Relationship between Egr-1 gene expression and apoptosis in esophageal carcinoma and precancerous lesions

Ming-Yao Wu, Ying-Rui Liang, Xian-Ying Wu, Chu-Xiang Zhuang

INTRODUCTION
Esophageal carcinoma is one of the most common malignant tumors in China[1-5]. Its pathogenesis and development are closely related to the expression of some proto-oncogenes and their products and apoptosis of the cancer cells[6-10]. The expression of oncogenes and tumor suppressor genes in esophageal carcinoma has been studied; the relationship between Egr-1 and carcinoma has been reported as well[20]. Our previous studies have indicated that Egr-1 gene inhibited the growth of Eca109 after the exogenous introduction of Egr-1 gene[21]. But the relationship between expression of Egr-1 and cell apoptosis in esophageal carcinoma is not well understood so far. In this paper, we have performed the examination of Egr-1 mRNA and Egr-1 protein expression, apoptosis related-protein Bcl-XL, expression and cell apoptosis of the carcinoma tissue, upper cut edge mucosa and paracancerous lesions from 66 cases esophageal carcinoma using In situ hybridization, immunohistochemistry and terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-digoxigenin nick end labeling method (TUNEL) respectively. The purpose is to understand the correlation of Egr-1 expression and cell apoptosis in esophageal carcinogenesis.

MATERIALS AND METHODS
Sample collecting and processing
Fresh esophagus specimens after operation including esophageal mucosa at the upper cut edge, cancer tissue and mucosa just adjacent to the tumor mass were taken from 66 patients of esophageal carcinomas who had not received accepted chemotherapy or radiotherapy before operation. All specimens were from Department of Pathology Shantou University Medical College, from January to December, 2000. The specimens were fixed in 40 mL/L neutral formalin; the specimens were from Department of Pathology, Shantou University Medical College, 22 Xinling Road, Shantou 515031, Guangdong Province, China. The plasmid was confirmed by amplification, purification and endonuclease cutting, then digoxigenin nick end labeling method (TUNEL) respectively.

Histopathology analysis
Histopathological diagnosis of esophageal para-cancer epithelia was made according to Liu et al's criteria including 39 cases of normal epithelium, 52 cases of simple hyperplasia and 41 cases of dysplasia. 66 cases of esophageal carcinoma were diagnosed using WHO histological tumor classification including 2 cases of carcinoma in situ, 18 cases of grade I squamous cell carcinoma, 33 cases of grade II and 13 cases of grade III. 22 cases had invaded the superficial muscular layer, and others had invaded the serosa. 36 of 66 cases had lymphatic metastasis.

In situ hybridization
Eukaryotic expression vector of PCMV-Egr-1 plasmid was donated by Dr RP Huang (Molecular Medicine, Northwest Hospital, WA, USA). The plasmid was confirmed by amplification, purification and endonuclease cutting, then...
retrieved the DNA. 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. Sections were dewaxed in xylene, then into ethanol, and then into 30 mL/L hydrogen peroxide for 30 min. Proteinase K at 37 °C for 20 min in 20 µg/ml and then fixed in 40 g/L PFA for 10 min in sequence. 90 % ethanol 5 min at -20 °C precooled, digoxigenin-labeled cDNA probe (1:40) were denatured in hybridization buffer at 95-100 °C for 10 min, then -20 °C for 3 min, added on tissues and cover slipped at 42 °C overnight. Sections were washed with SSC and then mouse anti-digoxigenin antibody, biotinylated goat anti-mouse and then streptavidin-biotin complex(SABC) for 30 min, finally, with 3,3’-diaminobenzidine (DAB) visualization. Human breast tissue and the mouse brain tissue were used as the positive control. Incubation solution instead of the probe and sections digested by RNase (10 µg/ml) before Egr-1 detection were designed for the negative control.

**Immunohistochemistry**

Egr-1 and Bcl-xL were analyzed by using Egr-1(588): cat#SC-110 rabbit polyclonal antiserum (1:200,Santa Cruz Biot Co, USA) and Bcl-xL(H-62): sc-7195 polyclonal antibody (ready to use) with the SABC method according to the manufacturer’s instructions (Boster company, China), and finally DAB visualization. The human breast tissue and the esophageal carcinoma tissue were used as the positive control. Negative control was designed by using PBS instead of Egr-1 antiserum or instead of Bcl-xL polyclonal antibody.

**Detection of cell apoptosis**

Apoptosis was detected by the TdT-mediated dUTP nick end labeling (TUNEL) method using a detection kit from Boster (H-62): sc-7195 polyclonal antibody (ready to use) with the SABC method according to the manufacturer’s instructions (Boster company, China), and finally DAB visualization. The human breast tissue and the esophageal carcinoma tissue were used as the positive control. Negative control was designed by using PBS instead of Egr-1 antiserum or instead of Bcl-xL polyclonal antibody.

**Judgement of the results**

The Egr-1 mRNA positive expression showed brown stained signal in the cytoplasm; the Egr-1 protein positive signal showed brown stained signal in the nucleus. Either Egr-1mRNA positive or Egr-1 protein positive was considered to be positive result. Bcl-xL positive expression showed brown stained signal in the cytoplasm; the result was considered positive if the positive cells accounted for more than 20 % in each slide. The nuclei of apoptotic cells were stained brown as detected under light microscopy(Olympus CHK). Apoptotic cells were counted according to the Scheop’s method. Ten optical fields which were the strongest positive areas were counted (×400, field diameter 0.545 mm, area 2.33 mm²) and the vicinity of the necrotic areas were not evaluated in each slide. The apoptosis index (AI) was the average of positive cells per mm² in the slide.

**Statistical analysis**

Statistical significance was determined by χ² test or Student’s t test.

**RESULTS**

**Changes of cell apoptosis in esophageal precancerous lesions and cancer tissues**

Apoptotic cells were observed in tissues with different lesions of esophageal epithelia and cancer tissues(Figure 1, 2). The AI and the frequency of apoptosis occurrence were low in the normal epithelia, but they were increased gradually from normal epithelia to dysplasia and to cancer tissues. The AI and the frequency of apoptosis occurrence of dysplasia lesions group was significantly higher than that of normal epithelia group ($P<0.01$, Table 1).

**Table 1** Changes of cell apoptosis in esophageal precancerous lesions and cancer tissues

| Groups                  | n  | Apoptosis cases (%) | AI (cells/mm²) |
|-------------------------|----|---------------------|----------------|
| Normal epithelia        | 39 | 8 (20.5)            | 8.2±3.1        |
| Simple hyperplasia      | 52 | 17 (32.7)           | 13.4±4.3       |
| Dysplasia               | 41 | 31 (75.6)           | 17.8±8.3       |
| Carcinoma in situ       | 2  | 2 (100)             | 20.3±5.1       |
| Invasive carcinoma      | 64 | 64 (100)            | 25.2±9.8       |

$^aP<0.01$, $^b\chi^2=24.29$ vs normal epithelia, $^c\chi^2=5.19$ vs normal epithelia, Student’s t test.

**The relationship between the expressions of Egr-1mRNA and Egr-1 protein and cell apoptosis in esophageal precancerous lesion and cancer tissues**

The expressions of Egr-1 mRNA and Egr-1 protein were observed in the cytoplasm and nuclei in different lesions of esophageal epithelia and cancer lesions respectively (Figure 3). The positivity results of Egr-1 ISH and Egr-1 IHC were nearly identical, but ISH showed slightly higher. The AI and the rate of Egr-1 positivity were increased gradually from normal epithelia to simple hyperplasia and to dysplasia; the AI and the rate of Egr-1 positive expression of dysplasia lesions group was significant higher than that of normal epithelia group ($P<0.01$, $P<0.01$); the Egr-1 positivity rate of invasive carcinoma group was significant lower than that of dysplasia lesions group ($P<0.01$, Table 2).

**Table 2** The relationship between the expressions of Egr-1 and cell apoptosis in esophageal precancerous lesions and cancer tissues

| Groups                  | n  | Egr-1 positive | Egr-1 negative |
|-------------------------|----|---------------|---------------|
|                         | n (%)| AI (cells/mm²) | n (%)| AI (cells/mm²) |
| Normal epithelia        | 39 | 9(23.1)       | 30(76.9)     | 3.8±2.5       |
| Simple hyperplasia      | 52 | 20(38.5)      | 30(60.0)     | 7.2±4.3       |
| Dysplasia               | 41 | 27(65.9)      | 14(34.1)     | 10.9±7.4      |
| Carcinoma in situ       | 2  | 1(50.0)       | 1(50.0)      | 11.5±3.7      |
| Invasive carcinoma      | 64 | 17(26.6)      | 47(73.4)     | 21.4±9.1      |

$^aP<0.01$, $^b\chi^2=14.78$ vs normal epithelia, $^c\chi^2=6.80$ vs normal epithelia, Student’s t test, $^dP<0.01$, $^e\chi^2=15.84$ vs Dysplasia, $^f\chi^2$ test.
The relationship between the protein expression of Bcl-X and cell apoptosis in esophageal precancerous lesions and cancer tissues

The expression of Bcl-X<sub>L</sub> protein was observed in tissues with different lesion of esophageal epithelia and cancer tissues. The AI and Bcl-X<sub>L</sub> immunostaining positivity rate were increased gradually as the lesion progressed. The AI and the rate of Bcl-X<sub>L</sub> positive expression of dysplasia lesions group was significantly higher than that of normal epithelia group (P<0.01, P<0.01). In the Bcl-X<sub>L</sub>(+) cases (Figure 4) the AI was low, while in the Bcl-X<sub>L</sub>(-) cases had higher AI in the cancer tissues (P<0.01, Table 3).

Table 3 The relationship between the protein expression of Bcl-X and cell apoptosis in esophageal precancerous lesions and cancer tissues

| Groups               | n (%| AI(cells/mm<sup>2</sup>) | n (%)| AI(cells/mm<sup>2</sup>) |
|----------------------|-----|--------------------------|-----|--------------------------|
| Normal epithelia     | 39  | 4(10.3) 1.4±2.2          | 35(90.7) 8.3±4.3 |
| Simple hyperplasia   | 52  | 11(21.2) 3.7±3.6          | 41(80.2) 14.5±3.7 |
| Dysplasia            | 41  | 28(68.3) 19.9±4.2         | 13(31.7) 20.6±6.3 |
| Carcinoma in situ    | 2   | 2(50.0) 11.8±3.3          | 1(50.0) 29.0±5.7 |
| Invasive carcinoma   | 64  | 46(71.9) 14.5±4.5         | 18(28.1) 28.6±8.6 |

<sup>a</sup>P<0.01, χ<sup>2</sup>=28.06 vs normal epithelia, χ<sup>2</sup> test; <sup>b</sup>P<0.01, t=10.53 vs normal epithelia, Student's t test; <sup>c</sup>P<0.01, t=6.10 vs compared with Bcl-X<sub>L</sub> protein negative group, Student's t test.

Table 4 The relationship between the expression of Egr-1 and cell apoptosis and biological behavior in esophageal squamous cell carcinoma

| Clinicopathologic data                  | n | Egr-1 positive (%) | AI(cells/mm<sup>2</sup>) |
|-----------------------------------------|---|--------------------|--------------------------|
| Age(years)                              |   |                    |                          |
| 50                                      | 20| 6(30.0)            | 21.5±7.9                |
| >50                                     | 46| 12(26.1)           | 24.3±8.8                |
| Tumor site                              |   |                    |                          |
| Upper segment                           | 4 | 0 (0)              | 20.4±6.2                |
| Middle segment                          | 50| 14(28.0)           | 23.7±9.3                |
| Lower segment                           | 12| 4(33.3)            | 21.5±8.4                |
| Lesion’s diameter(cm)                   |   |                    |                          |
| ≤5                                      | 45| 10(22.2)           | 22.6±8.5                |
| >5                                      | 21| 8(38.1)            | 22.3±8.2                |
| Differentiation degrees                 |   |                    |                          |
| Grade I                                 | 18| 7(38.9)            | 31.6±6.9                |
| Grade II                                | 33| 10(30.3)           | 28.5±8.9                |
| Grade III                               | 13| 0 (0)              | 7.2±4.5                 |
| Keratinization                          |   |                    |                          |
| Negative                                | 15| 1(6.7)             | 8.6±3.2                 |
| Positive                                | 51| 17(33.3)<sup>a</sup>| 34.7±9.4<sup>p</sup>   |
| Invasive depth                          |   |                    |                          |
| Upper muscular layer                    | 22| 5(22.7)            | 18.7±7.5                |
| Serosa                                   | 44| 13(29.5)           | 26.1±9.2                |
| Lymphatic metastasis                    |   |                    |                          |
| Negative                                | 30| 11(36.7)           | 23.4±8.6                |
| Positive                                | 36| 7(19.4)            | 20.8±7.5                |
| Bcl-X<sub>L</sub> protein               |   |                    |                          |
| Negative                                | 19| 10(52.6)           | 29.4±8.8                |
| Positive                                | 47| 8(17.0)<sup>d</sup>| 13.5±6.2<sup>d</sup>   |

<sup>a</sup>P<0.05, χ<sup>2</sup>=4.15 vs compared with non-keratinized squamous carcinoma group, χ<sup>2</sup> test; <sup>b</sup>P<0.01, t=16.79 vs compared with non-keratinized squamous carcinoma group, Student’s t test; <sup>c</sup>P<0.01, χ<sup>2</sup>=6.65 vs compared with Bcl-X<sub>L</sub> protein negative group, χ<sup>2</sup> test; <sup>d</sup>P<0.01, t=7.54 vs compared with Bcl-X<sub>L</sub> protein negative group, Student’s t test.
The relationship between the expression of Egr-1 and cell apoptosis and biological behavior in esophageal squamous cell carcinoma

All Egr-1 positive cases were of keratinized squamous cell carcinoma (grade I,II). The AI and Egr-1 positive rate of keratinized squamous cell carcinoma group were significantly higher than that of the non-keratinized squamous cell carcinoma group (P<0.05, P<0.01). In Bcl-X, protein positive group, the AI and the Egr-1 positive rate were lower significantly than those in Bcl-X−/−group (P<0.01, P<0.01, Table 4).

DISCUSSION

Modern molecular biology investigations have indicated that proliferative inhibition of some neoplasm cells is related to apoptosis induction by the oncogene expression of these cells[22-28]. Apoptosis, or programmed cell death, is a process in which a genetic program is activated, may be positively or negatively modulated by several oncogenes and tumor suppressor genes. The cancerogenic course of esophageal carcinoma has been identified as a successive course from simple hyperplasia of basal cell, dysplasia, carcinoma in situ to invasive carcinoma. Our studies suggest that The AI and the frequency of apoptosis occurrence increased gradually in esophageal carcinogenesis.

Egr-1 is one of the immediate early gene family, the Egr-1 gene was localized in human chromosome 5q31.1. Egr-1 is a nuclear protein that contains three zinc-finger domains, which regulate the cellular growth and differentiation by activating Cyclin D1 to promote the cell from the G0/G1 phase into the G2/M phase[29]. It was reported[30,31] that Egr-1 was originally in dormancy, and would be activated by the induction of stress, ischemia, hypoxia, bacterial toxin, cell factors, ionizing radiation and some oncogenic factors through membrane depolarization. We have detected tissue series of esophageal carcinogenesis using In situ hybridization and immunohistochemistry, and found that the rate of Egr-1 positive expression in dysplasia was the highest among all specimens (65.9 %). This might be explained that the paracancerous mucosa is much more stimulated, and this is in accordance with that Egr-1 expression is activated by many factors. High expression of Egr-1 might preserve the stabilization of chromosome and suppress proliferation, and also improve differentiation and apoptosis of the cell. Egr-1 expression is decreased significantly from dysplasia to cancer tissues[32-33].

Recent studies have shown[34] that there are several genes which participate in apoptosis regulation. Apoptosis regulation genes are divided into two groups: existence gene and death gene. Living gene includes cell promoting gene, while death gene includes proliferation suppressing gene. Studies using diverse tumor cells suggest that endogenous levels of Egr-1 act to impede proliferation[21,29]. Consistent with its anti-tumor role, Egr-1 has been identified an important gene for impeding proliferation, and apoptosis of certain tumor cells needs Egr-1. Some studies indicated that the mechanism of Egr-1 inducing apoptosis might be involved by the activation of some oncogenes, e.g. wild type P53 gene, TNF-α and the concentration of calcium ions[35,36]. Another gene that implicates apoptotic pathway is bcl-2. Bcl-2 has been identified to be an apoptosis inhibitor. Recently, a new bcl-2 related gene Bcl-X was identified. Alternative splicing results in two bcl-X-derived mRNA species, called bcl-XL and bcl-XS. Bcl-X appears to have functions similar to Bcl-2 in that it is capable of suppressing cell apoptosis in cancer cells[37,38].

Abnormal hyperplasia and cell apoptosis of cancer cell are the dynamic processes in the pathogenesis and development of esophageal carcinoma. Many studies indicated that cell hyperplastic cycle and cell apoptosis were two importance essential portions to maintain homostasis by many genes constituted complex modulation system. Our studies detected that cell apoptosis existed in normal epithelia and different lesion of esophagus, but AI and the frequency of apoptosis occurrence were increased gradually as the lesions progressed. In the similar lesions, the AI in Egr-1 positive group was higher than that in Egr-1 negative group. In contrast, the AI of Bcl-X, positive group was lower than that of Bcl-X, negative group. The AI and the expression of Egr-1 were not correlated with invasiveness and lymphatic metastasis of the cancer tissues. Our study suggests that cell apoptosis might be an important process in esophageal carcinogenesis. Egr-1 might promote apoptosis effect while Bcl-X, inhibit it. But further study is necessary to explore the mechanism and significance of Egr-1 and Bcl-X, improving or suppressing apoptosis in esophageal carcinogenesis.

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 Chaoan inhabitants of China. World J Gastroenterol 2001; 7: 657-661
2. 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
3. Qiao GB, Han CL, Jiang RC, Sun CS, Wang Y, Yang YJ. Overexpression of P53 and its risk factors in esophageal cancer in urban areas of Xi’an. World J Gastroenterol 1998; 4: 57-60
4. Chen KN, Xu GW. Diagnosis and treatment of esophageal cancer. Shijie Huanren Xiaohua Zazhi 2000; 8: 196-202
5. Zheng ZY, Wang LD, Shi ST, Yang YG, 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 Huanren Xiaohua Zazhi 1999; 7: 280-284
6. Wang LD, Zhou Q, Wei JF, Yang WC, Zhao X, Wang LX, Zou JX, Gao SS, Li YX, Yang CS. Apoptosis and its relationship with cell proliferation, p53, Waifp21, 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 relationship with pathomorphology 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 1994; 4: 277-279
10. Xu CT, Yan XJ. P53 anti-cancer gene and digestive system neoplasms. Shijie Huanren 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-DQBL alleles in esophageal cancer of Hubei Chinese Huns. Shijie Huanren 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 Huanren Xiaohua Zazhi 2000; 8: 782-785
13. Wang LD, Chen H, Guo LM. Alteration of tumor suppressor gene p53-Rb and human esophageal carcinogenesis. Shijie Huanren Xiaohua Zazhi 2001; 9: 367-371
14. Yu GQ, Zhou Q, Ivan D, Gao SS, Zheng ZY, Zou JX, Li YX, Wang LD. Changes of p53 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

15 Zhou YA, Gu ZP, Wang XN, Ma QF. WA1 gene suppresses growth of human esophageal carcinoma cell line EC109. Shijie Huaren Xiaohua Zazhi 2002; 10: 628-632

16 Zhou YA, Gu ZP, Wang XN, Ma QF, Huang LJ. Re-expression of p16INK4a gene suppresses growth of human esophageal carcinoma cells. Shijie Huaren Xiaohua Zazhi 2003; 9: 877-881

17 Liu J, Chen SL, Zhang W, Su Q. P21WAF1 gene expression with P53 mutation in esophageal carcinoma. Shijie Huaren XIAohua Zazhi 2000; 8: 1350-1353

18 Tan LJ, Jiang W, Zhang N, Zhang XR, QiU DH. Fas/FasL expression and apoptosis of colon adenocarcinoma cell lines. World J Gastroenterol 2001; 7: 88-92

19 Si XH, Yang LJ. Extraction and purification of TGFlg and its effect on the induction of apoptosis of hepatocytes. World J Gastroenterol 2001; 7: 527-531

20 Xu HY, Yang YL, Guan XL, Song G, Jiang AM, Shi LJ. Expression of regulation apoptosis gene and apoptosis index in primary liver cancer. World J Gastroenterol 2000; 6: 721-724

21 Li J, Yang XK, Yu XX, Ge ML, Wang WL, Zhang J, Hou YD. Overexpression of p27KIP1 induced cell cycle arrest in G1 phase and subsequent apoptosis in HCC-922 cell line. World J Gastroenterol 2000; 6: 513-521

22 Nair P, Muthukumkaram S, Sells SF, Han SS, Sukhatme VP, Rangnekar VM. Early growth response-1-dependent apoptosis is mediated by p53. J Biol Chem 1997; 272: 2031-2038

23 Yan SF, Lu J, Zou YS, Soh WJ, Cohen DM, Buttrick PM, Cooper DR, Stainberg SF, Mackman N, Pinsky DJ, Stern DM. Hypoxia-associated induction of early growth response-1 gene expression. J Biol Chem 1999; 274: 15030-15040

24 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

25 Huang RP, Fan Y, 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

26 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

27 Peter ME, Heufelder AE, Hengartner MO. Advances in apoptosis research. Proc Natl Acad Sci U S A 1997; 94: 12736-12737

28 Ahmed MM, Sells SF, Venkatasubbarao K, Fruitswala SM, Muthukumkaram 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

29 Woronicz JD, Calnan B, Ngo V, Winoto A. Requirement for the orphan steroid receptor Nur77 in apoptosis of T-cell hybridomas. Nature 1994; 367: 277-281

30 Schott AF, Apel IJ, Nunez G, Clarke MF. Bcl-XL protects cancer cells from p53-mediated apoptosis. Oncogene 1995; 11: 1389-1394

Edited by Xu JY