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c-erbB-2 EXPRESSION IN INVASIVE TRANSITIONAL AND SQUAMOUS CELL CARCINOMA OF THE SCHISTOSOMAL URINARY BLADDER:AN IMMUNOHISTOCHEMICAL AND CLINICAL STUDY

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Overexpression of c-erbB-2 gene, found in 30% of human cancers, significantly correlates with number of lymph node metastasis, chemoresistance and early relapse. In the present study, this oncoprotein has been investigated in invasive transitional (TCC) and squamous (SCC) cell carcinoma of the urinary bladder with chronic schistosomiasis. From January to December 1997, 13 patients were diagnosed and except one, underwent cystectomy and were followed up till May 2000. The tumours were graded and staged using a modified TNM and WHO systems. The tumour cell type, grade and stage and patients survival were correlated with the result of the immunohistochemical study. Strong positive staining for c-erbB-2 was detected in all TCC (n=5, 100%) and exhibited 1+ (n=3) and 2+ (n=2) with over 52% and 74% staining intensity respectively. The 1+ patients were G_2/G_3, T_2/T_3 and N_0/N_3. Two patients survived for 10 and 24 months respectively and the third remained disease free for 36 months. 2+ positive tumours were grade G2 and T2 with nodal metastasis and the patients survived 14 and 12 months respectively. The SCC sections (n=8) were c-erbB-2 negative. One patient refused surgery, another died 12 months after surgery due to heart failure. The other patients had G_1/G_2, T_2/T_4 and N_0M_0 tumours and remained disease free. It appeared that the oncogenic changes in the urothelium and the biological behavior of invasive TCC, with and without chronic schistosomiasis, deserves a comparative study to evaluate c-erbB-2, with other markers, as a prognostic factor. On the other hand, c-erbB-2 negative invasive SCC, complicating urothelial metaplasia and chronic schistosomiasis, had a biological behavior different from SCC elsewhere and may explain why it is an organ-confined disease.

Key words: Oncogens, Protooncogene, c-erbB-2, HER-2/neu, Bladder neoplasms, Transitional cell carcinoma, Squamous cell carcinoma, Chronic schistosomiasis.

INTRODUCTION

Tumour progression is strongly associated with specific changes in protooncogenes and tumour suppressor genes. One of the factors involved in tumorogenesis is protooncogene c-erbB-2[(1)]

The c-erbB-2 (also known as Her-2/neu) proto-oncogene encoding an epidermal growth factor (EGF) receptor-related protein is a potent oncogene [(2,3)] .It causes cancer cells to escape from host immune surveillance and reduces host defenses against neoplasia [(4)].

Overexpression of the c-erbB-2 gene was found in 30% of human cancers, including SCC of the Oropharynx [(5)], larynx [(1,6)] and oesophagus[(7)] as well as gastric[(8)], Gallbladder[(9)], hepatocellular[(10)] and endometrial[(11)] carcinoma. Comprehensive studies on breast cancer[(2)], with node positive, endometrial[(11)], ovarian[(2)], hepatocellular[(10)], and gastric[(8)], cancer patients, showed that c-erbB-2 gene amplification was a significant predictor of disease free and overall survival.

In urinary bladder TCC, overexpression of EGF and c-erbB-2 seemed to correlate with tumour grading, invasiveness and progression [(12,13,14,15,16,17)].

In Egypt, carcinoma of the urinary bladder is the commonest cancer and usually associated with chronic

schistosomal cystitis. TCC is an aggressive tumour necessitating early diagnosis, radical cystectomy and adjuvant poly-chemotherapy. The overall prognosis is poor. On the other hand, invasive SCC has always been considered a self-limited, organ confined disease with very low metastatic potentials and relatively good prognosis.[18,19]

It has been claimed that El A gene would downregulate c-erbB-2 expression by repressing the gene's transcription, thus reversing the malignant phenotype [20]. In addition, E1A is able to promote apoptosis induced by anticancer drugs and irradiation [21] Therefore, cerbB-2 overexpression is worth of investigation for the possible use of gene therapy in these tumours.

AIM OF THE WORK

The purpose of this study was to investigate the over-expression of c-erbB-2 in invasive TCC and SCC of the urinary bladder with chronic schistosomal cystitis, and correlate it with the clinical outcome.

PATIENTS AND METHODS

Clinical material: From January to December 1997, 13 patients with carcinoma of the urinary bladder and schistosomal cystitis were diagnosed by cystoscopic transurethral resection biopsy. The tumours were graded and staged using a modification of the WHO and the TNM system respectively. All patients, but one, underwent cystectomy and pelvic lymphadenectomy and were followed up until May 2000. The tumour grading, staging and patient's survival were correlated with the result of the immunohistochemical study (Table 1 and 2) Only invasive TCC patients received postoperative chemotherapy.

Immunohistochemical study: Staining was performed by the avidin-biotin-peroxidase complex using a polyclonal antibody directed to the c-erbB-2 oncoprotein.. Formalin-fixed and paraffin embedded specimens were deparaffinized through 2 changes of xylene and absolute ethanol. The specimens were blocked with normal goat serum at 1:60 in phosphate buffered saline and incubated at 4°C for 72 hours with the primary rabbit antipeptide antibody at a dilution of 1:20 then exposed to the biotinylated secondary antibody (goat antirabbit IgG 1:400) for 30minutes. The Sections were washed again and placed in the biotin-avidin complex for 30, visualized with diaminobenzidine and counterstained with Mayer's hematoxylin.

RESULTS

Tissue diagnosis by two independent pathologists revealed associated chronic schistosomal cystitis in all specimens with invasive TCC (n=5) or SCC (n=8). Tumours staging, grading and c-erb B-2 expression and patient's survival are shown in (Table 1 and 2).

Immunohistochemical results; All invasive TCC sections were c-erbB-2 positive and exhibited 1+ (n=3) and 2+(n= 2) with over 52% and 74% staining intensity respectively. The invasive SCC sections were c-erbB-2 negative (n=8) irrespective of grade and stage of the tumour.

Clinical follow up: One patient with 1+ c-erbB-2 positive had invasive TCC (G_2, $T_2N_0M_0$) and was disease free 36 months after radical cystectomy until May 2000. The other two patients were G_3, $T_3N_3M_0$ and G_2,$T_3N_1M_0$ and survived for 10 and 24 months respectively. Patients with 2+ c-erbB-2 positive (n=2) had tumours G_2 and T_2 with nodal metasasis, developed further metastasis, and survived for 14 and 12 months respectively after surgery.

In SCC group (n=8), one patient refused surgery, and 7 patients underwent total cystectomy and pelvic lymphadenectomy. After surgery, one patient died of heart failure that was considered not related to the urinary bladder cancer, and six patients were disease free until May 2000. They had G_1/G_2, T_2/T_4 and N_0M_0 tumours.

Table (1): ***Results of the postoperative metastasis and survival and c-erbB-2 over-expression in patients with TCC.***

Patient #	*Grade*	*Stage*	*Postoperative*		*c-erb-2*		
			Metastases	*Survival*	*% cells>0*	*Intensity of staining*	*Positivety*
1- S.M	G_2	$T_2N_2M_0$	Yes	14 months	82%	2+	Positive
2- A.M	G_3	$T_3N_3M_0$	Yes	10 months	67%	1+	Positive
3- A.Z	G_2	$T_2N_0M_0$	No	36 months, alive	52%	1+	Positive
4- A.A	G_2	$T_3N_1M_0$	Yes	24 months	68%	1+	Positive
5- A.Z	G_2	$T_2N_2M_0$	Yes	12 months	74%	2+	Positive

Table (2): *Results of the postoperative metastasis and survival and c-erbB-2 over-expression in patients with SCC.*

Patient #	*Grade*	*Stage*	*Postoperative*		*c-erb-2*		
			Metastases	*Survival*	*% cells>0*	*Intensity of staining*	*Positivety*
6- A.S	G_2	oMo N_3T	No	40 months, alive	0%	1+	Negative
7- H.H	G_1	oMo N_3T	No	3l months, alive	0%	1+	Negative
8- S.S	G_2	oMo N_4T	No	28 months,alive	8%	1+	Negative
9- S.I	G_2	oMo N_3T	No	35 months, alive	0%	2+	Negative
10- S.Y	G_2	oMo N_3T	No	36 months, alive	18%	1+	Negative
11-M.M	G_2	oMo N_3T	No	38 months,alive	0%	1+	Negative
12- A.A	G_2	oMo N_2T	No	14 months	10%	1+	Negative
13-F.A	G_2	oMo N_4T	-	-	17%	1+	Negative

Patierit 12 died of heart failure

Patient 13 refused surgery and was lost to follow up

DISCUSSION

Mellon et al[22] reported a 21% of TCC over-expressing c-erbB-2, while others [13,16,22] reported different incidences mostly in superficial TCC of the urinary bladder. The present study showed that c-erbB-2 was over-expressed in all invasive TCC (100%) of the urinary bladder with chronic schistosomiasis. Beside the invasive nature of these tumours, presence of schistosomiasis may have had a role in this high percentage of over-expression. Over-expression of c-erbB-2 in invasive TCC denoted poor prognosis, development of metastasis and poor survival. A combinations of markers, including C-erbB-2 over-expression, and correlations between markers and tumour grades, may yield prognostic indices superior than that of histological grade alone.

One of the potential factors involved in tumorogenesis of squamous cell carcinomas is protooncogene c-erbB-2[1], In this cohort of patients, c-erbB-2 was negative irrespective of grade and stage of the tumour. It seems that, invasive SCC complicating urothelial metaplasia and chronic schistosomiasis had a biological behavior different from SCC elsewhere [1,5,6,7]. To the best of our knowledge, no study on c-erbB-2 over-expression in bladder SCC was reported previously.

This peculiar tumor, often seen after many years of chronic schistosomal infection or irritation, is a slowly progressive local disease and has a low metastatic potential [18,19]. It would be reasonable to say that the low incidence of metastases, in this organ-confined tumour, can be explained by the negative expression of c-erbB-2.

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