IMMUNOHISTOCHEMICAL STUDIES ON THE EXPRESSION OF CEA, C-ERBB-2 AND CATHEPSIN D IN TISSUE SECTIONS AND FINE NEEDLE ASPIRATES OF BENIGN BREAST LESIONS

Hasnan J, Mutum SS, Ismail AM, Hasbullah AS
Pathology Department
School of Medical Sciences
Universiti Sains Malaysia
16150 Kubang Kerian, Kelantan
Malaysia

Ruj Fail : FPP 2000/123
No. A/C : 304/PPSP/6131131

USM J/P-06

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Nama Penyelidik-Penyelidik Lain *(Jika berkaitan)* : A-Prof. Mutum Samarendra Singh
En. Ismail Abdul Manan
En. Hasbullah Abdul Samad

2) Pusat Pengajian/Pusat/Unit : Pusat Pengajian Sains Perubatan / Jabatan Patologi

3) Tajuk Projek: Immunohistochemical Studies on the Expression of CEA, c-erbB-2 and Cathepsin D in Tissue Sections and Fine Needle Aspirates of Benign Breast Lesions.

4) (a) Penemuan Projek/Abstrak

(Perlu disediakan makluman di antara 100 - 200 perkataan di dalam Bahasa Malaysia dan Bahasa Inggeris Ini kemudiannya akan dimuatkan ke dalam Laporan Tahunan Bahagian Penyelidikan & Pembangunan sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti).

Lihat Lampiran.

(b) Senaraikan Kata Kunci yang digunakan di dalam abstrak:

Bahasa Malaysia	Bahasa Inggeris
CEA	CEA
c-erbB-2	c-erbB-2
Cathepsin D	Cathepsin D
kesi payudara	breast lesion
benigna	benign

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(c) Latihan Gunatenaga Manusia

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Disertasi pelajar sarjana perubatan Patologi
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Tajuk: Immunohistochemical expression of p53 and c-erbB-2 in ductal carcinoma in situ and infiltrating carcinoma of breast

ii) Pelajar Prasiswazah:

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Uji latih pewarnaan c-erbB-2 oleh Juruteknologi Makmal Perubatan bagi tujuan laporan rutin kanser payudara.

6. Peralatan Yang Telah Dibeli:

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Chairman of Research & Ethics Committee
School of Medical Sciences
Universiti Sains Malaysia
16150 Kubang Kerian
KELANTAN, MALAYSIA.

TANDATANGAN PENGERUSI
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Pathology Department
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16150 Kubang Kerian, Kelantan
Malaysia

Abstract

The diagnosis of breast lesions in histopathology relies heavily on the morphology of the epithelial cells. This study aim at looking on the immunohistological expression of well-known prognostic markers of breast carcinoma i.e. CEA, c-erbB-2 and Cathepsin D on the epithelial cells of benign breast lesions in tissue sections and fine needle aspiration smears. Being strongly positive in malignant breast lesions, it is expected that the markers will be less positive or totally negative in benign epithelial cells.

91 cases of benign breast lesions composed of 46 cases of fibroadenoma, 18 cases of fibrocystic change, 10 cases of sclerosing adenosis, 9 cases of epithelial hyperplasia, 7 cases of papilloma and 1 case of lactating adenoma were identified in tissue sections. CEA was positive in 11 of the cases (12%) and Cathepsin D was positive in 20 cases (22%). Complete ring of epimembranous cytoplasmic C-erb-B2 expression was absent in all the cases, therefore it is considered that all cases were negative for c-erb-B2 expression. 13 cases (14%) comprising of 9 fibroadenoma, 2 fibrocystic change and 2 epithelial hyperplasia showed incomplete ring of epimembranous staining by c-erb-B2.

It appeared that CEA and c-erbB-2 expression is low in benign breast lesion and the expression is specific in the epithelial cells. However, CEA at the same time also showed strong positivity to the adjacent myoepithelial cells and this may give a higher result of positivity in such cases. For Cathepsin D expression, on top of showing strong positivity for myoepithelial cells and epithelial cells, it showed a non-specific positivity in the surrounding stromal cells. These findings indicate that c-erbB-2 and CEA rather than Cathepsin D are more likely to give a better distinguishing expression when comparing benign and malignant breast lesion in tissue sections.

In 46 cases of fibroadenoma studied, CEA was positive in 10 cases (21.7%) and Cathepsin D in 9 cases (19.6%). In 18 cases of fibrocystic change studied, none showed expression for CEA and c-erbB-2 ; and 3 cases were positive for Cathepsin D. The same pattern is observed in sclerosing adenosis and papilloma cases studied. CEA and c-erbB-2 were not expressed in 10 cases of sclerosing adenosis and 7 cases of papilloma. Cathepsin D was positive in 3 cases of sclerosing adenosis and 2 cases of papilloma.

51 cases of fine needle aspirates of benign breast lesion were also studied. 28 cases comprising of 21 fibroadenoma, 4 benign proliferative lesion, 2 fibrocystic change and 1 non-specific inflammatory lesion were subjected to CEA staining while another 23 cases comprising of 18 fibroadenoma, 3 benign proliferative lesion, 1 fibrocystic change and 1 non-specific inflammatory lesion were subjected to c-erbB-2 staining. For the CEA staining, 6 cases (21%) comprising of 5 fibroadenoma and 1 fibrocystic change were positive. C-erbB-2 expression is only seen in one case (4%) out of 23 cases subjected to the staining and that single case was a fibroadenoma. In the study of the fine needle aspirate, c-erbB-2 appears to be less expressed compare to CEA.

In conclusion, it can be seen that c-erbB-2 marker is significantly less expressed or not expressed at all compare to CEA in both tissue sections as well as in fine needle aspiration smears. Therefore c-erbB-2 marker is a potential tool that can be used to differentiate benign from malignant breast lesions. Cathepsin D due to its non-specific staining and increased background staining make it unsuitable for usage to distinguish benign from malignant breast lesions.

Key words : CEA, c-erbB-2, Cathepsin D and benign breast lesions

Introduction

Lump in the breast can be benign or malignant. A benign lump will be amendable to localized surgery or lumpectomy and has a good prognosis. However a malignant lump may require removal of the breast tissue as well as the axillary tail of the breast. A malignant breast lesion may metastasize to other part of the body such as the lungs and bone and this will have a very unfavourable outcome to the patient. Therefore, for a proper and appropriate treatment approach offer to the patient, the surgeon requires the help of the pathologist to make the correct diagnosis.

There are two available methods of tissue biopsy that can be done to ascertain whether the breast lump is benign or malignant. These are tissue core biopsy and fine needle aspiration biopsy.

Tissue core biopsy needs to be done under anaesthesia and the biopsy specimen will be fixed in 10% formalin before it undergoes tissue processing. The tissue will then be subjected to a series of chemicals and later will be embedded in a paraffin wax. The end result is a tissue block. This tissue block will be cut into tissue section using a microtome and stained with Haematoxylin and eosin stain. The tissue section will be examined under the microscope by the pathologist and by analyzing the tumour cell morphology as well as the cell's interaction with the surrounding tissue an appropriate diagnosis will be offered.

On the hand, fine needle biopsy can be done as an outpatient procedure. It requires the insertion of a 23 gauge needle into the breast lump and the tissue material is aspirated under vacuum created in the attached syringe. The specimen is then spread onto glass slides and immediately fixed in 95% alcohol solution as well as air-dried. The alcohol fixed smear will be stained with Papaniculaou stain while the air-dried smear is stained with May Grunwald Giemsa stain. The pap stain smear allow the pathologist to analyse the nuclear features of the tumour cells while the MGG stain will be able to highlight the cytoplasmic details of the cells. However, the pathologist will not be able to relate the cells morphology with the surrounding tissue as in core biopsy specimen as the smear does not show the tissue architecture of the tumour.

In almost 90% of cases, an appropriate diagnosis can be reached. However, sometimes it is difficult to differentiate and distinguish a benign from a malignant lesion. This occur when either the benign lesion show a bizarre cell morphology or tissue reaction that mimics a malignant lesion; or a malignant lesion showing a bland cell morphology or well differentiated glands that mimics a benign lesion instead. These situations require a great patient and experience on the part of the pathologist to interpret the biopsy.

Benign versus malignant lesion

Benign lesions of the breast include fibroadenoma and fibrocystic change. The diagnosis of these lesion are straight forward most of the time. They occur mainly in young women in the reproductive age group. Fibroadenoma presents as a firm discreet mobile painless lump. Histologically, there is proliferation of the benign breast glands and also the

stroma. In patient above 40 years old, fibrocystic change of the breast become more prominent. Fibrocystic change may present as a vague painless lump in the breast and it shows fibrosis, glands proliferation some of which are cystically dilated and also apocrine metaplasia. The presence of epithelial hyperplasia of the lining cells of the glands increases the risk of developing carcinoma in later years. When stromal fibrosis is prominent with many of the proliferating glands being compressed another benign lesion called sclerosing adenosis occurs. Sclerosing adenosis have bizarre tissue architecture that it may mimic the infiltrating nature of malignant breast lesion namely infiltrating ductal carcinoma. Benign lesion occurring in the lactiferous duct such as ductal papilloma may sometimes be difficult to differentiate from papillary carcinoma of the breast.

Apart from looking at cells morphology of the tumour and its pattern of interaction of the surrounding tissue, one important feature of malignancy that need to observe is the breakage of the basement membrane and invasion of the tumour cells into the surrounding stroma. This is done for it patiently and diligently and also by highlighting the basement membrane using special stain such as PAS stain or immunostain for laminin.

CEA, c-erb-B2 and Cathepsin D

CEA, c-erb-B2 and Cathepsin D are tumour markers that are found to be highly expressed in malignant breast tumour. In addition c-erb-B2 is strongly expressed in malignant cells that have the capacity to metastasize while Cathepsin D in invading cells.

Cathepsin D is a lysosomal enzyme involved in intracellular protein turnover in all cells. It is synthesized as a 54kD precursor and normally secreted in its mature and active form of approximately 46kD. In breast cancers, the Cathepsin antibody showed granular staining of variable intensity and occasionally stained the surrounding stromal tissues. Stained fibroblasts and macrophages provide built-in positive controls. Rarely, nuclei of some cancer cells were also stained.

Objectives

We aim to study the expression of breast tumour markers that have been shown to have a strong correlation with malignancy, invasion and metastasis which include CEA, c-erbB-2 and Cathepsin D on benign breast lesions. The study will be conducted with a hypothesis that these markers should be expressed nil or low in the benign lesions. Studies that have been done so far on the expression of CEA and c-erbB-2 on benign lesions showed conflicting results. There was no study done on the expression of Cathepsin D on benign breast lesions.

We hope to identify a reliable marker that will consistently be able to differentiate benign breast lesion from a malignant one directly, without having to go through tediously searching for evidence of tumour cells breaching their basement membrane.

Materials and method

A total of 91 histopathology cases and 51 fine needle cases of benign breast lesions were identified from the registry book at the Pathology Laboratory, School of Medical Sciences, USM Kelantan.

The histopathology cases came from either tissue biopsy or lumpectomy specimens. The fine needle cases were selected from archive materials that have more than one satisfactory smear. For all these cases, the patient data was recorded, the report and histology slides or fine needle smears were reviewed.

The tissue blocks were retrieved and cut. The tissue sections produced were subjected to the standard three-stage immunoperoxidase staining – ABC (Complex Avidin-Biotin) method using the primary antibody CEA (Dako A 0115), c-erb-B2 (Dako A 0485) and Cathepsin D (Dako A 0561). Antigen retrieval methods with microwave treatment were used. Sections were immersed in citrate buffer (pH 6.0) and heated in the microwave oven (600 watt) until boiling for 20 minutes.

The fine needle slides selected for the study were de-stained and re-stained using the above staining method. Examination of the slides were done using light microscope at high magnification of 400X.

The expression of the tumour was scored either positive or negative according to the following scoring scheme.

For CEA and Cathepsin D expression, the presence of granular brown deposits in the cytoplasm of the epithelial mammary cells is considered as positive.

For c-erb-B2 expression, cytoplasmic granular deposit AND complete ring of circumferential epimembranous staining is considered positive. An incomplete ring of epimembranous staining or no epimembranous staining at all are considered negative.

For the sake of quantitative analysis, the percentage of positive staining by all tumour markers and incomplete circumferential staining for c-erb-B2 is also counted by observing 100 ductal epithelial cells in the tissue sections.

Results

Of the 91 cases of benign breast lesions diagnosed from trucut biospy and lumpectomy specimen; 46 fibroadenoma, 18 fibrocystic change, 10 sclerosing adenosis, 9 epithelial hyperplasia, 7 papilloma and 1 lactating adenoma were identified. CEA was positive in 11 of the 91 cases (12 %) and Cathepsin D was positive in 20 cases (22 %). C-erb-B2 was not positive in any of the benign lesions. However, 13 cases (14 %) of these cases showed incomplete ring of epimembranous staining.

In 46 cases of fibroadenoma studied, CEA is positive in 10 cases (21.7%) and Cathepsin D is positive in 9 cases (19.6%). All 18 cases of fibrocystic change studied showed no expression for CEA and c-erbB-2; and only 3 cases were positive for Cathepsin D. The same pattern is observed in sclerosing adenosis and papilloma cases studied. CEA and c-erbB-2 were not expressed in 10 cases of sclerosing adenosis and 7 cases of papilloma. Cathepsin D was positive in 3 cases of sclerosing adenosis and 2 cases of papilloma. 7 cases comprising of 5 fibroadenoma and 2 fibrocystic change showed apocrine metaplasia. In the metaplastic cells, 3 out of 7 cases (42.8%) were positive for CEA, 1 case (14.3%) was positive for c-erb-B2 and all 7 cases were positive for cathepsin D.

A total of 51 cases of fine needle aspirates of benign breast lesion were also studied. 28 cases comprising of 21 fibroadenoma, 4 benign proliferative lesion, 2 fibrocystic change and 1 non-specific inflammatory lesion were subjected to CEA staining while another 23 cases comprising of 18 fibroadenoma, 3 benign proliferative lesion, 1 fibrocystic change and 1 non-specific inflammatory lesion were subjected to c-erbB-2 staining. For the CEA staining, 6 cases (21%) comprising of 5 fibroadenoma and 1 fibrocystic change were positive. C-erbB-2 expression is only seen in one case (4%) out of 23 cases subjected to the staining and that single case was a

fibroadenoma. In the study of the fine needle aspirate, c-erbB-2 appears to be less expressed compare to CEA.

Table 1 : Positive expression of CEA, c-erb-B2 and Cathepsin D in tissue sections of 91 cases of benign breast lesion

Diagnosis	N	CEA	C-erb-B2		Cathepsin D
			Complete	Incomplete	
Fibroadenoma	46	10	0	9	9
Fibrocystic change	18	0	0	2	3
Sclerosing adenosis	10	0	0	0	3
Epithelial hyperplasia	9	1	0	2	2
Papilloma	7	0	0	0	2
Lactating adenoma	1	0	0	0	1
Total	91	11	0	13	20

Table 2 : Positive expression in fine needle aspiration smears of 28 cases of benign breast lesion

Diagnosis	No. of cases	Positive CEA
Fibroadenoma	21	5
Fibrocystic change	2	1
Benign Proliferative Lesion	4	0
Inflammatory Lesion	1	0
Total	28	6

Table 3 : Positive expression in fine needle aspiration smears of 23 cases of benign breast lesion

Diagnosis	No. of cases	Positive c-erb-B2
Fibroadenoma	18	1
Fibrocystic change	1	0
Benign proliferative lesion	3	0
Inflammatory lesion	1	0
Total	23	1

Discussion

Distinguishing benign from malignant breast lesions is an important challenge for a pathologist. While cell morphology, pattern of organization and surrounding tissue reations help to achieve this task most of the time, occasion does arise where the distinction is not clear cut. The breakage and invasion of the basement membrane by epithelial cells is the only true evidence of malignancy. Pathologist used to apply PAS stain to visualise the basement membrane but sometimes this can be difficult to interpret. A few researchers have gone identifying basement membrane markers such as type IV collagen, heparan sulphate proteoglycan and fibronectin to assist pathologist in identifying malignant cells.(Birembaut et al 1985; Raymond WA and Leong AS 1991). Researchers have also suggested the use of staining for laminin and smooth muscle actin to identify the basement membrane and myoepithelial cells.(Liotta LA et al 1984; Tsubura A et al, 1988; Raymond WA and Leong AS 1991; Guelstein VI et al 1993). This

has help a lot for the pathologist. However, these markers cannot be applied to fine needle aspiration specimens as the tissue architecture and basement membrane are absent.

The tumour markers of CEA, c-erb-B2 and Cathepsin had been shown in various studies to correlate well with breast carcinoma aggressiveness.

Duffy et al 1983 reported the presence of CEA—like material in 51 out of 62 primary human breast carcinomas and in only 2 out of 12 fibroadenomas. CEA expression is also low in preneoplastic in-situ carcinoma according to Matsukuma A et al.

However, Bhatavdekar JM et al 1987 while studying preoperatively plasma CEA concentration in 128 patients with breast cancer and Robertson et al 1989 studying the expression of CEA in 180 primary breast cancer showed no significant correlation between CEA and prognosis of the disease studied.

In a study done by Nap M et al (1984) to see the expression of CEA and NCA on 52 benign and 92 malignant lesions of the breast, they found out that CEA was positive in 42% of cases of malignancy and totally negative in fibroscystic disease and fibroadenomas. This result differs from our study that showed CEA being positive in 21.7% of fibroadenomas even though the fibrocystic disease cases also were totally negative as theirs. They suggested that CEA is cancer specific in breast lesions but our findings did not support that. Another important feature of CEA expression on benign lesions was the fact that myoepithelial cells also expressed the marker and this will make interpretation difficult and may give rise to false positive cases.

Papotti M et al (1983) reported that CEA was positive in 85% of papillary carcinoma and not expressed in papilloma. Our study on 7 cases of papilloma also had shown negative expression of CEA. In this regard, we agree with Papotti M et al that CEA can be used as a reliable marker to differentiate papilloma from papillary carcinoma of the breast. Our study on 28 cases of benign lesions in FNA smear showed that 6 cases stained positive for CEA. This indicates that CEA has a potential to differentiate benign from malignant lesions in FNA breast smear.

C-erb-B2 protein expression had been found to be strongly correlated with prognosis of patient (Keshgegian AA and Cnaan A,1997; Fontana X et al 1994; Tsuda H et al 1990) and especially to nodal metastasis (Zhang GJ et al 1997). Metastatic potential of tumour cells which expressed c-erb-B2 protein is high. Supanaranond K et al in 1997 studied the amplication of this gene in 90 benign breast lesions and 66 malignant cases. They found out that no gene amplification occur in the benign cases while 28.8% of malignant cases showed c-erb-B2 gene amplification and this was strongly correlated with positive nodal metastasis.

Kalogeraki A et al 1996 studied expression of c-erb-B2 in FNA specimens of 20 breast carcinoma, 20 fibroadenomas and 20 atypical fibrocystic lesions and found expression of 60%, 30% and 25% in the respective lesions. This finding on fibroadenoma differs from our finding in 46 fibroadenomas that we studied which none of them showed positivity for the marker. C-erb-B2 expression was negative in all 50 cases of fibrocystic disease studied by Goussia A et al 1995 and they stated that their results implied that benign

lesions were negative for c-erb-B2. These findings are supported by our study also. Alexiev BA et al also noted that none of the 58 cases of benign breast lesions studied stained with c-erbB-2 while 48.06% of 129 sporadic breast carcinoma were positive.

McCann A et al studied 200 breast carcinoma and 46 benign lesions for c-erb-B2 expression and found out that with exception of one case of apocrine metaplasia, none of the remaining benign breast samples (n=45) were positive for this oncoprotein. Our result showed similar finding where all the benign cases were negative for c-erbB-2 except for one case showing positivity in its apocrine metaplastic component. This fact indicates that c-erbB-2 expression has a powerful distinguishing power to differentiate benign from malignant cells in tissue sections. The other important beneficial staining quality of the c-erbB-2 is the fact that it was not expressed by the myoepithelial cells and the stromal cells. This will greatly prevent false positive cases.

Our study on 23 cases of FNA specimen of benign cases also implied that c-erbB-2 can be used in FNA material also for differentiating benign from malignant lesions. This is more specific (only 4% expression) compare to that of CEA expression of 21% in benign FNA breast smear.

Cathepsin D is an enzyme that appears to be markedly increased in malignant breast tumour and strongly associated with prognosis of breast carcinoma (Losch A. et al 1998; Ioachim EE et al 1998; Fulco RA et al 1998). According to Garcia M et al, Cathepsin D appears to facilitate cell growth at distant sites rather than increase cancer cell escape

from primary tumour through basement membrane degradation as proposed for neutral proteinases. This protein is expressed in the surrounding stromal cells and a study done by Nadji M et al suggested that the Cathepsin D in stromal cells, but not in tumour cells, is associated with aggressive behaviour in node-negative ductal carcinoma of the breast. To our knowledge, there is no study been done on Cathepsin D expression on benign breast lesions. In our study, Cathepsin D was expressed in 20 out of 91 benign breast lesions (22%) and its expression is seen in all type of benign cases studied. This indicates that Cathepsin D is not suitable to be used to differentiate benign from malignant cases. Furthermore, we found out that the expression in the stromal cells add up to the difficulty in visualizing the reactivity in the epithelial cells and this is especially so in cases where there are prominent stromal reactions. At the same time, Cathepsin D also stained up the myoepithelial components of the benign lesions. The marker was also strongly expressed in apocine metaplasia and all our 7 cases of apocrine metaplasia stained strongly positive for Cathepsin D.

In conclusion, we found that c-erbB-2 is a reliable marker that is totally absent in benign cases. It can be used in difficult cases such as differentiating sclerosing adenosis from infiltrating carcinoma or papilloma from papillary carcinoma. CEA can also be used in the same manner however it has a limitation where it stained myoepithelial cells and this will affect the results. We found that Cathepsin D expression is unsuitable for differentiating benign from malignant breast lesions due to background stromal cell staining reaction. We have not looked into the possibility of using these markers to differentiate preneoplastic lesions such as Ductal Carcinoma In Situ from malignant

lesions and the potential of these markers can be explored in this aspect. Lastly, a larger study with more samples are required to validate our findings.

Acknowledgement

This study was supported by funds from USM Short Term Grant
(No: 304/PPSP/6131131)

Correspondence

Dr Hasnan Jaafar MD, MPath
Pathology Department
School of Medical Sciences
Universiti Sains Malaysia
16150 Kubang Kerian, Kelantan
Malaysia

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