Chinese Journal of Cancer Research 7(2): 103 - 107, 1995.

EXPRESSIONS OF ESTROGEN OCCUPIED RECEPTOR(EoR) AND PROGESTERONE OCCUPIED RECEPTOR(PoR) AND C - *erb*B - 2 ONCOPROTEIN IN HUMAN NASOPHARYNGEAL CARCINOMAS

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It is first reported here that estrogen occupied receptor (EoR) and progesterone occupied receptor (PoR) expressed in cancerous tissues (59.57% and 82.98% respectively) and morphologically normal epithelium (50—77.78% and 70—88.89% respectively) in nasopharyngeal carcinomas (NPCs) with insignificant difference ($P > 0.05$). Positive rates of EoR and PoR increased greatly in clinical stage Ⅲ and Ⅳ, compared with in Ⅱ ($P < 0.05$), and exhibited insignificant difference between female cases and male ones ($P > 0.05$). Positive rate of C - *erb*B - 2 was 19.15% in cancerous cells, and 9.68% in stage Ⅲ and 66.67% in Ⅳ in NPCs($P < 0.05$). Significant difference of C - *erb*B - 2 expression was observed between bilateral cervical lymph node metastasis(BCLM) and unilateral ones ($P < 0.005$) but not for EoR or PoR ($P > 0.05$). These findings suggest that EoR or PoR may be correlated with aggravation but not genesis and node metastasis in NPCs and that C - *erb*B - 2 may be correlated with aggravation and promotion of formation of node metastasis in NPCs.

Key words: Female hormone receptors, C - *erb*B - 2 oncoprotein, Clinical stage, Node metastasis, Nasopharyngeal carcinoma.

Female hormone receptors, estrogen receptor (ER) and progesterone receptor (PR), have been found to express in tumors from target organs, such as breast cancer[1] and also in tumors from nontarget organs, such as brain tumors,[2] lung cancer,[3] gastrointestinal cancer,[4] others.[5] C - *erb*B - 2 oncoprotein was found to express in breast cancer and be correlated with metastasis, relaps and survival in breast cancer.[6] Other finding showed that ER inhibits the C - *erb*B - 2 expression in breast cancer cell lines.[7] However, little has been known about expressions of ER and PR, or their correlation with C - *erb*B - 2 in nasopharyngeal carcinoma (NPC). ER binds to estrogen into estrogen - receptor complex in cytoplasm and then the complex translocates to the neucleus and activates transcription of factors which are important for the growth and metabolism of the cell,[8] which indicates that EoR, perhaps PoR, is more value to be detected for clinic than non - occupied type ER and PR. For these cases, we studied the expressions of both EoR and PoR, and C - *erb*B - 2 in human NPC by immunohistochemistry.

MATERIALS AND METHODS

Tissues

Accepted December 18, 1994

Of 50 cases of nasopharyngeal biopsied tissues, 3 is chronic nasopharyngitis (CNPs) and 47, NPCs, including 45 foorly-differentiated squamous cell carcinomas and one foorly - differentiated adenocarcinoma and carcinoma with vesicular nuclei, were obtained from three cases of patients with CNP and 47 with NPC from Department of Radiotherapy in our Hospital from May 2 to August 29, 1994. Of 47 cases of patients with NPC, 35 are male and 12, female, aged 20—63 years (mean, 42 years). All biopsied tissues were conventionally fixed in formalin and embedded in paraffin.

Immunohistochemical Agents

Polyclonal antibody (PAb) RAB - 9301 against estradiol, RAb 9302 against progesterone and RAb 0046 against C - *erb*B - 2, and the streptavidin biotin(S - P) kit, produced by Zymed Laboratories, CA, were purchased from Fuzhou Maxim Biotech Inc.

Immunohistochemical Methods

As previously described[9] but with some modifications. Procedure was described briefly as follows:

1. Added 50 μl hydrogen peroxide (agent A) to block endogenous peroxidase activity and incubated for 10 min at room temperature (RT) after dewaxed in xylene and then rehydrated in graded alcohols.

2. Added 50 μl unimmunized animal serum (agent B) and incubated for 10 min at RT

3. Added 50 μl primary antibody and incubated for 60 min at RT. Did not pretreat sections with estrogen or progesterone for detection of occupied receptor before addition of primary antibody.

4. Followed by incubation with 50 μl biotiny - lated antibody (agent C) and with 50 μl strepavidin - peroxidase (agent D), both for 10 min at RT.

5. Added DAB solution (agent E) and incubated for 10 min at RT and then wased in running water, counterstained lightly with haema - toxylin, dehydrated, cleared and mounted with coverslips.

All primary antibodies and agents A, B, C, D, E are "ready to use" types. Washed in PBS for 5 min × 3 in the end of step 1, 3, 4. Negative control slides were carried out by replacement of primary antibody with PBS. Immunostaining intensity was defined as follows, according to counting 200 cells in four separate fields of each section: +, less than 30% positive cells; + +, 30—60% and + + +, more than 60%.

Statistical Analysis

Significant difference of datas in this study was judged by x^2 test.

RESULTS

Expression of EoR or PoR in Neoplastic and Non - neoplastic Epithelium in NPC

As showed Table 1, expressions of EoR and PoR were observed in cancerous and non - neoplastic epithelium, includinng stratified squamous and columnar epithelium in NPCs, with insignificant difference between them ($P > 0.05$). EoR was found to localize in cytoplasm in all cases (100%), and PoR, in cytoplasm 89.36% (42/47) and in concurrence of cytoplasm and nucleuses, 10.64%(5/47) (Figure 1,2).

Expressions of EoR and PoR and Their Relation to Sex of Patients with NPC

As showed Table 2, gentle elevated frequencies of expressions of EoR and PoR were present in female cases without significant difference, compared with male ones ($P > 0.05$).

Expressions of EoR and PoR and C - *erb*B - 2 and Their Relation to Clinical Stages in NPCs

AS showed Table 3, significant decline of

frequency of EoR or PoR expression was observed in clinical stage Ⅱ, compared with stage Ⅲ or Ⅳ ($P > 0.05$). Significant differences of EoR or PoR expressions were found in various intensities among various clinical stages ($P < 0.05$). Markedly elevated frequency of C - *erb*B - 2 expression was noted in clinical stage Ⅳ, compared with Ⅲ, but absence of its expression in stage Ⅱ. Strong intensity (+ + +) of C - *erb*B - 2 expression was presence in only clinical stage Ⅳ (Figure 3).

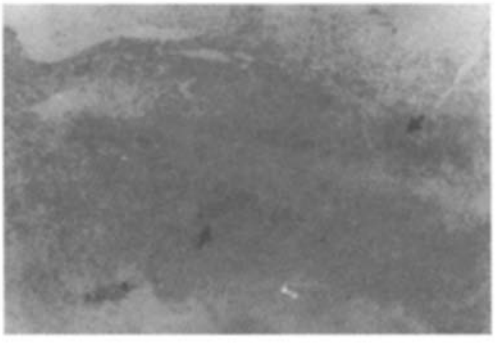

Fig. 1. Nasopharyngeal carcinoma section. EoR localized in cytoplasm of cancer cells (arrow) (S - P immunohistochemistry × 10)

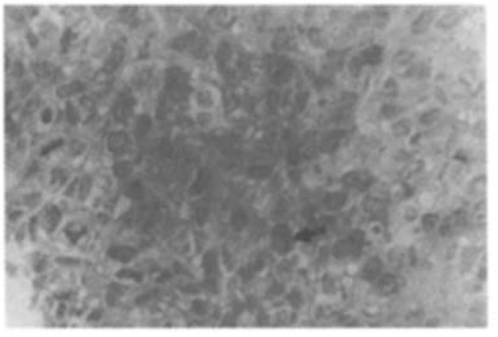

Fig. 2. Nasopharyngeal carcinoma section. PoR localized in nuclear of cancer cells (arrow) (S - P immunohistochemistry × 40)

Synergic Expressions of EoR and PoR in NPCs

As showed Table 4, concurrent expressions of EoR and PoR was observed in half of the cases (51.06%), but concurrent absence of them in few of cases (8.51%).

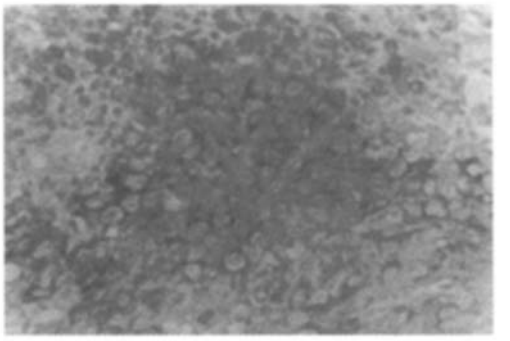

Fig. 3. Nasopharyngeal carcinoma section. C - *erb*B - 2 localized in membrane of cancer cells. (S - P immunohistochemistry × 40)

Expressions of EoR, PoR and C - *erb*B - 2 and Their Relation to Cervical Lymph Node Metastasis (CLM) in NPCs

Insignificant difference of EoR or PoR existed among various forms of CLM. C - *erb*B - 2 expression was presence in only BCLM (Table 5).

DISCUSSION

Because binding capacities of ER and PR could not retain at the temperature which tissues was exposed to during paraffin embedding,[10] it would seem unlikely that EoR or PoR are detected in paraffin embedded tissues by immunohistochemistry under pretreatment with estradiol or progesterone prior to addition of primary antibody against estrogen or progesterone. Removement of the pretreatment makes it possible for EoR orr PoR in the paraffin embedding to be detected by immunohistochemistry. Our results showed that EoR and PoR exhibited insignificant difference in expressive frequency between cancerous tissues and non - neoplastic ones ($P > 0.05$) or between female

Table 1. Expession of EoR or PoR in neoplastic - and non - neoplastic tissues in NPCs

Tissue types	Cases	EoR				Positive rate (%)	PoR				Positive rate (%)
		+	+ +	+ + +	-		+	+ +	+ + +	-	
Cancerous tissues	47	17	5	6	19	59.57	6	8	25	8	82.98
Non - neplastic -											
Squamous tissues	30	8	4	3	15	50	5	7	9	9	70
Columnar tissues	9	2	2	3	2	77.78	4	2	2	1	88.89
P value*						>0.05					>0.05

* Comparison of positive rates between cancerous tissues and non - neoplastic ones

Table 2. Expression of EoR or PoR and their relation to sex of patients with NPC

Sex	cases	EoR		Positive rate (%)	PoR		Positive rate (%)
		P	N		P	N	
Female	12	6	6	50	9	3	75
Male	35	25	10	71.43	31	4	88.57
P value*				>0.05			>0.05

* Comparison of positive rates between females and males, P: positive; N: negative.

Table 3. Expressions of EoR, PoR and C - erbB - 2 in various clinical stages in NPCs

Clinical stage	cases	EoR				Positive rate (%)	PoR				Positive rate (%)	C - *erb*B - 2				Positive rate (%)
		+	+ +	+ + +	-		+	+ +	+ + +	-		+	+ +	+ + +	-	
Ⅱ	7	2	0	0	5	28.57	0	2	2	3	57.14	0	0	0	7	0
Ⅲ	31	12	3	4	12	61.29	5	4	18	4	87.1	3	0	0	28	9.68
Ⅳ	9	3	2	2	2	77.78	1	2	3	1	88.89	4	0	2	3	66.67
P value						<0.05*					<0.05*					<0.005

* Comparison of positive rates between stage Ⅲ or Ⅳ and Ⅱ, but $P > 0.05$ between Ⅲ and Ⅳ.

Table 4. Synergic expression of EoR and PoR in cancerous tissue in NPC

	ER + PR +	ER - PR -	ER + PR -	ER - PR +
Cases	24	4	4	15
Positive rate (%)	51.06	8.51	8.51	31.91

Table 5. Expressions of EoR, PoR and C – erbB – 2 in various forms of CLM in NPCs

Forms of CLM	Cases	EoR		Positive rate	PoR		Positive rate	C – *erb*B – 2		Positive rate
		P	N	(%)	P	N	(%)	P	N	(%)
Bilateral –	13	8	5	61.54	11	2	84.62	9	4	69.23
Unilateral –	32	19	13	59.38	27	5	84.38	0	32	0
Non –	2	1	1	50	1	1	50	0	2	0
P value				> 0.05			> 0.05			< 0.005

P: Positive; N: Negative.

patients and male ones with NPC ($P > 0.05$), and but not between clinical stage Ⅲ or Ⅳ and Ⅲ ($P < 0.05$). ER or PR was defined previously to localize partly in cytoplasm or nuclear or entire cells, or nuclear.[11] In our studies, EoR localized only in cytoplasm and PoR mainly in nuclear and partly cytoplasm in NPCs. EoR and PoR were concurrent fresence frequently (51.06%) and concurrent abscence occasionally (8.15%).

C – *erb*B – 2 was presence in only cancerous cells in a small percentage (19.15%) in cancer cells, 9.68% in stage Ⅲ and 66.67% in Ⅳ, and strong intensity (+ + +) of its expression was presence in stage Ⅳ, indicating that C-*erb*B-2 overexpression was correlated with aggravation of NPC.

Significant difference of C-*erb*B-2 expression was observed between BCLM and UCLM ($P < 0.005$) but not for EoR or PoR ($P > 0.05$), suggesting that C – *erb*B – 2 but not EoR or PoR could plays a role in formation of CLM in NPC.

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