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SIGNIFICANCE OF EXPRESSION OF SMMHC (SMOOTH MUSCLE MYOSIN HEAVY CHAIN), A MYOEPITHELIAL MARKER, IN VARIOUS BENIGN AND MALIGNANT LESIONS OF BREAST.

Pathology

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ABSTRACT

The morphological distinction between benign and malignant or in situ and invasive breast lesions can be problematic at times due to considerable interobserver disagreement in the interpretation of difficult breast lesions based on histology alone. The presence of myoepithelial layer characterizes all normal, benign as well as in situ lesions of breast whereas the loss of myoepithelial layer is the hallmark of invasive carcinoma which can be demonstrated by immunohistochemical techniques.

In this study, we have made an effort to study the significance of expression of SMMHC (Smooth Muscle Myosin Heavy Chain), a myoepithelial marker, in various benign and malignant lesions of breast.

KEYWORDS

Myoepithelial Cells, SMMHC, Benign, Malignant.

INTRODUCTION

The breasts are an important feature of female anatomy and integral part of the female reproductive system(1). The morphofunctional unit of the organ is composed of two major parts: the terminal duct-lobular unit (TDLU) and the large duct system. Histologically, in the normal breast, the ductal and acinar units are lined by two cell layers. The inner layer of luminal cells and an outer layer of contractile myoepithelial cells. These two have distinctive ultrastructural and immunohistochemical features(2).

Mammary myoepithelial cells have been largely ignored since most of the tumors arise from the luminal cells. But emerging data in recent years confirms that myoepithelial cells play a key role in breast tumor progression(3). Better understanding of myoepithelial cell function and their role in tumor progression may lead to their use for diagnostic, therapeutic and preventive measures. An intact MEC layer is seen in both benign and in situ lesions where as loss of the MEC layer is considered the gold standard for the diagnosis of invasive cancer(4).

Various methods are used to separate ME cells from luminal epithelial cells. These include histopathological examination, ME cells appear spindly, hyperchromatic and are seen outside the luminal epithelial cells, along basement membrane. (5). Other methods include Immunomagnetic separation techniques using monoclonal Abs to epithelial membrane antigen(EMA) and CALLA antigen (common acute lymphoblastic leukemia antigen), by analysis and identification of protein expression from two cell types, using Proteonomic technique(9), by cloning myoepithelial cells(6,7) and by using immunohistochemical techniques using myoepithelial markers like S100, SMA, Calponin, SMMHC, H-Caldesmon, p63, CD10, HMW cytokeratin, Maspin, CD 109, CD29 & 14-3-3Sigma,p75 neurotrophic receptor and cytokeratin 14 & 17. Given the mixed epithelial and smooth muscle phenotype of MEC, and the need to distinguish the MEC layer from the epithelial cell layer, most of the immunological markers are detected against smooth muscle related antigens. SM-MHC is a cytoplasmic structural protein and is major component of the contractile apparatus in smooth muscle cells. Two isoforms of smooth muscle myosin heavy chain have been identified, designated MHC-1 and MHC-2. This antibody reacts with MHC-1 (205kDa) and MHC-2 (200kDa) isoforms, and it stains the intact myoepithelial cell layers present in benign and in situ breast lesions(8).

MATERIAL AND METHODS

The study was performed on forty cases (twenty benign and twenty malignant lesions) selected randomly, received as lumpectomy, mastectomy tissues in the department of Pathology, Govt. Medical College, Amritsar. The relevant clinical history, family history, investigations and gross examination were recorded. The tissues were formalin fixed, paraffin embedded and stained with routine haematoxylin and eosin and histological diagnosis was made. Normal breast tissue adjacent to the pathological area was taken as control.

Then, Immunohistochemistry (IHC) was performed using avidin-biotin-peroxidase complex (ABC) technique. New sections of 3 µm-5 µm thickness for each case were then cut from paraffin-embedded blocks and mounted on freshly prepared 0.01% poly-1-lysine-coated slides. Slides were dried overnight at 37°C, dewaxed in xylene, and then gradually rehydrated. Endogenous peroxidase activity was blocked using 0.3% hydrogen peroxide in methanol for 10 min followed by three washings in phosphate buffered saline (PBS), and then antigen retrieval was achieved in a pressure cooker (4 times, 5 min each; 0.1 M citrate buffer, pH 6.0). The sections were then brought to room temperature. For detection of SMMHC, mouse monoclonal antibody clone 131 was procured. The sections were then incubated with primary antibody for SMMHC at 1:100 dilutions for 1 hr at room temperature. Following this, sections were incubated with biotin-conjugated secondary antibody for 30 min and then incubated using streptavidin-biotin system for 30 min at room temperature. Each step was followed by PBS wash twice. The reactions became visible after immersion of sections in 3,3′-diaminobenzidine hydrochloride solution. Sections were then counterstained with Mayer's hematoxylin stain for 2 to 5 min, dehydrated, cleared and mounted with mounting media. Normal breast tissue adjacent to the pathological area was taken as control.

Positive control showed brown colored end product at the site of target antigen. Negative control did not have any color end product. Staining of myoepithelial cells was scored on the scale of 0 to 3 and the staining distribution was documented as focal, partial and circumferential.

Staining intensity
0-Negative
1-Weak
2-Medium
3-Intense

Staining pattern

Focal	< 10% of target cells
Partial	10-90% of target cells
Circumferential	>90% of target cells

Staining intensity of normal breast was taken as intense positive.

Immunohistochemical study for SMMHC was performed on all forty cases of breast and results were interpreted in terms of intensity of myoepithelial (ME) cell staining (0 to 3) around ducts and lobules. SMMHC was consistently positive in myoepithelial cells of all ducts & acini, but was exclusively negative in luminal epithelial cells and stroma of normal and benign lesions of breast.

Table 1: Comparison of immunohistochemical expression of SMMHC in benign lesions of breast

LESIONS	NO.	ME CELL STAINING			
		0	1	2	3
FIBROCYSTIC	3	-	-	1	2
FIBROADENOMA	8	-	-	1	7
BENIGN PHYLLODES	2	-	-	2	-
LOBULAR HYPERPLASIA	1	-	-	-	1
SCLEROSING ADENOSIS	4	-	-	2	2
FIBROADENOSIS	2	-	-	2	-
TOTAL	20	-	-	8	12
PERCENTAGE	100	-	-	40	60

All benign lesions, showed positive results and majority (60%) showed intensity of Grade 3, rest (40%) showed intensity of grade 2 with circumferential pattern.

Table 2: Comparison of immunohistochemical expression of SMMHC in malignant lesions of breast

GROUP	NO. OF CASES	STAINING INTENSITY			
		0	1	2	3
DCIS	2	0	0	1	1
IDC	18	18	0	0	0
TOTAL	20	0	0	1	1
PERCENATGE	100	90	0	5	5

Amongst malignant lesions, two cases (10%) showed staining index of 2 and 3. Both of these cases were of ductal carcinoma in situ and showed complete circumferential to partial staining of the ducts involved. Infiltrating ductal carcinomas did not show staining.

On applying chi square test, the **p value** obtained was <0.001 (highly significant).

DISCUSSION

SMMHC is a very good myoepithelial marker, as it strongly stains myoepithelial cells and shows less heterogeneity of staining (no epithelial cell staining and only weak myofibroblastic positivity), making interpretation easy.

All the benign lesions in our study showed circumferential staining of myoepithelial cells around the ducts. The staining intensity varied between 2 & 3.

Similarly, Kalof et al, also demonstrated uniform 3+ circumferential in all the benign lesions taken (3 cases of sclerosing adenosis & 5 cases of apocrine metaplasia)(9). Werling et al found that all the benign lesions i.e. 11 cases of sclerosing adenosis, showed staining for myoepithelial cells(10). Cheryl B. Hill and I-Tien Yeh also reported similar results and observed that all (25 cases) of benign intraductal papilloma gave continuous positive staining with SMMHC(11).

In present study, amongst DCIS cases, 50% cases showed staining index of 3 with circumferential pattern and rest (50%) showed staining index of 2 with partial disruption. Kalof et al studied 25 cases of DCIS and revealed hetergenous staining pattern with 48% of the ducts showing circumferential staining whereas MEC was not detected in 12.7% of ducts using SMMHC(9). Werling et al studied 33 cases of ductal carcinoma in situ and demonstrated that myoepithelial cells were present in all cases but a small minority of cases (7% cases) showed incomplete positivity resulting in apparent gaps(10). Cheryl B. Hill and I-Tien Yeh also demonstrated heterogenous staining pattern in nine cases of intraductal papillary carcinomas varying from 0 to 3 which indicated a spectrum of progression intermediate between in situ and invasive disease(11).

Hilson further worked on ME cell and expression of various markers for MEC in normal breast and in DCIS lesions and concluded that although ME cells are retained in insitu lesions but their IHC expression when compared to normal adjacent breast tissue was variable with variable intensity of staining from grade 0 to 3 whereas intensity was of grade 3 in normal breast tissue. This reduced intensity was attributed to associated stromal inflammation, altered ME cell differentiation state because of surrounding microenvironment conductive to invasive cancer(12).

All the cases of infiltrating carcinoma in our study were negative for staining with SMMHC. Kalof et al also demonstrated absence of MEC in all cases of invasive carcinoma (21 cases)(9). Similarly Werling et al revealed that all the 35 cases of infiltrating ductal carcinoma were negative for SMMHC(10).

Blood vessels were consistently positive in all the cases, thus serve as a good internal control.

SUMMARY AND COCLUSIONS

In the present study it was seen that benign lesions as well as in situ lesions retained myoepithelial cell layer, which was highlighted by staining with SMMHC. All the cases of benign lesions as well as cases of insitu carcinoma demonstrated staining of variable intensity, whereas in the cases of invasive breast cancer this myoepithelial cell layer was not preserved and thus no staining was seen.

Thus we can conclude that SMMHC, as an immunohistochemical marker for myoepithelial cells, can play an important role in differentiating noninvasive (benign as well as insitu) from Invasive breast lesions as invasive breast lesions are characterized by loss of myoepithelial cell layer.

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