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

World J Gastroenterol 2004;10(4):476-480
World Journal of Gastroenterology
 ISSN 1007-9327

• ESOPHAGEAL CANCER •

Expression of Egr-1, c-fos and cyclin D1 in esophageal cancer and its precursors: An immunohistochemical and *in situ* hybridization study

Ming-Yao Wu, Chu-Xiang Zhuang, Huan-Xing Yang, Ying-Rui Liang

Ming-Yao Wu, Huan-Xing Yang, Department of Pathology, Shantou University Medical College, Shantou 515031, Guangdong Province, China
Chu-Xiang Zhuang, Department of Physiology, Shantou University Medical College, Shantou 515031, Guangdong Province, China
Ying-Rui Liang, Department of Pathology, The First People' s Hospital of Foshan, Foshan 528000, Guangdong Province, China
Supported by the National Natural Science Foundation of China, No. 39670298
Correspondence to: Ming-Yao Wu, Department of Pathology, Shantou University Medical College, 22 Xinling Road, Shantou 515031, Guangdong Province, China. mywu@stu.edu.cn
Telephone: +86-754-8900486 **Fax:** +86-754-8557562
Received: 2003-06-05 **Accepted:** 2003-09-24

Abstract

AIM: To examine the expression of Egr-1, c-fos and cyclin D1 at both transcript and protein levels in esophageal carcinoma and to correlate the level of their expressions with precancerous and paracancerous esophageal lesions and esophageal carcinoma.

METHODS: *In situ* hybridization and immunohistochemistry were used respectively to detect the expression of mRNA and proteins of Egr-1, c-fos and cyclin D1 in 70 cases of esophageal squamous cell carcinoma and their corresponding para-cancerous mucosa and upper cut edge mucosa.

RESULTS: *In situ* hybridization and immunohistochemistry showed positive staining of all three mRNAs in the cytoplasm and those of the proteins in nuclei. Overexpression of Egr-1, c-fos and cyclin D1 mRNAs and their proteins was found in dysplasia and squamous carcinomas. The expression level of Egr-1 and c-fos was high, and cyclin D1 was low in dysplasia mucosa, whereas the expression of Egr-1 was decreased, c-fos was maintained and cyclin D1 was increased in the cancers. The expression of both c-fos and cyclinD1 was consistent between the mRNA and protein in their corresponding high expression lesions.

CONCLUSION: The expression of Egr-1, c-fos and cyclin D1 varies in esophageal precancerous lesions and cancer tissues, suggesting an involvement of these genes in the development of esophageal carcinoma.

Wu MY, Zhuang CX, Yang HX, Liang YR. Expression of Egr-1, c-fos and cyclin D1 in esophageal cancer and its precursors: An immunohistochemical and *in situ* hybridization study. *World J Gastroenterol* 2004; 10(4): 476-480
http://www.wjgnet.com/1007-9327/10/476.asp

INTRODUCTION

Esophageal carcinoma is one of the most common malignant tumors in China[1,2]. Its pathogenesis and development are closely related to the expression of some proto-oncogenes and their products[3,4]. Our previous studies have shown that Egr-1 inhibited the growth of esophageal carcinoma cell line Eca109 after exogenous introduction of Egr-1 gene[5,6], but there has been no report on the expression of Egr-1, c-fos, and cyclin D1 mRNAs and their proteins so far. In this study, we examined the expression of Egr-1, c-fos and cyclin D1 mRNAs by *In situ* hybridization and their proteins by immunohistochemistry in 70 specimens from esophageal carcinoma, upper cut edge mucosa and para-cancerous tissues. The purpose was to understand the expression of Egr-1, c-fos and cyclin D1 in esophageal carcinoma and their association with the development of tumor.

MATERIALS AND METHODS

Sample collecting and processing

Fresh surgical resection specimens of esophagus including tumor mass, the upper cut edge mucosa and adjacent mucosa of the tumor mass were taken from 70 patients with esophageal carcinomas who had not received chemotherapy or radiotherapy before the operation. All specimens were collected from Department of Pathology, Shantou University Medical College, between January and December of 2001. The specimens were fixed in 10% neutrally buffered formalin containing 1/1000 of diethyl pyrocarbonate (DEPC, Sigma Chemical Co., USA), paraffin embedded, sectioned to 4 μm thickness, and HE stained.

Histopathology analysis

The diagnosis of esophageal epithelial para-cancer was made by histopathology according to the criteria of Liu *et al.*, which identified 42 cases of normal epithelium, 54 cases of simple hyperplasia and 44 cases of dysplasia. Seventy cases of esophageal carcinoma were diagnosed using WHO histological tumor classification. These included 2 cases of carcinoma *in situ*, 23 cases of grade I, 33 cases of grade II and 12 cases of grade III squamous cell carcinoma. There were 23 cases with invasion of the tumor into the superficial muscular layer, and others into the serosa. In addition, 26 of 70 cases showed lymphatic metastasis.

In situ hybridization

Eukaryotic expression vector of PCMV-Egr-1 plasmid was donated by Dr. Huang RP (Molecular Medicine, Northwest Hospital, WA, USA). The final construct contains the neogene (5.5 kb fragment) driven by the respiratory syncytial virus (RSV) promoter and the Egr-1 gene (2.1kb fragment) driven by the human cytomegalovirus (CMV) promoter. The plasmid was confirmed by gene amplification, purification and double endonuclease cutting, then the products were determined by agarose gel electrophoresis, the 327 bp DNA fragments was recovered by promega DNA purification kit and was labeled with digoxigenin using random priming method (Boehringer Mannheim Biochemica, Germany). The expression of Egr-1

was detected by enhanced sensitive *in situ* hybridization detection kit I (POD) from a commercial Kit (Boster Company, China) according to the manufacturer' s instructions. Sections were dewaxed in xylene, hydrated in graduated ethanol, and then incubated in 3% hydrogen peroxide in methanol for 30 min. The tissue was then digested in 20 μg/ml proteinase K at 37℃ for 20 min, fixed in 40 g/L PFA for 10 min, and cooled in 90% ethanol at -20℃ for 5 min. The digoxigenin-labeled cDNA probe was denatured in hybridization buffer (1:40) at 95-100℃ for 10 min and cooled at -20℃ for 3 min. The tissues were then overlaid with the probe, covered with a coverslip and incubated at 42℃ overnight. The expressions of c-fos mRNA and cyclin D1mRNA were also detected by *in situ* hybridization with digoxigenin-labeled gene probes, which were supplied in commercial kits (Boster Company, China), according to the manufacturer' s instructions. Following hybridizations, the sections were washed with SSC and incubated with mouse anti-digoxigenin antibody, biotinylated goat anti-mouse and then streptavidin-biotin complex (SABC) for 30 min respectively. The staining was visualized with 3.3' -diaminobenzidine (DAB). The human breast tissue and the known positive esophageal carcinoma tissue were used as positive controls. The hybridization buffer without the probe and sections pre-digested by RNase (10 μg/ml) before Egr-1, c-fos and cyclin D1 detection were used as negative controls.

Immunohistochemistry

The expression of Egr-1, c-fos and cyclin D1 proteins was analyzed using Egr-1 (SC-110) and c-fos (SC-52) rabbit polyclonal antiserum (1:200) and cyclin D1 (A-12) monoclonal antibody (1:100) (Santa Cruz Biot Co, USA) and the SABC and DAB visualization methods according to the manufacturer' s instructions (Boster Company, China). The human breast tissue and the known positive esophageal carcinoma tissue were used as positive controls. Negative controls were designed by using PBS instead of Egr-1, c-fos antiserum or cyclin D1 monoclonal antibody.

Assessment of the staining

In situ hybridization showed brown signals of Egr-1, c-fos and cyclin D1 mRNAs in the cytoplasm. The positive immunostaining of Egr-1, c-fos and cyclin D1 proteins was shown as brown signals in the nuclei. The percentage of positively stained cells was evaluated for each tissue section by counting approximately 1000 cells at a high power field. The cases having positive cancer cells accounting for more than 75% of all cancer cells on the slide were defined as a score of 3+ (strong), about 25-75% of all cancer cells were defined as a score of 2+ (moderate), and less than 25% were defined as a score of 1+ (weak). The score of - (negative) was given to cases having no positive cancer cells seen.

Statistical analysis

Statistical analyses were performed using χ^2 test and χ^2 rectified test. A P value less than 0.05 were considered to be statistically significant.

RESULTS

Expression of Egr-1, c-fos and cyclin D1 in esophageal precancerous lesions and cancer tissues

In normal epithelia of esophageal mucosa, the expression of Egr-1 mRNA and protein was found in the basal layer of mucosa (Figure 1). The level of expression increased gradually from simple hyperplasia epithelia to dysplasia, but decreased significantly in cancer tissues in which only a few cases of well-differentiated squamous cell carcinoma had Egr-1 expression (Figure 2). A very few cases of normal epithilia and simple hyperplasia epithelia showed c-fos mRNA and protein expression, and the highest expression level was seen in dysplasia of para-cancerous area and squamous cell carcinomas (Figure 3). The cyclin D1 mRNA and protein were found in a few simple hyperplasia epithelia and dysplasia epithelia, but showed the highest expression level in cancer tissues (Figure 4). The expression levels between mRNA and protein in both c-fos and cyclinD1 genes were consistent in their correspondent highest expression lesions. However, the expression levels of both Egr-1 and cyclin D1 were significantly different between cancer group and dysplasia epithelia group (P<0.01, P<0.01). The expressions of Egr-1, c-fos and cyclinD1 mRNAs and their proteins in esophageal precancerous lessions and cancer tissues are shown in Table 1.

Table 1 Expression of Egr-1, c-fos and cyclin D1 mRNAs and their proteins in esophageal precancerous lesions and cancer tissues

Groups	*n*	Egr-1 positivity		c-fos positivity		cyclin D1 positivity	
		ISH(%)	IHC(%)	ISH(%)	IHC(%)	ISH(%)	IHC(%)
Normal epithelia	42	13 (31.0)	3 (7.1)	2 (4.8)	1 (2.4)	2 (4.8)	0(0.0)
Simple hyperplasia	54	18 (33.3)	8 (14.8)	3 (5.6)	3 (5.6)	5 (9.3)	3 (5.6)
Dysplasia	44	27 (61.4)	21 (47.7)	30 (68.2)	29 (65.9)	6 (13.6)	6 (13.6)
Carcinoma *in situ*	2	1 (50.0)	1 (50.0)	1 (50.0)	1 (50.0)	0 (0.0)	0 (0.0)
Invasive carcinoma	68	12 (17.6)	9 (13.2)[b]	35 (51.5)	36 (52.9)	35 (51.5)	33 (48.5)[d]

[b]P<0.01, χ^2=16.206, compared with dysplasia group, [d]P<0.01, χ^2=16.367, compared with dysplasia group.

Table 2 Association of expression of c-fos and cyclin D1mRNAs and their proteins with differentiation degree of cancer tissues

Groups	*n*	c-fos expression								cyclin D1 expression							
		ISH				IHC				ISH				IHC			
		-	+	++	+++	-	+	++	+++	-	+	++	+++	-	+	++	+++
Squamous carcinoma																	
Grade I	23	10	4	5	4	6	4	4	9	13	6	4	0	13	4	5	1
Grade II	33	14	7	8	4	16	3	5	9	17	7	6	3	19	6	5	3
Grade III	12	9	0	2	1	10	1	0	1	3	0	3	6	3	1	2	6[b]

[b]P<0.01, χ^2=11.256, compared with squamous cell carcinoma grades I and II.

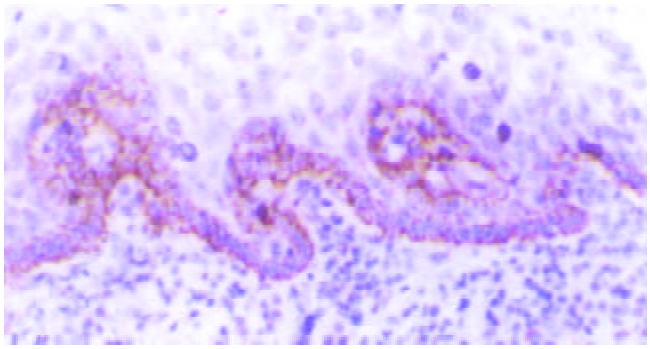

Figure 1 Egr-1 mRNA expression in basal mucosal layer in normal epithelia of esophagus. ISH ×200.

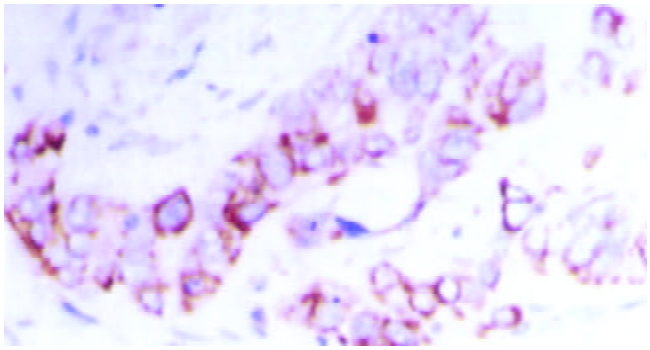

Figure 2 Positive expression of Egr-1mRNA in cytoplasm of esophageal squamous cell carcinoma. ISH ×400.

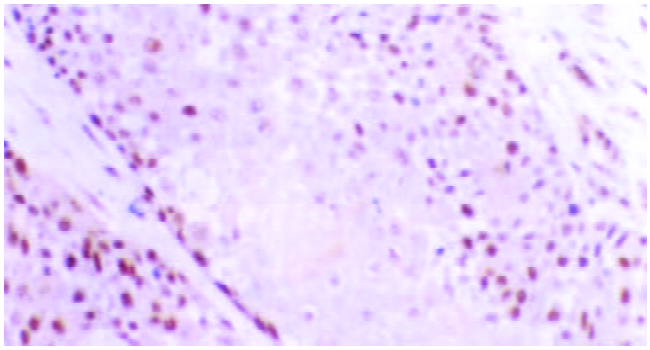

Figure 3 Positive expression c-fos proteins in nuclei of esophageal squamous cell carcinoma. IHC×200.

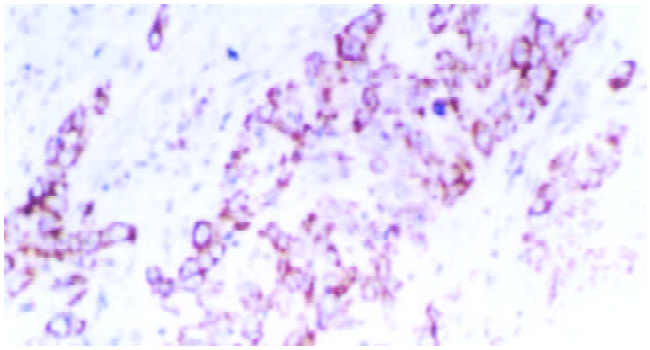

Figure 4 Positive expression of cyclin D1 mRNA in cytoplasm of esophageal squamous cell carcinoma. ISH×200.

Association of expression of c-fos and cyclin D1 with differentiation degree of cancer tissues

Of the three genes examined, the expression of Egr-1 mRNA and protein was low and the expression of c-fos and cyclin D1mRNAs and their proteins was high in esophageal cancer tissues. Furthermore, the expression of c-fos and cyclin D1 was different in different grades of esophageal carcinoma. The positive cases of c-fos appeared to be predominantly those with well or moderately differentiated squamous cell carcinoma, whereas the positive cases of cyclin D1 were mostly those with poorly differentiated squamous cell carcinoma. The cyclin D1 expression level in poorly differentiated squamous cell carcinoma group was significantly higher than that in well, or moderately differentiated squamous cell carcinoma group ($P<0.01$). The expression status of c-fos and cyclin D1mRNAs and their proteins in differently differentiated cancer tissues are shown in Table 2.

Association of expression of c-fos and cyclin D1 with lymphatic metastasis in esophageal carcinoma

Esophageal carcinomas were grouped according to different metastasis status and no association was found between the presence of lymphatic metastasis and the expression of c-fos and cyclin D1mRNAs and their proteins in esophageal carcinomas ($P>0.05$, $P>0.05$, Table 3).

Table 3 Association of expression of c-fos and cyclin D1 with lymphic metastasis in esophageal carcinomas

Groups	*n*	c-fos positivity		cyclin D1 positivity	
		ISH	IHC	ISH	IHC
Lymphatic metastasis					
Negative	42	23	20	21	20
Positive	26	12	16[a]	14	13[c]

[a]$P>0.05$, $\chi^2=0.767$, compared with lymphatic metastasis negative group; [c]$P>0.05$, $\chi^2=0.003$, compared with lymphatic metastasis negative group.

DISCUSSION

Regulations of cell growth are dependent on a number of gene families including proto-oncogene, growth factor, growth factor receptor and immediate early transcription factor gene. Egr-1, located on chromosome 5q31.1, is one of the immediate early gene families, and a nuclear protein that contains three zinc-finger domains and could regulate cell growth and differentiation by activating cyclin D1 to promote the progression of cells from G0/G1 phase into G2/M phase[7-9]. It has been reported that Egr-1 was originally in dormancy but might be activated through membrane depolarization by induction of stress, ischemia, hypoxia, bacterial toxin, cell factors, ionizing radiation and some oncogenic factors[10-12]. Using *in situ* hybridization and immunohistochemistry on a series of esophageal precancerous lesions and cancer tissues, Egr-1 mRNA and protein were detected in tissues of normal epithelia and simple hyperplasic epithelia of esophagus. However, the level of Egr-1 mRNA and protein expression was much higher (61.4% and 47.7%, respectively) in dysplasia but significantly decreased in cancerous tissues. It is possible that the paracancerous mucosa might be subjected to a higher level of stimulation, which was consistent with the findings that Egr-1 expression could be activated by many factors[13,14]. High expression of Egr-1 might preserve the stability of chromosome and suppress proliferation, and also improve differentiation and apoptosis of cells[15,16].

C-fos proto-oncogene has been found belonging to a class of cellular genes known as one of the immediate early genes and its protein product is a transcription factor, which could be strongly induced by a number of mitogens[17-19]. The present work found that the expression level of c-fos mRNA and

protein was very low in normal esophageal epithelia and simple hyperplasia epithelia, but was high in dysplasia mucous epithelia and cancer tissues of the esophagus. This finding was similar to those in some previous reports[20-22]. The development of esophageal carcinoma has been identified as a successive course from simple hyperplasia of basal cells, dysplasia, and carcinoma *in situ* to invasive carcinomas. In the cases of c-fos expression, the abnormal expression might be an early molecular event of the pathogenesis and development of esophageal carcinoma. To a certain degree, the expression level could determine the progress or regression of the pathological changes.

Cyclins are a family of cell-cycle-associated nuclear proteins and cyclin D1 is a member of this family, which could contribute to cell cycle progression through G1 phase and has been found to be closely related to the regulation and control of cell cycle[23]. The role of cyclin D1 in cell cycle control appeared to be mediated through the cyclin-dependent kinase (Cdk)/cyclin D1 complex. Some studies have indicated that cyclin D1 was involved in esophageal carcinoma and that its overexpression might be a useful prognostic factor[24-28]. Others argued that the overexpression of cyclin D1 in esophageal cancer tissues might not predict the prognosis independently[29-32]. In the current study, we found that cyclin D1 mRNA and protein expression was high in esophageal carcinoma compared with that in pre-cancerous tissue and the degree of positive expression was stronger in poorly differentiated squamous cell carcinomas. It seemed that the high level of expression of cyclin D1 indicated the poor prognosis. However, no correlation was found between cyclin D1 expression and lymphatic metastasis of squamous cell cancer. Our study suggested that overexpression of cyclin D1 could be used to help clinicians take more rational measures for post-operation patients who showed no metastasis to lymph nodes.

The pathogensis of esophageal carcinoma is a course in which the oncogenes and tumor suppressor genes act or counter act to each other. This interation could determine the development of esophageal carcinoma[33-35]. The progress from normal esophageal mucous epithelia to simple hyperplasia, atypical hyperplasia and cancer is usually atributed to the decreased expression of tumor suppressor genes and increased expression of oncogenes. Our studies found that the expression of Egr-1 was decreased, c-fos was remained and cyclin D1 was increased during the progress of esophageal mucous epithelia from precancerous lesions to cancer. The activity of Egr-1 was reduced, but the activity of c-fos and cyclin D1 was enhanced. This resulted in abnormal regulation and control of cell cycle in the basal layer cells of esophageal mucous epithelia leading to the development of esophageal carcinoma. Neverthless, the mechanism of the involvment of these genes in the development and progress of esophageal carcinoma remains to be further investigated.

REFERENCES

1 **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

2 **He YT,** Hou J, Qiao CY, Chen ZF, Song GH, Li SS, Meng FS, Jin HX, Chen C. An analysis of esophageal cancer incidence in Cixian County from 1974 to 1996. *World J Gastroenterol* 2003; **9:** 209-213

3 **Su H, Hu N,** Shih J, Hu Y, Wang QH, Chuang EY, Roth MJ, Wang C, Goldstein AM, Ding T, Dawsey SM, Giffen C, Emmert-Buck MR, Taylor PR. Gene expression analysis of esophageal squamous cell carcinoma reveals consistent molecular profiles related to a family history of upper gastrointestinal cancer. *Cancer Res* 2003; **63:** 3872-3876

4 **Nie Y,** Liao J, Zhao X, Song Y, Yang GY, Wang LD, Yang CS. Detection of multiple gene hypermethylation in the development of esophageal squamous cell carcinoma. *Carcinogenesis* 2002; **23:** 1713-1720

5 **Wu MY,** Chen MH, Liang YR, Meng GZ, Yang HX, Zhuang CX. Experimental and clinicopathologic study on the relationship between transcription factor Egr-1 and esophageal carcinoma. *World J Gastroenterol* 2001; **7:** 490-495

6 **Hao MW,** Liang YR, Liu YF, Liu L, Wu MY, Yang HX. Transcription factor EGR-1 inhibits growth of hepatocellular carcinoma and esophageal carcinoma cell lines. *World J Gastroenterol* 2002; **8:** 203-207

7 **Nagamura-Inoue T,** Tamura T, Ozato K. Transcription factors that regulate growth and differentiation of myeloid cells. *Int Rev Immunol* 2001; **20:** 83-105

8 **Thiel G,** Cibelli G. Regulation of life and death by the zinc finger transcription factor Egr-1. *J Cell Physiol* 2002; **193:** 287-292

9 **Krishnaraju K,** Hoffman B, Liebermann DA. Early growth response gene 1 stimulates development of hematopoietic progenitor cells along the macrophage lineage at the expense of the granulocyte and erythroid lineages. *Blood* 2001; **97:** 1298-1305

10 **Kaufmann K,** Thiel G. Epidermal growth factor and platelet-derived growth factor induce expression of Egr-1, a zinc finger transcription factor, in human malignant glioma cells. *J Neurol Sci* 2001; **189:** 83-91

11 **Kaufmann K,** Bach K, Thiel G. The extracellular signal-regulated protein kinases Erk1/Erk2 stimulate expression and biological activity of the transcriptional regulator Egr-1. *Biol Chem* 2001; **382:** 1077-1081

12 **Nishi H,** Nishi KH, Johnson AC. Early Growth Response-1 gene mediates up-regulation of epidermal growth factor receptor expression during hypoxia. *Cancer Res* 2002; **62:** 827-834

13 **Meyer RG,** Kupper JH, Kandolf R, Rodemann HP. Early growth response-1 gene (Egr-1) promoter induction by ionizing radiation in U87 malignant glioma cells *in vitro*. *Eur J Biochem* 2002; **269:** 337-346

14 **Pawlinski R,** Pedersen B, Kehrle B, Aird WC, Frank RD, Guha M, Mackman N. Regulation of tissue factor and inflammatory mediators by Egr-1 in a mouse endotoxemia model. *Blood* 2003; **101:** 3940-3947

15 **Wu MY,** Liang YR, Wu XY, Zhuang CX. Relationship between Egr-1 gene expression and apoptosis in esophageal carcinoma and precancerous lesions. *World J Gastroenterol* 2002; **8:** 971-975

16 **Pignatelli M,** Luna-Medina R, Perez-Rendon A, Santos A, Perez-Castillo A. The transcription factor early growth response factor-1 (EGR-1) promotes apoptosis of neuroblastoma cells. *Biochem J* 2003; **373**(Pt 3): 739-746

17 **Berthoud HR,** Patterson LM, Zheng H. Vagal-enteric interface: vagal activation-induced expression of c-Fos and p-CREB in neurons of the upper gastrointestinal tract and pancreas. *Anat Rec* 2001; **262:** 29-40

18 **Nephew KP,** Choi CM, Polek TC, McBride R, Bigsby RM, Khan SA, Husseinzadeh N. Expression of fos and jun proto-oncogenes in benign versus malignant human uterine tissue. *Gynecol Oncol* 2000; **76:** 388-396

19 **Weisstein JS,** Majeska RJ, Klein MJ, Einhorn TA. Detection of c-fos expression in benign and malignant musculoskeletal lesions. *J Orthop Res* 2001; **19:** 339-345

20 **Pacheco MM,** Kowalski LP, Nishimoto IN, Brentani MM. Differential expression of c-jun and c-fos mRNAs in squamous cell carcinoma of the head and neck: associations with uPA, gelatinase B, and matrilysin mRNAs. *Head Neck* 2002; **24:** 24-32

21 **de Sousa SO,** Mesquita RA, Pinto DS Jr, Gutkind S. Immunolocalization of c-Fos and c-Jun in human oral mucosa and in oral squamous cell carcinoma. *J Oral Pathol Med* 2002; **31:** 78-81

22 **Feng DY,** Zheng H, Tan Y, Cheng RX. Effect of phosphorylation of MAPK and Stat3 and expression of c-fos and c-jun proteins on hepatocarcinogenesis and their clinical significance. *World J Gastroenterol* 2001; **7:** 33-36

23 **Shinohara M,** Aoki T, Sato S, Takagi Y, Osaka Y, Koyanagi Y, Hatooka S, Shinoda M. Cell cycle-regulated factors in esophageal cancer. *Dis Esophagus* 2002; **15:** 149-154

24 **Sarbia M,** Stahl M, Fink U, Heep H, Dutkowski P, Willers R,

Seeber S, Gabbert HE. Prognostic significance of cyclin D1 in esophageal squamous cell carcinoma patients treated with surgery alone or combined therapy modalities. *Int J Cancer* 1999; **84:** 86-91

25 **Shamma A,** Doki Y, Shiozaki H, Tsujinaka T, Yamamoto M, Inoue M, Yano M, Monden M. Cyclin D1 overexpression in esophageal dysplasia: a possible biomarker for carcinogenesis of esophageal squamous cell carcinoma. *Int J Oncol* 2000; **16:** 261-266

26 **Toyoda H,** Nakamura T, Shinoda M, Suzuki T, Hatooka S, Kobayashi S, Ohashi K, Seto M, Shiku H, Nakamura S. Cyclin D1 expression is useful as a prognostic indicator for advanced esophageal carcinomas, but not for superficial tumors. *Dig Dis Sci* 2000; **45:** 864-869

27 **Natsugoe S,** Nakashima S, Matsumoto M, Sakita H, Sakamoto F, Okumura H, Baba M, Yoshinaka H, Takao S, Aikou T. Biologic and imaging diagnosis of lymph node metastasis in esophageal carcinoma. *J Surg Oncol* 2002; **81:** 25-32

28 **Shiozaki H,** Doki Y, Yamana H, Isono K. A multi-institutional study of immunohistochemical investigation for the roles of cyclin D1 and E-cadherin in superficial squamous cell carcinoma of the esophagus. *J Surg Oncol* 2002; **79:** 166-173

29 **Ikeda G,** Isaji S, Chandra B, Watanabe M, Kawarada Y. Prognostic significance of biologic factors in squamous cell carcinoma of the esophagus. *Cancer* 1999; **86:** 1396-1405

30 **Kuwahara M,** Hirai T, Yoshida K, Yamashita Y, Hihara J, Inoue H, Toge T. p53, p21(Waf1/Cip1) and cyclin D1 protein expression and prognosis in esophageal cancer. *Dis Esophagus* 1999; **12:** 116-119

31 **Shiozaki H,** Doki Y, Kawanishi K, Shamma A, Yano M, Inoue M, Monden M. Clinical application of malignancy potential grading as a prognostic factor of human esophageal cancers. *Surgery* 2000; **127:** 552-561

32 **Arber N,** Gammon MD, Hibshoosh H, Britton JA, Zhang Y, Schonberg JB, Roterdam H, Fabian I, Holt PR, Weinstein IB. Overexpression of cyclin D1 occurs in both squamous carcinomas and adenocarcinomas of the esophagus and in adenocarcinomas of the stomach. *Hum Pathol* 1999; **30:** 1087-1092

33 **Mandard AM,** Hainaut P, Hollstein M. Genetic steps in the development of squamous cell carcinoma of the esophagus. *Mutat Res* 2000; **462:** 335-342

34 **Xu SH,** Qian LJ, Mou HZ, Zhu CH, Zhou XM, Liu XL, Chen Y, Bao WY. Difference of gene expression profiles between esophageal carcinoma and its pericancerous epithelium by gene chip. *World J Gastroenterol* 2003; **9:** 417-422

35 **Zhou J,** Zhao LQ, Xiong MM, Wang XQ, Yang GR, Qiu ZL, Wu M, Liu ZH. Gene expression profiles at different stages of human esophageal squamous cell carcinoma. *World J Gastroenterol* 2003; **9:** 9-15

Edited by Liu HX and Wang XL