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EXPRESSION OF ONCOGENE AND ANTI-ONCOGENE IN MALE BREAST CARCINOMA

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Expression of oncogene and anti-oncogene products in 12 cases of male breast carcinoma was studied. Positive staining was seen in 6 cases for c-*myc*, 6 cases for EGFR, 4 cases for c-*erb*B-2, 2 cases for N-*ras*, 5 cases for Rb and 3 cases for P53. One case was positive and 4 cases were negative for all above mentioned oncogene and anti-oncogene products. In addition, Cathepsin D (Cath-D), ER, PR, AR, PCNA and AgNOR were also assayed. In all the cases showed c-*erb*B-2 or P53 positive were Cath-D positive. The significance of expression of c-*erb*B-2, c-*myc*, Cath-D, ER and PR in male breast carcinoma was emphatically discussed.

Key words: Male breast carcinoma, Oncogene, Anti-oncogene.

Few data were available in male breast carcino-ma, because of the rarity of the disease. The incidence of the male breast carcinoma was 1.29%–1.46% of the breast cancer in China. From 1950–1977, 22 cases (1.29%) were found in 1704 cases of operable breast cancer in our hospital.[1] In various populations around the world, the incidence of the disease rarely exceeds 1/100,000 males/year. However, as is the case for female, breast cancer incidence is relatively high in Jewish males (2.3/100000)[2] and relatively low in Chinese males (0.5/100,000). There is much interest in the range of genetic aberrations which occur in human malignancies. The present work was to study the frequency and distribution of the expression of some oncogenes and anti-oncogenes in male breast carcinomas and to evaluate any similarities with or differences from female breast cancers.

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MATERIALS AND METHODS

Over 19 years (1974–1993), 12 cases of male breast carcinoma were treated in the Cancer Hospital of Shanghai Medical University. Patients' age was ranged from 57 to 82 with a median of 64. Specimens were obtained from the primary tumor of male breast carcinoma undergoing mastectomy. None of the patients had prior anti-cancer therapy. Following sampling, all specimens were fixed in 10% formalin for routine diagnostic histopathology. According to the World Health Organization Histological Typing,[3] 10 cases of invasive ductal carcinoma, 1 of medullary carcinoma and 1 of undifferential carcinoma (unclassified) were identified. Tissue sections were processed with ABC immunostaining procedure.[4] Monoclonal and polyclonal antibodies used in this study were listed in

Table 1. Cytoplasm or nuclei staining in more than 50% of the cells counted was determined as positive. Omission of the primary antibody was used as a negative control and a known positive tissue such as the breast tissue from Paget's disease for c-*erb*B-2 was taken as a positive control. The silver staining method of nucleolar organizer regions (AgNOR) was described elsewhere.[5]

Table 1. Characteristics and origins of monoclonal and polyclonal antibodies

Name	Characteristics	Origin
c-erbB-2 (M)	Oncogene protein	Oncogene science Inc.
c-myc (M)	Oncogene protein	Oncogene science Inc.
N-ras (M)	Oncogene protein	Oncogene science Inc.
EGFR (M)	Oncogene protein	Oncogene science Inc.
P53 (M)	Anti-oncogene protein	Oncogene science Inc.
Rb (M)	Anti-oncogene protein	PharMingen Co.
PCNA (M)	Proliferating cell nuclear antigen	Dako Co. Denmark
ER (P)	Estrogen receptor	Shanghai Endocrinology Institute
PR (P)	Progesterone receptor	Shanghai Endocrinology Institute
AR (P)	Androgen receptor	Shanghai Endocrinology Institute
Cath-D (P)	Estrogen induced lysosomal protease	Biodesign Inc.

M: monoclonal antibody P: polyclonal antibody

RESULTS

Expression of Oncogene and Anti-oncogene

Immunoreactivity was seen in the neclei for c-*myc* oncoprotein and in the cytoplasm for N-*ras*. Immunohistochemical staining for EGFR and c-*erb*B-2 was predominantly localized at the cell membrane, but faint cytoplasmic staining was also seen in some tumor cells and this was frequently accentuated at the periphery of the cytoplasm. Positive staining for anti-oncoprotein of P53 or Rb was located mainly in the cellular nuclei. The distribution of staining was heterogeneous but among groups of tumor cells rather than between individual cells. In 12 cases of male breast carci-noma, 6 cases were positive for onco-protein of c-*myc* and of EGFR. Positive staining for oncoprotein c-*erb*B-2 (Figure 1) and N-*ras*, and anti-oncoproteins Rb and P53 was also observed (Table 2). In 4 of the 12 cases, all oncoproteins and anti-oncoproteins examined were negative. These 4 cases were of invasive ductal carcinoma. Positive for all oncoproteins and anti-oncoproteins assayed was seen in one case of invasive ductal carcinoma. Some patients had two simultaneously overexpressed oncoproteins or two anti-oncoproteins.

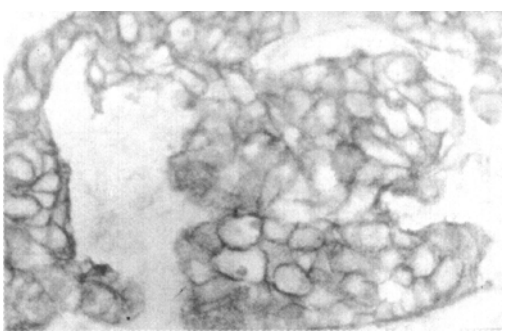

Fig. 1. Male breast carcinoma, c-*erb*B-2 positive. Positive staining was predominantly localised at the cell membrane × 400.

Table 2. Expression of oncogene and anti-oncogene products and assay of related factors in male breast carcinoma

Case No.	Age	Pathologic diagnosis	Oncogene				Anti-oncogene		Cath-D	ER	PR	AR	PCNA	AgNOR**	Follow-up
			c-*erb*B-2	N-*ras*	c-*myc*	EGFR	P53	Rb							
1	67	Invasive ductal carcinoma	-	-	-	-	-	-	-	-	-	-	1	7.06	dead
2	82	Invasive ducal carcinoma*	-	-	+	+	+	-	+	+	+	+	2	4.56	alive (8 years)
3	61	Invasive ducal carcinoma	-	-	+	+	-	-	-	-	-	-	2	15.26	alive (9 years)
4	60	Invasive ducal carcinoma	-	-	-	-	-	-	-	-	-	-	0	12.74	relapse and metastasis
5	70	Invasive ducal carcinoma	-	-	+	-	-	-	+	-	-	-	6	4.61	alive (19 years)
6	60	Invasive ducal carcinoma	+	-	+	+	-	+	+	-	+	-	20	3.49	alive (19 years)
7	64	Invasive ducal carcinoma	-	-	-	-	-	-	-	+	+	-	5	6.14	dead
8	57	Invasive ducal carcinoma	+	+	+	+	+	+	+	+	+	+	7	5.55	dead
9	60	Invasive ducal carcinoma	+	+	-	-	+	+	+	+	+	-	15	4.63	alive (15 years)
10	67	Invasive ducal carcinoma	-	-	-	+	-	-	+	-	-	-	0	7.93	alive (8 years)
11	60	Medullary carcinoma	+	-	+	+	-	+	+	-	-	-	2	10.06	alive (13 years)
12	60	Undifferential carcinoma	-	-	+	+	-	+	-	+	+	-	17	6.23	relapse and metastasis

*A few areas displayed small cell carcinoma

** Number of AgNORs/Nucleus

Assay of Related Factors

In 3 of the 10 cases of invasive ductal carcinoma, which showed negative staining for oncoproteins and anti-oncoproteins, Cath-D ER, PR and AR were all negative. Positive staining of ER (Figure 2) and AR was of low frequency in invasive ductal carcinoma (Table 2). In all the cases showed c-*erb*B-2 or P53 positive were Cath-D positive (Figure 3). Positive staining of Cath-D was seen in most cases which showed c-*myc*, EGFR and Rb positive (Figure 4) (Table 2). In 12 cases, the mean number of AgNORs per nucleus was 7.35, ranging from 3.4 to 15.2, and the mean number of PCNA was 6.4%, ranging from 0 to 20% (Table 2).

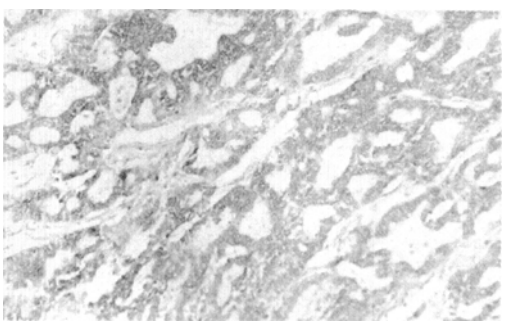

Fig. 2. Male breast carcinoma, estrogen receptors positive × 100.

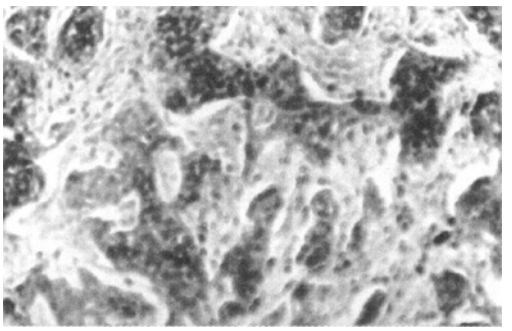

Fig. 3. Strong staining of Cath-D in the male breast carcinoma cells × 100.

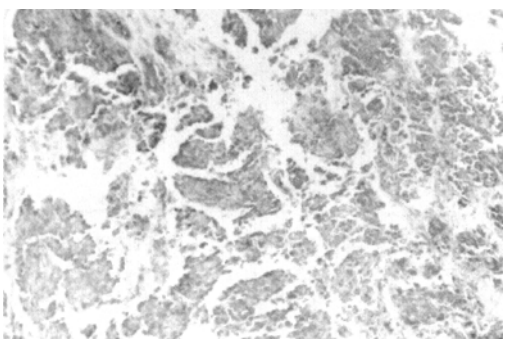

Fig. 4. Male breast carcinoma, Rb positive. Positive staining was located mainly in the cellular nuclei × 100.

DISCUSSION

Our previous paper reported that the positive rate of c-*erb*B-2 in 109 cases of female breast carcinoma was 58.7%.[6] As survival is little different between male and female breast carcinomas,[7] similar expression of c-*erb*B-2 might have been expected, our results revealed that there was no significant difference (P>0.05) (Table 3). In contrast to the data between from Fox et al.,[8] where male breast carcinoma was lack of c-*erb*B-2 oncoprotein expression, 33.3% of our cases was positive. The difference might be from different monoclonal antibodies used and not due to different sex.

Cath-D is an acidic lysosomal aspartyl endopeptidase with a molecular weight of approximately 42 KD which is synthesized as a 52 KD glycoprotein precursor.[9] Cath-D has been shown to be an independent predictor of prognosis in patients with primary breast cancer, particularly those with node negative disease.[10] Winstanley et al.[11] reported that specimens from 359 patients of breast cancer, cytoplasmic staining for Cath-D was present in 75% of tumors. In present study, positive rate of Cath-D was 58.3% in male breast carcinomas (Table 3). Roux- Dosseto et al.[12] showed an amplification rate of 25% for c-*myc* in 65 cases of breast cancer, and in our results expression rate was 50%. Both c-*myc* and Cath-D are associated with cell proliferation and related to malignancy. The positive

rates of Cath-D and c-*myc* in male breast carcinoma are similar to female breast carcinoma. It is consistent with the investigation[7] that little difference was found in malignancy between these two breast carcinomas.

Table 3. Some data between male and female breast carcinoma collected

Item	Male		Female	
	Number of cases	Positive rate (%)	Number of cases*	Positive rate (%)
c-*erb*B-2	12	33.3	109	58.7
N-*ras*	12	16.6	45	31
c-*myc*	12	50	65	25
EGFR	12	50	59	30
P53	12	25	200	15.5
Cath-D	12	58.3	359	75
ER	12	41.6	162	61.1
PR	12	50	162	60.4

*The number of cases were quoted from the data of references as follows: c-*erb*B-2[6], N-*ras*1[6], c-*myc*[12], EGFR[17], P53[18], Cath-D[11], ER[13], PR[13].

The estrogen receptor gene is normally present in the fetus mammary tissue and it is not influenced by age and sex. When the cell has malignant transformation, it may be still preserved whole or part receptors. In the mammary tissue positive relationship is present between ER level and the degree of cell differentiation. Positive rates of ER and PR were 61.1% and 60.4% respectively in female breast carcinoma[13] and 41.6% and 50% respectively in male breast carcinoma, no statistically significant difference being found ($P>0.05$). Everson et al. showed an ER positive rate of 85.2% in 34 cases of male breast carcinoma,[14] and Ribeiro reported a positive rate of 86% in 22 cases.[15] It seems that the ER positive rate in China was lower than that in western countries, but the number of patients in present study was small. For the analysis of prognosis, more cases and biological factors need to be investigated.

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