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• ESOPHAGEAL CANCER •

Transcription factor EGR-1 inhibits growth of hepatocellular carcinoma and esophageal carcinoma cell lines

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Abstract

AIM: **The transcription factor EGR-1 (early growth response gene-1) plays an important role in cell growth, differentiation and development. It has identified that EGR-1 has significant transformation suppression activity in some neoplasms, such as fibrosarcoma, breast carcinoma. This experiment was designed to investigate the role of *egr-1* in the cancerous process of hepatocellular carcinoma (HCC) and esophageal carcinoma (EC), and then to appraise the effects of EGR-1 on the growth of these tumor cells.**

METHODS: **Firstly, the transcription and expression of *egr-1* in HCC and EC, paracancerous tissues and their normal counterpart parts were detected by *in situ* hybridization and immunohistochemistry, with normal human breast and mouse brain tissues as positive controls. *egr-1* gene was then transfected into HCC (HHCC, SMMC7721) and EC (ECa109) cell lines in which no *egr-1* transcription and expression were present. The cell growth speed, FCM cell cycle, plate clone formation and tumorigenicity in nude mice were observed and the controls were the cell lines transfected with vector only.**

RESULTS: **Little or no *egr-1* transcription and expression were detected in HCC, EC and normal liver tissues. The expression of *egr-1* were found higher in hepatocellular paracancerous tissue (transcription level *P*=0.000; expression level *P*=0.143, probably because fewer in number of cases) and dysplastic tissue of esophageal cancer (transcription level *P*=0.000; expression level *P*=0.001). The growth rate of *egr-1*-transfected HHCC (HCC cell line) cells and ECa109 (EC cell line) cells was much slower than that of the controls. The proportion of S phase cell, clone formation and tumorigenicity were significantly lower than these of the controls' (decreased 45.5% in HHCC cells and 34.1% in ECa109 cells; 46.6% and 41.8%; 80.4% and 72.6% respectively). There were no obvious differences between SMMC7721 (HCC) *egr-1*-transfected cells and the controls with regard to the above items.**

CONCLUSION: **The decreased expression of *egr-1* might play a role in the dysregulation of normal growth in the cancerous process of HCC and EC. *egr-1* gene of transfected HHCC and ECa109 cells showed obvious suppression of the cell growth and malignant phenotypes, but no suppression in SMMC7721 (HCC cell line) cells.**

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INTRODUCTION

The tumor suppressorgene therapy research has become increasingly interested. Tumor suppressor genes have two categories: Type 1, they undergo mutation or deletion, such as *p*53, Rb, WT1 and BRCA1 etc. Type 2, they express little or nil and with rare mutation or deletion, such as cx34, maspin and integrin α6 etc[1]. In designing the tumor gene therapy, the type IItumor suppressor genes are evidently more advantageous. We only need to induce or to promote more expression of the genes to get rid of many technical difficulties such as replacing the deleted gene or knocking out the mutated gene.

The early growth response gene, *egr-1* (Zfp-6 in standardized genetic Nomenclature for mice[2], also known as NGFI-A, Krox-24, Zif-268, or TIS-8) is one of the important members of the immediate early gene family. It encodes a protein EGR-1 with 3 adjacent zinc-finger motifs, and structures that are present in many DNA-binding transcription factors. Its special zinc-finger structure can bind with "GC-rich" DNA regulatory elements and can control that particular gene to transcribe[3]. The expression of *egr-1* is closely related to cell growth[4] and differentiation[5]. Some researching results have revealed that EGR-1 can reverse the malignant phenotype of HT-1080 fibrosarcoma and ZR-75-1 mammary carcinoma cell lines, and can also EGR-1 inhibit the tumorigenicity and transforming growth of these cells[6-8]. *egr-1* may act as a type II tumor suppressor gene.

Both hepatocellular carcinoma (HCC) and esophageal carcinoma (EC) are common malignant tumors in China. Many research works on HCC and EC[9-24], especially on the effects of the tumor suppressor genes such as *p*53, *p*16 and *p*21 etc[25-38] had been reported, but the reports about *egr-1* for these two cancers are still lacking. In this paper, the transcription and expression of *egr-1* in HCC,EC tissues and their normal counterparts were detected by *in situ* hybridization and immunohistochemistry in order to investigate the role of *egr-1* in the cancerous process of HCC and EC. The constructed *egr-1* eukaryo-expressing vector was transfected into HCC and EC lines, where there was absence of expression of *egr-1*, with a view to observe how the high level of exogenous expression of EGR-1 in the cancer cell influenced their growth, clone formation and tumorigenicity. The inhibitory action of *egr-1* on HCC and EC is to be probed so as to provide an experimental evidence for the study of gene therapy of the two cancers.

MATERIALS AND METHODS

Materials

The plasmids, pAC-h-*egr-1* (fragment of single and double enzyme digestion by BamH I and/or SalI was determined by $10g \cdot L^{-1}$ agarose gel electrophoresis. The size of the fragments was identical to that of

plasmid map), containing the whole cDNA fragment of human wild-type *egr-1* gene and pAC-Φ eukaryo-expressing vectors, were kindly granted by Dr. JG Monore, Pennsylvania University School of Medicine[39].

Human hepatocellular, esophageal carcinomas and breast tissue specimens were collected from the Pathology Department of Fourth Military Medical University and Medical College of Shantou University. All patients providing the specimens were not treated by radiotherapy or chemotherapy before operation. The specimens were fixed with 40g·L^{-1} formaldehyde solution (for*in situ*hybridization) and Carnoy solution (for immuno-histochemistry). Mouse brain tissues were from Balb/c mice.

The cell lines HHCC and SMMC7721 of HCC were purchased from Shanghai Cytology Institute, China. The EC cell line ECa109 was from Cytology Institute of Chinese Medical Academy. All the cell lines were cultured in RPMI 1640 medium supplemented with 100mL·L^{-1} new born bovine serum and grown in the circumstance of 37℃, 50mL·L^{-1} CO_2 and saturated humidity. Cell numbers were determined by Coulter counting method.

The Lipofectamine™, G418 and TRIzol™ total RNA extraction kit were purchased from Gibco/BRL Co. The Wizard™ plus Minipreps DNA purification kit was the product of Promega Co. The polyclonal rabbit anti-human EGR-1 (C$^{\#}$-19) antibody was from Santa Cruz Co.*in situ*hybridization (POD) detection kit and SABC immunohistochemistry kit were from Boster Co, Wuhan,China. The Advantage™ PCR purification kit was from Clontech Co, and γ-^{32}P-dATP was provided by Yahui Biomedical Engineering Co, Beijing.

Methods

Probe preparation and labeling

The plasmid pAC-h-*egr-1* was digested by both *Sal* I and *Bam*H I, and the products were determined by agarose gel electrophoresis; the 400 bp DNA fragments were recovered by promega DNA purification kit. The recovered DNA was dissolved in 20ul sterile three-distilled water and ultraviolet spectrophotometry was taken for quantitation analysis. γ-^{32}P-dATP 5'end Label method was used for probe labeling. After alcohol precipitation, the probe was dissolved in TE; the specific radiation activity was determined by TAC method, and stored at -20℃.

In situ hybridization and Immunohistochemistry

In situ hybridization was performed according to the kit instructions on the formaldehyde-fixed and paraffin-embedded sections. Human normal breast and mouse brain tissue sections were used as positive controls. According to the SABC stain instructions, immunohistochemistry was done on the Carnoy-fixed or frozen section. Positive control was frozen section of human normal mammary adenosis, and negative control was primary antibody-blank.

Gene transfection and identification after transfection

Transfection was performed according to the legend of Lipofectamine™ kit, and 48h later selective culture was added with 600mg·L^{-1} G418.2 weeks later, the cells and clones appeared all (the cells of blank control died). The concentration of G418 was changed to 400mg·L^{-1} G418 for maintenance. The clones were digested *in situ*, and transferred to a 6-well plate for amplification, which were used for detection of every item.

mRNA dot hybridization: 2×10^6 cells were collected and total RNA was extracted according to TRIzol™ total RNA extraction kit. The extracted RNA was dissolved in the RNase-free water. A absorbance value was determined, RNA quantity was calculated and purified was identified; stored at -80℃; procedures of RNA dot blot was according to the directions.

Western blot hybridization and immunohistochemistry: Western blot hybridization was performed according to the directions. The "cell crawling slide" in logarithm stage was collected, rinsed with PBS×2, fixed in 700mL·L^{-1} alcohol and stained by SABC kit. The positive control was frozen section of mammary adenosis, and the negative control was primary antibody-blank.

Assay of cell phenotype

FCM cell cycle examination: The transfected cells were digested by 2.5g·L^{-1} trypsin to a single cell suspension when they were 80% confluent, counted, 1×10^6 cells were fixed in 700mL·L^{-1} alcohol and FCM was performed.

Cell growth curve: cells of transfected and blank vector groups were digested by trypsin and counted in 1×10^3 cells. Each group was inoculated in 24-well plate, and 1ml RPMI 1640 medium containing 100mL·L^{-1} FCS was added. In the culture period, cells of 3 wells were digested and counted every day, and the mean values were also calculated. Of the remainder, the medium was changed every 3-4d and counted every 6-7d according to the cell growth conditions, growth curves were then made.

Examination of plate cloning: HCC and EC lines, which had been transfected with *egr-1* gene, G418 selected and amplified were digested to a single cell suspension. Cells counted were inoculated into 6-well tissue culture plates; each well contained 100 cells. The control cells were transfected with blank vector. Changing the medium 1～2 times per wk; by about 2 wk plates were taken out to observe the clone formation; fixed in methyl alcohol, Giemsa stained, clones over 50 cells were counted, and the mean value was adopted and clone formation rates were made.

Nude mice tumorigenicity: After trypsin digestion, the cells of the experimental and control groups were inoculated into nude mice subcutaneously with 1×10^7 cell (about 0.1-0.2mL) for each mouse. About 3wk later the mice were killed according to the tumor growth, and then pictures were taken. The tumor mass was separated carefully and weighed, and the daily growing mass was calculated then. Tumor tissues were fixed, paraffin embedded, HE stained and observed microscopically.

Statistical analysis

Results were expressed as mean±SE. To compare the mean values, Stata statistical software was used. $P<0.05$ was considered significant.

RESULTS

Egr-1 mRNA and protein expression in HCC and EC

In situ hybridization and SABC immunohistochemistry staining for *egr-1* mRNA and protein in human HCC, EC tissues and their normal counterparts were performed. Little or no *egr-1* transcription was detected in HCC and normal liver tissues. Higher transcription of *egr-1* was detected in hepatocellular paracancerous tissues such as large cell hyperplasia (LCD) and "atrophic-like liver plate (reluctant LCD)". *egr-1* mRNA signal was present in the cytoplasm. EGR-1 expression in HCC, normal liver and hepatocellullar paracancerous tissues was consistent with the transcription (When EGR-1expression in HCC compared with the expression in the paracancerous tissue, P=0.143. Table 1, Figure 1). EGR-1 protein signal was in the nucleus. Little or no *egr-1* transcription was detected in the aggressive EC and normal esophageal epithelial tissues (not including basal layer cells). Higher transcription of *egr-1* was detected in dysplastic esophageal epithelium. EGR-1 expression in aggressive EC, normal esophageal epithelium and dysplastic tissues was also consistent with the transcription (Table 2, Figure 2).

Table 1 *egr-1* transcription and expression in HCC tissue and its normal counterparts

Tissue	*n*	mRNA (%)	*n*	Protein (%)
Normal liver	8	0(0.0)	2	0(0.0)
Hepatic paracancerous tissue	22	21(95.5)	5	4(80.0)
Liver carcinoma	24	6(25.0)[a]	3	0(0.0)[b]

[a]*P*=0.000, [b]*P*=0.143, *vs* hepatic paracancerous tissue

Table 2 *egr-1* transcription and expression in EC tissue and its normal counterparts

Tissue	*n*	mRNA(%)	Protein (%)
Agressive carcinoma	47	8(17.0)[a]	6(12.8)[b]
Dysplasia	29	18 (62.1)	14 (48.3)
Normal epithelium	28	9(32.1)	2(7.1)

[a]*P*=0.000, [b]*P*=0.001, *vs* dysplasia

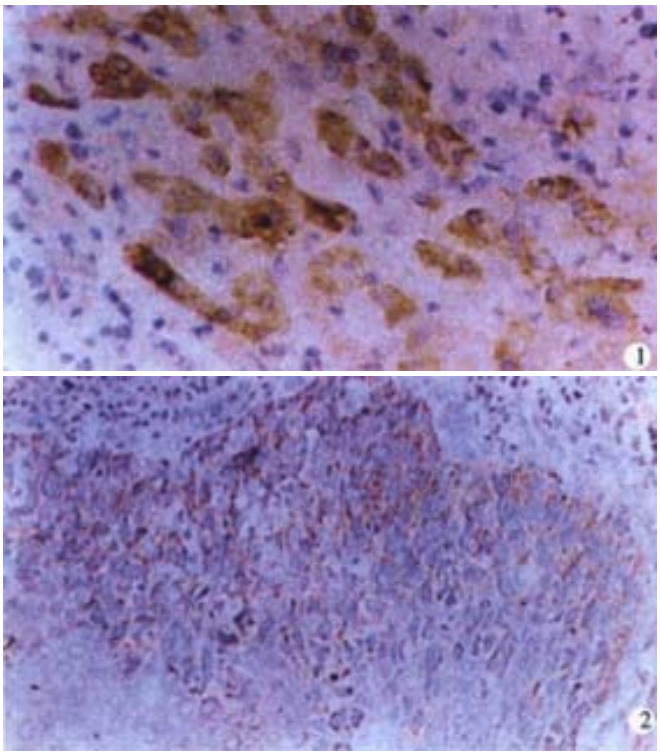

Figure 1 Positive signal of *egr-1* mRNA in the cytoplasm paracancerous "atrophic-like liver plate" tissue of HCC. SP×400
Figure 2 Positive signal of EGR-1 mRNA in the cytoplasm of moderately dysplastic esophageal epithelium. SP×400

Egr-1 mRNA and protein detection in transfected tumor cells

mRNA level (mRNA dot blot): The total RNA of *egr-1* and vector transfected HCC and EC cells were dot hybridized by ^{32}P labeled egr-1 probe. The result revealed that *egr-1* group had stronger signals than that of the control.

Western blot and immunohistochemistry: The proteins of *egr-1* and vector transfected HCC and EC cells were extracted and quantitatively analyzed. Then both groups with identical quantitative proteins were determined by SDS-PAGE; electrical transfer onto the nitro-cellulose membrane was made, and Western blot hybridization was performed. The results revealed that in *egr-1* transfected groups of both HCC and EC cells, there were strongly stained bands just at the molecular mass of 59ku, which was identical to that of EGR-1, whereas in all the controls there were no such bands present. This suggested that the tumor cells transfected by *egr-1* could strongly express EGR-1 protein. The results of immunohistochemistry by primary antibody EGR-1 and SABC method showed 50-70% positive staining of *egr-1* transfected cells were, while in the controls the results were all negative; which was consistent with that of the Western blot.

Malignant phenotype examination

Curves of cell growth

The growth rates of the *egr-1* transfected HHCC liver carcinoma cells and EC109 esophageal carcinoma cells were obviously slower than that of the controls. But the difference was not significant in the SMMC7721 liver carcinoma cell line (Figure 3).

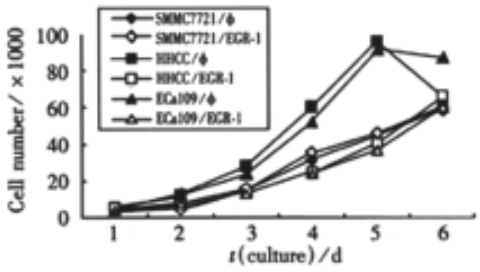

Figure 3 Cell growth curve of liver and esophageal carcinoma cells transfected *egr-1* and vector

FCM cell cycle assay

In *egr-1* transfected HHCC, the number of fraction of S phase accounted for 0.15, lower than that of the controls (0.28, *P*=0.038); i. e., the proportion of S phase was 0.46 lower than that of the control (Table 3). In ECa109, the number of fraction of S phase accounted for 0.18 (control 0.27, *P*=0.048), and was 0.34 lower than that of the control. In SMMC7721 (HCC), there was no marked difference between the experimental and the control groups (0.21 to 0.22, *P*=1.000). As for the G_1 and G_2/M phases, there were neither significant differences between experiment groups and control groups.

Table 3 Cell cycle of HCC and EC cells expressing EGR-1 with FCM (number of fraction)

Cell clone	G_1	G_2/M	S
HHCC/EGR-1	0.77	0.08	0.15[a]
HHCC/φ	0.64	0.08	0.28
SMMC7721/EGR-1	0.70	0.09	0.21[b]
SMMC7721/φ	0.70	0.09	0.22
ECa109/EGR-1	0.73	0.11	0.18[c]
ECa109/φ	0.64	0.10	0.27

[a]*P*=0.038, [b]*P*=1.000, [c]*P*=0.048, *vs* their controls

Plate cloning formation

In HHCC cell line, the clone forming rate of transfected cells was 49.0±10.6%, 42.7% lower than that of control 91.7±6.1%. In ECa109 line, the clone forming rate of transfected cells was 45.0±5.3%, 32.3% lower than that of control 77.3±3.1%. In SMMC7721, There were no statistical difference between the experimental group 26.0±2.6% and the control group 23.3±2.1% (Figure 4).

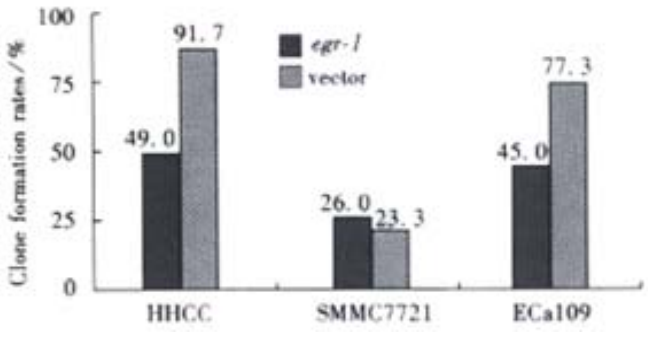

Figure 4 Clone formation rates of exogenous high EGR-1 expression HCC and EC

Nude mice tumorigenicity

All the inoculated nude mice had tumor mass growth except in one case (one of the three SMMC7721/φ inoculated mice). In HHCC cell line, the tumor growth rate of transfected group was 80.7%, lower than that of the control (P=0.001). In ECa109 cell, the growth rate of transfected group was 72.6%, also lower than that of the control (P=0.001). In SMMC7721, there was no growth rate difference between the experimental and the control group(P=0.681)(Table 4).

Table 4 Nude mice tumorigenicity of gene transfected groups in HCC and EC cells

Cell clone	n	Tumorigenicity rate	Tumor growth rate ($x\pm s$, mg·d^{-1})
HHCC/EGR-1	3	3/3	13.9±5.2[a]
HHCC/φ	3	3/3	70.9±7.8
SMMC7721/EGR-1	3	3/3	18.7±4.3[b]
SMMC7721/φ	3	2/3	15.2±13.0
ECa109/EGR-1	4	4/4	12.9±2.1[c]
ECa109/φ	4	4/4	47.1±11.3

[a]P=0.001, [b]P=0.681, P=0.001, *vs* their controls

DISCUSSION

Althrough *egr-1* is a member of immediate early genes, its expression is also elevated during the process of growth[4] and differentiation[5]. Observations suggest that EGR-1 have pleiotropic roles such as growth, development, differentiation etc. This is Supported by the finding that the GC-rich DNA-binding element for *egr-1* (GCE) is present in a large number of gene regulating regions, including growth factors, signal transduction genes, other transcription factors and oncogenes. In this report, we showed that *egr-1* transcription and expression decreased obviously in HCC and EC tissues compared with their paracancerous tissues. Another (large) study also indicated that human small cell lung tumors express little or no *egr-1* mRNA compared with adjacent normal tissues[40], further supporting our findings. Except that the *egr-1* gene was deleted only in all 5q$^-$ syndrome cases[41], *egr-1* expression was profoundly decreased in a number of other human tumor cell lines such fibroblastoma, glioblastoma, osteosarcoma and lung cancer[6].Therefore it seems likely that the inactivation of *egr-1* expression is a generalized phenomenon in the developmental process of a number of tumors.

Egr-1 is ubiquitously expressed at low levels but accumulates to relatively high levels in only a few adult organs as brain, heart, lung, kidney[42] and breast[1]. We showed here that normal liver and esophageal epithelial tissues expressed undetectable level of *egr-1*, but the *egr-1* gene was activated in the tissues of hepatic paracancerous tissue such as LCD and "atrophic liver plate" (reluctant LCD) and dysplastic tissue of esophageal carcinoma. During regeneration of normal liver tissue, increased *egr-1* expression is induced[43]. In mouse fibroblast NIH3T3 cells, *egr-1* expression increases two-fold 10min after UV irradiation, which rises to a maximum (eightfold induction) after 2h[6]. Human HT1080 fibrosarcoma subclone, cells H4, express little or no *egr-1*. Phorbol ester treatment only can elicit a small increase in *egr-1* expression in H4, in contrast to the normally rapid, high transient expression of *egr-1* observed after the addition of a wide range of stimulating agents to normal or immortalized cell lines[7]. Therefore, the meaning of little or no *egr-1* expression in liver and esophageal carcinomas is different to their counterparts. *egr-1* gene in the hepatic paracancerous tissue such as LCD and dysplasia of esophageal carcinoma can be activated when they are stimulated, but the stimulations is invalidated in carcinomas. The decreased expression of *egr-1* may play a role in the dysregulation of normal growth in the cancerous process of HCC and EC.

To confirm the development of liver and esophageal carcinomas involves the inactivation of *egr-1*. It would deserve further investigation of the effects of exogenous EGR-1 on the growth and malignant phenotypes of the cancer. Like tumor suppressor gene WT1, EGR-1 can also bind with GCE elements[44], this suggests that EGR-1 might have inhibitory activity on cancer growth. In the experimental report[8], EGR-1 could reverse the malignant phenotype of *v-sis* transformed NIH3T3 cells. The results of this paper also showd that stably expressed EGR-1 could inhibit the cancer's growth rate, reduce the number of fraction of S phase, decrease the plate clone formation rate and reduce the tumorigencity of HHCC and ECa109 cell lines. In recent years, studies have revealed that in human tumors, EGR-1 expression suppressing tumor cell growth is a common phenomenon. In human HT-1080 fibrosarcoma cells, the growth rates in 6 groups of expressing different EGR-1 level are inhibited proportionately and are dose-dependent. The total inhibition rate of these cells' tumorigencity is over 50%. The growth of human ZR-75-1 breast carcinoma, U251 glioblastoma and SAOS-2 osteosarcoma cells has also been demonstrated to be suppressed by EGR-1 in different degree[6]. However, in this experiment EGR-1 did not suppress the malignant phenotypes of SMMC7721, HCC cell line, this might be due to different transformation mechanism in this cell line, which needs investigation further.

The mechanism of EGR-1 inhibition on cancer growth is complicated, which interacts with TGF-β1, PDGF and WT1. TGF-β1 specifically inhibits PDGFβ-dependent cells' growth[4], while EGR-1 inhibits the malignant phenotype and growth of the PDGFβ/v-sis that were transformed from NIH3T3 cells. This suggests that EGR-1 is closely related with TGF-β1 and PDGF. Meanwhile, EGR-1 can also activate the transcription of TGF-β1 in a dose-dependent manner, this activation might be one of the potential mechanisms of EGR-1 of inhibiting tumor growth[45]. Interestingly, WT1 can bind with the GC element of TGF-β1 promoter, and then inhibit the activity of that promoter (but this reaction is weaker than that of inhibition on PDGFβ). One recent study has shown that the inhibitory reaction of EGR-1 on cancer growth is also by inducing the expression of TGF-β1, fibronectin, p21 and focal adhesion kinase (FAK) in human fibrosarcoma cell line[46]. EGR-1 may also regulate cell interaction with the extracellular matrix by synergistic induction of TGF-beta1, FN, and PAI-1 in human HT-1080 glioblastoma cells[47] and directly transactivates the fibronectin gene and enhances attachment of human glioblastoma cell line U251[48]. Other reports show that the suppression by *egr-1* also involves down-regulation of bcl-2[49] and stimulates apoptosis by transactivation of the p53 gene[50].

Recent studies[6, 8, 50] as well as this experiment suggest that EGR-1 can act as a tumor suppressor factor. EGR-1 has been identified to be little or no expression in several human malignant tumors such as fibroblastoma, glioblastoma, osteosarcoma, lung cancer, breast carcinoma (including cancer cells and tumor tissues)[1,6], as well as hepatocellular carcinoma and esophageal carcinoma in this experiment. Another (larger) study also indicates that human small cell lung tumors express little or no *egr-1* mRNA compared with adjacent normal tissues[40]. Furthermore, 5q-syndrome is the exclusive tumor with *egr-1* gene deletion[41]. Hence we suspect that *egr-1* might be belonged to typeII tumor suppressor genes as mentioned above. The reintroduction of *egr-1* gene products by drugs might be a promising approach to normalize growth regulation, which can avoid the difficult problem of replacing defective gene DNA.

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REFERENCES

1 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

2 Darland T, Samuels M, Edwards SA, Sukhatme VP, Adamson ED. Regulation of Egr-1 (Zfp-6) and c-fos expression in differentiating embryonal carcinoma cells. *Oncogene* 1991;6:1367-1376

3 Gashler AL, Swaminathan S, Sukhatme VP. A novel repression module, an extensive activation domain, and a bipartite nuclear localization signal defined in the immediate-early transcription factor *egr-1*. *Mol Cell Biol*

1993;13:4556-4571
4 Liu C, Calogero A, Ragona G, Adamson E, Mercola D. EGR-1, the reluctant suppression factor: EGR-1 is known to function in the regulation of growth, differentiation, and also has significant tumor suppressor activity and a mechanism involving the induction of TGF-beta1 is postulated to account for this suppressor activity. *Crit Rev Oncog* 1996;7:101-125
5 Nguyen HQ, Hoffman Liebermann B, Liebermann DA. The zinc finger transcription factor *egr-1* is essential for and restricts differentiation along the macrophage lineage. *Cell* 1993;7:197-209
6 Huang RP, Liu C, Fan Y, Mercola D, Adamson ED. *egr-1* negatively regulates human tumor cell growth via the DNA-binding domain. *Cancer Res* 1995;55:5054-5062
7 Liu C, Adamson E, Mercola D. Transcription factor EGR-1 suppresses the growth and transformation of human HT-1080 fibrosarcoma cells by induction of transforming growth factor beta1. *Proc Natl Acad Sci USA* 1996;93:11831-11836
8 Huang RP, Darland T, Okamura D, Mercola D, Adamson ED. Suppression of v-sis-dependent transformation by the transcription factor, *egr-1*. *Oncogene* 1994;9:1367-1377
9 Tang ZY. Schmid. Advances in clinical research of hepatocellular carcinoma in China. *World J Gastroenterol* 1998;4(Suppl 2):4-7
10 Wu XY, Zhang XF, Yin FS, Lu HS, Guan GX. Clinical study on surgical treatment of esophageal carcinoma in patients after subtotal gastrectomy. *World J Gastroenterol* 1998;4(Suppl 2):68-69
11 Bai YM, Wang LD, Seril DN, Liao J, Yang GY, Yang CS. Expression of hMSH2 in human esophageal cancer from patients in a high incidence area in Henan, China. *World J Gastroenterol* 1998; 4(Suppl 2): 107
12 Qi YJ, Wang LD, Nie Y, Cai C, Yang GY, Xing EP, Yang CS. Alteration of p19 mRNA expression in esophageal cancer tissue from patients at high incidence area in northern China. *World J Gastroenterol* 1998; 4(Suppl 2): 108-109
13 Qian QJ, Xue HB, Qu ZQ, Fang SG, Cao HF, Wu MC.*in situ*detection of tumor infiltrating lymphocytes expressing perforin and fas ligand genes in human HCC. *World J Gastroenterol* 1999; 5: 12-14
14 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
15 Liang YR, Zheng SY, Shen YQ, Wu XY, Huang ZZ. Relationship between expression of apoptosis related antigens in hepatocellular carcinoma and in situ end labeling. *World J Gastroenterol* 1998; 4: 99
16 Xiao L, Zhou HY, Luo ZC, Liu J. Telomeric associations of chromosomes in patients with esophageal squamous cell carcinomas. *World J Gastroenterol* 1998; 4: 231-233
17 Huang B, Wu ZB, Ruan YB. Expression of nm23 gene in hepatocellular carcinoma tissue and its relation with metastasis. *World J Gastroenterol* 1998; 4: 266-267
18 Sun HC, Li XM, Xue Q, Chen J, Gao DM, Tang ZY. Study of angiogenesis induced by metastatic and non metastatic liver cancer by corneal micropocket model in nude mice. *World J Gastroenterol* 1999; 5: 116-118
19 Luo YQ, Wu MC, Cong WM. Gene expression of hepatocyte growth factor and its receptor in HCC and nontumorous liver tissues. *World J Gastroenterol* 1999; 5: 119-121
20 Liu CG, Zhu MC, Chen ZN. Preparation and purification of F(ab') 2 fragment from anti hepatoma mouse IgG1 mAb. *World J Gastroenterol* 1999; 5: 522-524
21 Ma XD, Sui YF, Wang WL. Expression of gap junction genes connexin 32, connexin 43 and their proteins in hepatocellular carcinoma and normal liver tissues. *World J Gastroenterol* 2000; 6: 66-69
22 Tang YC, Li Y, Qian GX. Reduction of tumorigenicity of SMMC-7721 hepatoma cells by vascular endothelial growth factor antisense gene therapy. *World J Gastroenterol* 2001; 7: 22-27
23 Feng DY,Zheng H,Tan Y,Cheng RX.Effects of phosphorylation of MARK and Stat3 and expression of c-fos and c-jun proteins on hepatocarcinogenesis and their clinical significance. *World J Gastroenterol* 2001; 7: 33-36
24 Fan ZR, Yang DH, Cui J, Qin HR, Huang CC. Expression of insulin-like growth factor II receptor in hepatocellular carcinogenesis. *World J Gastroenterol* 2001; 7: 285-288
25 Yang SM, Zhou H, Chen RC, Wang YF, Chen F, Zhang CG, Zhen Y, Yan JH, Su JH. Sequencing of *P*53 mutation in established human hepatocellular carcinoma cell line of HHC4 and HHC15 in nude mice. *World J Gastroenterol* 1998; 4: 506-510
26 Luo F, Kan B, Lei S, Yan LN, Mao YQ, Zou LQ, Yang YX, Wei YQ. Study on P53 protein and C erbB2 protein expression in primary hepatic cancer and colorectal cancer by flow cytometry. *World J Gastroenterol* 1998; 4(Suppl 2): 87
27 Zhou Q, Wang LD, Gao SS, Li YX, Zhao X, Wang LX. P53 immunostaining positive cells correlated positively with S phase cells as measured by BrdU in the esophageal precancerous lesions from the subjects at high incidence area for esophageal cancer in northern China. *World J Gastroenterol* 1998; 4 (Suppl 2): 106
28 Feng DY, Chen RX, Peng Y, Zheng H, Yan YH. Effect of HCV NS3 protein on P53 protein expression in hepatocarcinogenesis. *World J Gastroenterol* 1999; 5: 45-46
29 Casson AG. Molecular biology of Barrett's esophagus and esophageal cancer: role of *P*53. *World J Gastroenterol* 1998;4:277-279
30 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, *P*53, 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
31 Yu GQ, Zhou Q, Ding I, Gao SS, Zheng ZY, Zou JX, Li YX, Wang LD. Changes of *P*53 protein blood level in esophageal cancer patients and normal subjects from a high incidence area in Henan, China. *World J Gastroenterol* 1998; 4: 365-366
32 Deng ZL, Ma Y. Aflatoxin sufferer and *P*53 gene mutation in hepatocellular carcinoma. *World J Gastroenterol* 1998; 4: 28-29
33 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
34 Luo D, Liu QF, Gove C, Naomov NV, Su JJ, Williams R. Analysis of N ras gene mutation and *P*53 gene expression in human hepatocellular carcinomas. *World J Gastroenterol* 1998; 4: 97-98
35 Peng XM, Peng WW, Yao JL. Codon 249 mutations of *P*53 gene in development of hepatocellular carcinoma. *World J Gastroenterol* 1998; 4:125-127
36 Jiao LH,Wang LD,Xing EP,Yang G,Yu Y,Yang CS.Frequent inactivation of *P*16 and *P*15 expression in human esophageal squamous cell carcinoma detected by RT-PCR. *World J Gastroenterol* 1998;4(Suppl 2):105
37 Liu LH, Xiao WH, Liu WW. Effects of 5-Aza-2'-deoxycytidine on the *P*16 tumor suppressor gene in hepatocellular carcinoma cell line HepG2. *World J Gastroenterol* 2001; 7: 131-135
38 Qin LL, Su JJ, Li Y, Yang C, Ban KC, Yian RQ. Expression of IGFII, p53, *P*21 and HBxAg in precancerous events of hepatocarcinogenesis induced by AFB1 and/or HBV in tree shrews. *World J Gastroenterol* 2000; 6: 138-139
39 Maltzman JS, Carman JA, Monroe JG. Role of EGR1 in regulation of stimulus-dependent CD44 transcription in B lymphocytes. *Mol Cell Biol* 1996; 16: 2283-2294
40 Levin WJ, Press MF, Gaynor RB, Sukhatme VP, Boone TC, Reissmann PT, Figlin RA, Holmes EC, Souza LM, Slamon DJ. Expression patterns of immediate early transcription factors in human non-small cell lung cancer. The Lung Cancer Study Group. *Oncogene* 1995; 11: 1261-1269
41 Le Beau MM, Espinosa R, Neuman WL, Stock W, Roulston D, Larson RA, Keinanen M, Westbrook CA. Cytogenetic and molecular delineation of the smallest commonly deleted region of chromosome 5 in malignant myeloid diseases. *Proc Natl Acad Sci USA* 1993; 90: 5484-5488
42 Herms J, Zurmohle U, Schlingensiepen R, Brysch W, Schlingensiepen KH. Developmental expression of the transcription factor zif268 in rat brain. *Neurosci Lett* 1994; 165: 171-174
43 Columbano A, Shinozuka H. Liver regeneration versus direct hyperplasia. *FASEB J* 1996; 10: 1118-1128
44 Rauscher FJ 3d, Morris JF,Tournay OE, Cook DM, Curran T. Binding of the Wilms' tumor locus zinc finger protein to the EGR-1 consensus sequence. *Science* 1990; 250: 1259-1262
45 Dey BR,Sukhatme VP,Roberts AB,Sporn MB,Rauscher FJ 3rd,Kim SJ. Repression of the transforming growth factor-beta 1 gene by the Wilms' tumor suppressor WT1 gene product. *Mol Endocrinol* 1994;8:595-602
46 Belle I, Huang RP, Fan Y, Liu C, Mercola D, Adamson ED. *P*53 and *egr-1* additively suppress transformed growth in HT1080 cells but *egr-1* counteracts *P*53-dependent apoptosis. *Oncogene* 1999; 18: 3633-3642
47 Liu C, 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-beta1, fibronectin, and plasminogen activator inhibitor-1. *J Biol Chem* 1999; 274: 4400-4411
48 Liu C, Yao J, Mercola D, Adamson E. The transcription factor EGR-1 directly transactivates the fibronectin gene and enhances attachment of human glioblastoma cell line U251. *J Biol Chem* 2000; 275: 20315-20323
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 down-regulation of Bcl-2. *Int J Cancer* 1998;77:880-886
50 Liu C, Rangnekar VM, Adamson E, Mercola D. Suppression of growth and transformation and induction of apoptosis by EGR-1. *Cancer Gene Ther* 1998; 5: 3-28

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