RESEARCH ARTICLE

Associations between Single Nucleotide Polymorphisms of COX-2 and MMP-2 Genes and Colorectal Cancer Susceptibility in the Saudi Population

Manal Ali Shalaby[1], Howaida Attia Nounou[2], Mohammad Saud Alanazi[3], Othman Alharby[4], Nahla Azzam[4], Hesham Mahmoud Saeed[5]*

Abstract

Background: It has been reported that COX-2 expression is associated with MMP-2 expression in thyroid and breast cancers, suggesting that MMPs are linked to COX-2-mediated carcinogenesis. Several polymorphisms within the *MMP2* promoter region have been reported in cases with oncogenesis and tumor progression, especially in colorectal carcinogenesis. Materials and Methods: This research evaluated risk of association of the SNPs, including genes for COX-2 (A/G transition at +202) and MMP-2 (C/T transition at-1306), with colorectal cancer in 125 patients and 125 healthy controls. Results and Conclusions: Our data confirmed that *MMP2* C-1306 T mutations were significantly more common in colon cancer patients than in our control Saudi population; p=0.0121. On the other hand in our study, there was no significant association between genotype distribution of the *COX2* polymorphism and colorectal cancer; p=0.847. An elevated frequency of the mutated genotype in the control group as compared to the patients subjects indeed suggested that this polymorphism could decrease risk in the Saudi population. Our study confirmed that the polymorphisms that could affect the expressions of MMP-2 and COX-2 the colon cancer patients were significantly higher than that in the COX-2 negative group. The frequency of individuals with *MMP2* polymorphisms in colon cancer patients was higher than individuals with combination of *COX2* and *MMP2* polymorphisms. Our study confirmed that individuals who carried the polymorphisms that could affect the expressions of *COX2* are more susceptible to colon cancer. *MMP2* regulatory polymorphisms could be considered as protective; further studies need to confirm the results with more samples and healthy subjects.

Keywords: Colorectal cancer - single nucleotide polymorphism - matrix metalloproteinase - cyclooxygenase

Asian Pac J Cancer Prev, **15 (12)**, 4989-4994

Introduction

Colorectal cancer (CRC) is one of the major causes of mortality worldwide. It is ranked the third most common form of cancer worldwide in terms of incidence (Brown and DuBois, 2005), with unanimous findings that prostaglandins are important for both carcinogenesis and progression (Greenhough et al., 2009; Gustafsson et al., 2007a; 2007b). Clinical and experimental studies reported that Cyclooxygenase-1/-2 inhibition attenuates appearance of epithelial cancer (Ruud et al., 2013). While induced prostaglandin production, particularly PGE2, is known to be involved in cell signaling through prostanoid receptors, other observations suggested subtype EP2 receptor expression in colon cancer tissue to predict reduced survival (Gustafsson et al., 2007a). Gene expression studies in colon cancer showed that the appearance and progression of colorectal cancer seems to be related to increased production of PGE2 secondary to *COX-2* induction (Hull et al., 2004; Gustafsson et al., 2007a; Cahlin et al., 2008; Ruud et al., 2013).

In an original observational study, Chan et al., (2007) observed that aspirin's preventive role was effective only in the subgroup of colon cancers overexpressing *COX-2* (a member of the cox family of enzymes) in a dose and treatment duration dependent manner. The COX family consists of two isozymes: COX-1and *COX-2*. While COX-1 is constitutively expressed in a wide range of organs and responsible for tissue homeostasis *COX-2* is induced under inflammatory and tumor promotion stimuli, being overexpressed in approximately 50% of adenomas and 85% of colon adenocarcinomas (Eberhart et al.,

*[1]Genetic Engineering and Biotechnology Research Institute (GEBRI), City for Scientific Research and Technology Applications, [2]Department of Medical Biochemistry, Faculty of Medicine, [5]Institute of Graduate Studies and Research, Department of Bioscience and Technology, Alexandria University, Alexandria, Egypt, [3]Department of Biochemistry, College of Science, [4]Department of Internal Medicine, Division of Gastroenterology, King Khalid University Hospital, King Saud University, Riyadh, KSA *For correspondence: hesham25166@hotmail.com, hsaeed1@ksu.edu.sa*

1994). Animal studies have shown that pharmacological inhibition or genetic deletion of *COX-2* leads to reduced tumour formation in the rodent gastrointestinal tract, (Oshima et al., 1996; Chulada; et al., 2000; Thiel et al., 2012; Chun and Surh, 2014) whereas expression of *COX-2* directly induces tumour growth (Oshima et al., 2004).

Identification of individuals who express higher levels of *COX-2* through the interaction between the genetic background and environmental exposure could be useful for personalized treatment (Markowitz, 2007). The involvement of *COX-2* genetic variations in colorectal tumor development has been proposed in many reports (Pereira et al., 2009). The-1195A>G (rs689466) and-765G>C (rs20417) polymorphisms, identified in gene's promoter region, are expected to modulate *COX-2* expression by altering the recognition binding site for specific nuclear proteins, thus influencing the genetic susceptibility for CRC onset (Papafili et al., 2002; Zhang et al., 2005) In contrast, the 8473T>C (rs5275) polymorphism in an AU-rich elements region (30UTR) might contribute to cancer development by influencing *COX-2* mRNA stability (Campa et al., 2004; Langsenlehner et al., 2006; Song et al., 2013).

MMPs are large family of zinc-dependent endopeptidases secreted as latent pro-enzymes by both stromal and cancer cells (Wang et al., 1995). They are activated by proteolytic removal of an N- terminal domain and function in the degradation of ectracellular matrix proteins that constitutes connective tissues. They can be classified into four groups on the basis of sequence homology and substrate specificity: collagenases, gelatinases [including gelatinase A (*MMP-2*) and gelatinase B (*MMP-9*)], stromelysins and membrane- type metalloproteinases (Wastson SA et al., 1995; Wastson SA et al., 1996). The overexpression of MMPs is known to be associated with tumor invasion, metastasis, and a worse prognosis (Kessenbrock et al., 2010).

Several polymorphisms within the MMP2 promoter regions have been reported in cases with oncogenesis and tumor progression, especially in colorectal carcinogenesis (Decock et al., 2008) Inhibition of MMPs provides one attractive target for a novel class of therapeutic agents to control tumor progression and metastatic spreading. (Wastson et al., 1995; 1996; Zervos et al., 1997)

It has been reported that *COX-2* expression is associated with *MMP-2* expression in thyroid and breast cancers, suggesting that MMPs are linked to *COX-2*-mediated carcinogenesis (Siironen et al., 2004; Sivula et al., 2005). All the downstream cellular effects of *COX-2* are not yet established. *COX-2*-derived prostanoids may, however, induce production of MMPs, in tissue culture experiments (Callejas et al., 2001).

COX-2 inhibitors may reduce synthesis of active MMPs. In addition, inhibition of *COX-2* causes reduction in pro-*MMP-2* expression in ovarian carcinoma cells and *MMP-9* expression is reduced in gastric cancer cells by the blocking of *COX-2* (Church et al., 2003; Symowicz et al., 2005; Wu et al., 2005).

This research will investigate the risk of association of the SNPs, including genes for *COX-2* (A/G transition at +202) and *MMP-2* (C/T transition at -1306), with colorectal cancer in 125 colorectal cancer patients and 125 healthy controls.

Materials and Methods

Chemicals

All chemicals used were of analytical reagent, molecular biology, or chromatographic grade as appropriate. Water was deionized and distilled.

Samples collection

This study was conducted after review and approval of the Institutional Review Board of the Ethics Committee at King Khalid University Hospital in Riyadh, Kingdom of Saudi Arabia. Blood samples were collected from 90 confirmed colon cancer patients (age range, 33-77 years; mean age, 49 years) and 92 age matched healthy controls. The diagnosis of cancer was based on standard clinical, endoscopic, radiological, and histological criteria.Clinical and demographic characteristics were recorded, including age at diagnosis, estrogen receptor, progesterone receptor, HER status, family history, smoking habits, disease behavior, disease location, and need for surgery.

Genomic DNA isolation and purification

Genomic DNA was isolated from whole blood samples using DNeasy Blood kit (Qiagen) where samples were first lysed, then the lysate was loaded onto the DNeasy Mini spin column. Concentrations and purity of DNA samples were assessed using NanoDrop 8000 (Thermo Scientific). Genotyping for *MMP-2* and *COX-2*

Genotyping for the C-1306 T (rs 243865) in the 5'-flanking region of *MMP-2* gene (Lacchini et al., 2012) was performed by real time polymerase chain reaction (RTPCR) using TaqMan Allele. Discrimination assay (Applied Biosystems, Carlsbad, CA, USA). Probes and primers used for the C-1306T genotyping assay were customized as follows: forward 5'-GCCATTGTCAATGTTCCCTAAAACA-3'; reverse 5'-TGACTTCTGAGCTGAGACCTGAA-

3' and probes 5'-CAGCACTC [T/C] ACCTCT-3'. Genotyping for *COX-2* the +202 A/G (rs2745557) in the 3'-flanking region of *COX-2* gene (Ozhan et al., 2011) was performed by real time polymerase chain reaction (RTPCR) using TaqMan Allele. Discrimination assay (Applied Biosystems, Carlsbad, CA, USA). Probes and primers used for the +202 A/G genotyping assay were customized as follows: forward 5'-CCCTATTTAGGAGGTGAGAG-3'; reverse 5'-TCAGCCATACAGGTGAGTAC-3'and probes 5'-AACAGAAAATCTGAGAAAACATATC [A/G] TTATTCAAGCACAGCTTGGTACTTC- 3'

TaqMan PCR was performed in a total volume of 20 µl containing 20 ng of genomic DNA, 1x TaqMan master mix and 1x assay mix placed in 96-well PCR plate. The PCR conditions were 1 cycle at 95°C for 5 min followed by 30 cycles at 95°C for 40 seconds, 54°C for 40 seconds, and 68°C for 1 min. The final extension step was carried out at 72°C for 5 min. Fluorescence from PCR amplification was detected using Chromo 4 detector (Applied Biosystems 7500 Fast Real Time PCR System) and analyzed with the manufacturer's software.

TaqMan Real Time PCR assay is based TaqMan probes consist of a fluorophore covalently attached to the 5' end of the oligonucleotide probe and a quencher at the 3' end. TaqMan probes are designed such that they anneal within a DNA region amplified by a specific set of primers. The Taq polymerase extends the primer and synthesizes the nascent strand, the 5' to 3' exonuclease activity of the polymerase degrades the probe that has annealed to the template. Degradation of the probe releases the fluorophore from it and breaks the close proximity to the quencher, thus relieving the quenching effect and allowing fluorescence of the fluorophore. Hence, fluorescence detected in the real-time PCR thermal cycler is directly proportional to the fluorophore released and the amount of DNA template present in the PCR.

PCR sequencing

Products sequencing was carried out according to Sanger et al., (1977) using the MegaBACE

1000 DNA Sequencing System (Pharmacia/Amersham Co.). The chain termination sequencing reaction was conducted utilizing the DYEnamic ET terminator kit as an integral part of the MegaBACE 1000 DNA sequencing system. DNA template, unlabeled primer, buffer, the four dNTPs, the four fluorescently labeled ddNTPs, and AmpliTaq DNA Polymerase are added to the reaction tube. Fluorescent fragments are generated by incorporation of dye-labeled ddNTPs. Each different ddNTP (ddATP, ddCTP, ddGTP, or ddTTP) will carry a different color of dye. All terminated fragments (those ending with a ddNTP), therefore, will contain a dye at their 3' end. Big-Dye terminators utilize single energy transfer molecules, which include an energy donor and acceptor dye connected by a highly efficient energy transfer linker. In the structure of the Big Dye molecule, the acceptor is a dichlororhodamine dye. These brighter, cleaner dyes result in a sequencing chemistry suiTable for most applications. The sequencing reaction products were purified using DyeEx 2.0 Spin Kit (63206 Qiagen) and applied to MegaBace 1000 Sequencing machine.

Statistical analysis

Fisher's exact test and the odds ratio (OD) with 95% confidence interval (CI) were used to test the association between cancer and the studied genetic polymorphisms and to describe the strength of the association. The associations were considered to be statistically significant if the Fisher's exact p-value was less than 0.05 and if the 95% CI excluded the value 1.0. All statistical calculations were done using MedCalc Software (Version 11.3.1.0© 2010 MedCalc Software bvba).

Results

It should be noted that the molecular tools used for the genotyping do not determine whether other polymorphisms in the same gene are also present in the tested subject. The wild type (wt) denotation thus refers to the wild type allele at the investigated polymorphic site only, regardless of other unstudied polymorphisms for that particular gene. Furthermore, in this study, there were no significant differences in the distribution of genotypes for male groups when compared with female group

A total of 125 colon cancer cases and 125 healthy controls were included in this case-control based study. Clinical characteristics of colon cancer cases and healthy controls are given in Table 1. Among the colon cancer patients, 80 males and 45 females, 55.2% were smokers; and 63.2% had the cancer in the colon, 36.8% in the rectum.

The genotype and allele frequencies of the C-1306 T in the 5'-flanking region of *MMP-2* gene in 125 CRC patients

Table 1. Clinical Characteristics of Study Subjects

Variable		Colorectal cancer	Healthy controls	p value
Age group	≤ 60	55	42	0.0923
	≥ 60	70	83	
Gender	Female	45	47	0.6044
	Male	80	78	
Smoking status	Smoker	69(55.2)	65	0.8861
	Nonsmoker	56(44.8)	60	
Tumor Location	Colon	79(63.2)		
	Rectum	46(36.8)		

Table 2. Genotype Frequencies of MMP2 Gene Polymorphism in Colon Cancer Cases and Controls

MMP2 Genotype	Cases	Controls	OR	95% CI	X^2	p value
CC (wild)	92 (73.6)	103 (82)	Ref			
CT	21 (17)	20 (16)	1.06	0.5887-2.2191	0.02	0.8644
TT (variant)	12 (9)	2 (1)	6.531	1.4304-29.8195	6.7	0.0154
CT+TT	33 (26.4)	24 (19.2)	1.8084	1.0009-3.2676	1.16	0.0497
C	205 (82	226 (90.4)	Ref			
T	45 (18)	24 (9.6)	2.0409	1.1689-3.5636	7.41	0.0121

Table 3. Genotyping Frequency of Cox-2 Gene Polymorphisms in Colon Cancer Cases and Controls

Cox 2 Genotyping	Cases	Controls	OR	95%CI	X^2	p value
GG(wild)	98 (77.6)	97 (77.6)	Ref			
GT	25 (20)	25 (20)	0.9895	0.5691-1.7203	0	0.9701
TT(variant)	2 (1.6)	3 (2.4)	0.752	0.1357-4.1678	0.2	0.7443
GT+TT	27 (21.6)	28 (22.4)	0.9669	0.5670-1.6488	0.01	0.9015
G	221 (88.4)	219 (87.6)	Ref			
T	29 (11.6)	31 (12.4)	1.0498	0.6407-1.7200	0.06	0.8471

Table 4. Comparisons between the Two Groups Studied according to Combinations of Mutations

	Patients	Control
None of the mutant alleles	73 (58.4)	74 (59.2)
Cox Only	19 (15.2)	23 (18.4)
MMP2 only	26 (20.8)	8 (6.4)
Cox/MMp2	7 (5.6)	20 (16)

and 125 healthy controls are shown in Table 2. The mutant genotype frequencies of *MMP-2* C-1306 T in the patient group were 9% compared with frequencies of 1% in the control group. The mutant allele frequencies of C-1306 T in the patient group were 18% compared with frequencies of 9.6% in the control group. The corresponding P values and Odd ratio (OR) and 95% confidence interval {95% CI} were as follows: p=0.0154, OR 6.531, CI (1.4304-29.8195) for the mutant genotype. The P values and OR {95% CI} for the mutant alleles were as follows p=0.0121, OR 2.0409, CI (1.1689 to 3.5636) (Table 2).

The mutant genotype frequencies of *Cox2* +202 A/G in the patient group were 1.6 % compared with frequencies of 2.4 % in the control group. The mutant allele frequencies of +202 A/G in the patient group were 11.6% compared with frequencies of 12.4% in the control group. The corresponding P values and Odd ratio (OR) and 95% confidence interval {95% CI} for the mutant genotype were as follows: p=0.7443, OR 0.752, CI (0.1357 - 4.1678). The P values and OR {95% CI} for the mutant alleles were as follows: p=0.847, OR 1.0498, CI (0.6407-1.7200) (Table 3). The frequency of individuals with MMP2 polymorphisms (20.8%) in colon cancer patients was significantly higher than individuals with combination of Cox2 and MMP2 polymorphisms (5.6%) (Table 4).

Discussion

In the recent years, interest in the genetic susceptibility to cancers has led to a growing attention to the study of polymorphisms of genes involved in tumourigenesis.

Cox2 and MMP2 mutations are well-known colon cancer-susceptibility alleles, but the risk associated with the mutations at the individual and population levels remains largely indeterminate. The data presented here and previous reports demonstrate that in different populations, large variations are observed for the frequencies of the common mutations.

Our data confirmed that *MMP-2* C-1306 T mutations are more common in colon cancer patients than in control Saudi population, additional genetic and/or environmental cofactors are required for disease development at the individual and population levels.

Polymorphisms within the *MMP-2* promoter regions have been reported in cases with oncogenesis and tumor progression, especially in colorectal cancer (Decock et al., 2008). Previous studies showed that the SNP C→T transition at -1306 is reported to interrupt the Sp1-type promoter site (CCACC box), causing lower promoter activity and also reducing the transcriptional activity (Xu et al., 2004). Price et al. (2001) identified the -1306C/T polymorphism in the MMP2 promoter and showed a strikingly lower promoter activity with the T allele via in vitro transient transfection experiment. It is likely that the - 1306CC genotype may be associated with a high transcription level and enzyme activity of *MMP-2*, and eventually, it might affect individual susceptibilities to neoplasms.

Increased prostanoid activity is a well recognized characteristic of colorectal cancer (Cahlin et al., 2005; Hull et al., 2004; Wang and Dubois, 2006; Asting et al., 2011; Ekambaram et al., 2011; Zhao et al., 2013). Colorectal cancer development and progression has been allied to the increased production of PGE2 secondary to *COX-2* induction in host stroma (Hull et al., 2004; Cahlin et al., 2005; 2008; Asting et al., 2011). The role of *COX-2* gene induction in colorectal cancer disease remain to be elucidated, although a lot of information is available on the regulation of *COX-2* gene expression (Chun and Surh, 2004; Tsatsanis et al., 2006).

COX-2 is an inducible enzyme that performs the rate-limiting step in the conversion of free arachidonic acid into prostaglandins which play a distinct role in cell proliferation and are potent mediators of inflammation. Substantial evidence has shown unregulated *COX-2* expression to be a contributing factor in many chronic diseases and cancer (Menter et al., 2010).

Many genetic variants within the *COX-2* gene have been identified and select polymorphisms have shown disease association. This study focuses on the genotype and allele frequencies of the +202 A/G in the 3'-flanking region for *COX-2* gene in 125 CRC patients and 125 healthy controls shown in Table 3.

In our study, there was no significant association between genotype distribution of the *Cox2* polymorphism and colorectal cancer. The elevated frequency of the mutated genotype in the control group other than patients' subjects suggests that this polymorphism could have a decreased risk in the Saudi population. Moreover, no significant difference was noted in *cox2*-+202 A/G polymorphism and clinico pathological parameters.

The functional impact of rs2745557 *cox2*-+202 A/G, an intronic variant, on *COX-2* activity is not yet known. Several studies were conducted to investigate the associations of *COX-2* rs2745557 *cox2*-+202 A/G with prostate cancer (PCa) susceptibility (Wu et al., 2011; Amirian et al., 2011; Salinas et al., 2010; Fradet et al., 2009; Dossus et al., 2010; Cheng et al., 2007; Shahedi et al., 2006). *COX-2* +202 A/G (rs2745557) polymorphism was associated with a trend of PCa risk under dominant model and allelic model in African Americans and American Caucasian men, however significant relation was absent in Asians.

The rs2745557 *cox2*-+202 A/G was associated with a lower Pca risk under dominant model. However, the significant association is completely lost under homogeneous co-dominant model or allelic model. Metanalysis data suggest that rs2745557 may associate with a lower PCa risk under over-dominant model. However, the significant association was absent. The reason for this phenomenon may be caused by a lack of sufficient genotype data in several studies.

Prostaglandin E2 has been reported to regulate the metastatic potential of cells through regulation of *MMP-2* (Masferrer et al., 2006; Hua et al., 2011). Thus, it is possible that *COX-2* expression is associated with *MMP-2* expression. Previous studies suggest that there is a direct link between *COX-2* and *MMP-2* as transfection of *COX-2* increases the amount of activated *MMP-2* in breast and colon cancer cells (Tsujii et al., 1997).

A direct link between *COX-2* and *MMP-2* has been shown to exist in several experimental models. Masferrer et al., 2006 have reported that *COX-2* stimulates endothelial cell migration and vessel tube formation, and these processes are inhibited by non-steroidal anti-inflammatory drugs. They also reported that *COX-2* affects the *MMP-2* levels and the activated collagenase levels. The present study found that the expressions of VEGF and *MMP-2* in the *COX-2* positive group were significantly higher than that in the *COX-2* negative group (Tsujii et al., 1997).

References

Amirian ES, Ittmann MM, Scheurer ME (2011). Associations between arachidonic acid metabolism gene polymorphisms and prostate cancer risk. *Prostate*, **71**, 1382-9.

Asting G, Carén H, Andersson M, Lönnroth C, Lagerstedt K, Lundholm K (2011). *COX-2* gene expression in colon cancer tissue related to regulating factors and promoter methylation status. *BMC Cancer*, **11**, 1-10.

Brown JR, DuBois RN (2005). *COX-2*: a molecular target for colorectal cancer prevention. *J Clin Oncol*, **23**, 2840-55.

Cahlin C, Lonnroth C, Arvidsson A, Nordgren S, Lundholm K (2008). Growth associated proteins in tumor cells and stroma related to disease progression of colon cancer accounting for tumor tissue PGE2 content. *Int J Oncol*, **32**, 909-18.

Callejas NA, Casado M, Diaz-Guerra M (2001). Expression of cyclooxygenase-2 promotes the release of matrix metalloproteinase-2 and -9 in fetal rat hepatocytes. *Hepatology*, **33**, 860-7.

Campa D, Zienolddiny S, Maggini V, et al (2004). Association of a common polymorphism in the cyclooxygenase 2 gene with risk of non-small cell lung cancer. *Carcinogenesis*, **25**, 229-35.

Chun KS, Surh Y (2004). Signal transduction pathways regulating cyclooxygenase-2 expression: potential molecular targets for chemoprevention. *Biochem Pharmacol*, **68**, 1089-1100.

Chun S, Surh J (2014). Cancer chemoprevention targeting *cox-2* using dietary phytochemicals, in 'Cancer and Inflammation Mechanisms: Chemical, Biological, and Clinical Aspects' (eds Y. Hiraku, S. Kawanishi and H. Ohshima), John Wiley & Sons, Inc., Hoboken, NJ, USA.

Church RD, Fleshman JW, McLeod HL (2003). Cyclo-oxygenase 2 inhibition in colorectal cancer therapy. *Br J Surg*, **90**, 1055-67.

Chan AT, Ogino S, Fuchs CS (2007). Aspirin and the risk of colorectal cancer in relation to the expression of *COX-2*. *N Engl J Med*, **356**, 2131-42.

Cheng I, Liu X, Plummer SJ, et al (2007). COX2 genetic variation, NSAIDs, and advanced prostate cancer risk. *Br J Cancer*, **97**, 557-61.

Chulada PC, Thompson MB, Mahler JF (2000). Genetic disruption of Ptgs-1, as well as Ptgs-2, reduces intestinal tumorigenesis in Min mice. *Cancer Res*, **60**, 4705-8.

Decock J, Paridaens R, Ye S (2008). Genetic polymorphisms of matrix metalloproteinases in lung, breast and colorectal cancer. *Clin Genet*, **73**, 197-211.

Dossus L, Kaaks R, Canzian F, et al (2010). PTGS2 and IL6 genetic variation and risk of breast and prostate cancer: results from the Breast and Prostate Cancer Cohort Consortium (BPC3). *Carcinogenesis*, **31**, 455-61.

Eberhart CE, Coffey RJ, Radhika A, et al (1994). Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. *Gastroenterology*, **107**, 1183-8.

Ekambaram P, Lambiv W, Cazzolli R, Ashton W, Honn V (2011). The thromboxane synthase and receptor signaling pathway in cancer: an emerging paradigm in cancer progression and metastasis. *Cancer and Metastasis Reviews*, **30**, 397-408.

Fradet V, Cheng I, Casey G, Witte JS (2009). Dietary omega-3 fatty acids, cyclooxygenase-2 genetic variation, and aggressive prostate cancer risk. *Clin Cancer Res*, **15**, 2559-66.

Greenhough A, Smartt HJ, Moore AE, et al (2009): The *COX-2*/PGE2 pathway: key roles in the hallmarks of cancer and adaptation to the tumour microenvironment. *Carcinogenesis*, **30**, 377-86.

Gustafsson A, Hansson E, Kressner U, et al (2007). EP1-4 subtype, COX and PPAR gamma receptor expression in colorectal cancer in prediction of disease-specific mortality. *Int J Cancer*, **121**, 232-40.

Gustafsson A, Hansson E, Kressner U, et al (2007). Prostanoid receptor expression in colorectal cancer related to tumor stage, differentiation and progression. *Acta Oncol*, **56**, 1-6.

Hull MA, Ko SC, Hawcroft G (2004). Prostaglandin EP receptors: targets for treatment and prevention of colorectal cancer? *Mol Cancer Ther*, **3**, 1031-39.

Hua H, Li M, Luo T, Yin Y, Jiang Y (2011). Matrix metalloproteinases in tumorigenesis: an evolving paradigm. *Cellular and Molecular Life Sciences*, **68**, 3853-68.

Kessenbrock K, Plaks V, Werb Z (2010). Matrix metalloproteinases: regulators of the tumor microenvironment. *Cell*, **141**, 52-67.

Lacchini, R, Jacob-Ferreira AL, Luizon MR, et al (2012). Common matrix metalloproteinase 2 gene haplotypes may modulate left ventricular remodelling in hypertensive patients. *J Hum Hypertens*, **26**, 171-7.

Langsenlehner U, Yazdani-Biuki B, Eder T, et al (2006). The cyclooxygenase-2 (PTGS2) 8473T >C polymorphism is associated with breast cancer risk. *Clin Cancer Res*, **12**, 1392-4.

Markowitz SD (2007). Aspirin and colon cancer-targeting prevention? *N Engl J Med*, **356**, 2195-8.

Masferrer JL, Leahy KM, Koki AT, et al (2006). Antiangiogenic and antitumor activities of cyclooxygenase-2 inhibitors. *Cancer Res*, **60**, 1306-11.

Menter DG, Schilsky RL, DuBois RN (2010). Cyclooxygenase-2 and cancer treatment: understanding the risk should be worth the reward. *Clin Cancer Res*, **16**, 1384-90.

Oshima M, Dinchuk JE, Kargman SL (1996). Suppression of intestinal polyposis in Apc delta716 knockout mice by inhibition of cyclooxygenase 2 (*COX-2*). *Cell*, **87**, 803-9.

Oshima H, Oshima M, Inaba K (2004). Hyperplastic gastric tumours induced by activated macrophages in *COX-2*/mPGES-1 transgenic mice. *EMBO J*, **23**, 1669-78.

Ozhan G, Lochan R, Leathart JBS, Charnley R, Daly AK (2011). Cyclooxygenase-2 polymorphisms and pancreatic cancer susceptibility. *Pancreas*, **40**, 1289-94.

Pereira C, Medeiros RM, Dinis-Ribeiro MJ (2009). Cyclooxygenase polymorphisms in gastric and colorectal carcinogenesis: are conclusive results available? *Eur J Gastroenterol Hepatol*, **21**, 76-91.

Papafili A, Hill MR, Brull DJ, McAnulty RJ, Marshall RP,

Humphries SE (2002). Common promoter variant in cyclooxygenase-2 represses gene expression: evidence of role in acute-phase inflammatory response. *Arterioscler Thromb Vasc Biol*, **22**, 1631-36.

Price SJ, Greaves DR, Watkins H (2001). Identification of novel, functional genetic variants in the human matrix metalloproteinase-2 gene: role of Sp1 in allele-specific transcriptional regulation. *J Biol Chem*, **276**, 7549-58.

Ruud J, Nilsson A, Ruud L, Wang W, et al (2013). Cancer-induced anorexia in tumor-bearing mice is dependent on cyclooxygenase-1. *Brain Behav Immun*, **29**, 124-35.

Sanger F, Nicklen S, Coulson AR (1977). DNA sequencing with chain terminating inhibitors. *Proc Natl Acad Sci U S A*, **74**, 5463-7.

Shahedi K, Lindstrom S, Zheng SL, et al (2006). Genetic variation in the *COX-2* gene and the association with prostate cancer risk. *Int J Cancer*, **119**, 668-72

Siironen P, Ristima¨ki A, Nordling S (2004). Expression of *COX-2* is increased with age in papillary thyroid cancer. *Histopathology*, **44**, 490-7.

Sivula A, Talvensaari-Mattila A, Lundin J (2005). Association of cyclooxygenase-2 and matrix metalloproteinase-2 expression in human breast cancer. *Breast Cancer Res Treat*, **89**, 215-20.

Song F, Wang L, HuangY, et al (2013). MiR-21 upregulation induced by promoter zone histone acetylation is associated with chemoresistance to gemcitabine and enhanced malignancy of pancreatic cancer cells. *Asian Pac J Cancer Prev*, **14**, 7529-36.

Thiel A, Narko K., Heinonen M, Hemmes A, Tomasetto C, Rio MC, Ristimäki A (2012). Inhibition of cyclooxygenase-2 causes regression of gastric adenomas in trefoil factor 1 deficient mice. *Int J Cancer*, **131**, 1032-41.

Tsatsanis C, Androulidaki A, Venihaki M, Margioris AN (2006). Signalling networks regulating cyclooxygenase-2. *Int J Biochem Cell Biol*, **38**, 1654-61.

Tsujii M, Kawano S, DuBois RN (1997). Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. *Proc Natl Acad Sci U S A*, **94**, 3336-40

Wang X, Fu X, Brown PD, Crimmin MJ (1995). Foffman RM. Matrix metalloproteinase inhibitor BB-94 (batimastat) inhibits human colon tumor growth and spread in a patient-like orthotopic model in a nude mice. *Cancer Res*, **54**, 4726-8.

Wang D, Dubois RN (2006). Prostaglandins and cancer. *Gut*, **55**, 115-22.

Wastson SA, Morris TM, Robinson G, et al (1995). Inhibition of organ invasion by the matrix metalloproteinase inhibitor BB-94 (batimastat) in two human colon carcinoma metastasis models. *Cancer Res*, **55**, 3629-33.

Waston SA, Morris TM, Parsons SL, Steele RJC, Brown PD (1996). Therapeutic effect of matrix metalloproteinase inhibitor, batimastat, in a human colorectal cancer ascites model. *Br J Cancer*, **74**, 1354-8.

Wu HC, Chang CH, Ke HL, et al (2011). Association of cyclooxygenase 2 polymorphic genotypes with prostate cancer in Taiwan. *Anticancer Res*, **31**, 221-25.

Wu CY, Wang CJ, Tseng CC (2005). Helicobacter pylori promote gastric cancer cells invasion through a NF-kappaB and *COX-2*-mediated pathway, *World J Gastroenterol*, **11**, 3197-203.

Xu E, Lai M, Lv B, et al (2004). A single nucleotide polymorphism in the matrix metalloproteinase-2 promoter is associated with colorectal cancer. *Biochem Biophys Res Commun*, **324**, 999-1003.

Zervos EE, Norman JG, Gower WR, Franz MG, Rosemurgy AS (1997). Matrix metalloproteinase inhibition attenuates human pancreatic cancer growth in vitro and decreases mortality and tumorigenesis in vivo. *J Sur Res*, **69**, 367-71.

Zhang X, Miao X, Tan W, et al (2005). Identification of functional genetic variants in cyclooxygenase-2 and their association with risk of esophageal cancer. *Gastroenterology*, **129**, 565-76.

Zhao F, Zhu H, Huang M, Yi C, Huang Y (2013). The 765G> C polymorphism in the cyclooxygenase-2 gene and gastric cancer risk: an update by meta-analysis. *Asian Pac J Cancer Prev*, **15**, 2863-8.