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

World J Gastroenterol 2001;7(4):490-495
World Journal of Gastroenterology
 ISSN 1007-9327

• ORIGINAL RESEARCH •

Experimental and clinicopathologic study on the relationship between transcription factor Egr-1 and esophageal carcinoma

Ming-Yao Wu, Mao-Huai Chen, Ying-Rui Liang, Guo-Zhao Meng, Huan-Xing Yang and Chu-Xiang Zhuang

Department of Pathology, Shantou University Medical College, Shantou 515031, 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@ncrc.stu.edu.cn
Tel: 0086-754-8900486, Fax: 0086-754-8557562
Received 2001-02-06 **Accepted** 2001-02-12

Abstract

AIM **To observe the growth suppression effect of exogenous introduction of early growth response gene-1 (Egr-1 gene) on esophageal carcinoma tissue as well as on esophageal carcinoma cell line Eca109 and to explore the potential application of Egr-1 gene in gene therapy of tumor.**

METHODS **Eukaryotic expression vector of PCMV-Egr-1 plasmid was introduced into Eca109 cell line which expressed no Egr-1 protein originally with lipofectamine transfection method. The introduction and expression of PCMV-Egr-1 plasmid into Eca109 cell line was confirmed by G418 selection culture, PCR amplification of neogene contained in the vector, Western blot analysis and immunocytochemical analysis. The cell growth curve, soft agar colony formation rate and tumorigenicity in SCID mice were examined to demonstrate the growth suppression effect of exogenous Egr-1 gene on Eca109 cell line. The Egr-1 mRNA and Egr-1 protein were also detected in 50 surgical specimens of esophageal carcinoma by *in situ* hybridization and immunohistochemistry.**

RESULTS **Exogenous Egr-1 gene was introduced successfully into Eca109 cell line and expressed Egr-1 protein stably. The transfected Eca109 cell line grew more slowly than control Eca109 as shown by cell growth curves, the soft agar colony formation rate (4.0% *vs* 6.9%, *P*<0.01) and the average growth rate of tumor in SCID mice (35.5±7.6 *vs* 65.8±7.6, *P*<0.05). The expression level of Egr-1 mRNA and protein significantly increased in dysplastic epithelia adjacent to cancer rather than in cancer tissues (65.8% *vs* 20.0% by ISH and 57.9% *vs* 14.0% by IHC, *P*<0.01).**

CONCLUSION **Exogenous Egr-1 gene shows the strong effect of growth inhibition in Eca109 cell line. Egr-1 in the cancer tissue shows down-regulated expression that supports the inhibited function of Egr-1 in cancer growth and suggests Egr-1 may have an important role in gene therapy of esophageal carcinoma.**

Subject headings esophageal neoplasms/pathology; tumor cells, cultured; genes, immediate early; gene expression; transfection

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(4):490-495

INTRODUCTION

Esophageal carcinoma is one of the most common malignant tumors in China[1-5]. Its pathogenesis and development are closely related with some of proto-oncogenes and their products[6-17]. Early growth response gene-1 (Egr-1 gene) is known as a member of immediate early gene (IEG) family characterized by rapid and transient expression in response to stimulation, modulating gene transcription positively or negatively depending on the cell types and playing an important role in the early cell growth. But its relationship with esophageal carcinoma is not well understood so far. In order to evaluate the growth inhibition in the cell line of esophageal carcinoma (Eca109) and analyze the expression of Egr-1 in the cancer tissue of esophageal carcinoma, the relationship between Egr-1 and esophageal carcinoma is explored by the methods of gene transfection, tumorigenicity in severe combined immunodeficient (SCID) mice and *in situ* hybridization.

MATERIALS AND METHODS

Human esophageal carcinoma cell line Eca109

Eca109 cell line from Chinese Academy of Medical Sciences was cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 100mL·L^{-1} fetal bovine at 37℃ and 50mL·L^{-1} CO_2 incubator. The cancer tissue with adjacent mucosa and the esophageal mucosa in the upper surgical margin was from the same surgical specimen of 50 patients with esophageal carcinomas who had received neither chemotherapy nor radiotherapy before surgery. These were fixed in 40mL·L^{-1} neutral formalin with 1/1000 diethyl pyrocarbonate (DEPC, Sigma Chemical Co, USA) and embedded in paraffin, the sections were cut in 5μm.

Eukaryotic expression vector of PCMV-Egr-1 plasmid

The plasmid was donated by Dr RP Huang (Molecular Medicine, Northwest Hospital, WA, USA). The final construct contains the neogene (5.5kb 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 amplification, purification and tested by cutting endonuclease.

Gene transfection

The eukaryotic expression vector of PCMV-Egr-1 plasmid was transfected to human esophageal carcinoma cell line Eca109 by the lipofectamine (Gibcobrl Company, USA) according to the manufacturer's instructions. After transfection, the cells were trypsinized and reseeded at 1:2 ratio for selection culture with G418 at 600mg·L^{-1}. Four weeks later, the resistant colonies were formed.

Detection of transfected cell neogene with polymerase chain reaction

Extracting the cell DNA followed by performing polymerase chain reaction (PCR) to amplify 327bp of neogene with primer 1 (5'ACAAGATGGATTGCACGC AGG3') and primer 2 (5'TTCTCGGCAGGAGCAAGGTGAG3'). The cycling procedure was: denatured at 95℃ for 1 min, annealed at 55℃ for 1.5 min, extended at 72℃ for 1 min, and after 30 cycles, lengthened at 72℃ for 5 min. The untransfected Eca 109 cell line was used as a negative control.

Western blot and immunocytocheimstry

Cells (3×10^5) were harvested and dissolved by the addition of sodium dodecyl sulfate (SDS)-containing lysis buffer. The lysate was used for SDS-polyacrylamide gel electrophoresis on a 75g·L^{-1} gel. The proteins were electrophoretically transferred from the gel to NC membrane. The transferred membrane was treated with polycolonal antiserum against Egr-1 protein (1:200, Santa Cruz Biot Co, USA) followed by detection with peroxidase-labeled goat anti-rabbit (1:1000, Dako, USA). The resulting complexes were detected with the ECL reagent (Amersham Company, UK) according to the manufacturer's instructions. The Egr-1 protein was detected by immunocytochemistry.The untransfected Eca 109 cell line was used as a negative control.

Detection of biological feactures in transfected cells

Growth curve assays Cells (1×10^4) were seeded in each well of a 24-well plate and were allowed to grow for varying periods. The trypsinized cells were counted by cell counter plate. The growth assays were made by counting in triplicate on every other day of culture for up to 7d followed by constructing the cell growth curves.

Soft agar culture The anchorage independent growth was examined by seeding 1000 cells in 3g·L^{-1} agar medium into 60mm plates previously lined with 5g·L^{-1} agar medium. The plates (in triplicate and repeated twice) were cultured at 37℃, 50mL·L^{-1} CO_2 incubator for 14d. The average number of colony formation (any colony containing >50 cells was counted as a colony) and the colony formation rate (the number of colonies/the number of seeded cells) were calculated.

Tumorigenicity in SCID mice Two groups were divided randomly. The experimental group that consisted of 6 mice was used for Eca 109 cells with transfected Egr-1 injection. The control group of 4 mice was used for Eca 109 cells without transfected Egr-1 injection. Trypsinized cells (5×10^6) were injected into the subcutaneous sites on the shoulders of SCID mice. Animals were inspected at regular intervals for the appearance of visible tumors to measure the time of first appearance. Thirty days later, the mice were sacrificed and the tumors were carefully removed by blunt dissection. The tumors were weighed and their average growth rates were measured as mg·d^{-1}.

Egr-1 in situ hybridization

The expression of Egr-1 was detected by digoxigenin-labeled gene probe from a commercial kit (Boster Company, China) according to the manufacturer's instructions. The human breast tissue and the mouse brain tissue were used as the positive control. Either the sections detected with incubation solution instead of the probe or the sections digested with ribonucleases (RNase) (10mg·L^{-1}) before Egr-1 detection was designed for the negative control. The positive expression showed the brown staining signal in the cytoplasm.

Immunohistochemistry

Egr-1 was analyzed by using Egr-1 rabbit polyclonal antiserum with the SABC method according to the manufacturer's instructions (Boster Company, China) following treatment with 3.3'-diaminobenzidine (DAB) staining. The human breast tissue and the mouse brain tissue were used as the positive control. Negative control was designed using phosphate-buffered saline (PBS) instead of Egr-1 rabbit polyclonal antiserum in detection. The positive expression showed the brown staining signal in nuclei.

Statistical analysis

The data were statistically analyzed using Student's *t* test and the difference of results was analyzed by *U* test and χ^2 test.

RESULTS

Identification of plasmid

The eukaryotic expression vector of PCMV-Egr-1 plasmid was tested by PCR amplification, purification and restriction endonuclease and confirmed to be consistent with the plasmid map. With single digestion of *Sac* II, a 7.6kb band was obtained which represents the whole lengh of the plasmid DNA. With double digestion of *Sac* II combining with *Sma* I, both 5.5kb and 2.1kb bands were obtained which represented the fragment of the vector and Egr-1 gene fragment respectively (Figure 1).

Gene transfection

Eca109 was transfected with PCMV-Egr-1 plasmid by lipofectamine transfection method. Four weeks after G418 selection culture, the resistant colonies were formed. PCR

indicated that 327bp of neogene was shown in transfected Eca109 compared to the negative band in control Eca109 (Figure 2), demonstrating that PCMV-Egr-1 plasmid had been introduced into Eca109 cell and integrated into the genomic DNA.

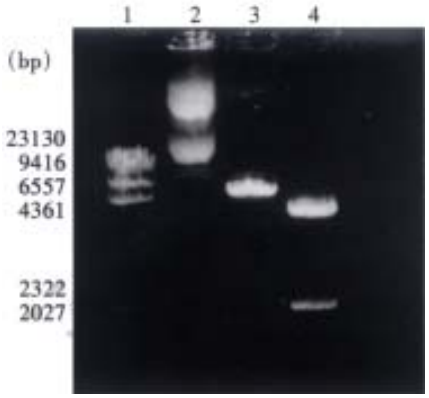

Figure 1 Identification of PCMV-Egr-1 plasmid.
1. Marker; 2. Uncut plasmid; 3. Cut with *Sac* II, showing 7.6kp fragment of whole plasmid; 4. Cut with *Sac* II and Sma I, showing 5.5kp fragment of vector and 2.1kp fragment of Egr-1 gene.

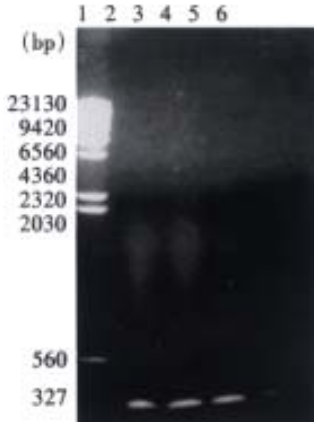

Figure 2 PCR amplification of neogene.
1. Marker; 2-5. For transfected Eca109, showing 327bp of neogene; 6. Negative control Eca109, no band was shown.

Detection of Egr-1 protein in transfected Eca109

Western blot The 80kd of Egr-1 protein was shown for transfected Eca109 compared to the negative band of the control Eca109.

Immunocytochemistry The transfected Eca109 was positively brown stained in the nucleus compared to the negative staining for control Eca109 (Figure 3). The results demonstrated that exogenous Egr-1 gene introduced into Eca109 expressed high level of Egr-1 protein.

Growth feature of transfected Eca109

Growth curves The transfected Eca109 grew much more slowly in DMEM medium than the control Eca109 cell (Figure 4).

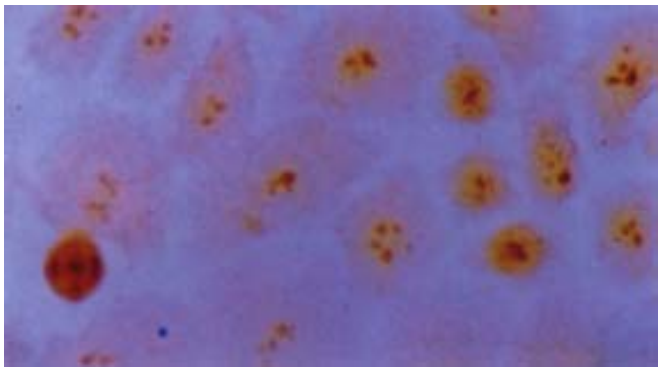

Figure 3 Positive Egr-1 protein in nuclei of transfected Eca109 cells. ICC ×400

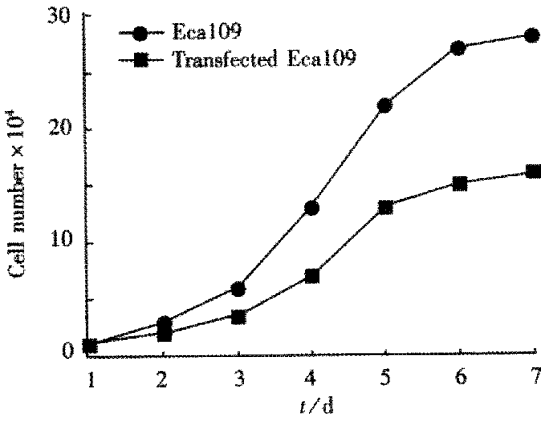

Figure 4 Cell growth curves showing lower growth rate in transfected Eca109 cell than in the control Eca109 cell.

Soft agar growth assay Small and few colonies were formed slowly in transfected Eca109 compared to large and numerous colonies formed quickly in control Eca109. The colony formation rates were 4.0% and 6.9% in transfected Eca109 and in control Eca109 respectively (Table 1) which demonstrated that the anchorage independent growth ability of Egr-1 expressing Eca109 was reduced.

Table 1 Soft agar assays in Eca109 cell line

Cell line	No. of seeded cell	No. of colonies				CFR
		1	2	3	Mean	
Eca109	1000	65	71	73	69±2.4	6.9%
Egr-1-Eca109	1000	40	43	39	40±1.2	4.0%[b]

[b]$P<0.01$, *vs* Eca109, *U* test.

Tumorigenicity in SCID mice The tumorigenicity test showed that the tumors started to appear on the 21st day after injection in the six SCID mice injected with transfected Eca109, and on the 14th day in four SCID mice injected with control Eca109. The tumors of Egr-1 expressing Eca109 grew slowly with an average growth rate of 35.5mg·d^{-1}, and the tumors of control Eca109 grew fast with an average growth rate of 65.8mg·d^{-1} (Figure 5, Table 2). The results demonstrated that the tumorigenicity in SCID mice of Egr-1 transfected Eca109 was inhibited.

Table 2 Tumorigenicity assays in Eca109 cell line

Cell line	Tumorigenicity rate	Growth rate of tumors(mg·d^{-1})						
		1	2	3	4	5	6	Mean
Eca109	4/4	83.3	70.0	46.6	63.3			65.8±7.6
Egr-1-Eca109	6/6	20.0	13.3	33.3	66.6	43.0	36.7	35.5±7.6[a]

[a]P<0.05, *vs* Eca109, Student's t test.

Egr-1 expression on esophageal tissues

In simple hyperplastic epithelia of esophageal mucosa, the expression of Egr-1 was found in the basal layer of the mucosa (Figure 6). In dysplastic epithelia, the expression of Egr-1 increased but significantly decreased in cancer tissues in which only a few cases of well-differentiated squamous cell carcinoma had the Egr-1 expression (Figure 7). The expression of Egr-1mRNA and proteins in the various pathological changes of esophagus are shown in Table 3.

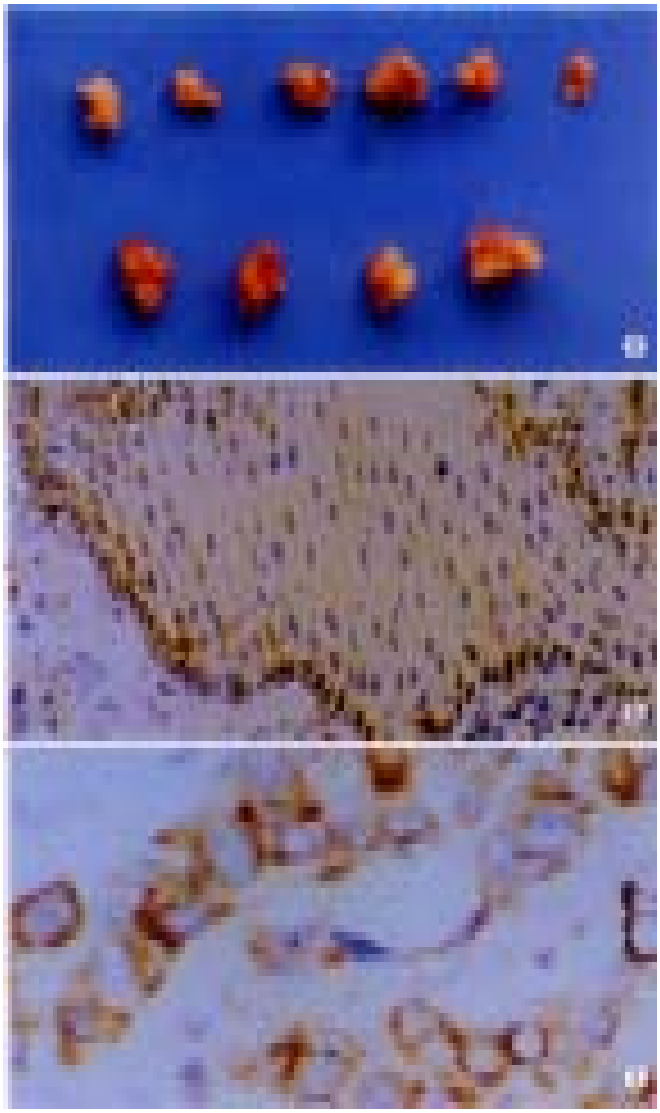

Figure 5 The tumors of transfected Eca109 are smaller than that of control Eca109, *in vitro*, 30 d after injection in tumorigenicity test in SCID mice.

Figure 6 Egr-1 protein expression in basal mucosal layer in simple hyperplastic epithelia of esophagus. IHC ×200

Figure 7 Positive Egr-1 mRNA in cytoplasm of esophageal squamous cell carcinoma. ISH ×400

Table 3 Expression of Egr-1mRNA and proteins in various pathological changes of esophagus, n(%)

Group	n	ISH	IHC
Simple hyperplasia	30	12 (40.0)	9 (30.0)
Dysplasia	38	25 (65.8)	22 (57.9)[a]
Esophageal cancer	50	10 (20.0)	7 (14.0)[b]

[a]P<0.05, *vs* simple hyperplasia, χ^2 test; [b]P<0.01, *vs* dysplasia,χ^2 test.

DISCUSSION

As we know, the oncogenes and tumor suppressor genes are involved in the pathogenesis and development of esophageal carcinoma[18-32]. Recent studies in dicate that Egr-1 gene as a tumor related-gene is correlated with the tumor development. It was reported that Egr-1 proteins were decreased and even disappeared in several kinds of cancer tissues. Furthermore, the Egr-1 mRNA expression was consistent with the expression level of Egr-1 protein which had been verified in the down-regulation of Egr-1 occurred at the transcription level in cancer cells[33]. Southern blot analysis indicated no deletion, no rearrangement or mutation of Egr-1 on DNA level. The exogenous introduction of Egr-1 could inhibit the growth of tumor cells accompanied in a dose-dependent manner, for example, the ability of anchorage independent growth and tumorigenicity in SCID mice of human HT1080 fibrosarcoma without original Egr-1 was significantly inhibited after the exogenous introduction of Egr-1. If the antisense Egr-1 was introduced to the cells, it would inhibit the endogenous Egr-1 expression and promote the malignant transformation of the cells. In the present study, eukaryotic expression vector of PCMV-Egr-1 plasmid was introduced into Eca109 cell line that expressed no Egr-1 protein originally with Lipofectamine transfection method. The introduction and expression of PCMV-Egr-1 plasmid into Eca109 cell line was confirmed by G418 selection culture by which colony formation persisted in next generations and by PCR amplification of neogene contained in the vector. Furthermore, the strong expression of Egr-1 protein in transfected Eca109 was detected by Western blot and immunocytochemistry, which verified the success of gene transfection. Growth inhibition of the transfected cells shown by the growth curves, the colony formation rates in the soft agar and tumorigenicity in SCID mice demonstrated that exogenous Egr-1 gene inhibited the growth of Eca109.

Recent studies suggest that the mechanism of suppression of tumor growth by Egr-1 is the Egr-1 protein with a zinc-finger domain which regulates the transcription of many downstream genes by binding to the GC rich element in the promoter region and modulates genes transcription and various biological effects[34-45]. Egr-1 can compete with transcription activator SP1 in binding to an overlapping consensus binding motif in the promoter region of GCE which commonly exists in oncogene and tumor suppressor gene and abolishes the function of SP1, leading to the transcription inhibition of downstream genes and growth inhibition of tumor cells[46,47]. In addition, the exogenous Egr-1 may inhibit the growth by binding to the GEE of TGF-β1 and activating the transcription of TGF-β1 and subsequently activating p21 gene or by down-regulating Bcl-2 gene to influence the tumor cells[48,49]. Some

studies indicated that the activation of some oncogenes e.g. wild type *p*53 gene, cell apoptosis, the TNF-α and the concentration of calcium may be involved in the mechanism of growth inhibition property of Egr-1[50,51]. On the whole, various and complicated mechanisms may be involved in the suppressive property of Egr-1 for tumor growth.

Egr-1 is one of the immediate early gene family which regulates the cellular growth and differentiation by activating Cyclin D1 to promote the cells from the G_0/G_1 phase into the G2/M phase[52]. The mechanism of down-expression of Egr-1 in cancer tissues is not clear up to now. It was hypothesized that the high concentration of Egr-1 proteins produced by overstimulation of Egr-1 interacted with the promoter region of EBS and subsequently the gene transcription was inhibited, which was concordant with the present results that showed strong expression of Egr-1 in dysplastic cells adjacent to esophageal carcinoma and weekly expression in cancer cells.

Since only downregulation expression without gene mutation occurred in Egr-1 DNA level, it was different from some tumor suppressor genes such as *p*3, *p*16 and Rb with mutation gene on DNA level. It is more convenient to introduce normal exogenous gene into the tumor cells than to repair mutation gene in gene therapy. The present study substantiates that exogenous Egr-1 as a target gene has a potential application in gene therapy of esophageal carcinoma.

REFERENCES

1 Gao SS, Zhou Q, Li YX, Bai YM, Zheng ZY, Zou JX, Liu G, Fan ZM, Qi YJ, Zhao X, Wang LD. Comparative studies on epithelial lesions at gastric cardia and pyloric antrum in subjects from a high incidence area for esophageal cancer in Henan, China. *World J Gastroenterol*, 1998;4:332-333
2 Qiao GB, Han CL, Jiang RC, Sun CS, Wang Y, Wang YJ. Overexpression of P53 and its risk factors in esophageal cancer in urban areas of Xi'an. *World J Gastroenterol*, 1998;4:57-60
3 Li J, Niu ZX, Wang LD. Advance in molecular studies on esophageal cancer. *Xin Xiaohuabingxue Zazhi*, 1997;5:189-191
4 Wang YB. Diagnosis and treatment of esophageal cancer. *Shijie Huaren Xiaohua Zazhi*, 2000;8:1017-1030
5 Zou JX, Wang LD, Shi ST, Yang GY, Xue ZH, Gao SS, Li YX, Yang CS. p53 gene mutations in multifocal esophageal precancerous and cancerous lesions in patients with esophageal cancer in high-risk northern China. *Shijie Huaren Xiaohua Zazhi*, 1999;7:280-284
6 Wang LD, Zhou Q, Wei JP, Yang WC, Zhao X, Wang LX, Zou JX, Gao SS, Li YX, Yang CS. Apoptosis and its relationship with cell proliferation, p53, Waf1p21, bcl-2 and c-myc in esophageal carcinogenesis studied with a high-risk population in northern China. *World J Gastroenterol*, 1998;4:287-293
7 Li J, Feng CW, Zhao ZG, Zhou Q, Wang LD. A preliminary study on ras protein expression in human esophageal cancer and precancerous lesions. *World J Gastroenterol*, 2000;6:278-280
8 Zhang LJ, Chen KN, Xu GW, Xing HP, Shi XT. Congenital expression of mdr-1 gene in tissues of carcinoma and its relation with patho-morphology and prognosis. *World J Gastroenterol*, 1999;5:53-56
9 Casson AG. Molecular biology of Barrett's esophagus and esophageal cancer: role of p53. *World J Gastroenterol*, 1998;4:277-279
10 Xu CT, Yan XJ. p53 anti-cancer gene and digestive system neoplasms. *Shijie Huaren Xiaohua Zazhi*, 1999;7:77-79
11 Lin J, Deng CS, Sun J, Zhou Y, Xiong P, Wang YP. Study on the genetic susceptibility of HLA-DQB1 alleles in esophageal cancer of Hubei Chinese Hans. *Shijie Huaren Xiaohua Zazhi*, 2000;8:965-968
12 Qin HY, Shu Q, Wang D, Ma QF. Study on genetic polymorphisms of DCC gene VNTR in esophageal cancer. *Shijie Huaren Xiaohua Zazhi*, 2000;8:782-785
13 Liu J, Chen SL, Zhang W, Su Q. P21-WAF1 gene expression with P53 mutation in esophageal carcinoma. *Shijie Huaren Xiaohua Zazhi*, 2000;8:1350-1353
14 Liu J, Su Q, Zhang W. Relationship between HPV-E6 P53 protein and esophageal squamous cell carcinoma. *Shijie Huaren Xiaohua Zazhi*, 2000;8:494-496
15 Tan LJ, Jiang W, Zhang N, Zhang XR, Qiu DH. Fas/FasL expression of esophageal squamous cell carcinoma, dysplasia tissues and normal mucosa. *Shijie Huaren Xiaohua Zazhi*, 2001;9:15-19
16 Gu HP, Shang PZ, Su H, Li ZG. Association of CD15 antigen expression with cathepsin D in esophageal carcinoma tissues. *Shijie Huaren Xiaohua Zazhi*, 2000;8:259-261
17 Wang LD, Chen H, Guo LM. Alterations of tumor suppressor gene system p53 Rb and human esophageal carcinogenesis. *Shijie Huaren Xiaohua Zazhi*, 2001;9:367-371
18 Kawaguchi H, Ohno S, Araki K, Miyazaki M, Saeki H, Watanabe M, Tanaka S, Sugimachi K. p53 polymorphism in human papillomavirus-associated esophageal cancer. *Cancer Res*, 2000;60:2753-2755
19 Wijnhoven BPL, Nollet F, De Both NJ, Tilanus HW, Dinjens WNM. Genetic alterations involving exon 3 of the β-catenin gene do not play a role in adenocarcinomas of the esophagus. *Int J Cancer*, 2000;86:533-537
20 Takubo K, Nakamura K, Sawabe M, Arai T, Esaki Y, Miyashita M, Mafune K, Tanaka Y, Sasajima K. Primary undifferentiated small cell carcinoma of the esophagus. *Hum Pathol*, 1999;30:216-221
21 Fong LYY, Pegg AE, Magee PN. α-difluoromethylornithine inhibits *N*-nitrosomethylbenzylamine-induced esophageal carcinogenesis in zinc-deficient rats: effects on esophageal cell proliferation and apoptosis. *Cancer Res*, 1998;58:5380-5388
22 Sarbia M, Bittinger F, Grabellus F, Verreet P, Dutkowski P, Willers R, Gabbert HE. Expression of Bax, a pro-apoptotic member of the Bcl-2 family, in esophageal squamous cell carcinoma. *Int J Cancer*, 1997;73:508-513
23 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
24 Van Lieshout EMM, Roelofs HMJ, Dekker S, Mulder CJJ, Wobbes T, Jansen JBMJ, Peters WHM. Polymorphic expression of the glutathione S-transferase P1 gene and its susceptibility to Barrett's esophagus and esophageal carcinoma. *Cancer Res*, 1999;59:586-589
25 Kumble S, Omary MB, Cartwright CA, Triadafilopoulos G. Src activation in malignant and premalignant epithelia of Barrett¡äs esophagus. *Gastroenterology*, 1997;112:348-356
26 Iwaya T, Maesawa C, Tamura G, Sato N, Ikeda K, Sasaki A, Othuka K, Ishida K, Saito K, Satodate R. Esophageal carcinosarcoma: a genetic analysis. *Gastroenterology*, 1997;113:973-977
27 Mori M, Mimori K, Shiraishi T, Alder H, Inoue H, Tanaka Y, Sugimachi K, Huebner K, Croce CM. Altered expression of Fhit in carcinoma and precarcinomatous lesions of the esophagus. *Cancer Res*, 2000;60:1177-1182
28 Dolan K, Garde J, Walker SJ, Sutton R, Gosney J, Field JK. LOH at the sites of the DCC, APC, and TP53 tumor suppressor genes occurs in Barrett's metaplasia and dysplasia adjacent to adenocarcinoma of the esophagus. *Hum Pathol*, 1999;30:1508-1514
29 Zur Hausen A, Sarbia M, Heep H, Willers R, Gabbert HE. Retinoblastoma-protein (pRb) expression and prognosis in squamous-cell carcinomas of the esophagus. *Int J Cancer*, 1999;84:618-622
30 Chen YJ, Chen PH, Lee MD, Chang JG. Aberrant FHIT transcripts in cancerous and corresponding non-cancerous lesions of the digestive tract. *Int J Cancer*, 1997;72:955-958
31 Muzeau F, Flejou JF, Thomas G, Hamelin R. Loss of heterozygosity on chromosome 9 and p16 (MTS1, CDKN2) gene mutations in esophageal cancers. *Int J Cancer*, 1997;72:27-30
32 Suzuki K, Daigo Y, Fukuda S, Tokino T, Isomura M, Isono K, Wainwright B, Nakamura Y. No evidence of mutation in the human PTC gene, responsible for nevoid basal cell carcinoma syndrome, in human primary squamous cell carcinomas of the esophagus and lung. *Jpn J Cancer Res*, 1997;88:225-228
33 Huang RP, Fan Y, De Belle I, Niemeyer C, Gottardis MM, Mercola D, Adamson ED. Decreased Egr-1 expression in human, mouse and rat mammary cells and tissues correlates with tumor formation. *Int J Cancer*, 1997;72:102-109

34 Yan SF, Lu JS, Zou YS, Soh-Won J, Cohen DM, Buttrick PM, Cooper DR, Steinberg SF, Mackman N, Pinsky DJ, Stern DM. Hypoxia-associated induction of early growth Response-1 gene expression. *J Biol Chem*, 1999;274:15030-15040
35 Bae SK, Bae MH, Ahn MY, Son MJ, Lee YM, Bae MK, Lee OH, Park BC, Kim KW. Egr-1 mediates transcriptional activation of IGF-II gene in response to hypoxia. *Cancer Res*, 1999;59:5989-5994
36 Mechtcheriakova D, Wlachos A, Holzmüller H, Binder BR, Hofer E. Vascular endothelial cell growth factor-induced tissue factor expression in endothelial cells is mediated by EGR-1. *Blood*, 1999;93:3811-3823
37 Jansen E, Ayoubi TAY, Meulemans SMP, Van De Ven WJM. Regulation of human prohormone convertase 2 promoter activity by the transcription factor EGR-1. *Biochem J*, 1997;328:69-74
38 Decker EL, Skerka C, Zipfel PF. The early growth response protein (EGR-1) regulates interleukin-2 transcription by synergistic interaction with the nuclear factor of activated T cells. *J Biol Chem*, 1998;273:26923-26930
39 Minc E, de Coppet P, Masson P, Thiery L, Dutertre S, Amor Gueret M, Jaulin C. The human copper-zinc superoxide dismutase gene (SOD1) proximal promoter is regulated by Sp1, Egr-1, and WT1 via Non-canonical binding sites. *J Biol Chem*, 1999;274:503-509
40 Cui MZ, Penn MS, Chisolm GM. Native and oxidized low density lipoprotein induction of tissue factor gene expression in smooth muscle cells is mediated by both Egr-1 and Sp1. *J Biol Chem*, 1999;274:32795-32802
41 Barroso I, Santisteban P. Insulin-induced early growth response gene (Egr-1) mediates a short term repression of rat malic enzyme gene transcription. *J Biol Chem*, 1999;274:17997-18004
42 De Belle I, Huang RP, Fan Y, Liu CT, Mercola D, Adamson ED. P53 and Egr-1 additively suppress transformed growth in HT1080 cells but Egr-1 counteracts P53 dependent apoptosis. *Oncogene*, 1999;17:3633-3642
43 Riva A, Wilson GL, Kehrl JH. *In vivo* footprinting and mutational analysis of the proximal CD19 promoter reveal important roles for an SP1/Egr-1 binding site and a novel site termed the PyG box. *J Immunol*, 1997;159:1284-1292
44 Peng Y, Du K, Ramirez S, Diamond RH, Taub R. Mitogenic Up-regulation of the PRL-1 Protein-tyrosine Phosphatase Gene by Egr-1. *J Biol Chem*, 1999;274:4513-4520
45 Dorn C, Ou Q, Svaren J, Crawford PA, Sadovsky Y. Activation of luteinizing hormone β gene by gonadotropin-releasing hormone requires the synergy of early growth response-1 and steroidogenic factor-1. *J Biol Chem*, 1999;274:13870-13876
46 Thottassery JV, Sun D, Zambetti GP, Troutman A, Sukhatme VP, Schuetz EG, Schuetz JD. Sp1 and Egr-1 have opposing effects on the regulation of the rat pgp2/mdr1b gene. *J Biol Chem*, 1999;274:3199-3206
47 Liu CT, Yao J, De Belle I, Huang RP, Adamson E, Mercola D. The transcription factor Egr-1 suppresses transformation of human fibrosarcoma HT1080 cells by coordinated induction of transforming growth factor-β1, fibronectin and plasminogen activator Inhibitor-1. *J Biol Chem*, 1999;274:4400-4411
48 Akagi M, Yasui W, Akama Y, Yokozaki H, Tahara H, Haruma K, Kajiyama G, Tahara E. Inhibition of cell growth by Transforming growth factor β1 is associated with p53 independent induction of p21 in gastric carcinoma cells. *Jpn J Cancer Res*, 1996;87:377-384
49 Huang RP, Fan Y, Peng A, Zeng ZL, Reed JC, Adamson ED, Boynton AL. Suppression of human fibrosarcoma cell growth by transcription factor, Egr-1, involves downregulation of Bcl-2. *Int J Cancer*, 1998;77:880-886
50 Nair P, Muthukkumar S, Sells SF, Han SS, Sukhatme VP, Rangnekar VM. Early growth response-1 dependent Apoptosis is mediated by p53. *J Biol Chem*, 1997;272:20131-20138
51 Ahmed MM, Sells SF, Venkatasubbarao K, Fruitwala SM, Muthukkumar S, Harp C, Mohiuddin M, Rangnekar VM. Ionizing radiation-inducible apoptosis in the absence of P53 linked to transcription factor Egr-1. *J Biol Chem*, 1997;272:33056-33061
52 Yan YX, Nakagawa H, Lee MH, Rustgi AK. Transforming growth factor-α Enhances cyclin D1 transcription through the binding of early growth response protein to a cis-regulatory element in the cyclin D1 promoter. *J Biol Chem*, 1997;272:33181-33190

Edited by Wu XN