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

ELSEVIER

World J Gastroenterol 2005;11(1):46-50
World Journal of Gastroenterology ISSN 1007-9327

• GASTRIC CANCER •

Expression of nitric oxide synthase in human gastric carcinoma and its relation to p53, PCNA

Yong-Zhong Wang, You-Qing Cao, Jian-Nong Wu, Miao Chen, Xiao-Ying Cha

Yong-Zhong Wang, You-Qing Cao, Jian-Nong Wu, Miao Chen, Xiao-Ying Cha, Department of Pathology, the Affiliated Hospital of Jiangsu University, Zhenjiang 212001, Jiangsu Province, China
Supported by the Scientific Foundation of Jiangsu University, No. 2631280055
Correspondence to: Dr. Yong-Zhong Wang, Department of Pathology, the Affiliated Hospital of Jiangsu University, Zhenjiang 212001, Jiangsu Province, China. zjwyzh@163.com
Telephone: +86-511-5038423
Received: 2003-12-19 **Accepted:** 2004-02-03

Key words: Gastric carcinoma; Nitric oxide synthases; P53 protein; PCNA

Wang YZ, Cao YQ, Wu JN, Chen M, Cha XY. Expression of nitric oxide synthase in human gastric carcinoma and its relation to p53, PCNA. *World J Gastroenterol* 2005; 11(1): 46-50
http://www.wjgnet.com/1007-9327/11/46.asp

Abstract

AIM: To investigate the expression of NOS in gastric carcinoma, and to explore the relationship between the expression of nitric oxide synthases (NOS) and p53, PCNA, pathological features and clinical staging of gastric cancer.

METHODS: The activity of NOS protein was investigated in 85 samples of human gastric carcinoma and 25 samples of normal gastric mucosal tissue by biochemical assay. We then examined the expression of NOS, p53, PCNA in 85 samples of human gastric cancer was examined by immunohistochemistry, and NOS mRNA expression in 85 gastric cancer tissue specimens by *in situ* hybridization.

RESULTS: Biochemical assay showed that the activity of NOS was significantly higher in gastric carcinoma than in normal gastric mucosal tissues (t = 0.4161, P<0.01). Immunohistochemistry revealed that endothelial nitric oxide synthase (eNOS) expressed in all samples of normal gastric mucosa, but only 6 cases of 85 gastric cancer specimens showed weak positive immunohistochemical reactions to eNOS (20%). Inducible nitric oxide synthase (iNOS) was expressed strongly in human gastric carcinoma (81.2%). *In situ* hybridization analysis showed that iNOS mRNA expression was significantly stronger than eNOS mRNA expression in gastric cancer tissue (χ^2 = 10.23, P<0.01). The expression of iNOS in gastric cancer was associated with differentiation, clinical stages or lymph node metastases (r = 0.3426, P<0.05). However, iNOS expression did not correlate with histological classifications and morphological types. The expression of iNOS was significantly correlated with p53 or PCNA expression (r = 0.3612, P<0.05). The expression of neuronal nitric oxide synthase (nNOS) was not examined by immunohistochemistry and *in situ* hybridization in gastric cancer specimens and normal gastric mucosa.

CONCLUSION: In human gastric cancer, there is an enhanced expression of iNOS, but not of eNOS. NOS promotes the proliferation of tumor cells and plays an important role in gastric cancer spread. Inactivation of antioncogene p53 and overexpression of iNOS might play a synergetic role in the process of carcinogenesis of human gastric carcinoma.

INTRODUCTION

Nitric oxide (NO) is a short-lived biomolecule with various biologic functions. Since its discovery as a biologically active molecule in the late 1980s, NO has been regarded to play a role as a signal molecule in organisms, immunological defense mechanisms and carcinogenesis[1-4]. This small molecule is a product of the conversion of L-arginine to L-citrulline by NOS. NOS can be classified into three isoforms: neuronal nitric oxide synthase (nNOS), inducible nitric oxide synthase (iNOS) and endothelial nitric oxide synthase (eNOS)[5-7]. nNOS is expressed mainly in neuronal cells, and NO functions as a neurotransmitter. eNOS mainly exists in endothelial cells and NO regulates blood pressure. Both isozymes are constitutively expressed and their activities are Ca^{2+}-dependent. iNOS activity is Ca^{2+}-independent and is induced in various types of cells by inflammatory cytokines, lipopolysaccharides, and other stimuli[8-10]. Thus, the distribution of NOS isoforms is tissue-specific and altered by pathological conditions.

In some tumor tissues, NO has been found to enhance tumor angiogenesis and induce vasodilatation, thus accelerating tumor growth[11]. In other tumors, including gastric and colon cancer, a decreased amount of NOS protein was demonstrated by immunohistochemistry, and there is a possible relationship between loss of NO and carcinogenesis[12,13]. Recent studies have demonstrated that NOS expression and its activities in gastric cancer are elevated[14,21,22]. However, the distribution and function of NOS isoforms in gastric tumor tissue have not been fully elucidated. This study investigated the expression of the constitutive isoform (cNOS), the inducible isoform (iNOS) in human gastric cancer tissue, and further explored the relationship between iNOS expression and the pathological features, clinical staging of gastric cancer.

Recent studies indicate that gastric cardia carcinogenesis is a multistep progressive process involving multiple genetic changes (accumulation or overlap). The accumulation of p53 protein and p53 gene mutation can be observed in the very early stage of gastric cardia carcinogenesis, with positive immunostaining and mutation rates increased with the progression of lesions[15,16]. In addition, other studies also show that PCNA protein overexpression can be observed in the carcinogenesis of gastric cardia adenocarcinoma[17]. In the present study, the expression levels of iNOS were examined to explore whether they correlated with the expression of p53 and PCNA in gastric cancer tissue.

MATERIALS AND METHODS

Tissue preparation

Eighty-five patients with gastric cancer (50 men, 35 women, ranging in age from 35 to 70 years, mean 52 years) underwent curative gastrectomy in our hospital from 1998 to 2001, including 38 cases of early gastric cancer and 47 cases of advanced gastric cancer. Histologically it included 28 cases of moderately differentiated gastric carcinoma, 25 cases of highly differentiated carcinoma and 32 cases of poorly differentiated carcinoma. Pathologists confirmed all gastric cancer tissues. Twenty-five samples of normal gastric mucosa were obtained through a gastroscope. Dissected samples were frozen in liquid nitrogen and stored at -79 ℃ until analyzed. Resected tissues were fixed in 40 g/L buffered neutral formaldehyde and embedded in paraffin and serially cut into 5 μm thick sections.

NOS activity

Activity of NOS was determined by a method based on the conversion of L-[^{3}H] arginine to L-[^{3}H] citrulline. Frozen tissues were homogenized in 4 volumes of 50 mmol/L Tris buffer (pH 7.4) containing 1 mmol/L dithiothreitol (DTT), 1 mmol/L EDTA, 0.1 mmol/L EGTA, 100 mg/L PMSF, and 5 mg/L leupeptin. Whole homogenates of the tissues were incubated for 30 min at 37 ℃ in the presence of 1 mmol/L NADPH, 100 μmol/L H_4B, 10 μmol/L FAD, 10 μmol/L FMN, 1.5 mmol/L $CaCl_2$, 2 μg calmodulin and 500 μmol/L L-[^{3}H] arginine. The reaction was terminated by adding 1 mL ice-cold Dowex-50 W equilibrated in 20 mmol/L sodium acetate buffer (pH 5.5) containing 1 mmol/L citrulline, 2 mmol/L EDTA and 0.2 mmol/L EGTA. The reaction mixtures were centrifuged at 10 000 *g* for 5 min. The supernatant was collected into a water miscible scintillate and the radioactivity was counted using a Beckman LS 2400 liquid scintillation counter.

Immunohistochemistry

Immunohistochemistry was performed using the streptavidin-peroxidase (SP) method. The following primary antibodies and kits were used: polyclonal antibodies against iNOS, eNOS, nNOS, p53, PCNA (Santa Cruz Inc. USA). Dewaxed sections were heated in a microwave oven (700 W) for 12 min to retrieve the antigens and cooled to room temperature. Endogenous peroxide was blocked by 3% hydrogen peroxide (H_2O_2) for 15 min in methanol. After washing with phosphate-buffered-saline (PBS. 0.01 mol/L), the sections were further blocked by 10% goat serum for 15 min to reduce the nonspecific antibody binding, and then incubated with the primary antibodies against iNOS (eNOS, nNOS, p53, PCNA) at 4 ℃ overnight. After washing with PBS for 2×5 min, the sections were incubated with the secondary anti-rabbit immunoglobulin (Ig, Santa Cruz Inc. USA) conjugated with biotin at room temperature for l5 min, washed again with PBS (0.01 mol/L), and incubated with streptavdin-peroxidase complex for l5 min. The reaction products of peroxidase were visualized by incubation with 0.05 mol/L Tris-HCl buffer (pH7.6) containing 20 mg 3.3' -diaminobenzidine (DAB, Maixin-Bio Co. China) and 100 μL 5% hydrogen peroxide per 100 mL. Finally, the sections were counterstained for nuclei by hematoxylin solution. The sections in the control group were stained according to the above method, with the first antibody substituted by PBS. The assessment of all the samples was conducted blindly by calculating the average ratio of positive cells in 10 vision fields (the plasma staining brown-yellow) under a 400× microscope. If the average positive cell ratio was more than 10%, this sample was considered positive.

In situ hybridization

In situ hybridization (ISH) was used to detect the expression of iNOS mRNA, eNOS mRNA and nNOS mRNA in gastric cancer tissue. NOS probes and kits were purchased from Boster Bio Co. (China). Dewaxed sections were incubated with 3% hydrogen peroxide for 30 min to reduce the non-specific binding and then with 1 μg/mL pepsin for 5-8 min to improve the penetration of the probe. Prehybridization was performed at 40 ℃ for 3 h to enhance the hybridization efficiency, and hybridization was conducted in 42 ℃ water bath with each section covered with a coverslip. The thorough washing procedure was as follows: 2×SSC (sodium chloride and sodium citrate) at 37 ℃ for 15 min, 0.5×SSC for 15 min, 0.2×SSC for 15 min. The sections were visualized according to the manufacturer's instructions of the kit. We counted the positive cells in total cells in 10 vision fields (the plasma was stained purplish blue) under 400× microscopes. If the average positive cell ratio was more than 10%, the sample was considered positive.

Statistics analysis

Statistical comparison of NOS immunoreactivity with clinico-pathological findings. p53 and PCNA overexpression was performed using chi-square test. *t* test was used for comparison of the activity of NOS. *P* values less than 0.05 were considered statistically significant.

RESULTS

NOS activity

The total NOS activity (pmole/min per mg protein) was measured in human gastric tumor tissues from surgically treated patients and normal tissues. The activity in gastric tumor tissues was about 75% higher than that in normal tissues ($P<0.01$) (Figure1). Analysis of gastric cancer samples by histological classification showed that poorly differentiated adenocarcinomas had 2.0 and 2.5 times higher activity than highly and moderately differentiated adenocarcinomas, respectively (Figure2).

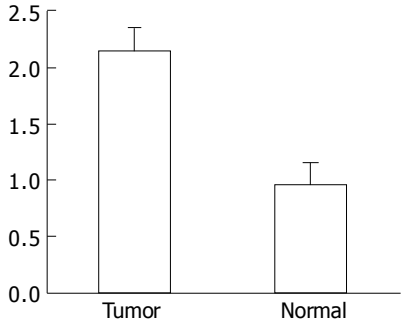

Figure 1 Total NOS activity in gastric adenocarcinoma and normal tissues [pmole/(min·mg) protein].

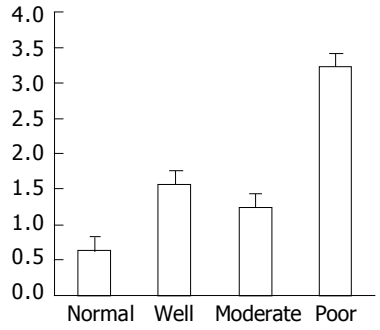

Figure 2 Total NOS activity of gastric cancer by histological classification [pmole/(min·mg) protein]. Normal: normal gastric mucosa Well: well differentiated Moderate: moderately differentiated Poor: poorly differentiated.

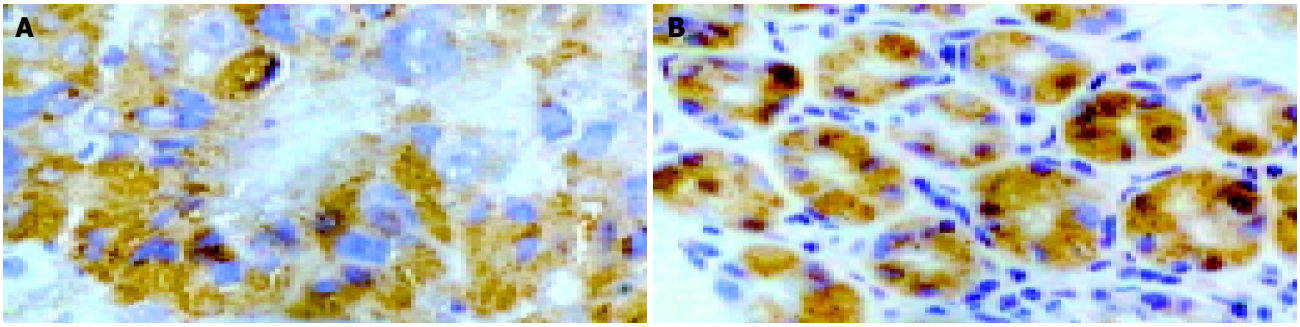

Figure 3 Immunohistochemical strong staining for NOS in cytoplasm (SP), ×400. A: Expression of iNOS in gastric cancer cells; B: Expression of eNOS in gastric mucosal cells.

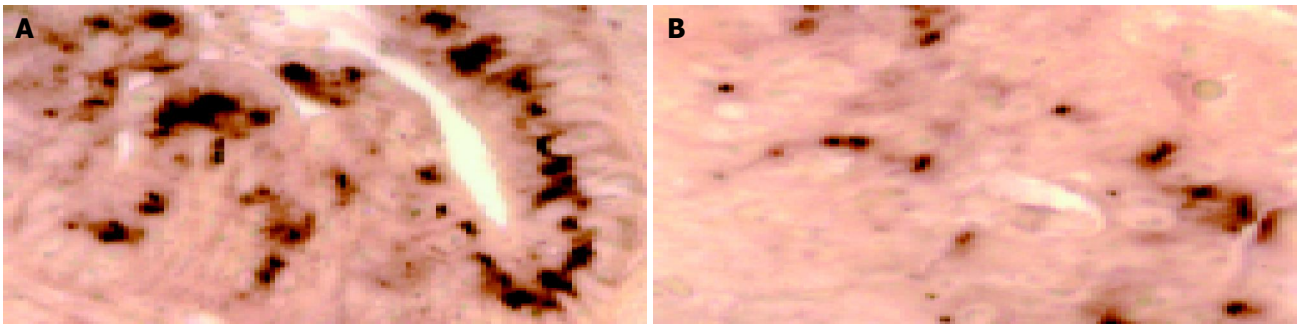

Figure 4 Expression of NOS mRNA in gastric cancer (ISH), ×400. A: Strong iNOS mRNA expression; B: Weak eNOS mRNA expression.

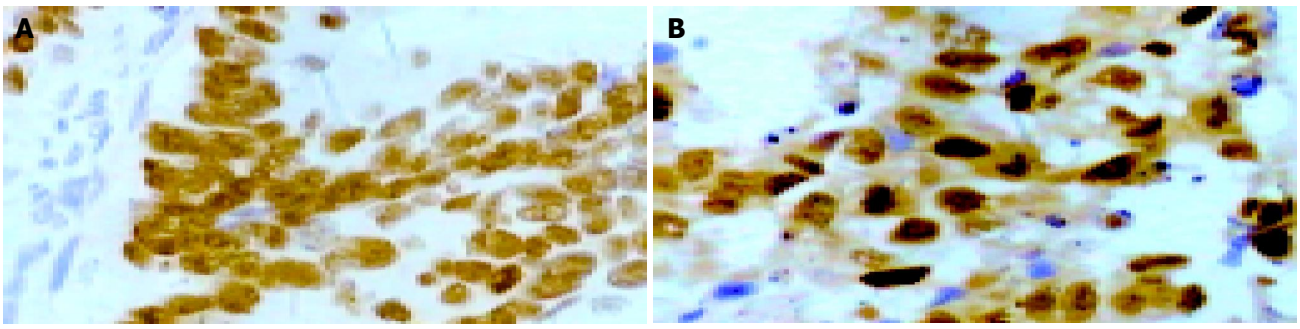

Figure 5 Immunohistochemical strong staining for p53 or PCNA in nuclei of gastric cancer cells (SP), ×400. A: p53 protein expression; B: PCNA protein expression.

Immunohistochemical analysis

Tumor cells in 69 out of 85 cases (81.2%) were positive for iNOS. Strongly positive reactions showed diffuse dark brown-yellow reaction products in cytoplasm of most tumor cells (Figure 3A). There was no apparent relationship between iNOS positivity and invasiveness of the tumor. Five cases out of 25 (20%) showed positive reaction to iNOS in normal gastric epithelial cells. The expression of iNOS protein was significantly higher in human gastric tumor tissues than in normal tissues. Statistically, there was a significant difference between gastric tumor tissues and normal tissues ($P<0.01$). However, the epithelial cells of gastric mucosa in all cases were strongly positive for eNOS (Figure 3B), only 6 out of 85 cases (7.1%) showed weakly positive reactions to eNOS in tumor cells. The nNOS was not detected by immunohistochemistry in gastric tumor tissues and normal tissues.

In situ hybridization

iNOS, nNOS and eNOS expressions were analyzed at mRNA level in gastric tumor tissues. The purple-blue hybridization signal was restricted to the cytoplasmic portion. Forty-two out of 69 iNOS-positive gastric tumor tissues had a strong staining (Figure 4A), while the rest had a less strong staining. Six eNOS-positive gastric tumor tissues had a weak staining (Figure 4B). Statistically, there were significant differences in gastric tumor tissues ($P<0.01$) between iNOS mRNA and eNOS mRNA expressions. nNOS mRNA was not detected by *in situ* hybridization in gastric tumor tissues.

Relationship between iNOS expression and clinico-pathological findings

There was a significant correlation between iNOS expression and tumor differentiation, clinical stages and lymph node metastases in gastric tumor tissues ($P<0.05$). However, the expression of iNOS was not associated with the patho-histological classifications and types of morphology (Table 1).

Relationship between iNOS and p53, PCNA expressions in gastric cancer

p53 and PCNA proteins were detected in a large number of tumor cells in 72 (84.7%) (Figure 5A) and 69 (81.2%) (Figure 5B) out of 85 gastric tumor tissues, respectively. As shown in Table

2, 59 out of 72 p53-positive tumor samples were positive for iNOS (81.9%) whereas, 5 out of 13 p53-negative tumor samples showed a positive reaction to iNOS (38.5%) ($P<0.05$). Likewise, the positive rate of iNOS (81.2%, 56/69) in PCNA-positive tumor group was higher than that (37.5%, 6/16) in PCNA-negative group ($P<0.05$).

Table 1 Relationship between iNOS expression and pathological parameter in gastric cancer tissues

Parameter	Cases *n*	iNOS expression		Positive rate(%)	*P*
		Positive	Negative		
Morphological type					
Polypoid or fungating type	30	22	8	73.3	
Ulcerative type	34	27	7	79.4	>0.05
Infiltrating type	21	16	5	76.2	
Histological classification					
Adenocarcinoma	31	23	8	74.2	
Scirrhous carcinoma	15	12	3	80.0	
Medullary carcinoma	17	13	4	76.5	>0.05
Mucoid carcinoma	22	16	6	72.7	
Degree of differentiation					
Poorly differentiated	35	30	5	85.7[a]	<0.05
Well differentiated	51	33	17	64.7	
Clinical stage					
Early gastric carcinoma	36	24	12	66.7	
Advanced gastric carcinoma	49	44	5	89.8[c]	<0.05
Lymph node metastasis					
Positive	51	44	7	86.3[e]	<0.05
Negative	34	19	15	55.6	

[a]$P<0.05$ *vs* the group of well-differentiated carcinomas; [c]$P<0.05$ *vs* the group of early gastric carcinomas; [e]$P<0.05$ *vs* the expression rate of iNOS in cases having no lymph node metastasis.

Table 2 Relationship between the expressions of iNOS and p53, PCNA in gastric cancer

	Cases *n*	iNOS expression		Positive rate(%)
		Positive	Negative	
P53				
Positive	72	59	13	81.9[a]
Negative	13	5	7	38.5
PCNA				
Positive	69	56	13	81.2[c]
Negative	16	6	10	37.5

[a]$P<0.05$, A comparison of iNOS expression between p53-positive and p53-negative groups; [c]$P<0.05$, A comparison of iNOS expression between PCNA-positive and PCNA-negative groups.

DISCUSSION

Gastric carcinoma tissues showed an increase of the overall NOS activity by about 75% when compared with the normal tissues in our studies. The increased NOS activity in the tumor was well reflected in the elevated level of NOS mRNA expression in the tumor tissues. The results coincide with the previous data obtained from colon[13], lung[18] and breast[19] tumors. However, our results differ from a previous report that showed a marked reduction of NOS expression in gastric tumor tissues than in gastric mucosa[20]. We assume that the difference in expression levels is due to the different determination methods employed by the authors. Our finding of the increased activity of NOS in gastric cancer supports the general hypothesis that excessive NO production may contribute to the pathogenesis of cancer progression. The expression of eNOS in our studies was strong in glandular epithelium of gastric mucosa by immunohistochemistry. This observation indicates a role of NO in the regulation of epithelial cell integrity or secretion. The iNOS was densely localized in tumor tissues, while normal gastric tissue weakly expressed it. These immunohistochemical results suggest that the increased NOS activity in gastric tumor tissues might be closely associated with the overexpression of the iNOS form, rather than the cNOS form. The localization of iNOS in the apical part of glandular epithelium of tumor tissues by Rajnakova *et al*[12] is confirmed by our results. Although some authors[19] have confirmed that iNOS in tumor tissues is localized in migrated macrophages, our results clearly show that iNOS is localized in gastric tumor epithelial cells but not in macrophages. Thus, we hold that NO comes from tumor cells rather than from macrophages in gastric tumor tissues. In this study, nNOS was not detected in human gastric tumor tissues and normal gastric mucosa by immunohistochemistry and *in situ* hybridization, which coincides with the findings of Koh *et al*[20].

In our studies, no correlation was found between iNOS expression and morphological types and histological classification of gastric tumor as observed by others in lung tumor[18]. We also found that the expression rate of iNOS was markedly higher in advanced than in early stages of gastric carcinoma. Our study has confirmed the hypothesis that NO produced by iNOS could promote tumor growth[21]. The reason is that NO produced by iNOS might increase the vascular permeability and accelerate the nutrient supply of tumor tissue and finally promote tumor growth. It was also found that the positive iNOS expression rate in gastric carcinoma with lymph node metastasis was higher than in that with no metastasis ($P<0.05$), suggesting NO produced by iNOS in gastric tumor tissues can promote its lymph node metastasis as observed by others[27]. In addition, poorly differentiated carcinomas of the stomach demonstrate a higher expression of iNOS than highly and moderately differentiated carcinomas ($P<0.05$). These results suggest that NO produced by iNOS plays an important role in gastric cancer growth and invasion.

Another interesting observation was a highly coincidental positive immunostaining rate of iNOS (81.9%) in p53-positive gastric tumor tissues and iNOS (81.2%) in PCNA-positive gastric tumor tissues. Our findings suggest that iNOS expression is significantly associated with p53 mutation and cell proliferation of gastric carcinoma ($P<0.05$). This result is consistent with the findings in human hepatocellular[22], head and neck[23], endometrial[24], pharyngeal[25], esophageal tumors[26]. Increased iNOS expression has been demonstrated in many tumors, such as gastric cancer[27], brain tumors[28] and colon cancer[13]. Tumor-associated NO production might modify DNA directly, or inhibit DNA repair activities[29]. Thus, we conclude that NO may lead to loss of cell proliferation control and p53 mutants in gastric cancer. Recent studies on gastric cancer patients have shown that increased expression of iNOS may promote gastric cancer progression by providing a selective growth advantage to tumor cells with non-functioning p53[30,31]. Thus, inactivation of antioncogene p53 and overexpression of iNOS play a synergetic role in the carcinogenesis of human gastric carcinoma.

In conclusion, the expression of iNOS is well correlated with the expression of PCNA and p53 protein. The molecular basis of the expression of iNOS and PCNA, p53 protein, and their roles in the progression of gastric cancer need to be investigated in follow-up studies.

REFERENCES

1 **Janssens MY,** Van den Berge DL, Verovski VN, Monsaert C, Storme GA. Activation of inducible nitric oxide synthase results in nitric oxide-mediated radiosensitization of hypoxic EMT-6 tumor cells. *Cancer Res* 1998; **58**: 5646-5648
2 **Goto T,** Haruma K, Kitadai Y, Ito M, Yoshihara M, Sumii K, Hayakawa N, Kajiyama G. Enhanced expression of inducible nitric oxide synthase and nitrotyrosine in gastric mucosa of gastric cancer patients. *Clin Cancer Res* 1999; **5**: 1411-1415
3 **Wink DA,** Vodovotz Y, Cook JA, Krishna MC, Kim S, Coffin D, DeGraff W, Deluca AM, Liebmann J, Mitchell JB. The role of nitric oxide chemistry in cancer treatment. *Biochemistry (Mosc)* 1998; **63**: 802-809
4 **Hamaoka R,** Yaginuma Y, Takahashi T, Fujii J, Koizumi M, Seo HG, Hatanaka Y, Hashizume K, Ii K, Miyagawa J, Hanafusa T, Matsuzawa Y, Ishikawa M, Taniguchi N. Different expression patterns of nitric oxide synthase isozymes in various gynecological cancers. *J Cancer Res Clin Oncol* 1999; **125**: 321-326
5 **Wolf H,** Haeckel C, Roessner A. Inducible nitric oxide synthase expression in human urinary bladder cancer. *Virchows Arch* 2000; **437**: 662-666
6 **Oyoshi T,** Nomoto M, Hirano H, Kuratsu J. Pathodynamics of nitric oxide production within implanted glioma studied with an *in vivo* microdialysis technique and immunohistochemistry. *J Pharmacol Sci* 2003; **91**: 15-22
7 **Hallinan EA,** Tsymbalov S, Dorn CR, Pitzele BS, Hansen DW, Moore WM, Jerome GM, Connor JR, Branson LF, Widomski DL, Zhang Y, Currie MG, Manning PT. Synthesis and biological characterization of L-N (6)-(1-iminoethyl) lysine 5-tetrazole-amide, a prodrug of a selective iNOS inhibitor. *J Med Chem* 2002; **45**: 1686-1689
8 **Hamaoka R,** Yaginuma Y, Takahashi T, Fujii J, Koizumi M, Seo HG, Hatanaka Y, Hashizume K, Ii K, Miyagawa J, Hanafusa T, Matsuzawa Y, Ishikawa M, Taniguchi N. Different expression patterns of nitric oxide synthase isozymes in various gynecological cancers. *J Cancer Res Clin Oncol* 1999; **125**: 321-326
9 **Doi C,** Noguchi Y, Marat D, Saito A, Fukuzawa K, Yoshikawa T, Tsuburaya A, Ito T. Expression of nitric oxide synthase in gastric cancer. *Cancer Lett* 1999; **144**: 161-167
10 **Kojima M,** Morisaki T, Tsukahara Y, Uchiyama A, Matsunari Y, Mibu R, Tanaka M. Nitric oxide synthase expression and nitric oxide production in human colon carcinoma tissue. *J Surg Oncol* 1999; **70**: 222-229
11 **Thomsen LL,** Miles DW. Role of nitric oxide in tumour progression: lessons from human tumours. *Cancer Metastasis Rev* 1998; **17**: 107-118
12 **Rajnakova A,** Goh PM, Chan ST, Ngoi SS, Alponat A, Moochhala S. Expression of differential nitric oxide synthase isoforms in human normal gastric mucosa and gastric cancer tissue. *Carcinogenesis* 1997; **18**: 1841-1845
13 **Ambs S,** Merriam WG, Bennett WP, Felley-Bosco E, Ogunfusika MO, Oser SM, Klein S, Shields PG, Billiar TR, Harris CC. Frequent nitric oxide synthase-2 expression in human colon adenomas: implication for tumor angiogenesis and colon cancer progression. *Cancer Res* 1998; **58**: 334-341
14 **Rajnakova A,** Moochhala S, Goh PM, Ngoi S. Expression of nitric oxide synthase, cyclooxygenase, and p53 in different stages of human gastric cancer. *Cancer Lett* 2001; **172**: 177-185
15 **Kim SS,** Bhang CS, Min KO, Chae HS, Choi SW, Lee CD, Lim KW, Chung IS, Park DH. p53 mutations and microsatellite instabilities in the subtype of intestinal metaplasia of the stomach. *J Korean Med Sci* 2002; **17**: 490-496
16 **Lee KE,** Lee HJ, Kim YH, Yu HJ, Yang HK, Kim WH, Lee KU, Choe KJ, Kim JP. Prognostic significance of p53, nm23, PCNA and c-erbB-2 in gastric cancer. *Jpn J Clin Oncol* 2003; **33**: 173-179
17 **Tao K,** Chen D, Tian Y, Lu X, Yang X. The relationship between apoptosis and the expression of proliferating cell nuclear antigen and the clinical stages in gastric carcinoma. *J Tongji Med Univ* 2000; **20**: 222-224
18 **Fujimoto H,** Ando Y, Yamashita T, Terazaki H, Tanaka Y, Sasaki J, Matsumoto M, Suga M, Ando M. Nitric oxide synthase activity in human lung cancer. *Jpn J Cancer Res* 1997; **88**: 1190-1198
19 **Thomsen LL,** Miles DW, Happerfield L, Bobrow LG, Knowles RG, Moncada S. Nitric oxide synthase activity in human breast cancer. *Br J Cancer* 1995; **72**: 41-44
20 **Koh E,** Noh SH, Lee YD, Lee HY, Han JW, Lee HW, Hong S. Differential expression of nitric oxide synthase in human stomach cancer. *Cancer Lett* 1999; **146**: 173-180
21 **Xu W,** Liu LZ, Loizidou M, Ahmed M, Charles IG. The role of nitric oxide in cancer. *Cell Res* 2002; **12**: 311-320
22 **Peng JP,** Zheng S, Xiao ZX, Zhang SZ. Inducible nitric oxide synthase expression is related to angiogenesis, bcl-2 and cell proliferation in hepatocellular carcinoma. *J Zhejiang Univ Sci* 2003; **4**: 221-227
23 **Gallo O,** Schiavone N, Papucci L, Sardi I, Magnelli L, Franchi A, Masini E, Capaccioli S. Down-regulation of nitric oxide synthase-2 and cyclooxygenase-2 pathways by p53 in squamous cell carcinoma. *Am J Pathol* 2003; **163**: 723-732
24 **Cinel L,** Polat A, Aydin O, Dusmez D, Egilmez R. Bcl-2, iNOS, p53 and PCNA expression in normal, disordered proliferative, hyperplastic and malignant endometrium. *Pathol Int* 2002; **52**: 384-389
25 **Pukkila MJ,** Kellokoski JK, Virtaniemi JA, Kumpulainen EJ, Johansson RT, Halonen PM, Kosunen AS, Nuutinen J, Kosma VM. Inducible nitric oxide synthase expression in pharyngeal squamous cell carcinoma: relation to p53 expression, clinicopathological data, and survival. *Laryngoscope* 2002; **112**: 1084-1088
26 **Matsumoto M,** Furihata M, Kurabayashi A, Araki K, Sasaguri S, Ohtsuki Y. Association between inducible nitric oxide synthase expression and p53 status in human esophageal squamous cell carcinoma. *Oncology* 2003; **64**: 90-96
27 **Feng CW,** Wang LD, Jiao LH, Liu B, Zheng S, Xie XJ. Expression of p53, inducible nitric oxide synthase and vascular endothelial growth factor in gastric precancerous and cancerous lesions: correlation with clinical features. *BMC Cancer* 2002; **2**: 8
28 **Kato S,** Esumi H, Hirano A, Kato M, Asayama K, Ohama E. Immunohistochemical expression of inducible nitric oxide synthase (iNOS) in human brain tumors: relationships of iNOS to superoxide dismutase (SOD) proteins (SOD1 and SOD2), Ki-67 antigen (MIB-1) and p53 protein. *Acta Neuropathol* 2003; **105**: 333-340
29 **Ohshima H,** Tatemichi M, Sawa T. Chemical basis of inflammation-induced carcinogenesis. *Arch Biochem Biophys* 2003; **417**: 3-11
30 **Rajnakova A,** Moochhala S, Goh PM, Ngoi S. Expression of nitric oxide synthase, cyclooxygenase, and p53 in different stages of human gastric cancer. *Cancer Lett* 2001; **172**: 177-185
31 **Aaltoma SH,** Lipponen PK, Kosma VM. Inducible nitric oxide synthase (iNOS) expression and its prognostic value in prostate cancer. *Anticancer Res* 2001; **21**: 3101-3106

Edited by Zhu LH and Wang XL