P.O.Box 2345, Beijing 100023, China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

World J Gastroenterol 2003;9(4):665-669
World Journal of Gastroenterology
 ISSN 1007-9327

• GASTRIC CANCER •

Expression of estrogen receptor and estrogen receptor messenger RNA in gastric carcinoma tissues

Xin-Han Zhao, Shan-Zhi Gu, Shan-Xi Liu, Bo-Rong Pan

Xin-Han Zhao, Shan-Zhi Gu, Shan-Xi Liu, Department of Medical Oncology, First Hospital of Xi' an Jiaotong University, Xi' an 710061, Shaanxi Province, China
Bo-Rong Pan, Department of Oncology, Xijing Hospital, Fourth Military Medical University, Xi' an 710032, Shaanxi Province, China
Supported by the First Hospital Scientific Foundation of Xi'an Jiaotong University, No.95012
Correspondence to: Dr. Xin-Han Zhao, Department of Medical Oncology, First Hospital of Xi' an Jiaotong University, Xi' an 710061, Shaanxi Province, China. zhxinhan@pub.xaonline.com
Telephone: +86-29-5324136 **Fax:** +86-29-5275472
Received: 2002-12-22 **Accepted:** 2003-01-16

Abstract

AIM: To study estrogen receptor (ER) and estrogen receptor messenger RNA (ERmRNA) expression in gastric carcinoma tissues and to investigate their association with the pathologic types of gastric carcinoma.

METHODS: The expression of ER and ERmRNA in gastric carcinoma tissues (15 males and 15 females, 42-70 years old) was detected by immunohistochemistry and *in situ* hybridization, respectively.

RESULTS: The positive rate of ER (immunohistochemistry) was 33.3 % in males and 46.7 % in females. In Borrmann IV gastric carcinoma ER positive rate was greater than that in other pathologic types, and in poorly differentiated adenocarcinoma and signet ring cell carcinoma the positive rates were greater than those in other histological types of both males and females ($P<0.05$). The ER was more highly expressed in diffused gastric carcinoma than in non-diffused gastric carcinoma ($P<0.05$). The ER positive rate was also related to regional lymph nodes metastases ($P<0.05$), and was significantly higher in females above 55 years old, and higher in males under 55 years old ($P<0.05$). The ERmRNA (*in situ* hybridization) positive rate was 73.3 % in males and 86.7 % in females. The ERmRNA positive rates were almost the same in Borrmann I, II, III and IV gastric carcinoma ($P>0.05$). ERmRNA was expressed in all tubular adenocarcinoma, poorly differentiated adenocarcinoma and signet ring cell carcinoma ($P<0.05$). The ERmRNA positive rate was related to both regional lymph nodes metastases and gastric carcinoma growth patterns, and was higher in both sexes above 55 years old but without statistical significance ($P>0.05$). The positive rate of ERmRNA expression by *in situ* hybridization was higher than that of ER expression by immunohistochemistry ($P<0.05$).

CONCLUSION: ERmRNA expression is related to the pathological behaviors of gastric carcinoma, which might help to predict the prognosis and predict the effectiveness of endocrine therapy for gastric carcinoma.

Zhao XH, Gu SZ, Liu SX, Pan BR. Expression of estrogen receptor and estrogen receptor messenger RNA in gastric carcinoma tissues. *World J Gastroenterol* 2003; 9(4): 665-669
http://www.wjgnet.com/1007-9327/9/665.htm

INTRODUCTION

Gastric carcinoma is the most common cause of cancer mortality in China[1-6] and is responsible for approximately 160 000 deaths annually. During the last 10 years, there has been no improvement in survival after the diagnosis of gastric cancer with an overall 5-year survival rate of 20 %. Surgery remains the primary treatment of choice. However, the disease is often advanced at first presentation, and only 30-40 % of patients undergoing surgery will have a curative resection. The failure of surgery on the disease has led to the use of chemotherapy and radiotherapy as adjuvant or palliative means, but their value is limited because of toxicity and lack of efficacy[7-12]. Since Jensen discovered the existence of estrogen receptor (ER) in the cytoplasm of human mammary cancer cells in 1960, many researchers have also discovered the presence of ER in some gastric cancer cells, suggesting that these cells can be controlled and regulated by sex hormones. From this we can infer that some cases of gastric cancer are hormone-dependent tumor, and this has stimulated the use of the anti-estrogen compound in its treatment. In this study, the expression of ER, ERmRNA in gastric cancer tissues was examined by immunohistochemistry and *in situ* hybridization, respectively, and the association of their expression and clinical significance at molecular pathological level was also investigated.

MATERIALS AND METHODS

Specimens

Thirty specimens of gastric cancer tissue were collected from The General Surgical Department and The Tumor Surgical Department of the First hospital of Xi' an Jiaotong University. All the cases were pathologically proved to be gastric carcinoma. Of the patients, 15 were females and 15 males. Their age ranged from 42 to 70 and the average age was 58.4. Pathologically 2 cases were papillary adenocarcinoma, 12 tubular adenocarcinoma, 13 poorly differentiated adenocarcinoma, and 3 signet ring cell carcinoma. According to Borrmann classfication, 6 cases were type I, 8 type II, 8 type III and another 8 type IV.

ERmRNA in situ Hybridization

The slides were treated with 3-amino propyltri-ethoxy saline (APES) and with polylysine. The slides were deparaffinized, hydrated and treated with 30 mL/L H_2O_2 at room temperature for 10 minutes to eliminate the endogenous peroxidase. The slides were incubated with freshly diluted protease K (1:1 000 with 0.01 mol/L Tris buffer saline (TBS)) at 37 ℃ for 5 to 15 minutes. After being washed with distilled water three times, the slides were treated with 2 g/L glycine for 5 minutes, washed with PBS for 5 minutes, fixed with 40 g/L polymethanol for 30 minutes, and washed again with PBS for 5 minutes, dehydrated with gradient alcohol, and then washed with DEPC, treated with digoxin-labeled probe in 90-100 ℃ water for 5 to 10 minutes, and then taken out and immediately put in shattered ice for 5 minutes. After the slides became dry in the air, 10 μL *in situ* hybridization solution containing digoxin-labeled probe was added onto each slide,

and the hybridization was conducted in a humidified box for 20 hours. The slides were then washed twice with 2×SSC at 20-30 ℃ for 5 minutes and with 1×SSC at 37 ℃ for 10miutes, incubated with mouse anti-digoxin at 20-37 ℃ for 30 minutes and washed with 0.5 mol/L PBS three times, each for 2 minutes. The slides were then incubated with anti-mouse biotin IgG at 20-37 ℃ for 20 minutes, washed with 0.5 mol/L PBS three times, each for 2 minutes and again incubated with SABC at 20-37 ℃ for 20 minutes, washed with 0.5 mol/L PBS four times, each for 5 minutes. The color reaction was developed with the addition of DAB, and the slides were counter-stained with hematoxylin and sealed with xylene.

Negative control: No estrogen receptor probe in the hybridization solution. The slices showed color directly without any solution added. Hybridization solution was replaced by reserve hybridization solution containing no probe.

Positive control: The specimens from 3 women with mammary cancer and 3 with ovarian cancer, all under 45 years old, were collected and treated in the same way as in the gastric cancer specimens.

ER Immunohistochemistry

Consecutive 5 μm thick sections were stained with HE and by immunohistochemistry separately. The deparaffinized sections were washed with PBS three times, soaked in 30 mL/L hydrogen dioxide solution for 10 minutes to eliminate the endogenous peroxidase, washed with PBS three times, digested with 10 g/L trypsin for 15 minutes (37 ℃), washed with PBS three times, heated to 95 ℃ in pH 6.0 citric acid buffer solution for 10 minutes before cooled down to room temperature, and then washed three times with PBS, and then blocked with serum (45 ℃). The sections were then incubated with the first antibody (1:50) over night, washed three times with PBS, incubated with biotin-labeled secondary antibody and then washed with PBS. The sections were finally incubated with streptavidin biotin peroxidase complex, the color reaction was developed with the addition of DAB, and the sections were counter-stained with hematoxylin and sealed transparently.

Positive cells from *in situ* hybridization appeared yellow and the positive stain was mainly located in the nuclei and cytoplasm around the nuclei. Immunohistochemically positive cells appeared brown yellow and the positive stain was located in the cytoplasm. The average positive rate in every case was calculated in 5 high-power fields. When 10 % or more of the cancer cells were stained positive in a labeled slice, it was defined as ER or ERmRNA positive.

Statistical analysis

All data were analyzed with SPSS 8.0 statistical software (including the accurate four square table probability method and similar χ^2 test) and $P<0.05$ was considered to have statistical significance.

RESULTS

Immunohistochemically stained positive cells looked brown yellow in cytoplasm. The distribution of ER positive cells and the intensity of positive reaction were uneven (Figure 1). The smooth muscle cells and the lymphocytes in the interstice and the mucosa membrane beside the cancer tissue appeared negative. The positively expressed ERmRNA were mainly located in cytoplasm and nuclei of cancer cells, next to the interstice (Figure 2). The number of positive cells was different in different fields. It was greater in some fields, with 34 positive cells in a high power field, but in other fields, the positive cells were scarce or absent. There were weakly hybridized positive signals in interstitial smooth muscle cells and lymphocytes. The tissue beside the cancer appeared negative.

ER positive gastric cancer tissues both in men and women were more common in Borrmann type IV, histologically it was more common in poorly differentiated adenocarcinoma and signet ring cell carcinoma ($P<0.05$). ERmRNA positive cells were found in Borrmann type I, II, III and IV ($P>0.05$). ERmRNA expression was also found in tubular adenocarcinoma, poorly differentiated adenocarcinoma and signet ring cells ($P<0.05$, Table 1).

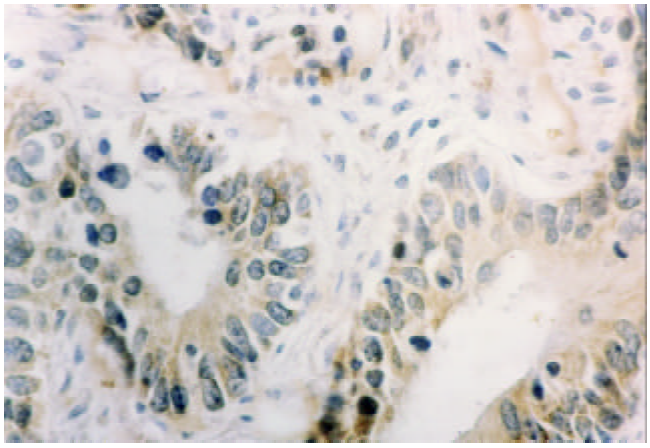

Figure 1 ER positive expression in gastric carcinoma tissue SABC×400.

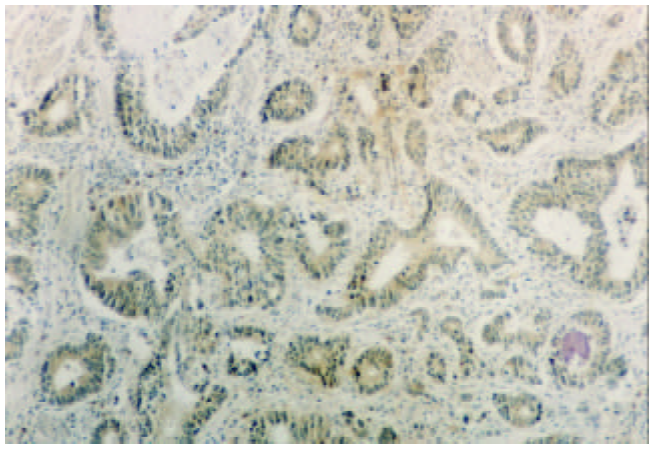

Figure 2 ER mRNA positive expression in gastric carcinoma tissue *in situ* hybridization ×100.

ER expression had a high positive rate in females above 55 years old and in males under 55 years old ($P<0.05$). And ERmRNA expression had a high positive rate in both males and females above 55 years old ($P>0.05$, Table 1).

Diffusely growing gastric cancer had a high ER positive rate ($P<0.05$). Both the diffusely and non-diffusely growing gastric cancers had a high positive expression rate of ERmRNA ($P>0.05$, Table 1). The increase of ER positive rate is associated with the increase of the involved regional lymph nodes including the upper and lower parts of pylorus, the greater and lesser curvatures of the stomach and the lymph nodes on both sides of cardia ($P<0.05$). There seemed to be a tendency that the increase of ERmRNA positive rate is associated with the increase of the number of the involved lymph nodes ($P>0.05$, Table 1).

To compare the immunohistochemistry results with *in situ* hybridization results, ER positive rate was 40.0 % (M/F: 33.3 % *vs* 46.7 %), and ERmRNA positive rate was 80.0 % (M/F: 73.3 % *vs* 86.7 %, $P<0.05$, Table 1).

Table 1 Relationship between ER, ERmRNA expression and pathology in gastric cancer

Pathology	Male					Female					Total				
	n	ER(+)	%	ERmRNA(+)	%	*n*	ER(+)	%	ERmRNA(+)	%	*n*	ER(+)	%	ERmRNA(+)	%
Borrmann I	3	1	33.3	1	33.3	3	0	0	2	66.7	6	1	16.7	3	50.0
II	4	1	25.0	3	75.0	4	2	50.0	3	75.0	8	3	37.5	6	75.0
III	4	1	25.0	3	75.0	4	2	50.0	4	100.0	8	3	37.5	7	87.5
IV	4	2	50.0	4	100.0	4	3	75.0	4	100.0	8	5	62.5	8	100.0
Papillary	1	0	0	0	0	1	0	0	0	0	2	0	0	0	0
Tubular	6	0	0	4	66.7	6	1	16.7	5	88.3	12	1	8.3	10	83.3
Poorly differentiated	7	4	57.1	6	85.7	6	4	66.7	6	100.0	13	8	61.5	11	84.6
Signet ring cell	1	1	100.0	1	100.0	2	2	100.0	2	100.0	3	3	100.0	3	100.0
Nondiffused	9	2	22.2	6	66.7	5	1	20.0	4	80.0	14	3	21.4	10	71.4
Diffused	6	3	50.0	5	83.3	10	6	60.0	9	90.0	16	9	56.3	14	87.5
Lymph node 0	3	0	0	1	33.3	4	0	0	3	75.0	7	0	0	4	57.1
Involvemen ≤3	4	1	25.0	3	75.0	5	3	60.0	4	80.0	9	4	44.4	7	77.8
4~6	6	2	33.3	5	83.3	5	3	60.0	5	100.0	11	5	45.5	10	90.9
>6	2	2	100.0	2	100.0	1	1	100.0	1	100.0	3	3	100.0	3	100.0
Age ≤55	6	3	50.0	4	66.7	9	3	33.3	7	77.8	15	6	40.0	11	73.3
>55	9	2	22.2	7	77.8	6	4	66.7	6	100.0	15	6	40.0	13	86.7
Total	15	5	33.3	11	73.3	15	7	46.7	13	86.7	30	12	40.0	24	80.0

DISCUSSION

In 1960 Jensen reported for the first time that after injecting a physiological dose of ^{3}H-E_2 into the hypoderm of a young mouse, the amount of ^{3}H-E_2 found in the tissues of uterus, vagina and other parts was far greater than that found in blood plasma. This proved for the first time that ER protein was present in the tissues of uterus and vagina. When estrogen enters target cells, it first combines with its receptor in cytoplasm, then forms a compound of receptor protein-estradiol which then enters cell nucleus, binds to the chromatin and affects the transcription of DNA. To sexual and non-sexual target organs, estrogen may be a hormone promoting splitting. There are proved documents that gastric cancer cells have sex hormone receptors and may be controlled and regulated by sex hormones, suggesting that gastric cancer in some cases is hormone-dependent tumor, but the molecular mechanisms underlying carcinogenesis are still largely unknown[13-17]. A lot of documents have proved that molecular biology plays an important role in the development and metastasis of some cancers, such as endometrial adenocarcinoma[18-20], lung cancer[21], breast cancer[22-37], apocrine carcinoma[38], leukemia[39] and prostate cancer[40,41]. But there are very few studies on the ERmRNA expression in gastric cancer tissues.

With ER examination on the specimens of ten primary gastric cancer patients (6 men and 4 women), Tokunaga discovered that 2 cases were ER(+) and the patients were women with histological undifferentiated cancer. Yanzuoshaner used the PAP method to analyze 140 specimens of primary gastric cancer after operation. The results showed that 23 cases were ER(+) (16.4 %), 6 cases were ER(±) (4.3 %), 111 cases were ER(-) (79.3 %). Recently, a new estrogen receptor, called estrogen receptor beta, has been found to be expressed in various tissues including normal gastrointestinal tract, the effect of estrogen in stomach cancer, as well as in normal stomach, might be mediated by ER beta, and the role of ER beta might differ by the subtype of stomach adenocarcinoma, specifically signet ring cell adenocarcinomas. But the conclusions needed more evidence to support[13]. Takano *et al* reported the expression of estrogen receptor-alpha and ER-beta mRNAs in human gastric cancers, and the results showed that the expression of estrogen receptor-alpha and ER-beta mRNAs were changed in 20 cases (49 %) and unchanged in 21cases (51 %). The incidences of lymph node metastasis and liver metastasis were significantly higher in changed cases than in unchanged cases[14]. One fourth gastric cancers were ER positive compared with breast cancers, and gastric cancer nuclear receptors were also smaller than that of breast cancers in number[22,23]. Of 95 cases of male patients with gastric cancers, 12 were ER(+) (12.6 %), of 45 cases of female patients 10 were ER(+) (22.2 %), 11.5 % of men under 50 years old were ER(+) and 14.5 % of men above 50 years old were ER (+). There was no marked difference between them, most of the ER(+) cases were Borrmann II, and most cases of the histological type were undifferentiated[15-16].

Our results of the present study showed that ER positive rate was 40.0 % and ER-mRNA positive rate was 80.0 % in gastric cancers. ER(+) was related to lymph node metastasis and gastric cancer growth patterns. Furthermore, we also discovered that the positive rates of gastric cancer ER, ERmRNA were higher in female than in male. Thus we can conclude that female gastric cancer patients are more easily affected by estrogen than male gastric cancer patients. Our results are similar to the results reported by other scholars.

Many foreign researchers think that compared with other methods in examining ER protein, the molecular hybridizations in examining ERmRNA has a higher sensitivity[13-17]. By using Northern hybridization method Hankin found that the ERmRNA positive rate of breast cancer was 87.0 %. But by using the ^{3}H-estradiol method to examine ER protein the positive rate was only 46.0 %. The two methods showed a marked difference. In our study, the immunohistochemical examination showed that the high expression of ER protein was most common in poorly differentiated adenocarcinoma and signet ring cell carcinoma. *In situ* hybridization showed

that ERmRNA had a high positive expression rate, which was also found in tubular adenocarcinoma and poorly differentiated adenocarcinoma of histological hype. What is more noteworthy is that in 13 cases of ER(-) gastric cancer tissues *in situ* hybridization examination showed that ERmRNA was (+). The reason may be that *in situ* hybridization probe could hybridize with mRNA which directs the synthesis of protein of irregular quality lacking function. The protein may lack the epitope that can be identified by the ER monoclonal antibody, and there may be defects present in ER protein synthesis after transcription. ERmRNA positive signal was also present in interstitial smooth muscle cells and lymphocytes, which suggests that estrogen can regulate not only epithelial cells but also interstitial cells.

We speculate that ERmRNA expression has greater value than ER protein expression in clinical application because of the high sensitivity of *in situ* hybridization and the strong ERmRNA expression in gastric cancer, which can be used to judge the prognosis of tumor and predict the effectiveness of endocrine therapy for gastric cancer.

REFERENCES

1 **Zhou Y,** Gao SS, Li YX, Fan ZM, Zhao X, Qi YJ, Wei JP, Zou JX, Liu G, Jiao LH, Bai YM, Wang LD. Tumor suppressor gene p16 and Rb expression in gastric cardia precancerous lesions from subjects at a high incidence area in northern China. *World J Gastroenterol* 2002; **8**: 423-425

2 **Cai L,** Yu SZ, Zhan ZF. Cytochrome p450 2El genetic polymorphism and gastric cancer in Changle, Fujian Province. *World J Gastroenterol* 2001; **7**: 792-795

3 **Su M,** Lu SM, Tian DP, Zhao H, Li XY, Li DR, Zheng ZC. Relationship between ABO blood groups and carcinoma of esophagus and cardia in Chaoshan inhabitants of China. *World J Gastroenterol* 2001; **7**: 657-661

4 **Cai L,** Yu SZ, Zhang ZF. Glutathione S-transferases M1, T1 genotypes and the risk of gastric cancer: a case-control study. *World J Gastroenterol* 2001; **7**: 506-509

5 **Xin Y,** Li XL, Wang YP, Zhang SM, Zheng HC, Wu DY, Zhang YC. Relationship between phenotypes of cell-function differentiation and pathobiological behavior of gastric carcinomas. *World J Gastroenterol* 2001; **7**: 53-59

6 **Wang RT,** Wang T, Chen K, Wang JY, Zhang JP, Lin SR, Zhu YM, Zhang WM, Cao YX, Zhu CW, Yu H, Cong YJ, Zheng S, Wu BQ. *Helicobacter pylori* infection and gastric cancer: evidence from a retrospective cohort study and nested case-control study in China. *World J Gastroenterol* 2002; **8**: 1103-1107

7 **Song ZJ,** Gong P, Wu YE. Relationship between the expression of iNOS, VEGF, tumor angiogenesis and gastric cancer. *World J Gastroenterol* 2002; **8**: 591-595

8 **Tao HQ,** Zou SC. Effect of preoperative regional artery chemotherapy on proliferation and apoptosis of gastric carcinoma cells. *World J Gastroenterol* 2002; **8**: 451-454

9 **Xao HB,** Co WX, Yin HR, Lin YZ, Ye SH. Influence of L-methionine-deprived total parenteral nutrition with 5-fluorouracil on gastric cancer and host metabolism. *World J Gastroenterol* 2001; **7**: 698-701

10 **Ma ZF,** Wang ZY, Zhang JR, Gong P, Chen HL. Carcinogenic potential of duodenal reflux juice from patients with long-standing postgastrectomy. *World J Gastroenterol* 2001; **7**: 376-380

11 **Miehlke S,** Kirsch C, Dragosics B, Gschwantler M, Oberhuber G, Antos D, Dite P, Lauter J, Labenz J, Leodolter A, Malfertheiner P, Neubauer A, Ehninger G, Stolte M, Bayerdorffer E. *Helicobacter pylori* and gastric cancer: current status of the Austrain Czech German gastric cancer prevention trial (PRISMA Study). *World J Gastroenterol* 2001; **7**: 243-247

12 **Wu K,** Shan YJ, Zhao Y, Yu JW, Liu BH. Inhibitory effects of RRR-alpha-tocophery1 succinate on benzo (a) pyrene (B(a)P)-induced forestomach carcinogenesis in female mice. *World J Gastroenterol* 2001; **7**: 60-65

13 **Matsuyama S,** Ohkura Y, Eguchi H, Kobayashi Y, Akagi K, Uchida K, Nakachi K, Gustafsson JA, Hayashi S. Estrogen receptor beta is expressed in human stomach adenocarcinoma. *J Cancer Res Clin Oncol* 2002; **128**: 319-324

14 **Takano N,** Iizuka N, Hazama S, Yoshino S, Tangoku A, Oka M. Expression of estrogen receptor-alpha and -beta mRNAs in human gastric cancer. *Cancer Lett* 2002; **176**: 129-135

15 **Tot T**. The role of cytokeratins 20 and 7 and estrogen receptor analysis in separation of metastatic lobular carcinoma of the breast and metastatic signet ring cell carcinoma of the gastrointestinal tract. *Apmis* 2000; **108**: 467-472

16 **Goldstein NS,** Long A, Kuan SF, Hart J. Colon signet ring cell adenocarcinoma: immunohistochemical characterization and comparison with gastric and typical colon adenocarcinomas. *Appl Immunohistochem Mol Morphol* 2000; **8**: 183-188

17 **Messa C,** Russo F, Pricci M, Di Leo A. Epidermal growth factor and 17beta-estradiol effects on proliferation of a human gastric cancer cell line (AGS). *Scand J Gastroenterol* 2000; **35**: 753-758

18 **Robertson JA,** Farnell Y, Lindahl LS, Ing NH. Estradiol up-regulates estrogen receptor messenger ribonucleic acid in endometrial carcinoma (Ishikawa)cells by stabilizing the message. *J Mol Endocrinol* 2002; **29**: 125-135

19 **Cobellis L,** Reis FM, Driul L, Vultaggio G, Ferretti I, Villa E, Petraglia F. Estrogen receptor alpha mRNA variant lacking exon 5 is coexpressed with the wild-type in endometrial adenocarcinoma. *Eur J Obstet Gynecol Reprod Biol* 2002; **102**: 92-95

20 **Horvath G,** Leser G, Hahlin M, Henriksson M. Exon deletions and variants of human estrogen receptor mRNA in endometrial hyperplasia and adenocarcinoma. *Int J Gynecol Cancer* 2000; **10**: 128-136

21 **Mollerup S,** Jorgensen K, Berge G, Haugen A. Expression of estrogen receptors alpha and beta in human lung tissue and cell lines. *Lung Cancer* 2002; **37**: 153-159

22 **Chearskul S,** Bhothisuwan K, Churintrapun M, Semprasert N, Onreabroi S. Estrogen receptor-alpha mRNA in primary breast cancer: relationship to estrogen and progesterone receptor proteins and other prognostic factors. *Asian Pac J Allergy Immunol* 2002; **20**: 13-21

23 **Zhao H,** Hart LL, Keller U, Holth LT, Davie JR. Characterization of stably transfected fusion protein GFP-estrogen receptor-alpha in MCF-7 human breast cancer cells. *J Cell Biochem* 2002; **86**: 365-375

24 **Bouras T,** Southey MC, Chang AC, Reddel RR, Willhite D, Glynne R, Henderson MA, Armes JE, Venter DJ. Stanniocalcin 2 is an estrogen-responsive gene coexpressed with the estrogen receptor in human breast cancer. *Cancer Res* 2002; **62**: 1289-1295

25 **Zheng WQ,** Lu J, Zheng JM, Hu FX, Ni CR. Variation of ER status between primary and metastatic breast cancer and relationship to p53 expression. *Steroids* 2001; **66**: 905-910

26 **Otto AM,** Schubert S, Netzker R. Changes in the expression and binding properties of the estrogen receptor in MCF-7 breast cancer cells during growth inhibition by tamoxifen and cisplatin. *Cancer Chemother Pharmacol* 2001; **48**: 305-311

27 **Yang X,** Phillips DL, Ferguson AT, Nelson WG, Herman JG, Davidson NE. Synergistic activation of functional estrogen receptor(ER)-alpha by DNA methyltransferase and histone deacetylase inhibition in human ER-alpha-negative breast cancer cells. *Cancer Res* 2001; **61**: 7025-7029

28 **Lacroix M,** Querton G, Hennebert P, Larsimont D, Leclercq G. Estrogen receptor analysis in primary breast tumors by ligand-binding assay, immunocytochemical assay, and northern blot: a comparison. *Breast Cancer Res Treat* 2001; **67**: 263-271

29 **Diel P,** Olff S, Schmidt S, Michna H. Molecular identification of potential selective estrogen receptor modulator(SERM) like properties of phytoestrogen in the human breast cancer cell line MCF-7. *Planta Med* 2001; **67**: 510-514

30 **Brouillet JP,** Dujardin MA, Chalbos D, Rey JM, Grenier J, Lamy PJ, Maudelonde T, Pujol P. Analysis of the potential contribution of estrogen receptor (ER) beta in ER cytosolic assay of breast cancer. *Int J Cancer* 2001; **95**: 205-208

31 **Fasco MJ,** Keyomarsi K, Arcaro KF, Gierthy JF. Expression of an estrogen receptor alpha variant protein in cell lines and tumors. *Mol Cell Endocrinol* 2000; **166**: 156-169

32 **Yang X,** Ferguson AT, Nass SJ, Phillips DL, Butash KA, Wang SM, Herman JG, Davidson NE. Transcriptional activation of estrogen receptor alpha in human breast cancer cells by histone deacetylase inhibition. *Cancer Res* 2000; **60**: 6890-6894

33 **Koduri S,** Poola I. Quantitation of alternatively spliced estrogen receptor alpha mRNAs as separate gene populations. *Steroids* 2001; **66**: 17-23

34 **Omoto Y,** Iwase H, Iwata H, Hara Y, Toyama T, Ando Y, Kobayashi S. Expression of estrogen receptor alpha exon 5 and 7 deletion variant in human breast cancers. *Breast Cancer* 2000; **7**: 27-31

35 **Anandappa SY,** Sibson R, Platt-Higgins A, Winstanley JH, Rudland PS, Barraclough R. Variant estrogen receptor alpha mRNAs in human breast cancer specimens. *Int J Cancer* 2000; **88**: 209-216

36 **Shao ZM,** Shen ZZ, Fontana JA, Barsky SH. Genistein's "ER-dependent and independent" actions are mediated through ER pathways in ER-positive breast carcinoma cell lines. *Anticancer Res* 2000; **20**: 2409-2416

37 **Koduri S,** Fuqua SA, Poola I. Alterations in the estrogen receptor alpha mRNA in the breast tumors of African American women. *J Cancer Res Clin Oncol* 2000; **126**: 291-297

38 **Bratthauer GL,** Lininger RA, Man YG, Tavassoli FA. Androgen and estrogen receptor mRNA status in apocrine carcinomas. *Diagn Mol Pathol* 2002; **11**: 113-118

39 **Cutolo M,** Carruba G, Villaggio B, Coviello DA, Dayer JM, Campisi I, Miele M, Stefano R, Castagnetta LA. Phorbol diester 12-O-tetradecanoylphorbol 13-acetate(TPA)up-regulates the expression of estropgen receptors in human THP-1 leukemia cells. *J Cell Biochem* 2001; **83**: 390-400

40 **Maruyama S,** Fujimoto N, Asano K, Ito A, Usui T. Expression of estrogen receptor alpha and beta mRNAs in prostate cancers treated with leuprorelin acetate. *Eur Urol* 2000; **38**: 635-639

41 **Li LC,** Chui R, Nakajima K, Oh BR, Au HC, Dahiya R. Frequent methylation of estrogen receptor in prostate cancer: correlation with tumor progression. *Cancer Res* 2000; **60**: 702-706

Edited by Pang LH