

Mitotically active AME (MA-AME) (Fig. 12) shows the same C-AME features, devoid of any atypia, but an increasing number of mitotic figures, up to 10 per 10 high power fields (HPF). The prognostic value of the high mitotic number is still debated. The AFIP book (84) suggests 3 mitoses per 10 HPF as cut-off to indicate a high recurrence risk. Nevertheless, this number has not been validated yet.

Malignant in situ AME (MIS-AME) (Fig, 13). This variant has been defined by Rakha et al. (78) as the presence of carcinoma in situ in AME. In MIS-AME the EC show features of ductal (DCIS) or lobular (LCIS) carcinoma in situ sometimes with apocrine differentiation, but no invasion of the stroma surrounding the AME mass is seen.
When DCIS and/or LCIS are seen outside the AME mass, they should not be diagnosed as MIS-AME. In practical terms, MIS-AME reflect DCIS and/or LCIS arising in/from AME.

Malignant AME (M-AME) (Fig. 14) is characterized by the malignant transformation of the EC and/or the MEC component.
M-AME can arise de-novo or result from malignant transformation of long standing C-AME (80). When malignant features involve both ECs and MECs, the term epithelial-myoepithelial carcinoma is applied (38). These lesions generally defeat the basic concept of malignancy in common breast lesions, where the presence of MECs is a feature against malignancy.
In cases showing malignant transformation of the MECs only, features are those of MME, being composed of spindle or epithelioid cells, with marked cytological malignancy features and frequent mitotic figures. Necrosis is often present.
A wide variety of patterns can be encountered, varying from different types of metaplastic carcinoma (39) from low-grade adenosquamous carcinoma to matrix-producing carcinoma, mucoepidermoid carcinoma, spindle cell carcinoma, carcinosarcoma, when malignant features involve the ECs (51).

The use of immunohistochemistry is of undoubted help to reach a correct diagnosis of AME, by the demonstration of the double EC/MEC components.
MEC express the typical markers, as p63, SMA, calponin, smooth muscle myosin heavy-chain, caldesmon and high molecular weight CKs as CK14 and CK 5/6. It is not uncommon to see loss of expression of some markers, and therefore a combination of markers is generally preferable. EC express the classical markers as EMA, low-molecular weight CKs (as CK7 and 8). Attention should be paid to the high molecular weight CK expression in EC (5).

AME can be ER/PR positive or negative, while HER2 is usually negative (24). AME molecular profile is characterized by mutations in phosphoinositide 3-kinase (PI3K) pathway genes and by mutations affecting the HRAS Q61 hotspot, these former being more frequent in ER negative AME (85,86,87). Recent data report EGFR gene amplification (88).

Prognosis is usually good in C-AME cases (38). However, C-AME with multinodular growth pattern or with papillary intraductal growth and satellite nodules can recur (89). In addition, very rare cases of metastasizing C-AME devoid of any atypia have been described (90,91).
Therefore, radical surgery with clear margins and follow-up is advised in all AME cases, comprising C-AME.
M-AME have higher recurrence and metastasizing risk. In M-AME cases prognosis depends on the features of the malignant component. More frequent metastatic sites are lungs, liver, bones and brain (89,92). Axillary lymph-node metastases are rare.

Low grade adenosquamous carcinoma (LGASC).

LGASC is considered a low aggressive variant of metaplastic carcinoma (38).

LGASC affects mainly adult women, arising in all breast quadrants. Size at presentation is usually small, with most of the cases being around 2 cm, but in a few cases, size can increase up to 8.6 cm (93).

On radiological examination, LGASC can be over-diagnosed (94) as conventional invasive carcinoma.

Preoperative diagnosis is quite difficult, both on FNAC and CNB. FNAC smears are quite cellular, thus simulating higher grade lesions (95). On CNB, the differential diagnosis with sclerosing papillomas and infiltrating epitheliosis remains a major issue to be solved.

On histology LGASC (Fig. 15) is characterized by an infiltrative pattern of growth, with neoplastic projection invading the surrounding breast tissue (96). It is composed of elongated glandular and tubular structures, with squamoid perls, laying in desmoplastic background richin spindle stromal cells. Neoplastic glands are lined by two cell layers, EC and MEC. Therefore, this is again a lesion challenging the basic concept of this double layer being a feature favouring benign lesions.

In rare cases LGASC was associated with intraductal papillomas (96); in addition, it can arise in AME (97).

Mitotic figures are rare, necrosis is absent.

Mature lymphocytes aggregates can be seen at the periphery of LGASC.

LGASC can be multifocal. However, apparently unifocal LGASC can have microscopic satellite neoplastic nodules at a distance from the main tumour area, separated by normal breast glandular parenchyma.

On immunohistochemistry, high molecular weight CKs, p40 and p63 are positive in the MECs and in areas of squamoid differentiation, while EMA and low molecular weight CKs are positive in the ECs.

LGASC is a TNBC of low aggressive potential, as it is ER/PR/HER2 negative. LGASC carries a distinct mutational profile, with high rates of PIK3CA mutations in absence of TP53 mutations (98).

Differential diagnoses include other metaplastic carcinomas, syringomatous tumor (syringoid adenoma) of the nipple and sclerosing lesions as infiltrating epitheliosis (IE). Conventional metaplastic carcinomas are easily excluded on the lack of marked atypia and low mitotic rate. On the contrary, differential diagnosis with syringomatous tumour of the nipple is more difficult. The two lesions share many similar features, but different locations, as LGASC arises in the deeper part of the parenchyma and syringoid adenoma typically affects the nipple area. IE is a benign condition originally described by Azzopardi (99), characterized by strands of UDH/EP invading the surrounding stroma. Most probably a relation exists between IE and LGASC, as they share similar molecular profile. Nevertheless, at the moment IE is a completely benign lesion, as no cases have been described with aggressive behaviour. Therefore, differential diagnosis between LGASC and IE is important in daily practice. At a variance of LGASC, IE is usually a circumscribed lesion, with well-defined margins. In addition, IE shows the focal ER positivity typically observed in UDH/EP, while LGASC is ER negative.

The prognosis of LGASC is usually good (92), when radical surgery with clear margins is achieved. Lymph-node metastases are extremely rare. Local recurrences are described in cases treated by local excision alone (96,100), with no attention paid to clear margins (92). One death is on record (96) related to local thorax invasion. Rare cases have been described to transform into conventional, aggressive metaplastic carcinoma (101).

Conclusions

MECs constitute an important component of the breast glandular tree. They can undergo a great variety of hyperplastic and neoplastic changes that can be of difficult interpretation in daily practice. Immunohistochemistry and molecular profile can help reaching a correct diagnosis, but numerous pitfalls can be encountered. Therefore, exact knowledge of the spectrum of MEC lesions is important to avoid misdiagnoses.

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Figure legends:

Fig. 1: Apocrine lesions devoid of MECs A: apocrine cyst lined by epithelium with no evidence of atypia; B) higher power view confirms absence of atypia, nevertheless there is no evidence of myoepithelial cells. C and D: absence of myoepithelial cells is confirmed by negativity with myoepithelial markers as CK14.

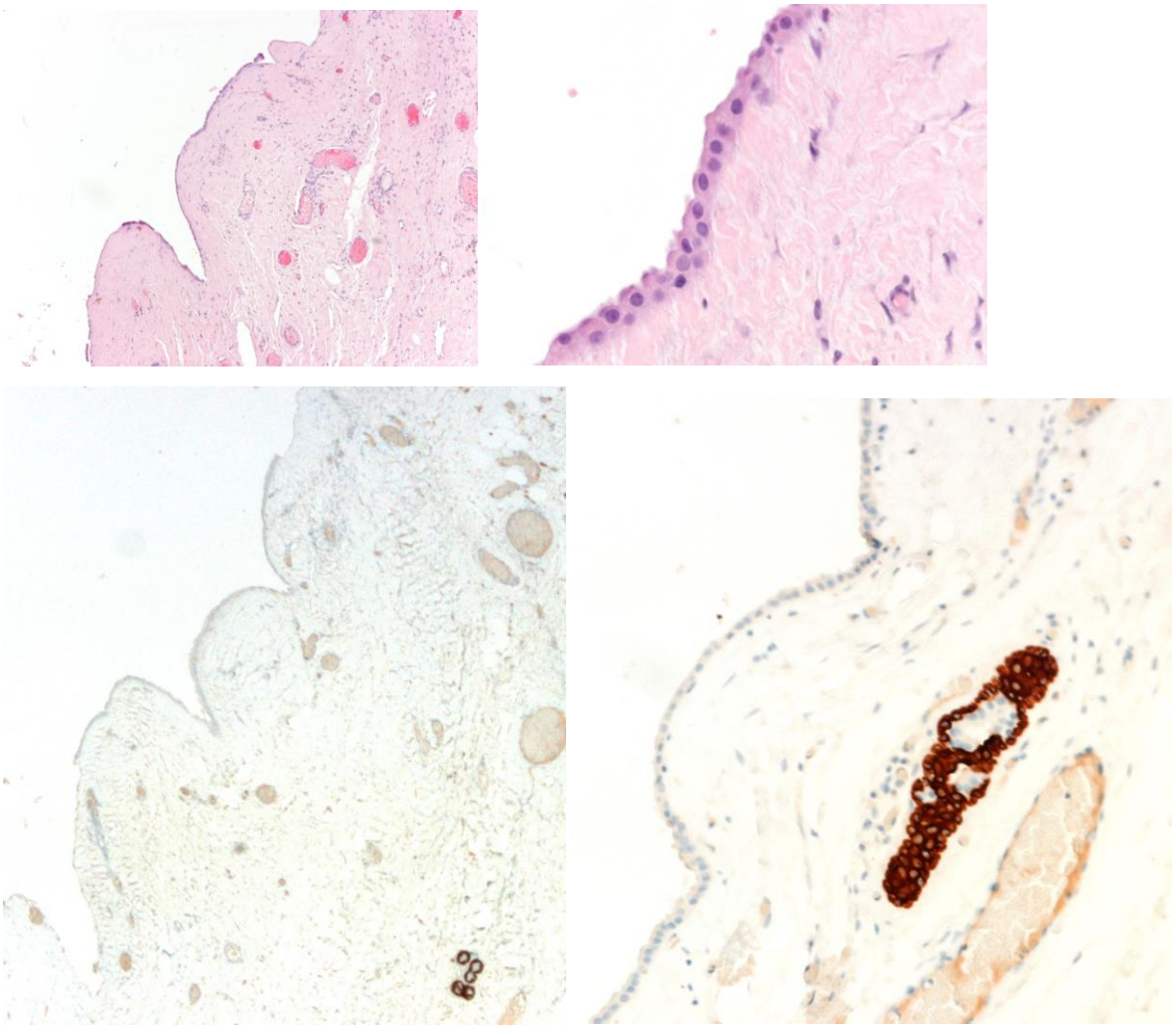

Fig. 2: Myoepitheliosis: a 53yrs old woman presented screen detected microcalcifications, classified as R3, Vacuum assisted biopsy was performed. On histology the lesion consisted in a TDLU with marked myoepithelial hyperplasia. A and B) On H&E Hyperplastic MECs were devoid of any atypia. C) Calponin confirmed the myoepithelial nature of the hyperplastic cells. D) Microcalcifications are localized on hyperplastic myoepithelial cells; E) as confirmed by Calponin.

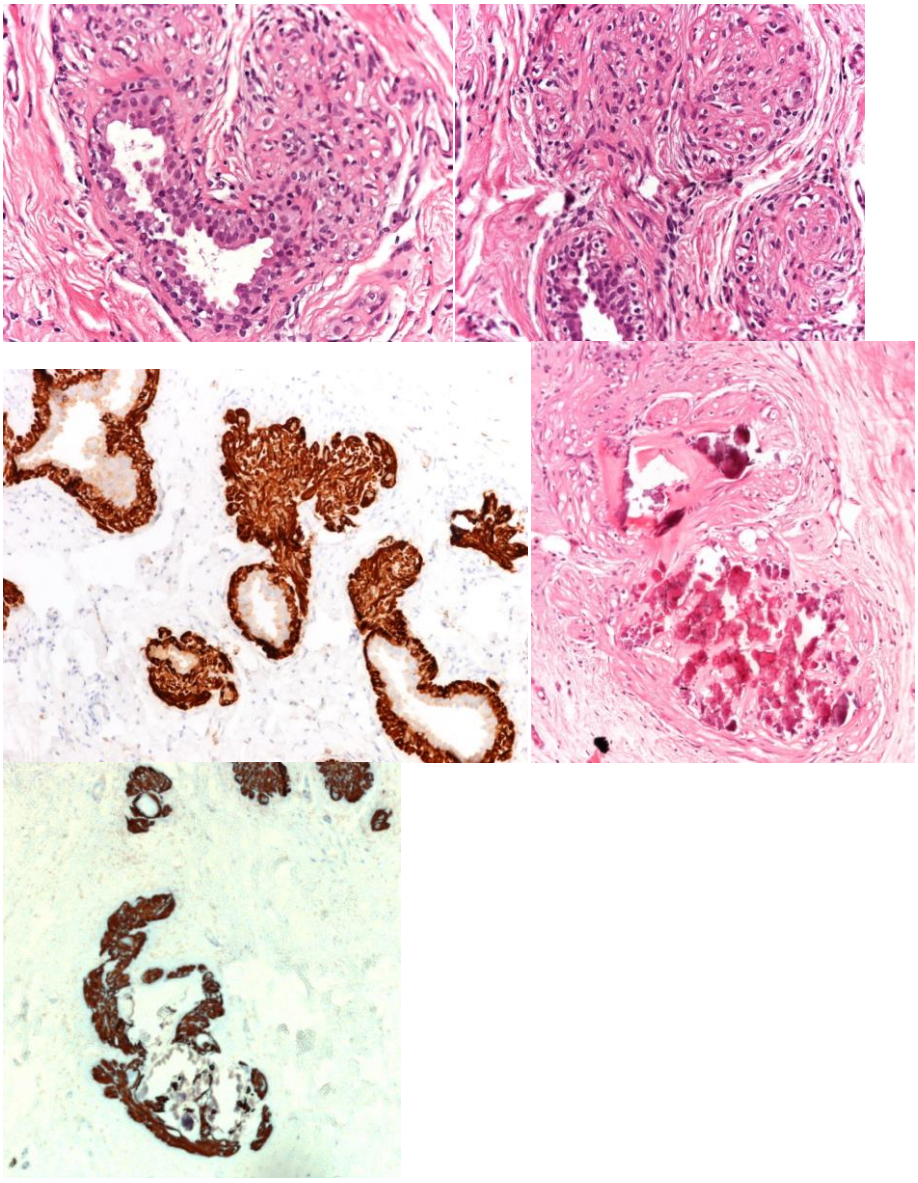

Fig. 3: Collagenous spherulosis: A) Collagenous spherulosis is characterized by enlarged TDLU, with cribriform appearance. Pseudoglandular spaces and filled with eosinophilic material, containing microcalcifications. B) CF at higher power, no atypia is seen. A thin rim of eosinophilic material lines the pseudo-glandular spaces; C: Immunostaining with calponin confirms the presence of myoepithelial cells; D: Collagen IV is strongly positive in the pseudoglandular spaces, thus confirming the presence of basal membrane.

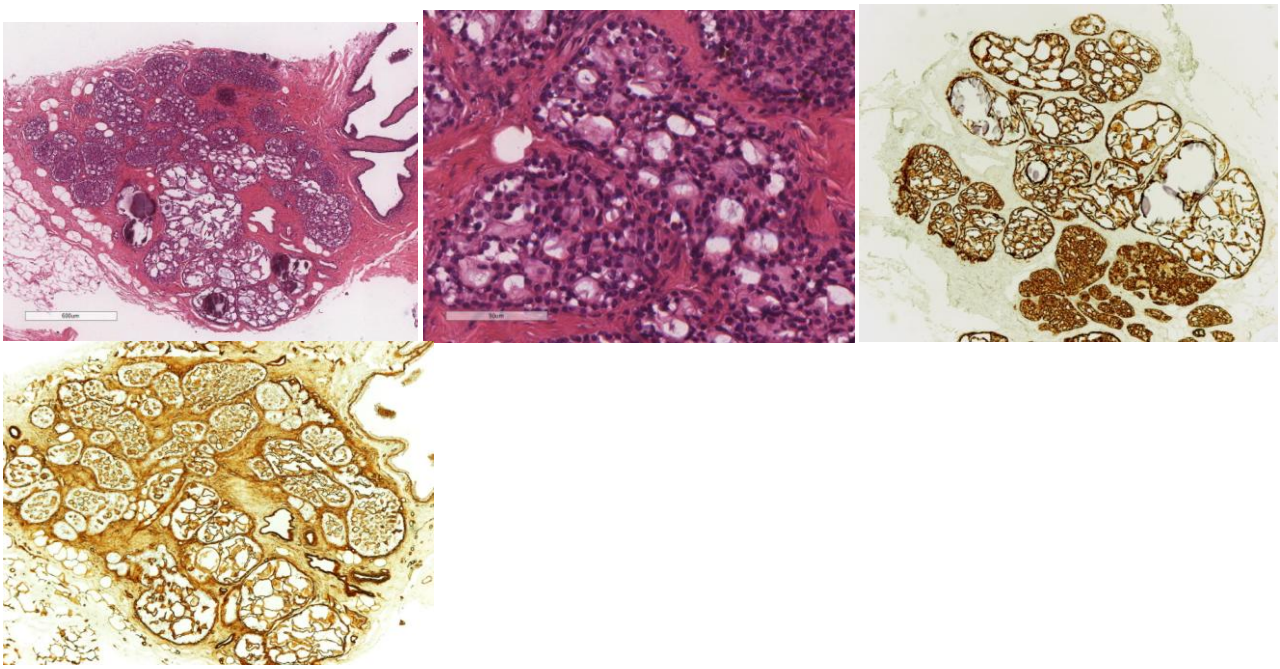

Fig. 4: Benign myoepithelioma: A: at low power is composed of spindle cells immersed in a myxoid stroma. It has well defined margins; B: at higher power the neoplastic cells are elongated and have eosinophilic cytoplasm; C: the neoplastic cells are strongly positive with myoepithelial markers as CK14; D: Calponin is also strongly positive.

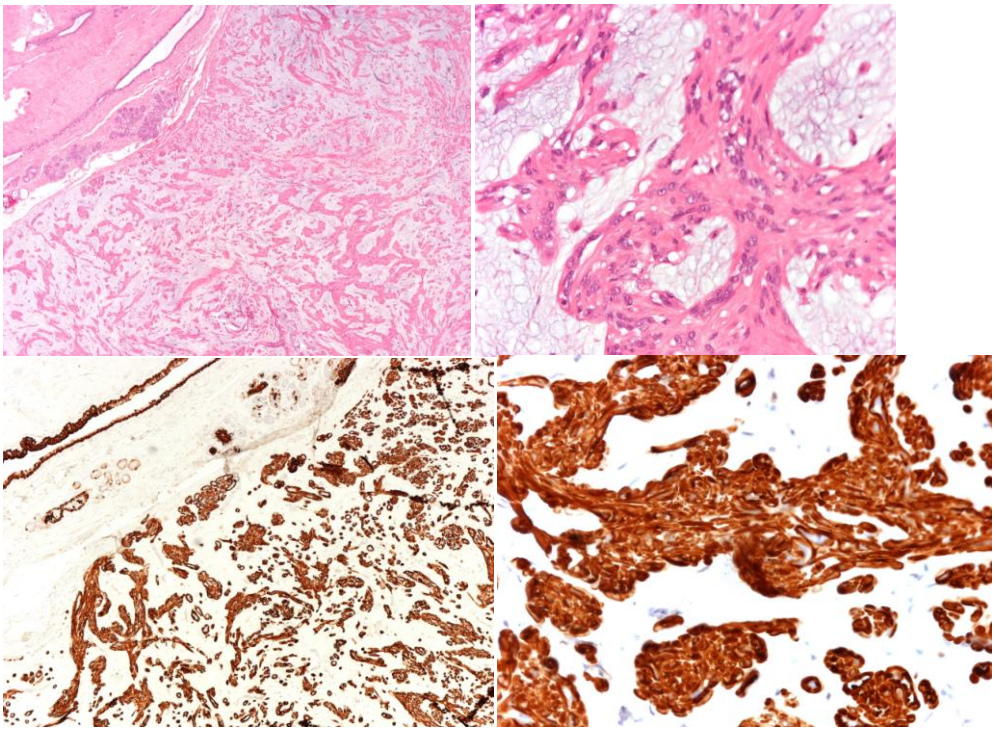

Fig. 5: Malignant myoepithelioma: A) this case is entirely composed of clear cells, with nuclear atypia. B) All the neoplastic cells were positive for myoepithelial markers as CK14; C) smooth muscle actin is also positive.

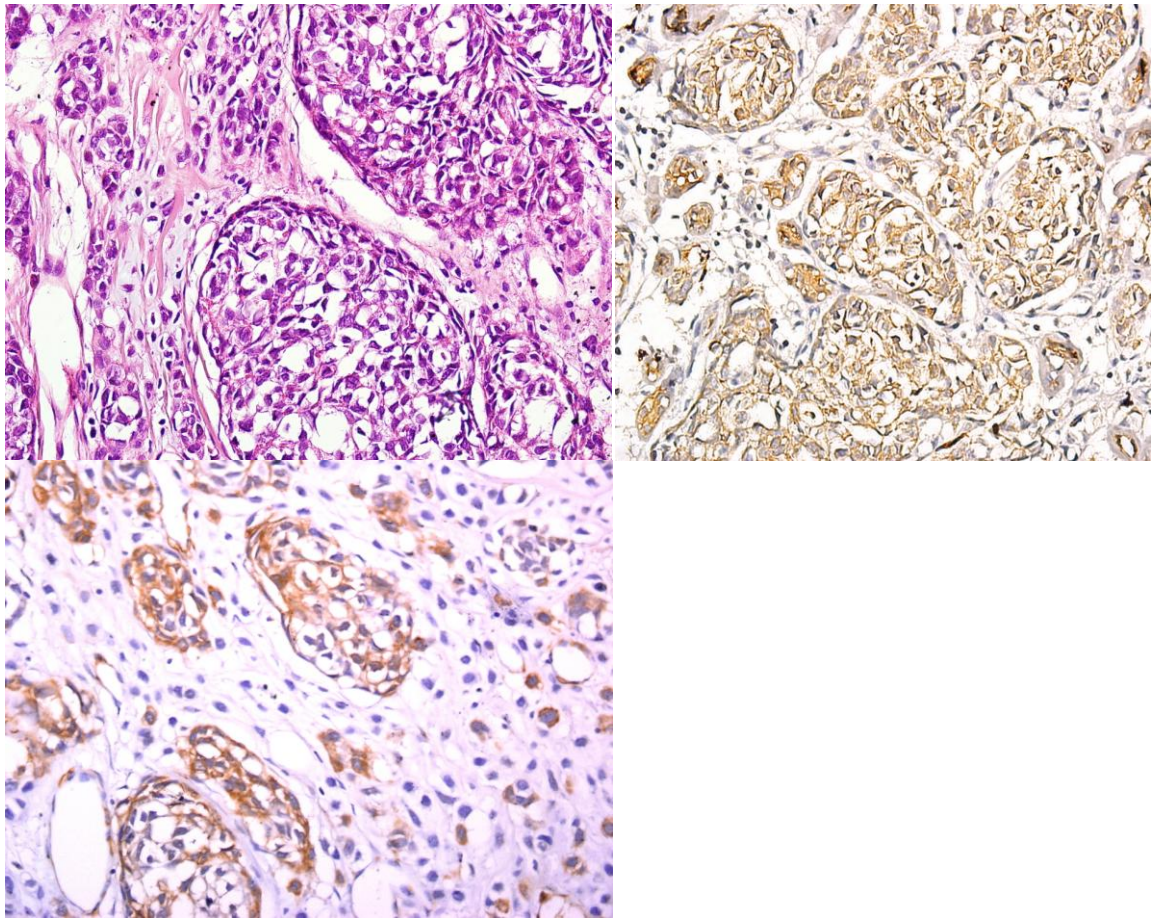

Fig. 6. AdCC classical type. A: at low power the cribriform area is surrounded by the tubular area.

B: at higher power the cribriform area shows the two types of mucins and the two types of cells, with epithelial cells surrounding glandular spaces (stars); C: At higher power the tubular area shows the same composition of the cribriform areas, with glands showing pseudoglandular spaces and glandular spaces (stars).

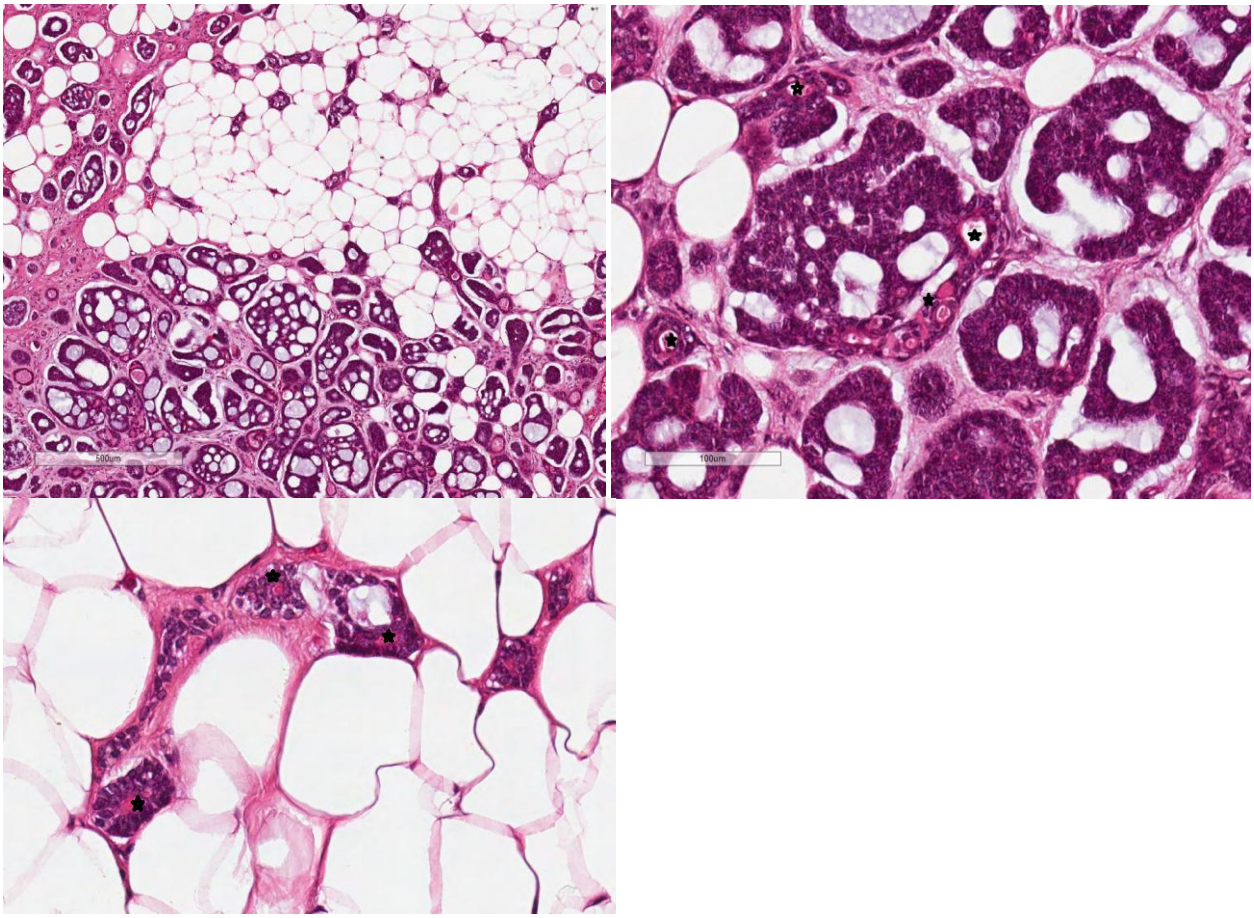

Fig. 7: SB-AdCC shows marked cellular atypia and necrosis.

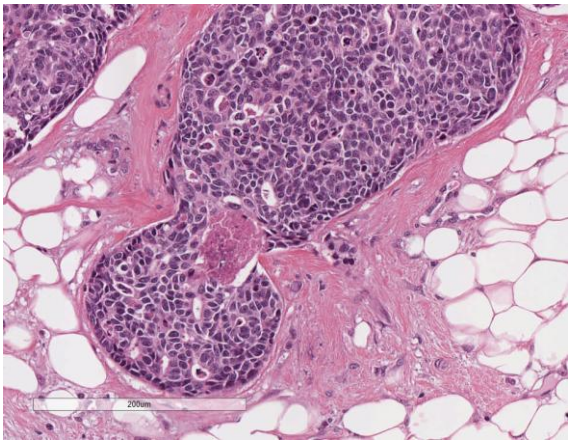

Fig. 8: HG AdCC shows markedly atypical cells and some remnants of AdCC features (arrow).

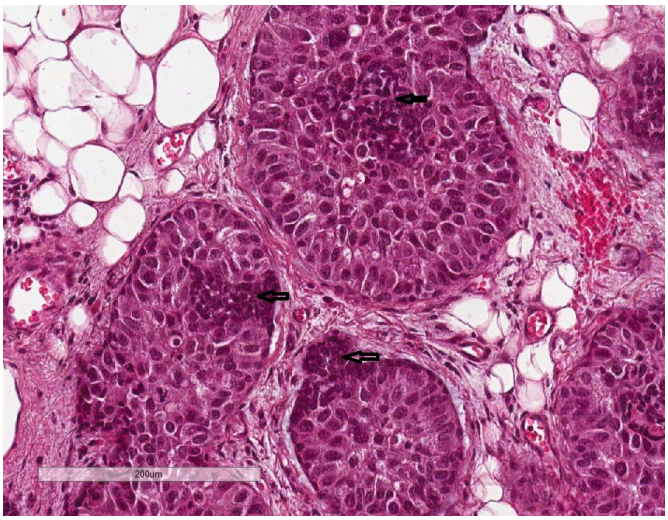

Fig. 9: C AME is characterized by glandular structures lined by double cell layer, ECs and MECs.

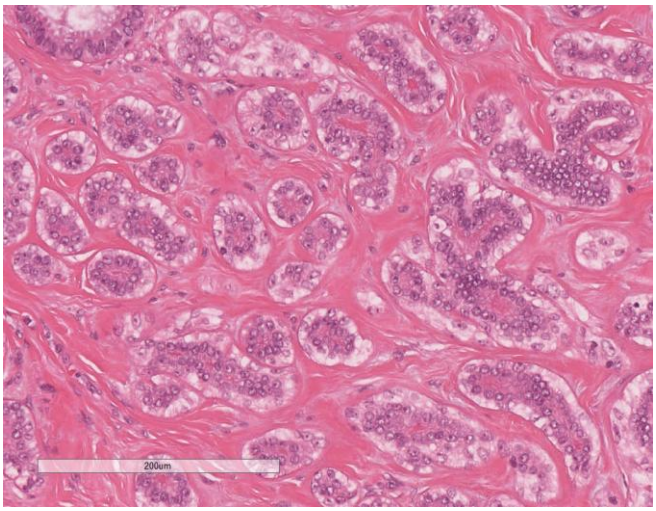

Fig. 10: Lobulated C-AME: A: on mammography the neoplasm appeared as a mass with well-defined margins and microcalcifications; B: the same lesion on H&E, shows well defined margins and a central area with basal membrane deposit and microcalcifications; C. At higher power the lesion shows a prominent myoepithelial layer, as evidenced by smooth muscle actin.

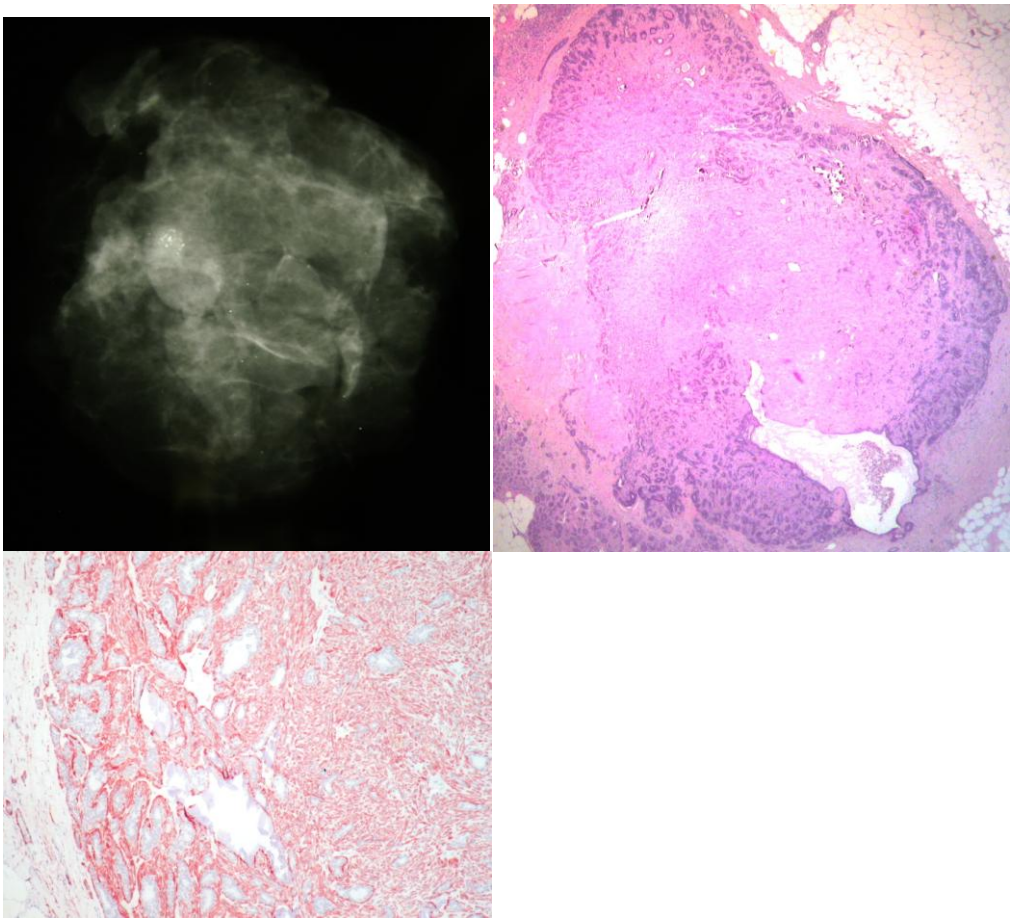

Fig. 11: Atypical AME: the cells are large; nuclei are atypical with prominent nucleoli.

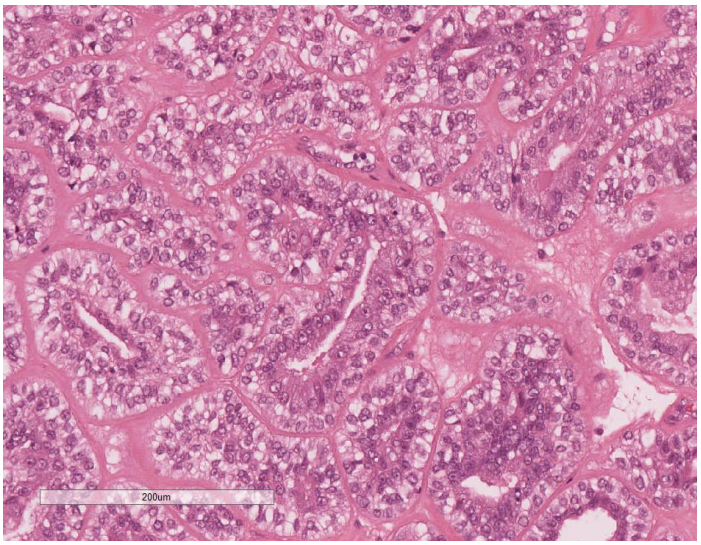

Fig. 12: Mitotically active AME: numerous mitotic figures are present (arrows) in absence of specific atypical features.

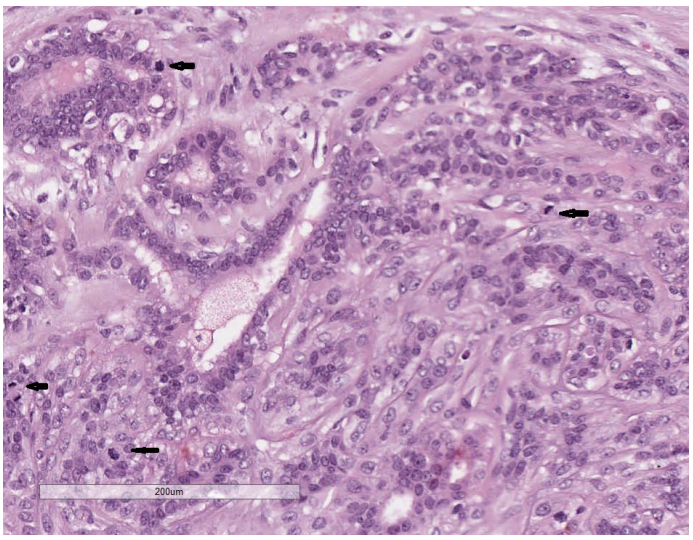

Fig. 13: MIS AME: A) Features of duct carcinoma in situ are present, associated with AME features. B) Calponin confirms the presence of prominent MEC layer.

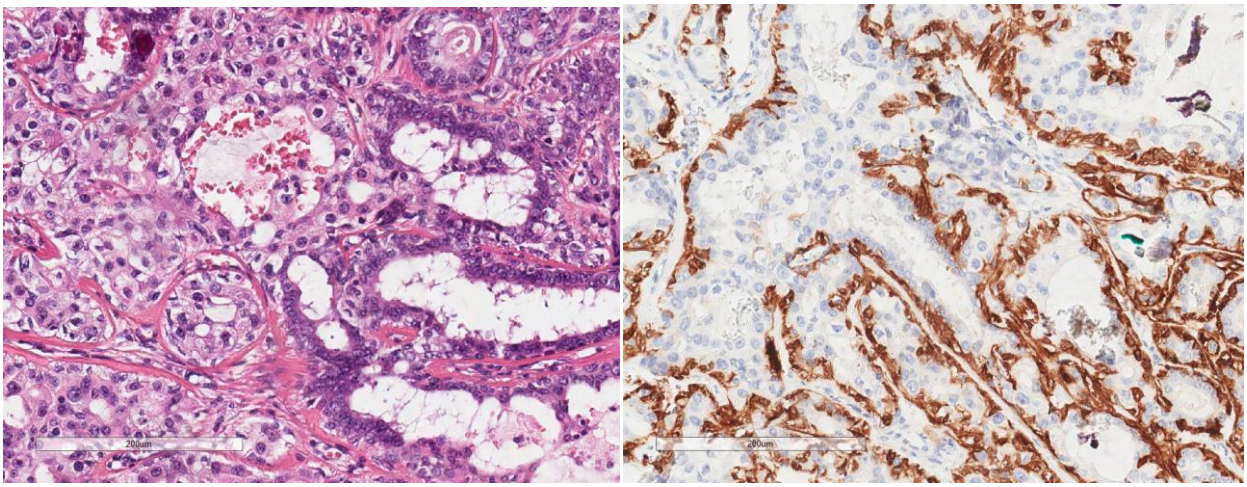

Fig. 14: Malignant AME: A) AME with malignant transformation in both epithelial and myoepithelial cells. Both components show marked nuclear atypia; B) same case presenting atypical mitotic figures in both epithelial cells and myoepithelial cells; C) same case showing focally a malignant spindle cells component; D) Double immunostaining with low molecular weight cytokeratin (CAM 5.2) in red and p63 in brown (Courtesy Dr. G. Collina, Ascoli Piceno, Italy) evidences the malignant spindle cells. E) CK 14 stains both ECs and MECs.

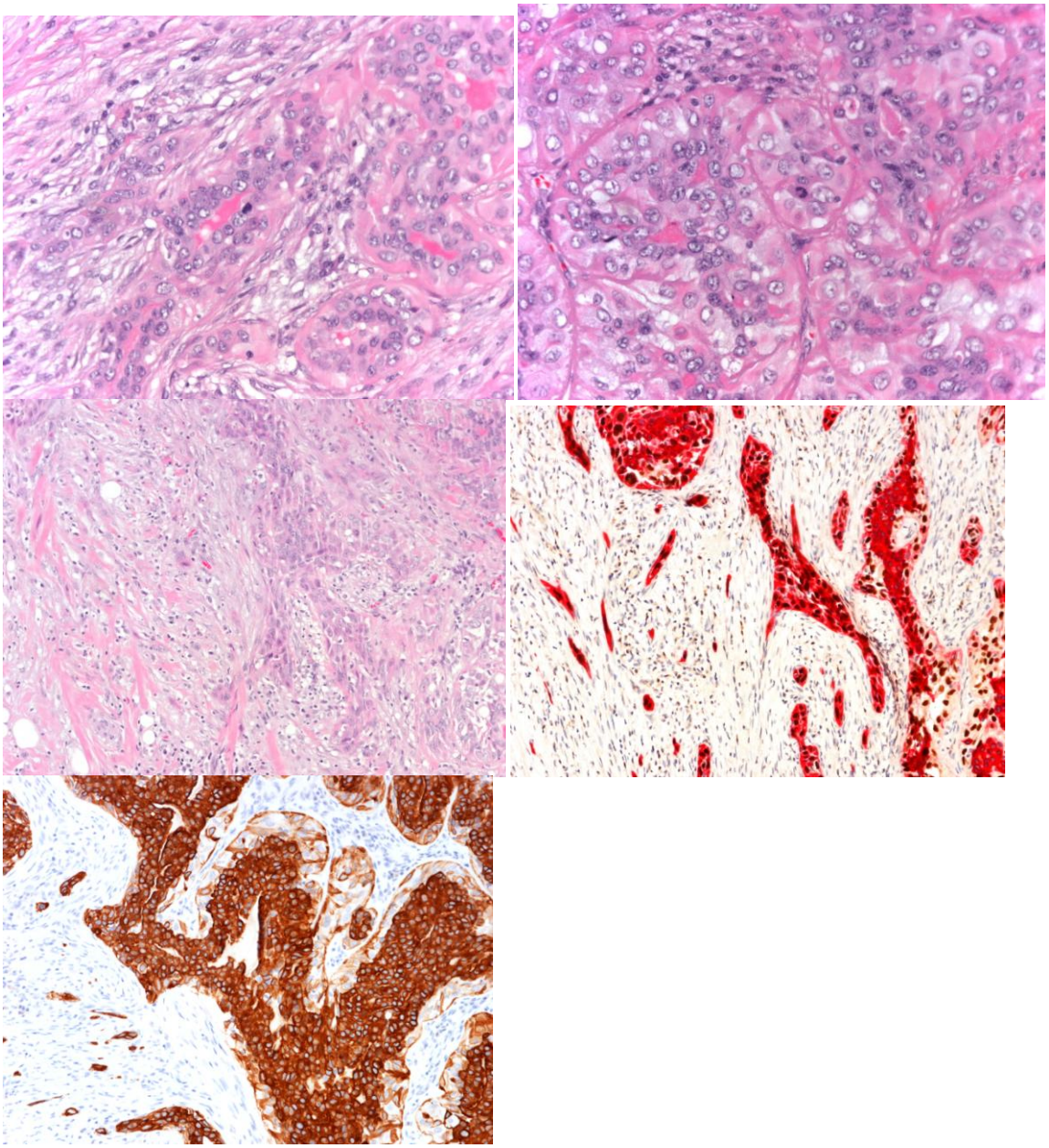

Fig. 15: LGASC: A) At low power it is evident that the lesion is composed of multiple neoplastic areas (arrows), separated by normal mammary glands; B) LGASC neoplastic glands are elongated, with rounded contours. They can contain mucoid material. C) neoplastic glands, focally contain keratin material; D) LGASC can be surrounded by a lymphocytic rim. Small neoplastic glands (arrow) are present outside the lymphocytic rim.

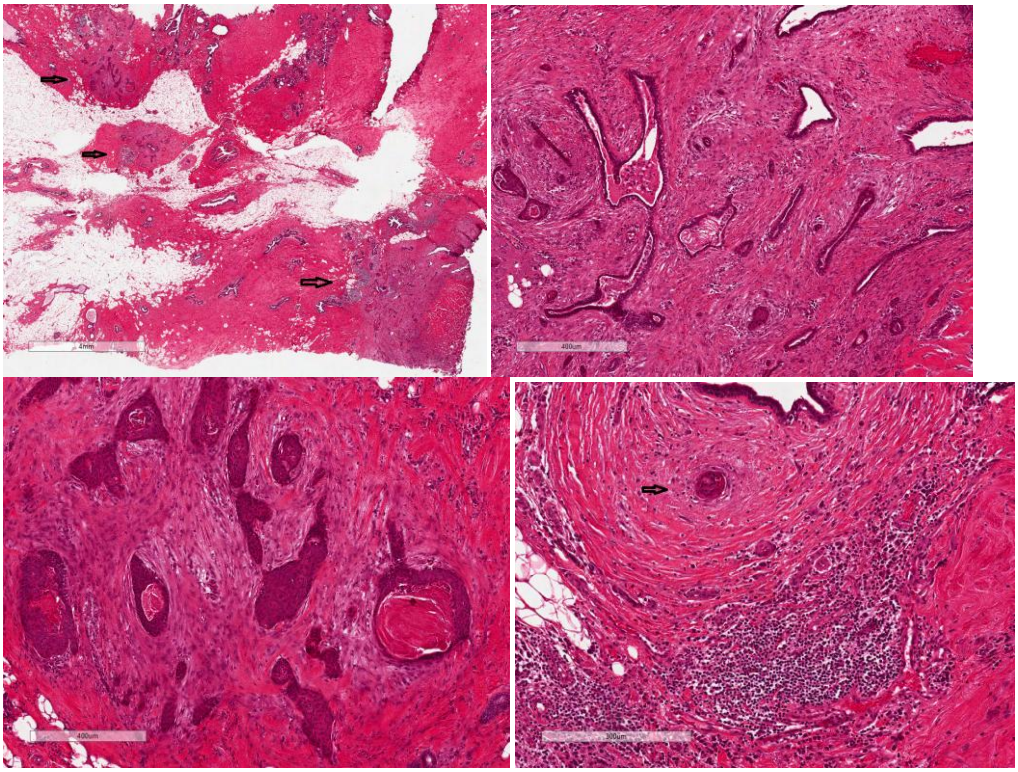

Fig. 1S: Apocrine cyst with papillary hyperplasia, devoid of myoepithelial cells: A: H&E, B: CK 14. C: at higher power the apocrine cells are devoid of any atypia and appear located directly on fat tissue, with no myoepithelial layer.

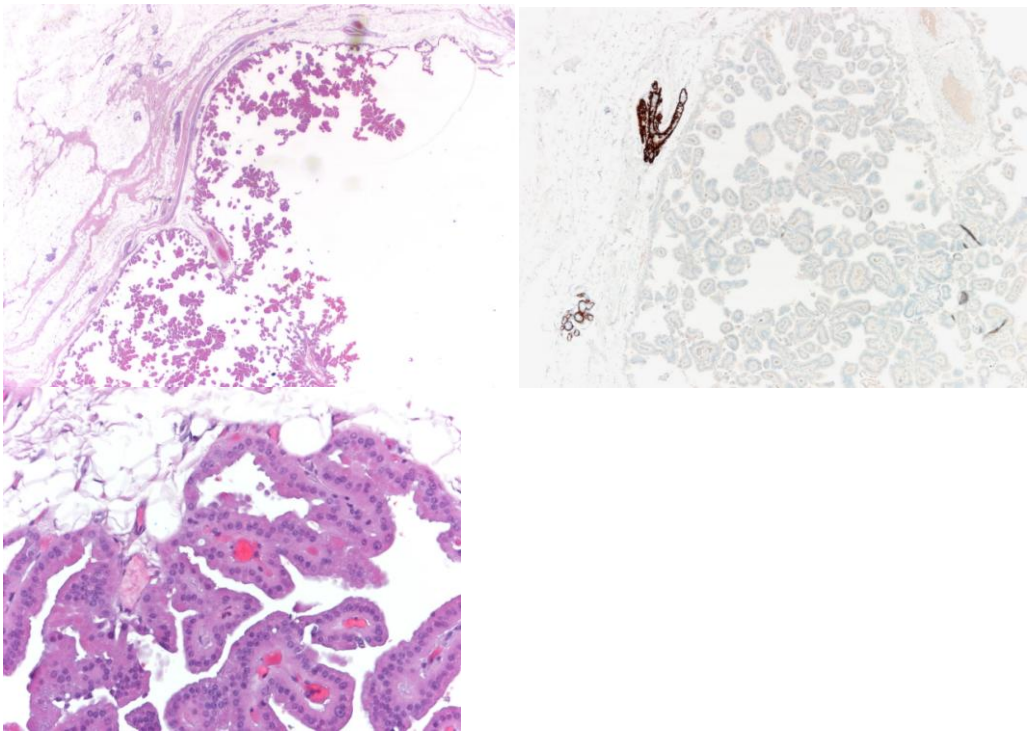

Fig. 2S: A,B: Focal MEC Hyperplasia in an otherwise typical intraductal papilloma.