Defining invasion in breast cancer: the role of basement membrane

Suzan Ghannam[1,2], Catrin Sian Rutland[3], Cinzia Allegrucci[3,4], Nigel P. Mongan[3,5], Emad Rakha[1,4]

[1]School of Medicine, The University of Nottingham, Nottingham, UK.

[2]Department of Histology and cell biology, Faculty of Medicine, Suez Canal University, Ismailia, Egypt.

[3]School of Veterinary Medicine and Sciences, University of Nottingham, Nottingham, UK. 5

[4]Nottingham Breast Cancer Research Centre, Biodiscovery Institute, University of Nottingham, Nottingham, UK

[5]Department of Pharmacology, Weill Cornell Medicine, New York, NY, USA.

Correspondence:

Professor Emad Rakha

Consultant Histopathologist

Department of Histopathology, Nottingham University Hospital NHS Trust,

City Hospital Campus, Hucknall Road, Nottingham, NG5 1PB, UK

Tel: (44) 0115-9691169, Fax: (44) 0115-9627768

Email: msxms8@nottingham.ac.uk

ORCID ID:

ABSTRACT

Basement membrane (BM) is an amorphous, sheet like structure separating the epithelium from the stroma. BM is characterised by a complex structure comprising collagenous and non-collagenous proteoglycans and glycoproteins. In the breast, the thickness, density, and composition of the BM around the ductal lobular system varies during differing development stages. In pathological conditions, the BM provides a physical barrier that separates proliferating intraductal epithelial cells from the surrounding stroma and its absence or breach in malignant lesions is a hallmark of invasion and metastases. Currently, diagnostic services often use special stains and immunohistochemistry (IHC) to identify the BM in order to distinguish *in situ* from invasive lesions. However, distinguishing BM on stained sections, and differentiating the native BM from the reactive capsule or BM-like material surrounding some invasive malignant breast tumours is challenging. Although diagnostic use of the BM is being replaced by myoepithelial cell IHC markers, BM is considered by many to be a useful marker to distinguish *in situ* from invasive lesions in ambiguous cases. In this review, the structure, function, biological and clinical significance of the BM are discussed in relation to the various breast lesions with emphasis on how to distinguish the native BM from alternative pathological tissue mimicking its histology.

INTRODUCTION

In the normal breast tissue, the duct lobular system comprises two cell types, epithelial and myoepithelial cells (MECs), which are separated from the surrounding stromal tissue by a thin, sheet like structure layer called the basement membrane (BM). MECs, which are located between the BM and the epithelial cells, play key roles in synthesising BM components in normal tissue. MECs secrete laminin 1, which is the major component of the BM and synthesise other BM components including collagen IV, laminin 5, nidogen and fibronectin. MECs produce matrix metalloproteinases (MMPs), which facilitate BM remodelling [1]. MECs also possess BM receptors, including integrins, which mediate cell-BM attachment. MECs attach to BMs via hemidesmosomes and to the adjacent myoepithelial and luminal epithelial cells using desmosomes. MECs in normal ducts form a continuous layer while those in the terminal duct lobular units (TDLUs) are discontinuous, allowing some luminal epithelial cells direct contact with the BM [2].

BMs are thin, pliable, and amorphous sheet like structures with an extracellular matrix component that provides cell support and acts as a platform for complex signalling. BMs are comprised of collagenous and non-collagenous components such as proteoglycans and glycoproteins [3]. However, BMs undergo dynamic transformations throughout life, and the molecular structure and arrangement of BM fibres differ in various tissues according to their functions and between normal, benign and malignant lesions [4]. In malignant lesions, the normal production and assembly of the BM is disrupted. In fact, BM changes further define the tumour microenvironment and provides host-derived regulatory signals during tumour progression. Animal models have indicated that

destruction of the BM is associated with genetic instability and tumorigenesis [5].

In the breast, the thickness, density, and composition of the BM around the normal duct lobular system (termed native BM) may vary slightly during different development stages [6]. In pathological conditions, including benign and malignant *in situ* lesions, the BM is typically preserved, but often exhibits altered thickness, structure, or continuity. In malignant breast lesions, the preservation of the BM is used to indicate the *in-situ* nature of the tumours (e.g., ductal carcinoma *in situ* (DCIS) or lobular carcinoma *in situ* (LCIS)) whereas the absence or breach of the BM denotes invasion [7].

In diagnostic practice, breast pathologists tend to use special stains and/or immunohistochemistry (IHC), rather than haematoxylin and eosin (H&E) staining, to distinguish one of more of the BM components that differentiate between *in situ* and invasive tumours in doubtful cases. Despite the advances in BM detection, distinguishing between native BM and reactive BM-like structures or pseudo-capsule surrounding some malignant breast tumours remains problematic [7]. Indeed, some invasive tumours show BM-like material, and it is known that the BM structure shares some components with the extracellular matrix of the interstitial stromal tissue and scar tissue, further complicating the differentiation between these types of tissues [8]. In addition, the expression of some of the BM components by invasive breast tumours makes interpretation of the findings challenging [9, 10].

Although the diagnostic use of BM is now being replaced by MEC IHC markers in routine breast pathology practice, some pathologists consider the

demonstration of a BM a specific finding, useful for distinguishing *in situ* from invasive lesions [7]. The presence of a BM-like reactive capsule around encapsulated papillary carcinoma (EPC) is also used as the main evidence to support its *in situ* nature [11]. In addition, the demonstration of BM in microglandular adenosis is used to indicate its benign nature despite the lack of myoepithelial cells, proliferating epithelial cell clonality and its infiltrative appearances [12]. Therefore, the diagnostic use of BMs to classify breast lesions remains controversial. In this review, we discuss the structure, function, biological and clinical significance of BMs in various breast lesions.

Composition of the BM

By electron microscopy (EM), the BM is well organised and comprises of three layers, (i) a linear, homogeneous, electron-dense region (lamina densa) and (ii) a clear zone (lamina lucida) directly beneath MECs, and epithelial cells within the breast ducts and lobules. These two layers constitute the basal lamina, which is located towards the MECs. (iii) The outer layer of BM is called reticular lamina, or lamina fibroreticularis, and is formed mainly of collagen III [13-15].

The lamina densa is known as the BM proper and is formed from densely packed fibrils embedded in dense matrix [16]. The lamina densa is between 20–300 nanometres (nm) thick, consisting of a network of collagen IV fibrils with proteoglycan perlecan, while the lamina lucida is ~40 nm thick and consists of laminin, nidogens (entactin) and integrins. A bridge of anchoring filaments of collagen VII connects the lamina densa and lucida. Hemidesmosomes, which are present on the basal aspect of MECs, are connected to the adjacent basal lamina by fine filaments [13-15].

Tissue preparation techniques, especially fixation methods, also influence the thickness and appearance of the BM structure [17]. Studies using other techniques such as cryofixation have shown that the BM is a homogenous layer, not laminated, and the lamina lucida is an artefact caused by fixation [17]. BMs show variable thickness, but have been measured at about 100nm by EM however this could be an underestimation due to dehydration caused during the preparation procedure, as proved by atomic force microscopy that showed two fold higher measurements [18]. The BM may be also be absent entirely, especially in embryonic tissues [19].

The molecular components of BMs are classified into major and minor. Major structures are collagen IV, laminin, nidogens and perlecan while minor components include fibronectin and collagens XV and XVIII [15, 20]. Collagen IV is the main constituent representing approximately 50% of the BM [15, 21]. It is a non-fibrillar collagen which presents in a network with different six alpha chains known as α1 (IV) through to α6 (IV) [22]. The chains α1 (IV) and α2(IV) which are known as the classical chains and are represented in BMs from all tissue types, whereas the other chains show variable distribution throughout differing tissues. The α5(IV) and α6(IV) chains are present in breast tissue [23-25]. Laminins are a family of large glycoproteins consisting of α, β, and γ chains. There are 15 different heterotrimeric isoforms of laminin resulting from 12 genetically different types of these chains. Laminin associates with other major structures of the BM to form the basic architecture of BMs and plays a key role in cell attachment and differentiation [20, 26]. Both laminin and collagen IV components are present diffusely along the basal lamina of the BM but are not localised to particular regions. Nidogen is a glycoprotein existing in two forms N1 and N2 which bridge with collagen IV and laminin for BM stability [15, 27,

28]. Perlecan is a heparan sulphate proteoglycan (HSPG) which contains binding sites for collagen IV and laminin, forming a bridging network and acting as a growth factor reservoir which affects adhesion and migration [15, 29, 30].

The minor components of the BM are variable according to the tissue and their functions and clinical significance remain unclear. Fibronectin is a glycoprotein present in lamina reticularis linking it with the basal lamina enhancing structural integrity. Collagen XV plays a role in BM maintenance, while collagen XVII is a nonfibrillar collagen binding with HSPGs which supports cell proliferation and migration. Collagens XV and XVIII are responsible for interconnecting the BM with the underlying connective tissue [31]. There are also other minor components such as SPARC (secreted protein acidic and rich in cysteine) and fibulins [15, 30, 32].

BM staining techniques and its pitfalls

Haematoxylin and eosin (H&E) stain, which is the gold standard stain pathologists use routinely in practice, can highlight the BM around the ducto-lobular system and around proliferating ducts, but it has limitations as some of BM structures do not stain well [33]. Periodic acid Schiff (PAS) is a special stain used for BM detection which is demonstrated as a magenta line lying under the epithelium. PAS staining is based on detection of glycogen and mucus, staining mucus or glycogen producing tissues stronger than the collagen rich BM. When diastase is added to breakdown glycogen (PAS-D), the BM appears as a pink line [33]. BMs also contain reticular fibres, which appear as black lines when silver-based stains are applied. Silver stains show greater levels of contrast than PAS stains as peridot oxidation results in aldehydes which are detected as black colloidal silver deposits following application of silver nitrate solution [34]. A

comparative study of the various BM stains showed that PAS and silver stains produce the strongest reactions and are therefore arguably better than other connective tissue stains including Masson's trichrome, Mallory stain, and Wilder's reticulum stain [35]. Another study compared H&E, PAS and acriflavine (fluorescent periodic acid) stains showing that BM continuity, contrast and pattern is better determined by acriflavine then PAS as it showed the fibrillar pattern of the BM in which reticular fibres appeared black while collagen fibres appeared golden brown [33]. IHC, alongside antibodies against the most ubiquitous BM components collagen IV and laminin, is now used more frequently in routine practice for BM identification [36]. However there are some limitations as cross reactivity, propensity to stain vasculature structure [37] and the expression of some of these markers in the malignant epithelial cells [9] can complicate interpretation.

BM in normal breast tissue

Mammary gland cellular anatomy varies through different stages of development including puberty, pregnancy, lactation, and menopause [6]. During puberty, the terminal buds develop at the tip of the ducts. The BM at the bulbous tip of the terminal bud is thin (around 104 nm) and it becomes thicker at the bud neck region reaching 1.4 µm [38].

EM was used in the past to detect BMs but it is technically challenging for routine use in breast pathology [39]. When visualised using H&E staining and light microscopy, BMs appear as a fine line at the periphery of the normal parenchymal elements of the breast (TDLUs and ducts). However, this is often difficult to demonstrate and special stains such as PAS are often used to identify BMs. Despite the availability of special stains and IHC, the detection of BMs in

breast lesions remains technically challenging, especially given the reduction in people who are experienced in interpretation of the staining findings. Therefore, the use of MEC markers has largely replaced the use of BM stains in routine practice. However, some pathologists still rely on BM markers and its diagnostic use remains valuable in certain situations where MEC markers provide limited value such as microglandular adenosis (MGA) [12].

The BM structure and composition are different in normal, reactive hyperplastic and neoplastic benign and malignant breast lesions [40]. Some malignant breast lesions secrete BM like material in the extracellular spaces in the breast [41, 42] and in the lymph nodes [43] and some tumours express some of the BM components in the malignant epithelial cells and these should be distinguished from the native BM that denote the *in situ* nature of breast lesions. This is observed more in tumours showing basal/myoepithelial cell phenotypes including some salivary gland like tumours. However, some conventional type ductal no special type carcinomas (NST) have a BM like structure around the invasive foci in the breast or in metastatic tumours particularly around DCIS like structures in the nodes (revertant DCIS) [44].

Comparisons between BM and its mimickers

BMs share many chemical and ultrastructural features with the extracellular matrix (ECM) in the interstitial stromal tissue [36] and with some reactive processes such as scar tissue and pseudocapsule. In addition, BM-like material is secreted by some invasive breast tumours (Table 1). The components of the BM are secreted by many types of cells in both normal and malignant conditions, and these can help to differentiate the native BM secreted by MECs from its histological mimickers (Table 2).

While the non-fibrillar collagen IV is the most abundant component in the BM, fibrillar collagens I, III and V are the major components of the ECM [45]. The ECM contains some collagens that act as anchoring fibrils (VII, VI, XXVI, and XXVIII), which play a key role in interconnecting matrix components and separate structural components within tissues. Chondroitin sulfate and glycoprotein fibronectin, which is a minor component of the BM, are major components of the interstitial ECM and has a bridging role in organising matrix and cell-matrix interactions [46]. Interstitial ECM contains matricellular proteins which are glycoproteins that can interact with matrix components, growth factors, and cell surface receptors which represent a regulatory function in matrix-cells communications [47, 48].

Two main families of proteoglycans (PGs) are represented in interstitial ECM, namely hyalectans and small leucine-rich PGs (SLRPs), which have a role in matrix organisation, cell signalling, inflammation processes and collagen fibrillogenesis. Perlecan and agrin are types of PGs which are found in BMs, and they are responsible for interactions with matrix components [31]. Unlike in BMs, elastin is a major component in ECM and accumulates as microfibrils composed of fibulins, fibrillin. These microfibrils insert into the BM binding to perlecan anchoring the BM to interstitial ECM [31, 49].

The interstitial ECM contains several key proteins which can be demonstrated by IHC and are different from those in the BM. These include collagen I, laminin1, thrombospondin 1 (THBS1), tenascin C (TNC), fibulin 1 and smooth muscle actin-α (α-SMA) [50, 51] in addition to the stromal cell markers such as CD73, CD90 and CD105 [52]. However, collagen IV and laminin are the main markers for BM,

and the latter is expressed in stromal cells [53, 54]. Other proteins, such as SPARC and fibronectin, are also expressed in the BM [55] and α-SMA is one of the markers for MECs [7]. Some collagens known as anchoring fibrils are found in the BM zone and have been used recently to demonstrate BMs, these include collagen XV, XIX [56] and VII [57]. The stroma of the breast also contains adipocytes, fibroblasts, blood vessels, and nerves. The blood vessels have their own laminin-rich BM. The stroma communicates with the epithelium despite their separation by the BM, and stromal changes are observed in the early stages of malignancy [36].

Reactive fibrotic stroma is defined as a new microenvironment due to stromal reactions in response to external stimuli, such as injury or cancer, results in ECM remodelling, inflammatory cells, angiogenesis, growth factors release and desmoplastic reactions [58]. If such reactive stromal elements are seen at the periphery of expansile breast lesions it can result in a band like collagenised tissue mimicking thickened BM or a capsule (pseudocapsule). Quantitative analysis of ECM components in scar tissue showed more collagen I than ECM, with thicker less organised bundles, instead of exhibiting a basket wave appearance, with decreased elastin and four-fold increases in glycosaminoglycans [59]. Histologically, scar tissue has a high mesenchymal density accompanied by high vascularity [60]. Similar to the BM, scar tissue is negative for CD34 [61], which is positive in normal intralobular mammary stroma [62]. The α-SMA marker remains negative in scar tissue [63] and normal stroma [64] while it is positive in reactive stroma [65]. Unlike native BMs, scar tissue and ECM show a negative reaction following silver staining [60], as does the reactive stroma in tumours [66]. Components of BM examined in scar tissue

revealed a BM-like structure with negative expression of collagen IV and positive laminin expression [60, 67].

It is also important to note that the various biochemical components of the BM can be detected in cancer cells and previous studies have linked such expression to tumour behaviour [9, 10, 36]. Proteomic analysis of breast cancers have shown that only stromal cells secrete laminin and collagen IV in poorly metastatic tumours however, in highly metastatic cancers both stromal cells and tumour cells produce these BM proteins [68]. Tumour associated BM materials and their structures are likely to be different from the native BM present around normal parenchymal structure in the breast and around benign and *in situ* lesions [69], not only in structure, arrangement, and anatomical localisation but also in terms of cell origin and functions. Despite this knowledge, data relating to the nature and functions of extracellular BM materials associated with invasive tumour foci remains to be elucidated.

Diagnostic use of the BM in breast pathology

The basic and original definition of invasive breast carcinoma is based on BM breaching with stromal infiltration, involving the migration of invasive tumour cells in the stroma with potential local and distant spread [7]. While the diagnostic use of BM is still valid, and some pathologists rely on BMs to distinguish *in situ* from invasive tumours at least in certain lesions [7], routine assessment of BM in practice is often challenging [70]. Here we consider the challenges involved in diagnostic applications of BM assessment in breast pathology.

BM in ductal carcinoma *in situ* (DCIS)

In situ carcinoma associated with microinvasion shows focal fragmentation and thinning, and disruption of the BM typically at the site of the microinvasion [71]. This may support the use of BMs to differentiate *in situ* from invasive tumours in the breast. Chen and colleagues studied the BM using multiphoton microscopy and reported that the BM is intact but enlarged compared to normal BM in atypical ductal hyperplasia (ADH), while the BM is similar to ADH with straighter collagen fibres resulting from duct expansion in low grade-DCIS. In contrast, high grade DCIS presents with varying collagen, thin in some areas and dense in others, with some ducts displaying disrupted BMs with microinvasion [72]. Despite the differences in the structure, composition, and arrangement of the BM components in DCIS compared to native BMs surrounding normal ducto-lobular system, it is believed that the DCIS BM is a modification of the native BM rather than a new structure. Therefore, their existence denotes the intraductal/intralobular nature of the proliferating epithelial cell components.

Some cases of high nuclear grade DCIS have exhibited thickened BMs together with inflammation and fibrosis. This has resulted in the perception that the well-developed layer of fibrous tissue (pseudocapsule) at the epithelial stroma interface around EPC may represent native BMs characteristic of *in situ* lesions [11, 73, 74]. However, there are several lines of evidence to indicate that this layer likely represents florid and abnormal proliferation of the BM-like material secreted either by the proliferating malignant cells or the surrounding stromal cells [11]. The presence of similar peripheral pseudocapsule around EPC at metastatic and recurrent sites outside the breast supports that such a structure is a reactive process initiated by the tumour itself [73, 74]. This likely reflects the interactions between such a slowly growing EPC with expansile growth and pushing margins and the surrounding stroma, perhaps exaggerated by the cystic

nature of EPC with leakage of cyst content into the stroma and a secondary stromal reaction. It is not infrequent to find stroma reactive changes similar to that seen at the previous biopsy site around benign and malignant intraductal papillary lesions across multiple areas, excluding the possibility of biopsy site related changes [11, 73, 74].

Studies have shown that EPC lesions express higher levels of TGFβ1 compared with conventional type DCIS and invasive carcinomas, which plays a role in the development of the thick fibrous capsule, supporting the hypothesis that the EPC capsule is a reactive rather than a thickened expanded native BM resulting from distention by the proliferation of neoplastic papillary [74]. Indeed, differentiation of a native BM from a reactive BM surrounding an invasive lesion is clinically important for early diagnosis and proper management of the patient, when considering implications of management *in situ*, and for benign versus invasive tumour diagnosis.

BM in other breast lesions

The BM is used more frequently when diagnosing MGA to differentiate it from tubular carcinoma. MGA is a clonal neoplastic proliferative lesion that shows infiltrating rounded glands surrounded by a well-developed BM [75]. MGA lacks peripheral MECs and has an infiltrative appearance, so it mimics invasive breast lesions. In this context, MECs markers do not help differentiate MGA from tubular or other low grade NST carcinomas with prominent tubule formation. In addition, due to the lack of the desmoplastic stromal reaction and the characteristic immunoprofile (oestrogen receptor negative and strong S100 positivity), BM markers are helpful, and they are frequently used in routine practice to distinguish MGA from invasive carcinoma with prominent tubule

formation. Perhaps this is currently the main diagnostic use of BM markers in the breast in routine practice due to ease of interpretation with the thickened BM layer around the tubules of MGA with an absence of BM structures in tubular carcinoma. Despite this, experience in undertaking interpretation of BM staining pattern is required and the role of the BM in differentiating atypical MGA from foci of acinic cell carcinoma (AC) is less well defined. The BM surrounding the glands of MGA can be identified with PAS/D-PAS, collagen IV, and laminin immunostaining [69]. The characteristic infiltrative nature and the growth patterns of MGA suggest that the BM layer is not native and is reproduced by the neoplastic cells [33]. This is an area for further research and a comparison of the ultrastructure and composition between MGA BM and native BM is warranted.

Lactational changes and apocrine metaplasia are two benign metaplastic changes in the breast that may show reduced or focal absence of MEC layers. Apocrine metaplasia can also exhibit cytological atypia (apocrine atypia) and lactational changes may also present with nuclear enlargement and irregularity raising suspicion of invasive carcinoma in cases with reduced or absent MECs. The use of BM markers can confirm the benign *in situ* nature of these lesions. Moreover, the BM has an important role in differentiating sclerosing adenosis from tubular carcinoma as IHC studies showed that continuous BMs surround the tubules of sclerosing adenosis despite perineural invasion in some cases however, BMs are absent in tubular carcinoma [40]. Collagenous spherulosis is a benign lesion characterised by multiple cysts composed of collagenous material surrounded by proliferative MECs and epithelial cells. It is associated with benign breast lesions and may be misdiagnosed as an adenoid cystic carcinoma (ACC). Studies have suggested that these spherules may represent

BM material [76]. Collagenous spherulosis exhibit positive staining for collagen IV and laminin, however, some showed that it contains only basal lamina which is duplicated [77].

Adenoid cystic carcinoma (ACC) is a rare breast carcinoma and is considered as a variant of the triple negative BC (TNBC) molecular subtype, however, it has a better prognosis. ACC presents with a biphasic morphological pattern and the glands are surrounded by BM-like material [78, 79]. IHC staining of fibronectin and laminin within ACC demonstrate pseudo cystic spaces containing disturbed BM like material mainly fibronectin and laminin that are lined by neoplastic cells. Both proteins were observed around the entire peripheral region of neoplastic cells clusters [80]. Ultrastructural EM studies showed ACCs were lined by an uninterrupted BM consisting of lamina lucida and lamina densa which were characterised by their extraordinary thicknesses [81]. These pseudocysts were surrounded by neoplastic cells, and as a result it was obvious that the BM lamina densa was produced by the tumour cells [81]. The BM like material within the pseudocyst contains the molecules of BM structures produced by the surrounding tumour cells which have basal/MECs characteristics. The presence of occasional vascular capillaries within these pseudocysts suggests invagination of the surrounding stroma into the tumour cribriform masses or solid structures. However, the ultrastructural features and the composition of the material in the pseudocysts are consistent with that of the BM and not that of the interstitial ECM stroma which support tumour secretion by cells having basal/myoepithelial cell differentiation, in a way mimicking the development of the native BM in normal tissue. The indolent behaviour of ACCs may also reflect the degree of differentiation of these tumours which tries to recapitulate the normal tissue and secrete BM like material. The correlation between anaplastic features and

BM disintegration supports the role of the BM in the process of invasion [82]. This may explain the better prognosis observed where the tumours have BM like material.

Acinic cell carcinoma (AC) is a rare type of salivary gland like breast carcinoma. AC with prominent well differentiated acinar like structures and may resemble atypical MGA. Although both lack a MEC layer, AC also lacks a BM structure [83]. Some cells in invasive breast carcinoma have shown intracytoplasmic staining of laminin [71, 84-87]. In addition, IHC studies have shown that BM components, especially heparan sulphate proteoglycan, are synthesised by the rough endoplasmic reticulum in some breast cancer cells [88].

In conclusion, the fundamental definition of cancer invasiveness is based on BM penetration, however some invasive BCs exhibit BM-like material surrounding the neoplastic cells and some invasive tumours create a reactive capsule that may mimic native BMs. These structures are different in both composition and structure from the native BM that surrounds the ducto-lobular system of the breast. Knowledge of such differences between native BMs and mimicking tissues are likely to help provide more accurate interpretation of histological findings. Further studies are needed to provide better explanations about the BM changes in DCIS and the early invasive process and the relationship between the development of BM like material in invasive lesions and tumour differentiation and behaviour.

REFERENCES

1. Adriance, M.C., et al., *Myoepithelial cells: good fences make good neighbors.* Breast Cancer Res, 2005. **7**(5): p. 190-7.
2. Fu, H.L., et al., *Ultrastructural localization of laminin and type IV collagen in normal human breast.* Ultrastruct Pathol, 2002. **26**(2): p. 77-80.
3. Kalluri, R., *Basement membranes: structure, assembly and role in tumour angiogenesis.* Nat Rev Cancer, 2003. **3**(6): p. 422-33.
4. d'Ardenne, A.J., *Use of basement membrane markers in tumour diagnosis.* J Clin Pathol, 1989. **42**(5): p. 449-57.
5. Sternlicht, M.D., et al., *The stromal proteinase MMP3/stromelysin-1 promotes mammary carcinogenesis.* Cell, 1999. **98**(2): p. 137-46.
6. Sternlicht, M.D., *Key stages in mammary gland development: the cues that regulate ductal branching morphogenesis.* Breast Cancer Res, 2006. **8**(1): p. 201.
7. Rakha, E.A., et al., *Invasion in breast lesions: the role of the epithelial-stroma barrier.* Histopathology, 2018. **72**(7): p. 1075-1083.
8. Frantz, C., K.M. Stewart, and V.M. Weaver, *The extracellular matrix at a glance.* J Cell Sci, 2010. **123**(Pt 24): p. 4195-200.
9. Ioachim, E., et al., *Immunohistochemical expression of extracellular matrix components tenascin, fibronectin, collagen type IV and laminin in breast cancer: their prognostic value and role in tumour invasion and progression.* Eur J Cancer, 2002. **38**(18): p. 2362-70.
10. Jansson, M., et al., *Prognostic Value of Stromal Type IV Collagen Expression in Small Invasive Breast Cancers.* Front Mol Biosci, 2022. **9**: p. 904526.
11. Esposito, N.N., D.J. Dabbs, and R. Bhargava, *Are encapsulated papillary carcinomas of the breast in situ or invasive? A basement membrane study of 27 cases.* Am J Clin Pathol, 2009. **131**(2): p. 228-42.
12. Tavassoli, F.A. and G.L. Bratthauer, *Immunohistochemical profile and differential diagnosis of microglandular adenosis.* Mod Pathol, 1993. **6**(3): p. 318-22.
13. Noonan, D.M., et al., *The complete sequence of perlecan, a basement membrane heparan sulfate proteoglycan, reveals extensive similarity with laminin A chain, low density lipoprotein-receptor, and the neural cell adhesion molecule.* J Biol Chem, 1991. **266**(34): p. 22939-47.
14. Paulsson, M., *Basement membrane proteins: structure, assembly, and cellular interactions.* Crit Rev Biochem Mol Biol, 1992. **27**(1-2): p. 93-127.
15. Mak, K.M. and R. Mei, *Basement Membrane Type IV Collagen and Laminin: An Overview of Their Biology and Value as Fibrosis Biomarkers of Liver Disease.* Anat Rec (Hoboken), 2017. **300**(8): p. 1371-1390.
16. Miosge, N., *The ultrastructural composition of basement membranes in vivo.* Histol Histopathol, 2001. **16**(4): p. 1239-48.
17. Chan, F.L. and S. Inoue, *Lamina lucida of basement membrane: an artefact.* Microsc Res Tech, 1994. **28**(1): p. 48-59.
18. Pastor-Pareja, J.C., *Atypical basement membranes and basement membrane diversity - what is normal anyway?* J Cell Sci, 2020. **133**(8).
19. Merker, H.J., *Morphology of the basement membrane.* Microsc Res Tech, 1994. **28**(2): p. 95-124.
20. Timpl, R., *Structure and biological activity of basement membrane proteins.* Eur J Biochem, 1989. **180**(3): p. 487-502.

21.   Boudko, S.P., et al., *Basement membrane collagen IV: Isolation of functional domains.* Methods Cell Biol, 2018. **143**: p. 171-185.

22.   Nakano, S., J. Tsuruta, and K. Iyama, *[Remodeling of basement membrane in association with cancer invasion].* Rinsho Byori, 2000. **48**(5): p. 451-7.

23.   Khoshnoodi, J., V. Pedchenko, and B.G. Hudson, *Mammalian collagen IV.* Microsc Res Tech, 2008. **71**(5): p. 357-70.

24.   Hudson, B.G., et al., *Structure and organization of type IV collagen of renal glomerular basement membrane.* Contrib Nephrol, 1994. **107**: p. 163-7.

25.   Tanjore, H. and R. Kalluri, *The role of type IV collagen and basement membranes in cancer progression and metastasis.* Am J Pathol, 2006. **168**(3): p. 715-7.

26.   Timpl, R., *Macromolecular organization of basement membranes.* Curr Opin Cell Biol, 1996. **8**(5): p. 618-24.

27.   Miosge, N., et al., *Ultrastructural triple localization of laminin-1, nidogen-1, and collagen type IV helps elucidate basement membrane structure in vivo.* Anat Rec, 1999. **254**(3): p. 382-8.

28.   Khalilgharibi, N. and Y. Mao, *To form and function: on the role of basement membrane mechanics in tissue development, homeostasis and disease.* Open Biol, 2021. **11**(2): p. 200360.

29.   Condomitti, G. and J. de Wit, *Heparan Sulfate Proteoglycans as Emerging Players in Synaptic Specificity.* Front Mol Neurosci, 2018. **11**: p. 14.

30.   LeBleu, V.S., B. Macdonald, and R. Kalluri, *Structure and function of basement membranes.* Exp Biol Med (Maywood), 2007. **232**(9): p. 1121-9.

31.   Theocharis, A.D., D. Manou, and N.K. Karamanos, *The extracellular matrix as a multitasking player in disease.* Febs j, 2019. **286**(15): p. 2830-2869.

32.   Hynes, R.O., *The dynamic dialogue between cells and matrices: implications of fibronectin's elasticity.* Proc Natl Acad Sci U S A, 1999. **96**(6): p. 2588-90.

33.   Pujar, A., et al., *Comparing The Efficacy of Hematoxylin and Eosin, Periodic Acid Schiff and Fluorescent Periodic Acid Schiff-Acriflavine Techniques for Demonstration of Basement Membrane in Oral Lichen Planus: A Histochemical Study.* Indian J Dermatol, 2015. **60**(5): p. 450-6.

34.   Kumar, G.L. and J. Kiernan, *Special stains and H&E.* Connection, 2010: p. 14.

35.   Jones, D.B., *Inflammation and repair of glomerulus.* Am J Pathol, 1951. **27**(6): p. 991-1009.

36.   Robertson, C., *The extracellular matrix in breast cancer predicts prognosis through composition, splicing, and crosslinking.* Exp Cell Res, 2016. **343**(1): p. 73-81.

37.   Hoda, R.S., E. Brogi, and H.Y. Wen, *Quality Issues in Diagnostic Immunohistochemistry in Breast Pathology.* Pathobiology, 2022: p. 1-10.

38.   Paine, I.S. and M.T. Lewis, *The Terminal End Bud: the Little Engine that Could.* J Mammary Gland Biol Neoplasia, 2017. **22**(2): p. 93-108.

39.   Mrak, R.E., *The Big Eye in the 21st century: the role of electron microscopy in modern diagnostic neuropathology.* J Neuropathol Exp Neurol, 2002. **61**(12): p. 1027-39.

40.   Willebrand, D., F.T. Bosman, and A.F. de Goeij, *Patterns of basement membrane deposition in benign and malignant breast tumours.* Histopathology, 1986. **10**(12): p. 1231-41.

41.   Vogel, H.P. and G. Mendelsohn, *Laminin immunostaining in hyperplastic, dysplastic, and neoplastic lesions of the endometrium and uterine cervix.* Obstet Gynecol, 1987. **69**(5): p. 794-9.

42.   Gusterson, B.A., et al., *Invading squamous cell carcinoma can retain a basal lamina. An immunohistochemical study using a monoclonal antibody to type IV collagen.* Lab Invest, 1984. **51**(1): p. 82-7.

43.   Visscher, D.W., et al., *Immunohistologic analysis of invasive phenotype in breast carcinoma. A clinicopathologic study.* Pathol Res Pract, 1993. **189**(8): p. 867-72.

44.   Barsky, S.H., et al., *'Revertant' DCIS in human axillary breast carcinoma metastases.* J Pathol, 1997. **183**(2): p. 188-94.

45.   Maller, O., H. Martinson, and P. Schedin, *Extracellular matrix composition reveals complex and dynamic stromal-epithelial interactions in the mammary gland.* J Mammary Gland Biol Neoplasia, 2010. **15**(3): p. 301-18.

46.   Zollinger, A.J. and M.L. Smith, *Fibronectin, the extracellular glue.* Matrix Biol, 2017. **60-61**: p. 27-37.

47.   Theocharis, A.D., et al., *Extracellular matrix structure.* Adv Drug Deliv Rev, 2016. **97**: p. 4-27.

48.   Sawyer, A.J. and T.R. Kyriakides, *Matricellular proteins in drug delivery: Therapeutic targets, active agents, and therapeutic localization.* Adv Drug Deliv Rev, 2016. **97**: p. 56-68.

49.   Thomson, J., et al., *Fibrillin microfibrils and elastic fibre proteins: Functional interactions and extracellular regulation of growth factors.* Semin Cell Dev Biol, 2019. **89**: p. 109-117.

50.   Wang, T., et al., *High expression of intratumoral stromal proteins is associated with chemotherapy resistance in breast cancer.* Oncotarget, 2016. **7**(34): p. 55155-55168.

51.   Bergamaschi, A., et al., *Extracellular matrix signature identifies breast cancer subgroups with different clinical outcome.* J Pathol, 2008. **214**(3): p. 357-67.

52.   Valkenburg, K.C., A.E. de Groot, and K.J. Pienta, *Targeting the tumour stroma to improve cancer therapy.* Nat Rev Clin Oncol, 2018. **15**(6): p. 366-381.

53.   Diaz, L.K., et al., *Beta4 integrin subunit gene expression correlates with tumor size and nuclear grade in early breast cancer.* Mod Pathol, 2005. **18**(9): p. 1165-75.

54.   Berry, S.D.K., R.D. Howard, and R.M. Akers, *Mammary Localization and Abundance of Laminin, Fibronectin, and Collagen IV Proteins in Prepubertal Heifers.* Journal of Dairy Science, 2003. **86**(9): p. 2864-2874.

55.   Schittenhelm, J., et al., *Patterns of SPARC expression and basement membrane intactness at the tumour-brain border of invasive meningiomas.* Neuropathol Appl Neurobiol, 2006. **32**(5): p. 525-31.

56.   Amenta, P.S., et al., *Loss of types XV and XIX collagen precedes basement membrane invasion in ductal carcinoma of the female breast.* J Pathol, 2003. **199**(3): p. 298-308.

57.   Wetzels, R.H., et al., *Distribution patterns of type VII collagen in normal and malignant human tissues.* Am J Pathol, 1991. **139**(2): p. 451-9.

58.   Silva, M.M., Jr., et al., *Characterization of reactive stroma in prostate cancer: involvement of growth factors, metalloproteinase matrix, sexual hormones receptors and prostatic stem cells.* Int Braz J Urol, 2015. **41**(5): p. 849-58.

59.   Sidgwick, G.P. and A. Bayat, *Extracellular matrix molecules implicated in hypertrophic and keloid scarring.* J Eur Acad Dermatol Venereol, 2012. **26**(2): p. 141-52.

60.   Yang, S.W., et al., *Comparison of the histological morphology between normal skin and scar tissue.* J Huazhong Univ Sci Technolog Med Sci, 2016. **36**(2): p. 265-269.

61.   Kamath, N.V., et al., *A light microscopic and immunohistochemical evaluation of scars.* J Cutan Pathol, 2002. **29**(1): p. 27-32.

62.   Catteau, X., et al., *Variable stromal periductular expression of CD34 and smooth muscle actin (SMA) in intraductal carcinoma of the breast.* PLoS One, 2013. **8**(3): p. e57773.

63.   Limandjaja, G.C., et al., *Hypertrophic and keloid scars fail to progress from the CD34(-) /α-smooth muscle actin (α-SMA)(+) immature scar phenotype and show gradient differences in α-SMA and p16 expression.* Br J Dermatol, 2020. **182**(4): p. 974-986.

64.   Nakayama, H., et al., *Alpha smooth muscle actin positive stromal cells in gastric carcinoma.* J Clin Pathol, 2002. **55**(10): p. 741-4.

65.   San Martin, R., et al., *Recruitment of CD34(+) fibroblasts in tumor-associated reactive stroma: the reactive microvasculature hypothesis.* Am J Pathol, 2014. **184**(6): p. 1860-70.

66.   George, J., R.S. Narang, and N.N. Rao, *Stromal response in different histological grades of oral squamous cell carcinoma: a histochemical study.* Indian J Dent Res, 2012. **23**(6): p. 842.

67.   Yang, S., et al., *Abnormalities in the basement membrane structure promote basal keratinocytes in the epidermis of hypertrophic scars to adopt a proliferative phenotype.* Int J Mol Med, 2016. **37**(5): p. 1263-73.

68.   Naba, A., et al., *Extracellular matrix signatures of human mammary carcinoma identify novel metastasis promoters.* Elife, 2014. **3**: p. e01308.

69.   Kim, D.J., et al., *Microglandular adenosis.* J Breast Cancer, 2011. **14**(1): p. 72-5.

70.   Rowe, R.G. and S.J. Weiss, *Breaching the basement membrane: who, when and how?* Trends Cell Biol, 2008. **18**(11): p. 560-74.

71.   Barsky, S.H., et al., *Loss of basement membrane components by invasive tumors but not by their benign counterparts.* Lab Invest, 1983. **49**(2): p. 140-7.

72.   Chen, Z., et al., *Label-Free Identification of Early Stages of Breast Ductal Carcinoma via Multiphoton Microscopy.* Scanning, 2020. **2020**: p. 9670514.

73.   Rakha, E.A., et al., *Encapsulated papillary carcinoma of the breast: an invasive tumor with excellent prognosis.* Am J Surg Pathol, 2011. **35**(8): p. 1093-103.

74.   Rakha, E.A., et al., *Encapsulated papillary carcinoma of the breast: a study of invasion associated markers.* J Clin Pathol, 2012. **65**(8): p. 710-4.

75.   Guerini-Rocco, E., et al., *Microglandular adenosis associated with triple-negative breast cancer is a neoplastic lesion of triple-negative phenotype harbouring TP53 somatic mutations.* J Pathol, 2016. **238**(5): p. 677-88.

76.   Resetkova, E., C. Albarracin, and N. Sneige, *Collagenous spherulosis of breast: morphologic study of 59 cases and review of the literature.* Am J Surg Pathol, 2006. **30**(1): p. 20-7.

77.   Bavle, R.M., *Collagenous spherulosis.* J Oral Maxillofac Pathol, 2013. **17**(3): p. 322-3.

78.   Bae, G.E., et al., *Clinicopathological and Molecular Characteristics of Mammary Adenoid Cystic Carcinoma With Adipocytic Differentiation With Emphasis on the Identification of a Novel BRAF Mutation.* Anticancer Res, 2019. **39**(1): p. 369-374.

79.   Barsky, S.H., et al., *Two human tumors with high basement-membrane-producing potential.* Cancer, 1988. **61**(9): p. 1798-806.

80.   d'Ardenne, A.J., et al., *Laminin and fibronectin in adenoid cystic carcinoma.* J Clin Pathol, 1986. **39**(2): p. 138-44.

81.   Koss, L.G., C.D. Brannan, and R. Ashikari, *Histologic and ultrastructural features of adenoid cystic carcinoma of the breast.* Cancer, 1970. **26**(6): p. 1271-9.

82.   Wu, X., et al., *Visualization of basement membranes in normal breast and breast cancer tissues using multiphoton microscopy.* Oncol Lett, 2016. **11**(6): p. 3785-3789.

83.   Conlon, N., et al., *Acinic cell carcinoma of breast: morphologic and immunohistochemical review of a rare breast cancer subtype.* Hum Pathol, 2016. **51**: p. 16-24.

84.   Siegal, G.P., et al., *Stages of neoplastic transformation of human breast tissue as monitored by dissolution of basement membrane components. An immunoperoxidase study.* Invasion Metastasis, 1981. **1**(1): p. 54-70.

85.   Albrechtsen, R., et al., *Basement membrane changes in breast cancer detected by immunohistochemical staining for laminin.* Cancer Res, 1981. **41**(12 Pt 1): p. 5076-81.

86.   Liotta, L.A., *Tumor invasion and metastases--role of the extracellular matrix: Rhoads Memorial Award lecture.* Cancer Res, 1986. **46**(1): p. 1-7.

87.   Birembaut, P., et al., *Usefulness of basement membrane markers in tumoural pathology.* J Pathol, 1985. **145**(4): p. 283-96.

88.   Kimura, S., et al., *Basement membrane heparan sulfate proteoglycan (perlecan) synthesized by ACC3, adenoid cystic carcinoma cells of human salivary gland origin.* J Biochem, 1999. **125**(2): p. 406-13.

89.   Watson, R.J., et al., *Ultrastructural observations on the basal lamina in the normal human breast.* J Anat, 1988. **156**: p. 1-10.

90.   Stirling, J.W. and J.A. Chandler, *The fine structure of ducts and subareolar ducts in the resting gland of the female breast.* Virchows Arch A Pathol Anat Histol, 1977. **373**(2): p. 119-32.

91.   Ghosh, L., B.C. Ghosh, and T.K. Das Gupta, *Ultrastructural study of stroma in human mammary carcinoma.* Am J Surg, 1980. **139**(2): p. 229-32.

92.   Hunter, J.A. and J.B. Finlay, *Scanning electron microscopy of normal human scar tissue and keloids.* Br J Surg, 1976. **63**(10): p. 826-30.

93.   Tsuji, T. and M. Sawabe, *Elastic fibers in scar tissue: scanning and transmission electron microscopic studies.* J Cutan Pathol, 1987. **14**(2): p. 106-13.

94.   Ormos, J. and A. Halász, *Electron microscopic study of adenoid cystic carcinoma.* Ultrastruct Pathol, 1991. **15**(2): p. 149-57.

95.   Leblond, C.P. and S. Inoue, *Structure, composition, and assembly of basement membrane.* Am J Anat, 1989. **185**(4): p. 367-90.

96.   Linares, H.A., et al., *The histiotypic organization of the hypertrophic scar in humans.* J Invest Dermatol, 1972. **59**(4): p. 323-31.

97.   Natal, R.A., et al., *Collagen analysis by second-harmonic generation microscopy predicts outcome of luminal breast cancer.* Tumour Biol, 2018. **40**(4): p. 1010428318770953.
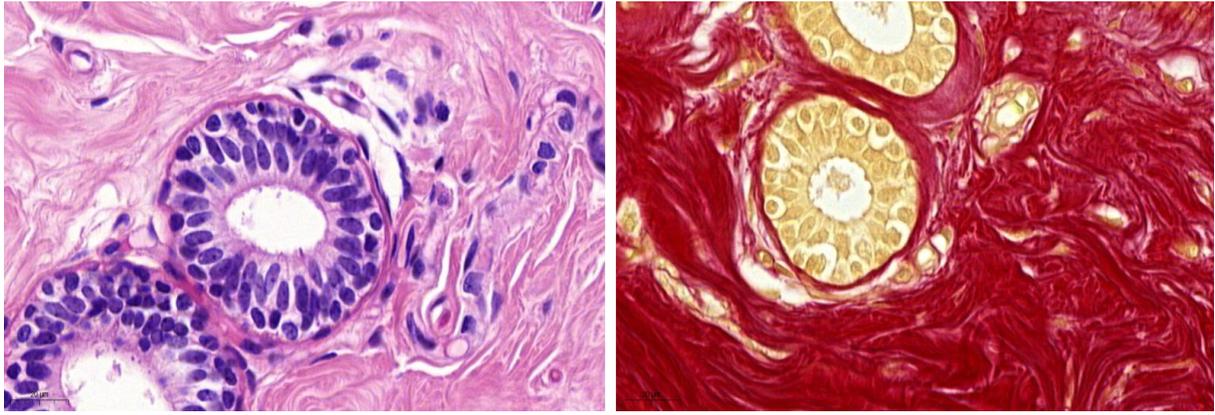
Figure 1. Photomicrographs of normal breast tissue basement membranes. a) H&E showing a well-defined basement membrane (BM) that surrounds the glands. b) Picaro Sirius red (PSR) staining shows the BM as a red definite layer surrounding a normal duct (Magnification x 63). Scale bar=20μm.
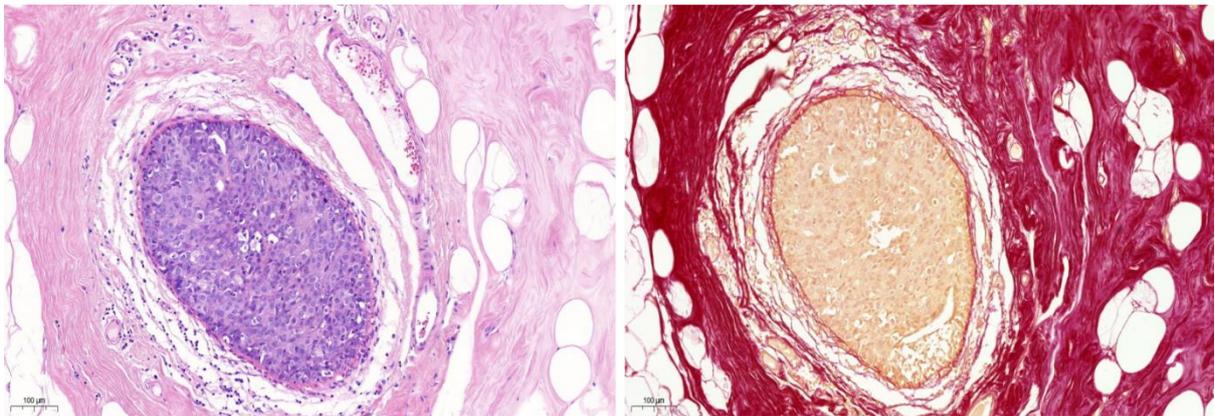

Figure 2. Ductal carcinoma in situ photomicrographs. a) H&E-stained tissue with a thickened BM surrounding the gland. b) PSR stained tissue showing a thickened BM surrounding the gland. Magnification x20. Scale bar=100μm.
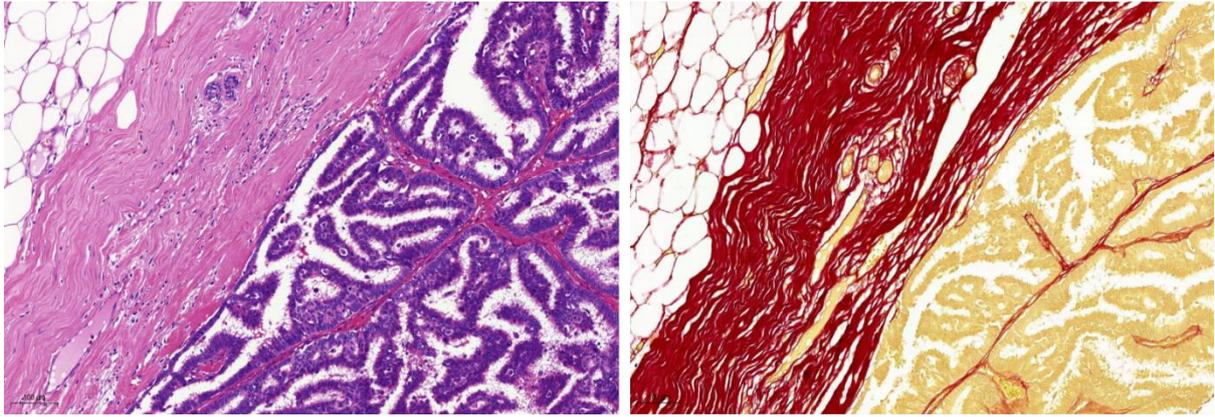
Figure 3. Encapsulated papillary carcinoma photomicrographs. a) H&E-stained tissue showing thick fibrous capsule surrounds the gland. b) PSR stained tissue showing a thick fibrous capsule, red in colour, surrounding the epithelial cells stained yellow. magnification x20. Scale bar=100μm.
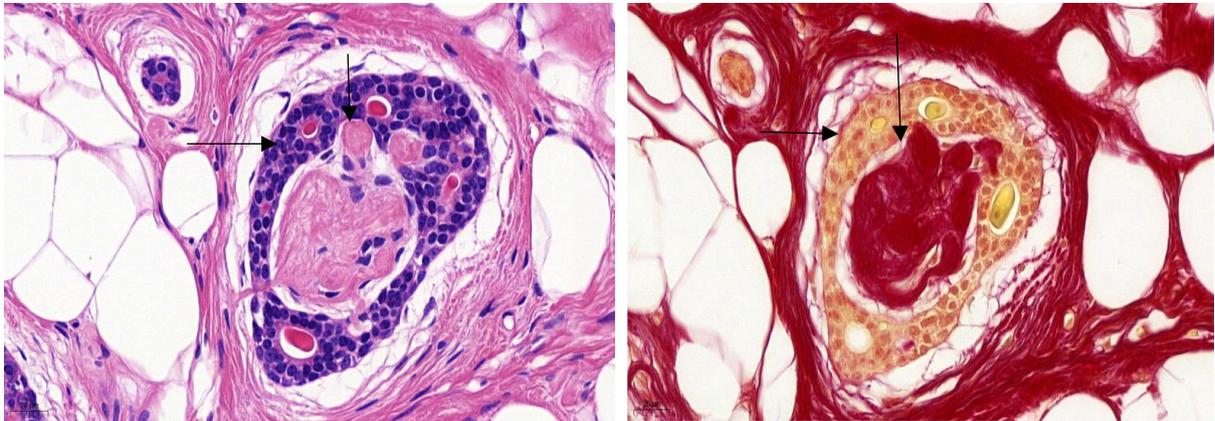

Figure 4. Adenoid cystic carcinoma tissue photomicrographs a) H&E stained showing clusters of epithelial cells in cribriform like pattern surrounded by eosinphilic BM like material (horzointal arrows) and microcysts filled with solid spheres of BM material (vertical arrows). **b)** PSR stain showing a cluster of epithelial cells in a cribriform pattern (yellowish colour) surrounded with red circular BM like material (horzointal arrow) and the microcysts shown as dark red spheres of BM material (vertical arrows). Magnification x40, Scale bar=20μm

Table 1. Characteristics of the breast basement membrane (BM) and its mimickers

Component	Basement membrane	Normal interstitial tissue stroma (ECM)	Tumour associated stroma	Scar tissue	BM-like material in invasive carcinoma
Chemical composition (main constituents)	Laminin 1, 5 Collagen IV Anchoring fibrils XV, XVIII, VII ,XIX Fibronectin PGs: Perlecan & agrin	Fibroblasts Laminin 1 Collagen I, III, V Anchoring fibrils VII, VI, XXVI, and XXVIII Fibronectin PGs: hyalectans and small leucine-rich Matrix metalloproteinases Elastic fibres	Myofibroblasts Laminin 1 Collagen I, III, V Anchoring fibrils VII, VI, XXVI, and XXVIII Fibronectin PGs: hyalectans and small Growth factors Matrix metalloproteinases Inflammatory cells	Collagen I Elastic fibres Laminin 5	Collagen iv Laminin 5 Fibronectin Hyaline material Heparan sulphate proteoglycan Entactin
Staining pattern					
Collagen IV	+	_	_	_	+
Laminin	+	_	+	+	+
CD+34	_	+	+	_	_
Alpha SMA	_	+	+	_	_
Special stains					
PAS	+	-	-		+
Silver stain	+	+	+	-	
Van Gieson	-	+	+	+	
Masson's trichrome	+	+	+	+	
acriflavine	+	+	+		
Electron microscopic features	Electron dense zone of lamina densa which is network of fibrils and electron lucent area called lamina lucida , both layers called basal lamina which may be duplicated in some areas with underlying lamina reticularis of reticular fibres[89]	Bundles of collagen fibres and elastin with many fibroblasts [90]	Collagen fibres with more prominent elastic fibres with size and pattern variation arranged in bands with some amorphous matrix. four cell types (fibroblasts (myofibroblasts), MECs, primitive mesenchymal cells and myelin like bodies) [91]	Meshwork of thick collagen bundles with few elastic fibres which are curled or tortuous numerous large fibroblasts with long processes within and between the bundles [92] [93]	Narrow basal lamina area or wider hyaline (loose BM material) area, lamina densa with variable thickness fine fibrillar split into many layers, lamina lucida, bands of fine fibres[81, 94]
Thickness and appearance	100nm, sheet like, homogeneous eosinophilic appearance	Wavy basket shaped collagen bundles	Dense collagen bundles	Thick bundles of disorganised collagen fibres	Eosinophilic BM like material, variable thickness
Fibre arrangements	Collagen fibres packed and arranged regularly in lamellas [95]	Parallel collagen fibre Loose packing of collagen [96]	Disorganised poorly aligned collagen fibres [97].	Disorganised collagen bundles with no specific orientation appear as whorl-like pattern [96]	

Table 2: Cells secrete basement membrane (BM) components.

Components	Secreting cells	Special features
Collagen IV	Myoepithelial cells [79] Fibroblasts [80] Endothelial cells[81] Pericytes [81]	Network [82]
Laminins	$\beta1$, $\gamma1$ →Epithelial cells $\alpha1$→ epithelial cells $\alpha2$ and $\beta3$ →Fibroblasts $\gamma2$→ epithelial & MSC [83] Tumour cells [84-86]	Network glycoprotein 11 isoforms[87]
Perlecan	Epithelium [83, 88] Endothelium [10] Mesenchymal cells[10]	Heparan sulphate proteoglycan[89]
Agrin	α-smooth muscle actin +ve (SMA+ve) cells [90] Motor neurons [91] Myofibroblasts [90]	proteoglycan[82]
Nidogen (entactin)	Mesenchymal cells[92, 93] Fibroblasts [83]	
BM-40/SPARC	Endothelium [10, 94]	Ca binding [82]
Fibulin-1	Epithelium[95, 96] Mesenchymal cells[95, 96] Tumour cells [97, 98]	Ca binding Glycoprotein[95]
Fibulin-2	Epithelium [95, 96] Fibroblasts [99] Mesenchymal cells[95, 96]	Ca binding Glycoprotein [95]
Collagen VII	Epithelial cells [100] Fibroblasts [80]	Fibrillar[80]

Table 3: Basement membrane (BM) and myoepithelial cells (MEC) in various breast lesions

Breast lesion	Classification	BM	MEC
Microglandular adenosis	Benign but infiltrative	Present	Absent
Infiltrating epitheliosis	Benign /focally infiltrative	Mainly present	Focally absent
Collagenous spherulosis	Benign	Present	present
Metaplastic apocrine changes	Benign	Present	Can be attenuated or absent in occasional cases
Lactational changes	Benign	Present	Can be attenuated or absent in occasional cases
Ductal Carcinoma in-situ (DCIS)	Malignant in situ	BM is preserved at the periphery but can be different from the normal BM around the TDLU and breast ducts	Present but can be focally attenuated
Encapsulated papillary carcinoma	Low-grade invasive	Typically, thick capsule like structure at the periphery	Absent in 70-80% of cases at the periphery
Solid papillary carcinoma	Low-grade invasive	Typically, thin capsule-like structure (BM) at the periphery	Absent in 50-70% of cases at the periphery
Adenoid cystic carcinoma	Malignant invasive	BM-like material at the periphery of the cribriform structures and within the false lumen (pseudocyst)	Absent