Original Research Article

Expression of p63 in Benign, In-Situ and Invasive Ductal Carcinoma of Breast

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Abstract

Background: p63 is a member of p53 family of transcription factors which have important function in tumorogenesis and oncogenesis. It is involved in cellular differentiation and exposed in nuclei of myoepithelial cells of normal breast. *Aim:* To study immunoexpresion of p63 in the benign, insitu, and invasive ductal carcinoma of breast. *Material and Methods:* The present study was conducted on 110 cases of formalin fixed paraffin embedded histological sections of surgically resected breast tissue specimens suspected to have neoplastic lesions and mastectomy specimens already diagnosed as carcinoma on cytology for a period of one year. *Result:* p63 is strongly expressed in all benign breast lesion in form of continuous peripheral rim around ductal epithelial cells, except atypical ductal hyperplasia in which moderate expression of p63 was noted in form of scattered as less continuous rim of myoepithelial cells similarly moderate to weak expression with focal gaps was described in ductal carcinoma of in situ and no expression was noted in infiltrative ductal carcinoma. *Conclusion:* p63 is a hallmark of very poorly differential cells or cells component with higher histological grade in breast carcinoma. Hence p63 can be used as an indicator of aggressiveness of breast carcinoma as a marker of choice in differentiating benign and malignant breast lesions.

Keywords: Breast Carcinoma; Myoepithelial Cells; p63.

Introduction

Disease of breast constitute a significant proportion of surgical cases seen in both developed and developing countries and frequently the need arise to distinguish benign and malignant lesions prior to definitive treatment [1] Breast cancer has ranked number one cancer among Indian females with age adjusted rate as high as 25.8% per 100,000 women and mortality 12.7% per 100,000 women [2]. There is a significant increase in the incidence and cancer associated morbidity and mortality in Indian subcontinent as described in global and Indian studies.

During the last few decades immunohistochemistry (IHC) has become an integral part of pathology. Although H & E stain remains the fundamental basis for diagnostic

pathology of breast, IHC stains provide useful and vital information. IHC can be a powerful tool for resolving many diagnostic problems in breast pathology [3].

In every day pathology practice the identification of myoepithelial cell in breast lesion is of great diagnostic value in differential diagnosis of breast lesion in discrimination between invasive and non invasive lesion, because they are retained in most benign breast while being lost in malignancy [4].

The nuclear protein p63 has attended much attention in recent reports. p63 positive myoepithelial cells have been shown to surround benign epithelial lesion and form a consistent, although discontinuous ring around epithelial cell in carcinoma in situ. No staining has been noted in infiltrative carcinoma.

The aim of present study was to establish histopathological diagnosis of benign, in situ and invasive ductal carcinoma of breast and to study immunoexpression of p63 in histopathological specimen of benign,in situ and invasive ductal carcinoma of breast.

Material and Methods

The present study was conducted on 110 formalin fixed liquid paraffin sections from various breast lesions including benign, insitu and invasive carcinoma cases were studied in the department of pathology during a period of one year from June 2016 to May 2017. Resected breast tissue specimen suspected to have neoplastic lesion and mastectomy specimens already diagnosed as carcinoma in cytology are included in the study. The tissue were processed for routine histopathological examination and then, the immunohistochemical expression of p63 was analysed in all the specimens using avidin-biotin peroxidase method. The reactivity of p63 antibody was scored semi quantitatively. p63 was normally expressed in the nucleus of the myoepithelial cells and when it was completely unstained or immunoreactivity observed in <5% of cell was assigned score 0 (negative), 1 (5-25% of the cells positive, 2 (26-75% of the cell stained) and 3 (>75% of the cell stained). According to Doina Ivon et al. (2004) [5], positivity of cells is defined regardless of staining intensity. By convention, we considered that >25% positive cells represented the cut off between negativity and positivity of p63 immunostaining. All relevant data was collected and appropriate statistical tools were applied to analyze the data.

Results

Out of 110 cases of breast lesions, 72 cases (65.5%) were benign and 38 cases (34.5%) were malignant. Fibroadenoma was the most common benign breast lesion followed by fibrocystic disease,while infiltrating ductal carcinoma (84.22%) was the most common malignant lesion. All the cases were in the age group between 11-70 years of age with maximum cases in age group of 31-40 years. Out of 110 cases, 108 cases (98%) were female and only 2 cases (2%) were male. Most of the benign lesion occurred in premenopausal women while maximum case of malignant lesion occurred in perimenopausal and postmenopausal women. The size of the lump ranged from 0.5cm to 12cm in maximum dimension. Majority of benign lesion were less than 5cm while most malignant lesions were of size >5 cm.

Most of the lesions were observed in left breast involving superolateral quadrant (63.3%) followed by inferolateral quadrant (16.8%). On analysis immunoreactivity pattern of p63, all of the benign breast lesions showed strong (+3) positivity except atypical ductal hyperplasia which showed moderartely positivity (+2) in 67% cases (Table 1).

All cases of fibroadenoma showed continuous immunoreactivity while fibrocystic disease showed

**Table 1:** Expression of p63 in benign breast lesions

Type of Lesion	Number of cases		p63 positivity		p63 negative
		+3 staining	+2 staining	+1 staining	
Fibroadenoma	40	40 (100%)	-	-	00%
Fibrocystic disease	18	18(100%)	-	-	00%
Atypical ductal hyperplasia	06	02(33.34%)	04(66.66%)	-	00%
Fibroadenosis	04	04(100%)	-	-	00%
Chronic mastitis	02	02(100%)	-	-	00%
Gynaecomastia	02	02(100%)	-	-	00%
Total	72	68(98.45%)	04(05.55%)	-	00%

Rajesh Kumar Rai, Shaila Kumari Mitra, Manjari Rao et al. \ Expression of
p63 in Benign, In-Situ and Invasive Ductal Carcinoma of Breast

1179

Table 2: Expression of p63 in malignant lesions of breast

Type of Lesions	Number of cases	p63 posivity			p63 negativity
		+3	+2	+1	
In situ ductal carcinoma	06	-	04(66.66%)	02(33.34%)	00(00.00%)
Invasive carcinoma	32	-	00(00.00%)	02(06.25%)	30(93.75%)
Total	38	-	04(10.52%)	04(10.52%)	30(78.94%)
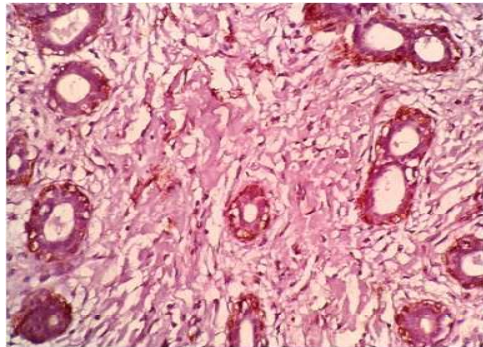
Fig. 1: Microphotograph of immunohistochemical staining of p63 in fibroadenoma (pericanalicular pattern) showing strong (+3) nucleus positivity.(X400)
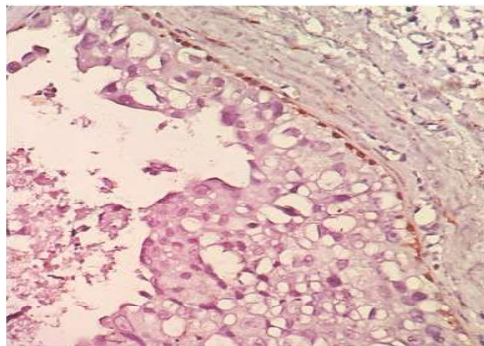
Fig. 2: Microphotograph of intermediate grade ductal carcinoma in situ showing focal or discontinuous moderate (+2) nuclear positivity of p63 immunostain.(X400)
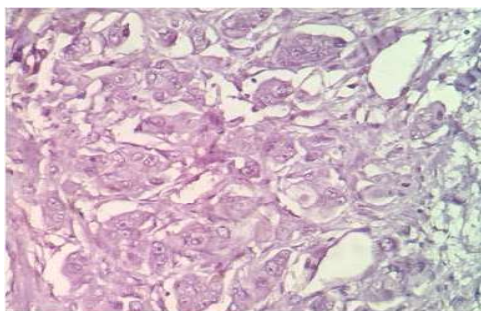
Fig. 3: Microphotograph of immunohistochemical staining of p63 in grade I or well differentiated infiltrating ductal carcinoma showing complete loss of p63 expression.(X400)

discontinuous positivity. Ductal carcinoma in situ showed moderate to weak (+1 to +2) positivity while all the infiltrating ductal carcinoma cases showed complete absence of p63 immunoexpression. (Table 2, Fig. 1)

On statistical evaluation, the difference of expression of p63 in benign and malignant lesion of breast was found to be highly significant. (p<0.0001) A highly significant association was also was found in differentiating in situ from invasive lesion (p<0.0001).

The sensitivity and specificity of p63 was 100% and 78.94% respectively with positive predictive value of 90% and negative predictive value of 100%. Diagnostic accuracy was 92.72%.

Discussion

Out of total 110 cases, 72 cases (65.5%) were benign and 38 cases (34.5%) were malignant lesions. Fibroadenoma was the commonest benign lesion (55%) while infiltrative ductal carcinoma being most common type of carcinoma (70%), followed by lobular, medullary, mucinous, comedo-carcinoma, paget's disease, papillary, tubular and inflammatory carcinoma among malignant lesions Ductal carcinoma in situ comprised of 15.78%.

Similar finding was seen in the study by D. Stenafou et al. (2004) [6] who observed P63 positive myoepithelial cells surrounding benign epithelial lesion, scattered and weakly positive P63 in almost half of ductal hypoplasia (22/48, 45%). Comparative results were also reported by Werling et al. [7], 2003. However, Wang et al [8] showed that p63 was expressed in myoepithelial cells of benign breast, partially expressed in ductal hyperplasia, rarely expressed in carcinoma in situ and expressed in invasive carcinoma.

Our study was in accordance with earlier reports of Gusterson et al. (1982) [9] who reported that there is definite difference in p63 staining between benign lesion and insitu carcinoma in one hand and invasive carcinoma on the other.

On statistical analysis, the expression of p63 between benign and malignant breast lesion was found to be highly significantly (p<0.0001). The association of p63 immunoreactivity between in situ and invasive carcinoma of breast was also found to be highly significant (p<0.0001).

The sensitivity and specificity of p63 was 100% and 78.74% respectively with positive predictive value of 90% and negative predictive value of 100%. Diagnostic accuracy was 92.72%. Thus, in our study p63 proved to be the sensitive marker for the detection of myoepithelial cells and a negative p63 staining is quiet specific for malignant cell. Kim S.K. et al. (2014) [10] reported overall sensitivity of 93.8% and specificity of 97% in identifying myoepithelial cells in his study. p63 immunostaining being intense nuclear, is superior to the often weak and cytoplasmic staining with other myoepithelial markers, making interpretation easier. Moreover, it appears that p63 is most sensitive and specific myoepithelial markers than those currently used, with no staining of secretory cells, stromal myofibroblast, smooth muscle cells or pericytes.

Conclusion

The present study demonstrates that p63 is strongly expressed in all benign breast lesions in the form of continuous peripheral rim around ductal epithelial cells except atypical ductal hyperplasia in which moderate expression of p63 was noted in the form of scattered or less continuous rim of myoepithelial cells. Similarly, moderate to weak expression with focal gaps was observed in ductal carcinoma in situ and no expression was noted in infiltrating ductal carcinoma. Thus, there is progressive loss of p63 expression with the progression from benign to malignant breast lesions, so it can be used as a highly sensitive and specific marker in differentiating benign and malignant breast lesions.

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