Role of cyclooxygenase-2 in gastric cancer development and progression

Jian Cheng, Xiao-Ming Fan

Although the incidence of gastric cancer has been declining in recent decades, it remains a major public health issue as the second leading cause of cancer death worldwide. In China, gastric cancer is still the main cause of death in patients with malignant tumors. Most patients are diagnosed at an advanced stage and mortality is high. Cyclooxygenase-2 (COX-2) is a rate-limiting enzyme in prostanoid synthesis and plays an important role in the development and progression of gastric cancer. The expression of COX-2 in gastric cancer is upregulated and its molecular mechanisms have been investigated. *Helicobacter pylori* infection, tumor suppressor gene mutation and the activation of nuclear factor-kappa B may be responsible for the elevated expression of COX-2 in gastric cancer. The mechanisms of COX-2 in the development and progression of gastric cancer are probably through promoting the proliferation of gastric cancer cells, while inhibiting apoptosis, assisting angiogenesis and lymphatic metastasis, and participating in cancer invasion and immunosuppression. This review is intended to discuss, comment and summarize recent research progress on the role of COX-2 in gastric cancer development and progression, and elucidate the molecular mechanisms which might be involved in the carcinogenesis.

© 2013 Baishideng Publishing Group Co., Limited. All rights reserved.

Key words: Cyclooxygenase-2; Gastric cancer; Prostaglandin; Carcinogenesis; Molecular mechanism

Core tip: Cyclooxygenase-2 (COX-2) plays an important role in gastric cancer development and progression. The present review aims to determine the molecular mechanism of COX-2 overexpression in gastric cancer and focus on the detailed information on COX-2 involved in carcinogenesis. By reviewing research progress, this may be helpful in clarifying the internal relationship of the afore-mentioned aspects.

INTRODUCTION

Gastric cancer is one of the most common malignant tumors worldwide, and the morbidity and mortality associated with this disease are ranked second highest of all malignant neoplasms[1-3]. In China, the incidence of gastric cancer has been declining in recent years[4]. Since 75% of gastric cancer patients are diagnosed at an advanced stage and cannot be cured merely by surgery, chemotherapy combined with surgery is often the primary treatment. There are many factors that affect the prognosis of gastric cancer, and of these factors invasion and metastasis are leading causes of death. The role of cyclooxygenase-2 (COX-2) in gastric cancer development and progression...
has been extensively studied. The expression of COX-2 is elevated in gastric cancer tissues, therefore, inhibition of COX-2 expression may prevent or reverse gastric carcinogenesis. This review focuses on the crucial role of COX-2 in gastric cancer development and progression. In addition, its mechanisms of action are illustrated.

**COX**

COX, also known as prostaglandin synthase, is the rate-limiting enzyme responsible for the conversion of arachidonic acid (AA) into the various prostaglandins (PGs), a family of lipid mediators that have widespread and diverse biological functions. This enzyme possesses both peroxidase activity in catalyzing prostaglandin G2 (PGG2) to prostaglandin H2 (PGH2) and COX catalytic activity in the conversion of PGG2 from AA. Members of the PGs family including PGD2, PGE2, PGF2. PGG2, and PGH2 are widely distributed in organic bodies and play different roles in metabolism. It is reported that PGE2 was overexpressed in tumor tissues and was involved in carcinogenesis.

COX, with a relative molecular mass of 71000, is a type of glycoprotein which is located on the surface of the nuclear membrane and microsomal membrane. Two COX isoforms have been found: COX-1 and COX-2. Although COX-1 and COX-2 share a high level of homology (65%), the activity and expression of these enzymes are different, and they can function independently within the same cell type.

The COX-1 gene, comprised of 11 exons and 10 introns, is a type of housekeeping gene, which is located at chromosome 9q22-33. The full length of the COX-1 gene is about 22.5 kb, and no hogness box and promoter elements are found. In most tissues, COX-1 is composed of 599-600 amino acid residues and expressed constitutively and continuously. The basic functions of COX-1 are not only promoting the synthesis of PGs, but also maintaining the homeostasis of an organism such as regulating the clotting mechanism, stabilizing renal blood flow and protecting gastric mucosa. COX-1 is expressed negatively or weakly in tumor tissues and is not involved in carcinogenesis.

The COX-2 gene, located at chromosome 1q25.2-25.3, is composed of 10 exons and 9 introns. With hogness box, CAAT enhancer binding protein (C/EBP) and CAMP response elements in the 5'-terminal nucleotide sequence, the gene is approximately 8.3 kb in size. There are also some binding sites in the gene sequence such as the activator protein-2 (AP-2) binding site and the nuclear factor-kappa B (NF-kB) binding site. COX-2 is composed of 604 amino acid residues and is expressed negatively in normal tissues and organs under physiological conditions, except the constitutive expression in kidney and brain. It is inducible in response to certain stimuli such as growth factors and cytokines. COX-2 is involved in many pathological processes such as inflammation and carcinogenesis. It was reported that more than 15% of malignant tumors are correlated with infection. Various inflammation networks have been confirmed to play crucial roles in the microenvironment of carcinogenesis, and the most important network is the COX-2/PGE2 pathway. In addition, it has been well established that COX-2 is up-regulated in a variety of cancers and promotes their growth.

**EXPRESSION OF COX-2 IN GASTRIC CANCER**

The first report on the expression of COX-2 in gastric cancer was from Ristimaki et al. Their study showed that human gastric adenocarcinoma tissues contained significantly higher levels of COX-2 mRNA when compared with paired gastric mucosal specimens devoid of cancer cells. Immunohistochemical staining detected COX-2 protein expression in the cytoplasm of gastric cancer cells, but not in the surrounding stroma. Uefuji confirmed the overexpression of COX-2 protein in human gastric adenocarcinomas by immunoblotting, and reported that overexpression of COX-2 protein was independent of the histologic type of gastric cancer.

A study further confirmed the significant difference in COX-2 protein expression between normal tissues and gastric cancer tissues. Researchers found that the overexpression of COX-2 protein was not related to the clinicopathological characteristics of gastric cancer patients but related to tumor node metastasis clinical stage, depth of invasion and metastasis. A series of studies showed that COX-2 protein expression was associated with intestinal histological subtype, proximal location, tumor size and advanced clinical stage and lymph node involvement. Importantly, the expression of COX-2 protein and mRNA was already detected in noninvasive gastric dysplasia. Thus, it seems likely that COX-2 plays a role in early gastric carcinogenesis.

There are controversial results in the association between COX-2 and survival rate. Although COX-2 played a crucial role in gastric carcinogenesis and was relevant to the degree of tumor differentiation, the expression of COX-2 protein was not correlated with survival rate. In addition, it made little sense in predicting gastric cancer prognosis. In contrast, other research results suggested that COX-2 was an independent prognostic factor for gastric cancer as the 5-year survival rate of COX-2 protein positively expressed patients was lower than that of negatively expressed patients. In addition, early-stage gastric cancer patients with high expression of COX-2 protein were at a higher risk for cancer-related death than those with a low level of COX-2 expression. Another study assessed the correlation between tumor progression and epithelial mesenchymal transition using multivariate analysis, and showed that COX-2 protein overexpression was an independent prognostic factor for poor survival, due to angiogenesis, cancer invasion and metastasis. Recently, scientists also found that COX-2 protein and p53 expression were independent prognostic factors.
factors for poor survival, in addition to late-stage disease and non-curative surgery[48]. Most of the findings illustrated above share the similar points, while the controversial points need to be further investigated to reach a consensus.

**MECHANISM OF ELEVATED COX-2 EXPRESSION IN GASTRIC CANCER**

**Helicobacter pylori infection**

Some studies suggested that *Helicobacter pylori* (*H. pylori*) infection was significantly related to COX-2 expression[46-48]. *H. pylori* infection can lead to a local inflammatory response, phenotypic change of epithelial cells, promotion of cell proliferation and inhibition of cell apoptosis, and ultimately an increased risk of gastric cancer[49].

*H. pylori* is classified as a class I carcinogen by the International Agency for Research on Cancer. Over-expression of COX-2 was detected in *H. pylori* positive gastritis compared with *H. pylori* negative gastritis[50]. COX-2 over-expression was found in 50%-80% of gastric cancer patients. Another study showed that 24 h after *H. pylori* infection of epithelial cells in mice, the expression of COX-2 and PGE2 were significantly elevated[51]. An in vitro study also obtained similar results[52]. After the co-culture of MKN 28 cell lines with *H. pylori* for 24 h, the COX-2 mRNA transcription level increased five-fold, and the expression of PGE2 increased three-fold, suggesting that synthesis of COX-2 and PGE2 was one of the factors for *H. pylori* associated gastric cancer[53].

The mechanism of COX-2 over-expression caused by *H. pylori* infection is not entirely clear. In an in vitro study, *H. pylori* induced the expression of COX-2 and inducible nitric oxide synthase by activating AP-1 of AGS cells[54]. Cytokines from the *H. pylori* associated inflammatory response also promoted the upregulation of COX-2[55]. In an in vitro study, *H. pylori* infection influenced the expression of 385 genes, and 160 of these genes were related to COX-2, including the inflammation genes (Icam1), the apoptosis genes (Ch1), the proliferation genes (Gdf3, Igf2), the gastric physiology genes (Galf-1) and the epithelial barrier function genes (Tjp1, Aqp5). After treatment with NS398, a COX-2 inhibitor, the expression of 140 genes changed, which indicated that COX-2 was correlated with the occurrence of gastritis[56]. Another study indicated that *H. pylori* could lead to the phosphorylation of p38 mitogen-activated protein kinase (p38 MAPK) and its downstream transcription factor ATF-2, and the expression of COX-2 could be inhibited by a p38 MAPK inhibitor. These results indicated that the p38/ATF-2 signal transduction pathway induced by *H. pylori* was the crucial mechanism involved in COX-2 expression[57]. Infection with *H. pylori* stimulated the secretion of gastrin which promoted the expression of COX-2 and extended the half-life of COX-2 mRNA[58]. In addition, the inhibitor of gastrin-releasing peptide decreased the expression of COX-2, which indicated that gastrin may be involved in COX-2 expression induced by *H. pylori*[58].

**Suppressor gene mutation**

An imbalance of oncogenes and suppressor genes is responsible for carcinogenesis. The mutation of a suppressor gene can lead to the occurrence of cancer. A study revealed that COX-2 expression in patients with *P53* mutation was higher than in those without *P53* mutation. This indicated that *P53* mutation might be related to COX-2 over-expression[59]. The protein of wild-type *P53* could inhibit the formation of a complex composed of TATA box binding proteins and promoters located upstream of the gene sequences, and eventually inhibited the expression of COX-2. In contrast, the product of mutant type *P53* could elevate COX-2 expression by the Ras/Raf/MAPK signal pathway. Moreover, COX-2 could reversibly induce mutation of *P53*, and both were co-expressed in gastric cancer tissues[60].

**p16** is a tumor suppressor gene located at chromosome 9p21. It can inhibit the function of cyclinD1/CDK4 and CDK6 complex, and cause p53-independent G1 arrest through the phosphorylation of pRB[61,62]. This gene is usually inactivated in human gastric cancers for different reasons. A study indicated that p16 was found to harbor promoter methylation associated with the loss of protein expression in cancer cells, suggesting that p16 inactivation due to promoter methylation may be important for gastric tumorigenesis[63]. Other mechanisms such as mutation and homozygous deletion, are also responsible for the inactivation of p16, which lead to the development and progression of tumors[64]. Researchers explored the expression of COX-2 protein and p16 protein in gastric cancer mucosa, and found that COX-2 protein expression was negatively related to p16 protein expression. There may be a relationship between the expression of COX-2 and p16. However, the mechanism involved needs to be clarified in further research[65,66].

**NF-κB**

NF-κB is a protein which has the ability to combine with the nucleotide sequence in the promoter region and enhancer region of some genes, and consequently activate or enhance the transcription of these genes. NF-κB is usually distributed in the cytoplasm in the inactive form under physiological conditions. It is then activated and enters the nucleus in response to outside stimuli. The NF-κB signal pathway is involved in many processes such as the inflammatory response, cell proliferation, apoptosis and carcinogenesis. Some inflammatory cytokines, such as tumor necrosis factor (TNF-α), can activate NF-κB, and this activated transcription factor can induce over-expression of inflammatory factors including COX-2 and TNF-α itself, forming the inflammatory network in the tumor microenvironment[67]. The COX-2 promoter region contains several elements, with the presence of two NF-κB consensus sites[68]. COX-2 expression decreased significantly when NF-κB was blocked by chondroitin sulfate[69,70]. Expression of COX-2 and NF-κB increased...
simultaneously during the process from chronic atrophic gastritis, dysplasia to gastric cancer. The co-expression of COX-2 and NF-κB played an important role in the angiogenesis of stomach tissues. In addition, NF-κB upregulated the expression of vascular endothelial growth factor (VEGF), which is an important promoter of angiogenesis[71].

MECHANISM OF COX-2 IN GASTRIC CARCINOGENESIS

Cell proliferation and apoptosis

Accumulating evidence indicates that inflammation plays an important role in the development of cancers[72,73]. TNF-α, which is a mediator of PGE2, plays a crucial role in mediating the inflammatory process through activation of NF-κB. It has been found that stromal NF-κB can enhance proliferation of epithelial cells by inducing cytokines, chemokines, and growth factors, such as IL-6, IL-1β, macrophage inflammatory protein-2 and TNF-α, while epithelial NF-κB can suppress apoptosis by inducing anti-apoptotic proteins, such as GADD45β, A1/Bfl1, and cIAP1[74,75]. As a product of AA catalyzed by COX-2, PGE2 is involved in gene mutation and cancer cell proliferation[76]. Protein encoded by COX-2 genes is a type of oncogenic protein, which could promote the high expression of PGE2. PGE2 can stimulate the growth of blood vessels, inhibit local immune function and regulate a variety of signal transduction pathways which ultimately influence the proliferation of cells and the growth of tumors[77]. It has also been confirmed that over-expression of COX-2 promoted cell proliferation by weakening the anti-proliferative effect of transforming growth factor-β (TGF-β)[78].

An in vitro study showed that cell proliferation was suppressed when gastric cells were treated with COX-2 small interfering RNA (siRNA)[79]. In MKN-45 cells, inhibition of COX-2 with NS-398 led to reduced proliferation and induction of apoptosis, connected with downregulation of Bcl-2 (an anti-apoptotic gene) and upregulation of Bax (an apoptotic gene)[80]. COX-2 was a regulatory factor in the Bcl-2 upstream sequences, which upregulated the expression of Mcl-1, a member of the Bcl-2 family, through the phosphatidylinositol 3-kinase (PI3K) signal pathway, and eventually inhibited the apoptosis of cancer cells[81]. Some studies confirmed that COX-2 could inhibit the apoptosis of cancer cells by inducing the mutation of P53[82]. Other researchers indicated that COX-2 weakened the apoptotic signal mediated by Fas protein. After adding the COX-2 inhibitor, they detected the elevated expression of caspase, a key enzyme of death receptor signaling and apoptotic signaling pathways[83].

Angiogenesis and lymphatic metastasis

The growth and metastasis of tumors depend on the formation of new blood vessels. PGE2 and PGF2α can promote vessel formation directly or indirectly[84]. Research has found that COX-2 over-expression was associated with increased PGE2 biosynthesis and angiogenesis in gastric cancer[85]. Furthermore, COX-2 can induce cells to produce VEGF and TGF-β, which could promote endotheliocyte migration and tubular morphogenesis. In addition, VEGF was an independent prognostic factor for gastric cancer prognosis[86]. After being transfected with COX-2 siRNA, the expression of VEGF was down-regulated and the growth of gastric cancer cells was significantly inhibited[87]. Other studies indicated that COX-2 upregulated the expression of Bcl-2 and Akt, which can inhibit the apoptosis of endothelial cells and promote vessel formation[88,89].

VEGF-C has been identified as a new member of the VEGF family and is considered a specific lymphangiogenic factor. It can promote the formation and dilation of lymphatic vessels, enhance the permeability of lymphatic vessels and facilitate lymphatic metastasis. A recent study confirmed a positive correlation between the expression of COX-2 and VEGF-C in gastric cancer patients, and both were related to gastric cancer prognosis[90].

Invasion and metastasis

Invasion is the premise of cancer metastasis which involves a variety of cytokines. Adhesion molecule is a type of cell surface glycoprotein that mediates cell adhesion. E-cadherin adhesion which inhibits the separation of cancer cells from tissue can prevent cancer cell invasion. COX-2 can lower the activity of E-cadherin, thus the invasiveness of cancer cells is enhanced for further metastasis. The activity of E-cadherin was enhanced after inhibition of COX-2[91]. It has been confirmed that the over-expression of matrix metalloproteinase (MMP) accelerated the decomposition of collagen in local tissues, which was beneficial to the spread of cancer cells. A correlation between COX-2 and MMP upregulation was also found[92]. COX-2 inhibitors reduce the expression of MMP[93]. CD44, which acts as the membrane receptor of hyaluronic acid and is expressed in cancer stem-like cells, played an important role in cancer metastasis. A large number of CD44(+) gastric glands was found in human adenocarcinomas and adjacent metaplasias, but not in normal gastric epithelium. In addition, CD44(+) tumor cell expansion is triggered by the cooperative actions of PGE2 and Wnt in gastric tumorigenesis[94]. Studies also confirmed that PGE2 could upregulate the expression of CD44[95], while COX-2 inhibitor could inhibit CD44 expression[96]. A study demonstrated that CD44v, a variant form of CD44, could protect tumor cells from oxidative stress in a mouse gastric cancer model, thus it plays an important role in tumor development[97]. Other possible mechanisms include the upregulation of urokinase-type plasminogen activator (uPA) in promoting the metastasis of cancer cells[98] and more potential pathways need to be further clarified.

Immunosuppression

It has been found that COX-2 was involved in the im-
munosuppression in gastric cancer, where effector T cells were suppressed by regulatory T cells. In Treg cells, expression of COX-2 was correlated with that of forkhead box p3. By using a COX-2 inhibitor, the immunosuppression of effector T cells was reversed. The possible mechanisms involved may be as follows: PGE2 disabled the function of dendritic cells in the tumor microenvironment and the cells could not present the tumor antigen effectively, and eventually the T cells did not recognize or kill the cancer cells. In addition, PGE2 may also reduce the immunosurveillance of the immune system on mutant cells by inhibiting the expression of human leucocyte antigen I and II, and by reducing the production of lymphokine. The immunosurveillance effect could be enhanced by using a COX-2 inhibitor and stimulating the activity of natural killer cells.

CONCLUSION

The COX-2/PGE2 pathway involved in the inflammatory response plays a critical role in the microenvironment of gastric tumorigenesis. Expression of COX-2 is elevated in gastric cancer and its over-expression is associated with H. pylori infection, mutation of suppressor genes and NF-kB. Over-expressed COX-2 participates in gastric carcinogenesis by promoting cell proliferation, inhibiting cell apoptosis, inducing vessel formation, and enhancing metastasis and immunosuppression. Although progress has been made in exploring the mechanism of gastric cancer development, some issues remain to be explored in further studies. As research continues, interventions in gastric cancer using COX-2 as a target might eventually become a specific treatment of choice.

REFERENCES

1. Bornschein J, Rokkas T, Selgrad M, Malfertheiner P. Gastric cancer: clinical aspects, epidemiology and molecular background. Helicobacter 2011; 16 Suppl 1: 45-52 [PMID: 21896085 DOI: 10.1111/j.1537-2473.2010.00880.x]
2. Jing J, Liu HY, Hao JK, Wang LN, Wang YP, Sun LH, Yuan Y. Gastric cancer incidence and mortality in Zhonghe, China, between 2005 and 2010. World J Gastroenterol 2012; 18: 1262-1269 [PMID: 22468091 DOI: 10.3748/wjg.v18.i11.1262]
3. Krejš GJ. Gastric cancer: epidemiology and risk factors. Dig Dis 2010; 28: 600-609 [PMID: 20884090 DOI: 10.1159/000320277]
4. Lin Y, Ueda J, Kikuchi S, Totsuka Y, Wei WQ, Qiao YL, Inoue M. Comparative epidemiology of gastric cancer between Japan and China. World J Gastroenterol 2011; 17: 4421-4428 [PMID: 22110269 DOI: 10.3748/wjg.v17.i39.4421]
5. Smyth EM, Grosser T, Wang M, Yu F, FitzGerald GA. Prostanoids in health and disease. J Lipid Res 2009; 50 Suppl: S423-S428 [PMID: 19095631 DOI: 10.1194/jlr.R800094-JLR200]
6. Yu Q, Purwahana P, Ni K, Sun C, Mallik S, Qian SY. Characterization of novel radicals from COX-catalyzed arachidonic acid peroxidation. Free Radic Biol Med 2009; 47: 568-576 [PMID: 19482075 DOI: 10.1016/j.freeradbiomed.2009]
7. Ruan YC, Zhou W, Chan HC. Regulation of smooth muscle contraction by the epithelium: role of prostaglandins. Physiology (Bethesda) 2011; 26: 156-170 [PMID: 21670162 DOI: 10.1152/physiol.00036.2010]
8. Hein AM, O'Banion MK. Neuroinflammation and memory: the role of prostaglandins. Mol Neurobiol 2009; 40: 15-32 [PMID: 19365736 DOI: 10.1007/s12035-009-8866-2]
9. Stratton R, Shiivon X. Role of prostaglandins in fibroblast activation and fibrosis. J Cell Commun Signal 2010; 4: 75-77 [PMID: 20531982 DOI: 10.1007/s12079-010-0089-8]
10. Gibb W. The role of prostaglandins in human parturition. Ann Med 1998; 30: 235-241 [PMID: 9677008 DOI: 10.3109/0785398980905850]
11. Wang D, Mann JR, Dubois RN. The role of prostaglandins and other eicosanoids in the gastrointestinal tract. Gastroenterology 2009; 136: 1245-1461 [PMID: 19587126 DOI: 10.1053/j.gastro.2004.09.080]
12. Brown JR, Dubois RN. COX-2: a molecular target for colorectal cancer prevention. J Clin Oncol 2005; 23: 2840-2855 [PMID: 15837998 DOI: 10.1200/jco.2005.09.051]
13. Hogenдорf P, Düryczynski A, Kumar A, Strzelczyk J. Prostaglandin E2 (PGE2) in portal blood in patients with pancreatic tumor—a single institution series. J Invest Surg 2012; 25: 8-13 [PMID: 22272632 DOI: 10.3109/08941939.2011.592569]
14. Li J, Feng G, Liu J, Rong R, Luo F, Guo L, Zhu T, Wang G, Chu Y. Renal cell carcinoma may evade the immune system by converting CD4+Foxp3- T cells into CD4+CD25+Foxp3+ regulatory T cells: Role of tumor COX-2-derived PGE2. Mol Med Rep 2010; 3: 959-963 [PMID: 21472340 DOI: 10.3892/mmr.2010.374]
15. Davidge ST. Prostaglandin H synthase and vascular function. Circ Res 2001; 89: 650-660 [PMID: 11597987 DOI: 10.1161/01.hsr.2001.088311]
16. Bamba H, Ota S, Arai S, Ban S, Shimizu M, Imai Y, Kawamoto C, Yoshida Y, Fujiwara K. Expression of cyclooxygenase-2 in human hyperplastic gastric polyps. J Exp Clin Cancer Res 2003; 22: 425-430 [PMID: 14582702]
17. Peskar BM, Sawka N, Ehrlich K, Peskar BA. Role of cyclooxygenase-1 and -2, phospholipase C, and protein kinase C in prostaglandin-mediated gastroprotection. J Pharmacol Exp Ther 2003; 305: 1233-1238 [PMID: 12666249 DOI: 10.1124/jpet.103.049650]
18. Tokuyama H, Hayashi K, Matsuda H, Kubota E, Honda M, Okubo K, Ozawa Y, Saruta T. Distinct role of intrarectal cyclooxygenase-1/2 in chronic unilateral renal ischemia. Neprhon 2002; 92: 183-191 [PMID: 12187101 DOI: 10.1159/000064479]
19. Lipsky PE. Role of cyclooxygenase-1 and -2 in health and disease. Am J Orthop (Belle Mead NJ) 1999; 28: 8-12 [PMID: 10193997]
20. Saukkonen K, Rintahaka J, Sivula A, Buskens CJ, Van Rees BP, Rio MC, Haglund C, Van Lanschot JJ, Offerhaus GJ, Ristimäki A. Cyclooxygenase-2 and gastric carcinogenesis. APMIS 2003; 111: 915-925 [PMID: 14616542 DOI: 10.1034/j.1600-0463.2003.1111001.x]
21. Sonoda R, Naomota Y, Shirakawa Y, Fujiwara Y, Yamatsui T, Noma K, Tanabe S, Takaoka M, Gunduz M, Tsujiigawa H, Nagatsuka H, Ohara N, Yoshino T, Takubo K, Vieth M, Tanaka N. Preferential up-regulation of heparanase and cyclooxygenase-2 in carcinogenesis of Barrett's oesophagus and intestinal-type gastric carcinoma. Histopathology 2010; 57: 90-100 [PMID: 20653782 DOI: 10.1111/j.1365-2559.2010.03594.x]
22. Yao L, Liu F, Hong L, Sun L, Liang S, Wu K, Fan D. The function and mechanism of COX-2 in angiogenesis of gastric cancer cells. J Exp Clin Cancer Res 2011; 30: 13 [PMID: 21266034 DOI: 10.1186/1756-9966-30-13]
23. Ristimäki A, Honkanen N, Jänkälä H, Sipponen P, Härkönen M. Expression of cyclooxygenase-2 in human gastric carcinoma. Cancer Res 1997; 57: 1276-1280 [PMID: 9102213]
24. Kargman S, Charleson S, Carterwright M, Frank J, Riendeau D, Mancini J, Evans J, O'Neill G. Characterization of Prostaglandin G/H Synthase 1 and 2 in rat, dog, monkey, etc.

Cheng J et al. COX-2 and gastric cancer
and human gastrointestinal tracts. *Gastroenterology* 1996; 111: 445-454 [PMID: 8690211 DOI: 10.1053/gast.1996.v111. pmid8690211]

25 Parkin DM. The global health burden of infection-associated cancers in the year 2002. *Int J Cancer* 2006; 118: 3053-3044 [PMID: 16404738 DOI: 10.1002/ijc.21731]

26 Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. *Cell* 2010; 140: 883-899 [PMID: 20303878 DOI: 10.1016/j.cell.2010.01.025]

27 Wang D, Dubois RN. The role of COX-2 in intestinal inflammation and colorectal cancer. *Oncogene* 2010; 29: 781-788 [PMID: 19946329 DOI: 10.1038/onc.2009.421]

28 de Heer P, Gensens MJ, de Bruin EC, Dekker-Ensink NG, Putter H, Marijnen CA, van den Brule AJ, van Krieken JH, Rutten HJ, Kuppen PJ, de Velde CJ. Cyclooxygenase-2 expression in rectal cancer is of prognostic significance in patients receiving preoperative radiotherapy. *Clin Cancer Res* 2007; 13: 2955-2960 [PMID: 17504966 DOI: 10.1158/1078-0432.ccr-06-1023]

29 Higashi Y, Kanekura T, Kanzaki K. Enhanced expression of cyclooxygenase (COX)-2 in human skin epidermal cancer cells: evidence for growth suppression by inhibiting COX-2 expression. *Int J Cancer* 2000; 86: 667-671 [PMID: 10797288 DOI: 10.1002/(sici)1072-0241(20000601)86:3<667::aid-ijc12>3.0.co;2-5>]

30 Faroqui M, Li Y, Rogers T, Poonawala T, Griffin RJ, Song CW, Gupta K. COX-2 inhibitor celecoxib prevents chronic morphine-induced promotion of angiogenesis, tumour growth, metastasis and mortality, without compromising analgesia. *Br J Cancer* 2007; 97: 1523-1531 [PMID: 17971669 DOI: 10.1038/sj.bjc.6600457]

31 Uefuji K, Ichikura T, Mochizuki H, Shinomiya N. Expression of cyclooxygenase-2 protein in gastric adenocarcinoma. *J Surg Oncol* 1998; 69: 168-172 [PMID: 9846504 DOI: 10.1002/(sici)1096-9098(199811)69:2<168::aid-sno>3.0.co;2-d>]

32 Lim HY, Joo HJ, Choi HY, Yi JW, Yang MS, Cho DY, Kim HS, Nam DK, Lee KB, Kim HC. Increased expression of cyclooxygenase-2 protein in human gastric carcinoma. *Clin Cancer Res* 2006; 6: 519-525 [PMID: 16605332]

33 Mao XY, Wang XG, Lv XJ, Xu L, Han CB. COX-2 expression in gastric cancer and its relationship with angiogenesis using tissue microarray. *World J Gastroenterol* 2007; 13: 3466-3471 [PMID: 17659693]

34 Murata H, Kawanoto S, Tsujii S, Tsuji M, Sawaoka H, Kimura Y, Shiozaki H, Horii M. Cyclooxygenase-2 overexpression enhances lymphatic invasion and metastasis in human gastric cancer. *Ann J Gastroenterol* 2001; 96: 423-439 [PMID: 10022645 DOI: 10.1111/j.1570-0215.1999.876.x]

35 Mrena J, Wiksten JP, Thiel A, Kokkola A, Pohjola L, Lundin J, Nordling S, Ristimäki A, Polkowski W. Cyclooxygenase-2 expression during carcinogenesis in the human stomach. *J Pathol* 2002; 196: 171-179 [PMID: 11793368 DOI: 10.1010/path.1033]

36 Jou YE, Oh WT, Rew JS, Park CS, Choi SK, Kim SJ. Cyclooxygenase-2 expression is associated with well-differentiated and intestinal-type pathways in gastric carcinoma. *Digestion* 2002; 66: 222-229 [PMID: 12592098 DOI: 10.1159/000068366]

37 Han SL, Tang HJ, Hua YW, Ji SQ, Lin DX. Expression of COX-2 in stomach cancers and its relation to their biological features. *Dig Surg* 2003; 20: 107-114 [PMID: 12866777 DOI: 10.1159/000069384]

38 Fanelli MF, Chinen LT, Begnami MD, Costa WL, Fregnani JH, Soares FA, Montagnini AL. The influence of transforming growth factor-beta, cyclooxygenase-2, matrix metalloproteinase (MMP)-7, MMP-9 and CXCR4 proteins involved in epithelial-mesenchymal transition on overall survival of patients with gastric cancer. *Histopathology* 2012; 61: 153-161 [PMID: 22582975 DOI: 10.1111/j.1365-2559.2011.04139.x]

39 Mrena J, Wiksten JP, Kokkola A, Nordling S, Ristimäki A, Haglund C. COX-2 is associated with proliferation and apoptosis markers and serves as an independent prognostic factor in gastric cancer. *Tumour Biol* 2010; 31: 1-7 [PMID: 20257896 DOI: 10.1159/00032779-00001-4]

40 Zhang JJ, Wang SY, Hao XH, Zhu ZL, Chu JK, Ma JC, Cui DS, Gu P, Zhao ZR, Wang MW, Yu J. Anti-Helicobacter pylori therapy followed by celecoxib on progression of gastric precancerous lesions. *World J Gastroenterol* 2009; 15: 2731-2738 [PMID: 19522023 DOI: 10.3748/wjg.v15.i17.2731]

41 Pero R, Peluso S, Angrisano T, Tuccillo C, Sacchetti S, Keller S, Tomaiuolo R, Bruni CB, Lembo F, Chiariotti L. Chromatin and DNA methylation dynamics of Helicobacter pylori-induced COX-2 activation. *Int J Med Microbiol* 2011; 301: 140-149 [PMID: 21053479 DOI: 10.1016/j.ijmm.2010.08.009]

42 Targosz A, Brzozowski T, Pierzchalski P, Szczeryk U, Prak-Belowska A, Konturek SJ, Pawlik W. Helicobacter pylori stimulates apoptosis, activates cyclooxygenase (COX)-2 and inhibits heat shock protein HSP70 in gastric cancer epithelial cells. *Inflamm Res* 2012; 61: 955-966 [PMID: 22610150 DOI: 10.1007/s00118-012-0847-x]

43 Asaka M. Helicobacter pylori infection and gastric cancer. * Intern Med* 2002; 41: 1-6 [PMID: 11835883 DOI: 10.2169/internalmedicine.41.1]

44 Fu S, Ramanujam KS, Wong A, Fantry GT, Drachenberg CB, James SP, Meltzer SJ, Wilson KT. Increased expression and cellular localization of inducible nitric oxide synthase and cyclooxygenase 2 in Helicobacter pylori gastritis. *Gastroenterology* 1999; 116: 1319-1329 [PMID: 10348815 DOI: 10.1016/S0016-5085(99)07046-8]

45 Xiao F, Furuta T, Takashina M, Shirai N, Hanai H. Involvement of cyclooxygenase-2 in hyperplastic gastritis induced by Helicobacter pylori infection in C57BL/6 mice. *Aliment Pharmacol Ther* 2001; 15: 875-886 [PMID: 11380326 DOI: 10.1046/j.1365-2056.2001.00965.x]

46 Romano M, Ricci V, Memoli A, Tuccillo C, Di Popolo A, Sommi P, Aquaviva AM, Del Vecchio Blanco C, Bruni CB, Zarrilli R. Helicobacter pylori up-regulates cyclooxygenase-2 mRNA expression and prostaglandin E2 synthesis in
Cheng J et al. COX-2 and gastric cancer

81 Chen XL, Su BS, Sun RQ, Zhang J, Wang YL. Relationship between expression and distribution of cyclooxygenase-2 and bcl-2 in human gastric adenocarcinoma. World J Gastroenterol 2005; 11: 1228-1231 [PMID: 15754411]

82 Han JA, Kim JJ, Ongusaha PP, Hwang DH, Ballou LR, Mahale A, Aaronson SA, Lee SW. F53-mediated induction of Cox-2 counteracts p53- or genotoxic stress-induced apoptosis. EMBO J 2002; 21: 5635-5644 [PMID: 12411481 DOI: 10.1093/emboj/cdf591]

83 Casado M, Mollá B, Roy R, Fernández-Martínez A, Cucarel la C,Mayoral R, Boscá L, Martín-Sanz P. Protection against Fas-induced liver apoptosis in transgenic mice expressing cyclooxygenase 2 in hepatocytes. Hepatology 2007; 45: 631-638 [PMID: 17326157 DOI: 10.1002/hep.21556]

84 Bamba H, Ota S, Kato A, Kawamoto M, Fujitaka K. Prostaglandins up-regulate vascular endothelial growth factor production through distinct pathways in differentiated U937 cells. Biochem Biophys Res Commun 2000; 273: 485-491 [PMID: 10873632 DOI: 10.1006/bbrc.2000.2969]

85 Uefuji K, Ichikura T, Mochizuki H. Cyclooxygenase-2 expression is related to prostaglandin biosynthesis and angiogenesis in human gastric cancer. Clin Cancer Res 2000; 6: 135-138 [PMID: 10656441]

86 Vidal O, Soriano-Izquierdo A, Pera M, Elizalde JJ, Palacín M, Castells A, Piqué JM, Volant A, Metges JP. Positive VEGF immunostaining independently predicts poor prognosis in curatively resected gastric cancer patients: results of a study assessing a panel of angiogenic markers. J Gastrointest Surg 2008; 12: 1005-1014 [PMID: 17972143 DOI: 10.1007/s11605-007-0336-3]

87 Kim N, Kim CH, Ahn DW, Lee KS, Cho SJ, Park JH, Lee MK, Kim JS, Jung HC, Song IS. Anti-gastric cancer effects of celecoxib, a selective COX-2 inhibitor, through inhibition of Akt signaling. J Gastroenterol Hepatol 2009; 24: 480-487 [PMID: 18825436 DOI: 10.1111/j.1440-1746.2008.05599.x]

88 Piggeon GP, Barr MP, Harmey JH, Foley DA, Boucher-Hayes DJ. Vascular endothelial growth factor (VEGF) upregulates BCL-2 and inhibits apoptosis in human and murine mammary adenocarcinoma cells. Br J Cancer 2001; 85: 273-278 [PMID: 11461089 DOI: 10.1054/bjoc.2001.1876]

89 Gou HF, Chen XC, Zhu J, Jiang M, Yang Y, Cao D, Hou M. Expressions of COX-2 and VEGF-C in gastric cancer: correlations with lymhangiogenesis and prognostic implications. J Exp Clin Cancer Res 2011; 30: 14 [PMID: 21272377 DOI: 10.1186/1477-5622-30-14]

90 Rao DS, Gui D, Koski ME, Popovicic LM, Wang H, Reiter RE, Said JW. An inverse relation between COX-2 and E-cadherin expression correlates with aggressive histologic features in prostate cancer. Appl Immunohistochem Mol Morphol 2006; 14: 375-383 [PMID: 17122632 DOI: 10.1097/01.pai.0000201477.61176.6c]

91 Dicken BJ, Graham K, Hamilton SM, Andrews S, Lai R, Listgarten J, Jiangri GS, Saunders LD, Damaraju S, Cass C. Lymphovascular invasion is associated with poor survival in gastric cancer: an application of gene-expression and tissue array techniques. Ann Surg 2006; 243: 64-73 [PMID: 16371738 DOI: 10.1097/01.sla.0000194087.96582.3e]

92 Zhao Y, Zhou S, Heng CK. Celecoxib inhibits serum amylo id-a-induced matrix metalloproteinase-10 expression in human endothelial cells. J Vasc Res 2009; 46: 64-72 [PMID: 18552508 DOI: 10.1159/000139134]

93 Ishimoto T, Oshima H, Oshima M, Kai K, Torii R, Masuko T, Baba H, Saya H, Nagano O. CD44+ slow-cycling tumor cell expansion is triggered by cooperative actions of Wnt and prostaglandin E2 in gastric tumorigenesis. Cancer Sci 2010; 101: 673-678 [PMID: 20028388 DOI: 10.1111/j.1349-7006.2009.01430.x]

94 Dohadwala M, Batra RK, Luo J, Lin Y, Krysan K, Pold M, Sharma S, Dubinett SM. Autocrine/paracrine prostaglandin E2 production by non-small cell lung cancer cells regulates matrix metalloproteinase-2 and CD44 in cyclooxygenase-2-dependent invasion. J Biol Chem 2002; 277: 50826-50833 [PMID: 12993872 DOI: 10.1074/jbc.M210707200]

95 Jia XQ, Zhong N, Han LH, Wang JH, Yan M, Meng FL, Zhang SZ. Effect of NS-398 on colon cancer cells. World J Gastroenterol 2005; 11: 353-356 [PMID: 15637743]

96 Ishimoto T, Nagano O, Yae T, Tamada M, Motohara T, Oshima H, Oshima M, Ikeda T, Asaba R, Yagi H, Masuko T, Shimizu T, Ishikawa T, Kai K, Takahashi E, Imamura Y, Baba Y, Ohmura M, Suehata M, Baba H, Saya H. CD44 variant regulates redox status in cancer cells by stabilizing the xCT subunit of system xc(-) and thereby promotes tumor growth. Cancer Cell 2011; 19: 389-400 [PMID: 21397861 DOI: 10.1016/j.ccr.2011.01.038]

97 Shin YY, Wu WK, Chu KM, Wong HP, Lam EK, Tai EK, Koo MW, Cho CH. Nicotine induces cyclooxygenase-2 and vascular endothelial growth factor receptor-2 in association with tumor-associated invasion and angiogenesis in gastric cancer. Mol Cancer Ther 2005; 3: 607-615 [PMID: 16317086 DOI: 10.1158/1535-7163.mct-05-0106]

98 Yuan XL, Chen L, Li MX, Dong P, Xue J, Wang J, Zhang TT, Wang XA, Zhang FM, Ge HL, Shen LS, Xu D. Elevated expression of Fopx3 in tumor-infiltrating Treg cells suppresses T-cell proliferation and contributes to gastric cancer progression in a COX-2-dependent manner. Clin Immunol 2010; 134: 277-288 [PMID: 19900843 DOI: 10.1016/j.clim.2009.10.005]

99 Ahmadi M, Emery DC, Morgan DJ. Prevention of both direct and cross-priming of antitumor CD8+ T-cell responses following overproduction of prostaglandin E2 by tumor cells in vivo. Cancer Res 2008; 68: 7520-7529 [PMID: 18794140 DOI: 10.1158/0008-5472.can-08-1060]

100 Chen TH, Fukuhara K, Mandai M, Matsumura N, Kariya M, Takakura K, Fujii S. Increased cyclooxygenase-2 expression is correlated with suppressed antitumor immunity in cervical adenocarcinomas. Int J Gynecol Cancer 2006; 16: 772-779 [PMID: 16681759 DOI: 10.1111/j.1525-1438.2006.00385.x]
