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A STUDY OF STROMAL EXPRESSION OF ALPHA SMOOTH MUSCLE ACTIN IN PROLIFERATIVE LESIONS OF BREAST.

Pathology

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ABSTRACT

Background: Breast lesions are one of the common problems encountered in women. Breast cancer is the most common malignant tumor and the leading cause of cancer death in women worldwide, with > 1 million cases occurring annually and 30,000 patients die annually from the disease. The stroma immediately adjacent to a tumor is a passive structural element that elicits an immune response in an attempt to reject the tumor. CD34 fibrocytes undergo morphologic and phenotypic alterations characterized by the adoption of a plump myofibroblast-like appearance and loss of CD34 expression, accompanied by the acquisition of a-smooth muscle actin (SMA) expression. The purpose of this study is to see the presence and distribution of smooth muscle actin positive cells in the stroma of proliferative lesions of breast. **Method:** A total of 65 cases out of 105 cases received over a period of three years from august 2018 to July 2021. Smooth muscle actin expression was studied in the stroma and graded accordingly. **Results:** This study showed Smooth muscle actin was strong positive in stroma of malignant lesions, whereas it was negative in the benign and non-neoplastic lesions. It showed Sensitivity of 100% and specificity of 89.17%. Positive predictive value was 83.87% and negative predictive value was 100%. P value was <0.001, hence significant in malignant lesions. **Conclusion:** Smooth muscle actin in the stroma of proliferated breast lesions is associated with aggressive behavior of the lesion. Owing to the significant differences in SMA expression between benign and malignant breast lesions, it can potentially be used to differentiate between the two and can serve as an important diagnostic marker.

KEYWORDS

alpha SMA, Proliferative breast lesions, Immunohistochemistry

INTRODUCTION

Breast lesions have different etiologic causes and can be either benign or malignant. Breast cancer is the most common malignant tumor and the leading cause of cancer death in women worldwide, with > 1 million cases occurring annually and 30,000 patients die annually from the disease.[1] Studies have demonstrated that the extracellular matrix (ECM) molecules and cells that compose the microenvironment modulate tissue-specificity in the normal breast, as well as the growth, survival, polarity, and invasive behavior of breast cancer cells. During tumorigenesis some data suggest that precancerous epithelial cells acquire multiple genetic mutations and the associated stroma become "activated", commonly expressing myofibroblastic markers.[2,3] The stroma immediately adjacent to a tumor is not a passive structural element that elicits an immune response in an attempt to reject the tumor, but an element that actively participates and contributes to tumor progression.[4] The data suggest that CD34 fibrocytes undergo morphologic and phenotypic alterations characterized by the adoption of a plump myofibroblast-like appearance and loss of CD34 expression, accompanied by the acquisition of a-smooth muscle actin (SMA) expression. The stromal loss of CD34 expression and acquisition of SMA myofibroblastic features may constitute a prerequisite for tumor invasiveness.[5,6] The purpose of this study is to see the presence and distribution of smooth muscle cell actin positive cells in the stroma of the normal mammary gland, benign and malignant tumors.

MATERIAL AND METHOD

A total of 65 cases of diagnosed cases of proliferative lesions of breast out of 105 cases received over a period of three years. The H&E stained slides were examined microscopically to study the morphology. The lesions were typed and subtyped according to latest WHO classification of Tumors of the Breast. The tissue specimens were further subjected to immunohistochemistry for alpha smooth muscle actin. The antibody used is mouse monoclonal antibody, Actin (smooth muscle)- 1A4, IgG2a from PathnSitu. Internal controls were used in all cases.

Alpha smooth muscle actin shows cytoplasm staining.

The percentage of stromal cells expressing antigen was graded as follows:

0 Upto 5 %
1 5% to 25%
2 25% to 50%
3 More than 50%

Exclusion Criteria: 1. Patients who have undergone radiotherapy or chemotherapy for any breast lesion. 2. Biopsies containing hemorrhage and necrosis. 3. All non-proliferative breast lesions and metastatic lesions. 4. Patients taking chemo and radiotherapy for any type of cancer.

The statistical data is analyzed by SPSS 20.0 software. In this study for data analysis Chi square test will be applied. P value <0.05 will be considered as significant.

RESULT

A total of 65 cases out of 105 cases of breast lesions were included in the study.

Maximum number of lesions 42 presented with 2—5 cm size. Malignant lesions in majority i.e., 13 presented with the size >5 cm. The mean size of lesions was 3.42 cm. Table 1 shows histopathological diagnosis and SMA grading. Table 2 shows grading of alpha SMA in various cases.

Table 1: Histopathological Diagnosis And SMA Grade.

	Histopathological diagnosis	Number of cases	SMA grade			
			0	1	2	3
Non Neoplastic	Fibrocystic disease	06	5	1		
	Gynecomastia	03	3			
	Microglandularadenosis	01			1	
Benign	Fibroadenoma	14	10	3	1	
	Juvenile fibroadenoma	02		2		
	Fibroadenoma with cystic change	03	2	1		
	Fibroadenoma with apocrine change	02	1		1	

	Fibroadenoma with ductal hyperplasia	01	1			
	Fibroadenoma with adenosis	02		2		
	Tubular adenoma	02	2			
	Adenomyoepithelioma	01	1			
	Phyllodes	02			2	
Malignant	Borderline phyllodes	01				1
	Malignant phyllodes	04				4
	IDC NOS	18			5	13
	Spindle cell carcinoma	01			1	
	Papillary carcinoma	01				1
	Squamous cell ca	01				1
Total	--	65	25	9	11	20

Table 2: Grading Of IHC Marker Alpha SMA In Various Cases In Adjacent Stroma

Grade	Number of cases	Non neoplastic	Benign cases	Malignant cases
0	21(32.3%)	8(12.3%)	13(20.0%)	0 (0.0%)
1	14 (21.5%)	2 (3.1%)	12 (18.5%)	0 (0.0%)
2	10 (15.4%)	0 (0.0%)	4(6.2%)	6(9.2%)
3	20 (30.8%)	0 (0.0%)	0 (0.0%)	20(30.8%)
Total	65(100.0%)	10 (15.4%)	29(44.6%)	26(40.0%)
Fisher Exact Test & Sig.		P = 0.0037, Highly significant		

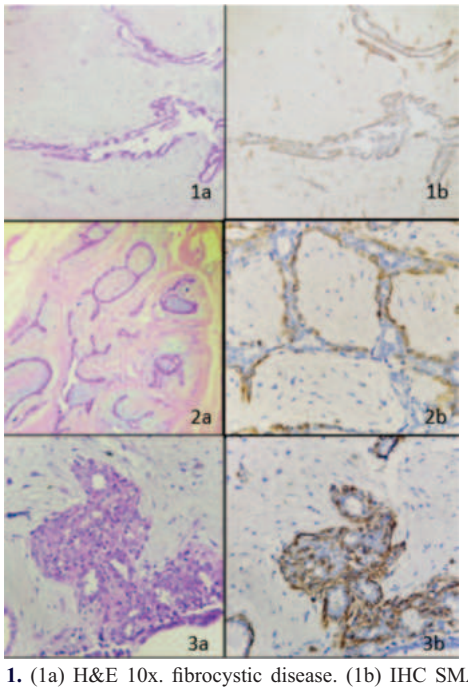

Figure 1. (1a) H&E 10x. fibrocystic disease. (1b) IHC SMA x10. Stromal positivity. Grade 1+.
Figure 2. (2a) H&E x10. Fibroadenoma. intracanalicular pattern. (2b) IHC SMA x40. Stroma between the slit like glands shows negative for SMA.
Figure 3. (3a) H&E x40. Fibroadenoma with apocrine change. (3b) IHC SMA x40. SMA positivity in hyperplastic myoepithelial cells and also stroma shows positivity.

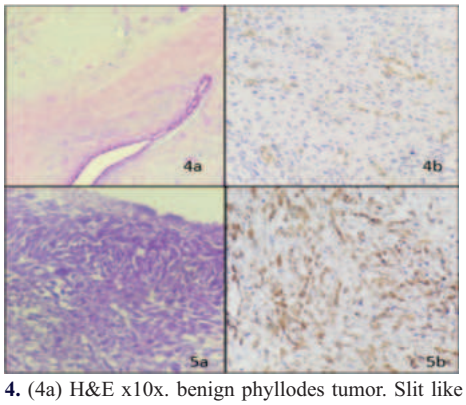

Figure 4. (4a) H&E x10x. benign phyllodes tumor. Slit like glands with increased fibrocollagenous stroma. (4b) IHC SMA x40. Stroma shows 2+ positivity for SMA.
Figure 5. (5a) H&E x40: Malignant phyllodes. (5b) IHC SMA x40: grade 3+.

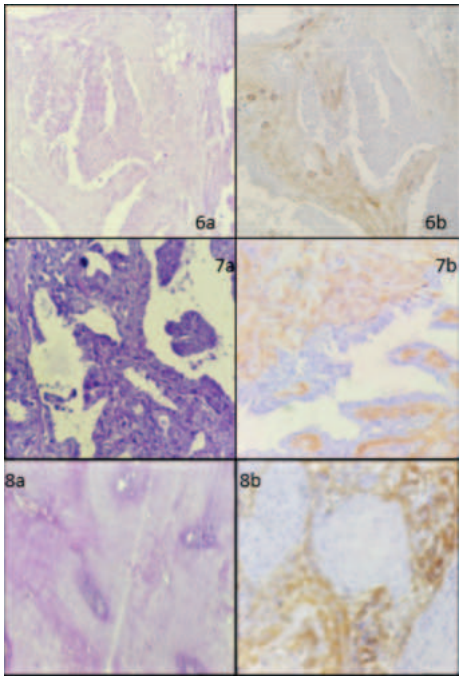

Figure 6. (6a) H&E x10. Infiltrating ductal carcinoma. (6b) IHC SMA x10. Tumor cells show negative for SMA whereas stroma shows positive for SMA.
Figure 7. (7a) H&E x40: malignant cells arranged in papillary fashion. (7b) IHC SMA x40: ductal cells negative while stromal cells are strong positive for SMA.
Figure 8. (8a) H&E x10: Squamous cell carcinoma (8b) IHC SMA x40: squamous cells not taking SMA whereas stroma shows strong positivity for SMA.

DISCUSSION

Immunohistochemistry has an expanding role in the diagnosis and the management of mammary gland lesions. A growing list of available antibodies, improved antigen retrieval techniques and a better understanding of biology have all contributed to the broader utility of IHC for solving everyday diagnostic problems in breast pathology.[7,8]

Myofibroblasts through the secretion of chemokines, cytokines, growth factors, inflammatory mediators, as well as extracellular matrix proteins and proteases, play an important role in organogenesis, oncogenesis, inflammation, repair and fibrosis in most organs and tissues.[9]

Smooth muscle actin (SMA) has long been used as a myoepithelial marker in breast pathology diagnosis as a sensitive marker of myoepithelial differentiation.[10] During breast stromagenesis there is downregulation of some stromal genes, such as CD34 and upregulation of other genes indicative of myofibroblastic differentiation, such as smooth muscle actin (SMA).[11]

Myoepithelial markers are useful in helping to distinguish invasive carcinoma from benign proliferations with a similar morphological appearance, benign proliferative lesions and most preinvasive lesions with an intact myoepithelium. Invasive carcinomas lack the myoepithelial cell layer that normally surrounds benign breast glands.[10]

Maximum number of cases i.e., 21(32.3%) showed grade "0" of which 13(20.0%) were benign and 8(12.3%) non neoplastic. Followed by 20 (30.8%) cases which showed grade 3 positivity. Non neoplastic cases were negative for SMA whereas benign cases show variable positivity 4 (6.2%). All malignant cases were positive for alpha SMA.

Of all non-neoplastic cases only one showed 2+ positivity (micro glandular adenosis). All 3 gynecomastia cases showed negative for SMA. There was no statistically significant difference in non-

neoplastic lesions with SMA (P>0.05). Only 1 case (fig a, b) out of 6 cases of fibrocystic disease showed grade 1 positivity compared with Cimpean et al which showed 2 cases with intermediate positivity.

Maximum number of benign cases showed grade 0 scoring for SMA, (fig c, d). Some fibroadenomas and its variants like juvenile fibroadenoma and fibroadenoma with apocrine change (fig e, f) showed 1+ positivity. The findings were in consistent with Cimpean et al study. The SMA positivity is associated with fibrosis in these lesions.

Benign phyllodes tumor showed 2+ positivity (fig g, h). As phyllodes tumor compromise of stroma which determines the biology of these tumors. These hypercellular stromal fibroblast becomes reactive and show positivity for Smooth muscle actin. Whereas, all Malignant phyllodes tumors show 3+ positivity (figure I and j). This shows strong statistical significance between invasion and SMA expression.

All malignant cases showed strong positivity 2+ and 3+ for SMA in the stroma; i.e., malignant phyllodes, invasive ductal carcinoma (figure k and l) and metaplastic carcinoma (figure m, n, o and p). There was statistically significant difference of distribution of malignant lesions with SMA Grades (P<0.05). The gain of Smooth muscle actin in the stroma shows the transformation of SMA negative fibroblast to SMA positive myofibroblast. These are the changes associated with invasion of the malignant cells in the stroma.

Sensitivity was 100% for SMA whereas specificity was 89.79%. Positive predictive value was 83.87% whereas negative predictive value was 100%. SMA showed a diagnostic accuracy of 92.3%. Our study was consistent with the findings of Yazhou et al which states that gain of Smooth Muscle Actin in the stromal myofibroblasts correlated significantly with pathological parameters associated with a poor prognosis.[14]

CONCLUSION

Smooth muscle actin in the stroma of proliferated breast lesions is associated with aggressive behavior of the lesion. Owing to the significant differences in SMA expression between benign and malignant breast lesions, it can potentially be used to differentiate between the two and can serve as an important diagnostic marker.

Declarations

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee of the Institute of MRMC, Kalaburgi.

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