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CYCLOOXYGENASE-2 AND HEPATOCELLULAR CARCINOMA: THE PROTEOMICS OF ASSOCIATION

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Abstract: Hepatocellular carcinoma represents one of the most common malignancies worldwide with a rising incidence in western countries. Chronic inflammation is recognised as a threat factor for cancer progression. Cyclooxygenase-2 is the major mediator of inflammation. Various studies on Cox-2 suggest its possible association with HCC differentiation. Sufficient genetic and pharmacologic evidences implicate its crucial role in neoplasia and it is also now clear that Cox-2 plays a crucial role in tumor progression. Cox-2 overexpression is associated with maintaining tumor microenvironment and has crucial implication for angiogenesis. Cox-2 operates in multifactorial fashion. Cox-2 selective inhibition has been reported as a successful tool in suppressing angiogenesis and metastasis. The pharmacological suppression of Cox-2 represents a bright future as a therapeutic tool for treatment of various malignancies. This review is an attempt to discuss the critical issue of overexpression of Cox-2 and its role in the development of HCC in particular and cancer in general.

***Keywords*:** Cox-2; PGE2; HCC; Tumorigenesis.

1. Introduction

Hepatocellular carcinoma represents one of the most common malignancies worldwide with a rising incidence in western countries. With advancement in science and medical facilities, early clinical diagnosis of HCC and its management is possible. Yet the complicated mechanisms involving infectious, genetic and epigenetic factors contributing to its development have not been completely understood and remain a serious medical issue (Cervello and Montalto, 2006). Causative agents like Hepatitis C Virus and Hepatitis B Virus infection, excessive alcohol consumption and aflatoxin ingestion lead to HCC progression (Abou-Shady *et al.*, 1999; Trujillo-Murillo *et al.*, 2007; Xue, 2005). Primary liver cancer caused by HCV has affected more than 170 million people world-wide (Xu *et al.*, 2001). In about eighty percent of infected people, HCV is responsible for acute and chronic liver diseases resulting in fibrosis, cirrhosis, and most importantly HCC (Choi *et al.*, 2004; Xu, 2002). HCC is a highly malignant tumor characterized by active angiogenesis and extracellular matrix formation (Abou-Shady *et al.*, 1999). Common clinical manifestations include severe abdominal pain and deteriorated hepatic function (Cervello and Montalto, 2006). Earlier incidences of HCC were reported to be more prevalent in east Asian countries, but in recent years the incidence is rising fast in the western world too and it has become the leading cause of liver transplant in USA and Europe (Chi-Man Tang *et al.*, 2005; Tang and Grise, 2009; Xu, 2002). This review is an attempt to look at the critical issue of overexpression of Cox-2 and its role in the development of HCC.

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2. HCC and Inflammation

HCC development is a multi-pathway process involving activation of proto-oncogenes, inactivation of tumor suppressor genes, dysregulation of RB1, p53 and Wnt pathways (Xue, 2005). Chronic liver inflammation coupled with active neovascularisation are characteristic

pathological changes associated with HCC (Chi-Man Tang *et al.*, 2005; Tang and Grise, 2009). Chronic inflammation is recognised as a threat factor for cancer progression. There is sufficient evidence to support its crucial role in pathogenesis related to several types of cancer including pancreatic, breast cancer, colorectal cancer, squamous cell carcinoma in head and neck, ovarian cancer, gastric adenocarcinoma, lung cancer and HCC (Cianchi *et al.*, 2001; Costa *et al.*, 2002; Fujiwaki *et al.*, 2002; Gallo *et al.*, 2002; Li *et al.*, 2003; Tang *et al.*, 2005; Wolff *et al.*, 1998; Yip-Schneider *et al.*, 2000). Inflammation is an immediate response of natural immunity and plays a key role in physiological and pathological conditions like pathogenic invasion or wound healing. It can be activated by various compounds such as lipopolysaccharides during bacterial confrontation, presence of toll like receptors that are produced to detect viruses, physical injury such as by UV radiation and chemical compounds like reactive oxygen species. When afflicted with pathogens, specific receptors are activated which trigger a multi-factorial network of signal cascade mediated by NFkB, p38 or MAPKs, which in turn regulate production and crosstalk between pro-inflammatory cytokines, chemokines and cell adhesion molecules. All these incidences dictate recruitment and activation of immune cells. Constant persistence of cytokines, chemokines, various immune cells like lymphocytes, macrophages, and dendritic cells potentiate production of tumor microenvironment. Tumor microenvironment orchestrated by inflammatory cells foster neoplastic growth, proliferation and metastasis (Coussens and Werb, 2002; Kulinsky, 2007; Sobolewski *et al.*).

3. Mediator of inflammation: Cyclooxygenase

Cyclooxygenase exist as two isoforms: Cox-1 and Cox-2

Production of various prostaglandins is directed by coordinated activity of eicosanoid forming enzymes named Cyclooxygenase. In humans, Cox is present as two isoforms designated as Cox-1 and Cox-2. Cox-1 has been reported to function as a housekeeping isoform of cyclooxygenase and is constitutively expressed to serve functions such as regulation of renal blood flow, imparting protection to stomach against ulcers, production of prostacyclin and PGE_2 to maintain coherence and structure of gastric mucosal surface, and production of prostanoid thromboxane in platelets (Leng *et al.*, 2003; Li *et al.*, 2002; Williams *et al.*, 1999). Cox isoforms differ a lot in their genomic structure, expression and regulation in spite of similar structure and enzymatic activity. In 1976, Cox-1 was purified and characterised for the first time from bovine vascular glands and was isolated in 1988. The molecular weight of unmodified and unglycosylated enzyme lacking signal peptide is 65 kDa, but after post translation modifications it increases to 70 kDa in fully functional enzyme (DeWitt and Smith, 1988; Merlie *et al.*, 1988). The cox-2 gene was cloned and characterised in 1993 (Jones *et al.*, 1993; Smith *et al.*, 1990). The genes encoding Cox-1 and Cox-2 proteins are located on separate chromosomes. Gene coding for Cox-1 is located on chromosome nine, contains 11 exons and generates mRNA of 2.8 kb (Cervello *et al.*, 2005; Funk *et al.*, 1991). It lacks TATA or CAAT box at transcription sites (Kraemer *et al.*, 1992). On the other hand, cox-2 is 7.5-9 kb in size and present on chromosome one. It consists of 10 exons. Its mRNA transcript is 4 kb long. Cox-1 consists of 602 residues while Cox-2 comprises 604 residues. The two isoforms share significant homology in sequence and enzymatic mode of action. They exhibit high similarity both structurally and mechanistically, and have almost identical size. The central part of both proteins consists of catalytic and substrate binding site but functional differences in their activity confers that Cox-2 has larger catalytic site. The analysis of amino-acid sequences of Cox proteins suggests noteworthy difference at N terminal. The signal peptide is comprised of 17 less amino acids in Cox-2 than in Cox-1. On the contrary, C terminal of Cox-2 has 18 more residues than Cox-1 (Bakhle and Botting, 1996; Gierse *et al.*, 1996) .

Cyclooxygenase-2 is the major mediator of inflammation

A number of reports by different groups have pointed to the involvement of Cox-2 prostanoid pathway in inflammation leading to hepatocellular carcinoma. Cox-2 differs markedly in expression and functioning from Cox-1. It is an inducible early response gene and is activated

in response to various extracellular or intracellular physiological stimuli. These factors comprise of lipopolysaccharide (LPS), interleukin-1 (IL-1), tumour necrosis factor (TNF), epidermal growth factor (EGF), platelet activating factor (PAF), serum, endothelin, and arachidonic acid. It is not expressed constitutively in all the tissues at all the time except in placenta, brain and kidney (Williams *et al.*, 1999). The up-regulation and over-expression of Cox-2 is mainly associated with inflammation, loss of apoptosis, uncontrolled cell proliferation, growth, metastasis, neovascularisation, and angiogenesis finally leading to cancer. Cox-2 generated prostaglandins have also been reported to function as immuno-suppressors. It has been shown that macrophage mediated and natural killer cell mediated cytotoxicity is suppressed by PGE_2 (Leng *et al.*, 2003; Williams *et al.*, 1999).

Transcriptional regulations of Cox-2

Transcriptional activation of Cox-2 occurs rapidly in response to broad spectrum of stimuli such as pathogen, cytokine, nitrous oxide (NO), growth factors, and extracellular ligands. Cross talks between various signalling pathways induced by pro-inflammatory and growth promoting stimuli converge to the activation of MAPK cascade which govern Cox-2 expression at transcription and post transcription level (Tsatsanis *et al.*, 2006). Highly specialised machinery is involved in the generation of these signalling molecules as they are highly specific for the stimulus and cell type (Kang *et al.*, 2007). Sequence analysis have shown numerous potential regulatory transcription factor sites including TATA box, C/EBP motif, AP-2, NF-KB sites present at 5'flanking region of cox-2 gene promoter region (Appleby *et al.*, 1994; Tsatsanis *et al.*, 2006). Consequences of chromatin remoulding, like differential acetylation status of histone and non-histone proteins also pose impact on transcriptional control of Cox-2. Literature has revealed decisive impact of p300, a histone acetyltransferase on Cox-2 translation through diverse mechanisms including acetylation of NF-kB. Elevated incidences of p300 binding to NK-kB play prominent role in cox-2 promoter activation (Deng *et al.*, 2003). Hypermethylation of CpG islands (cytosine-guanine rich dinucleotides) located on cox-2 promoter repress its efficiency of promoting transcriptional silencing (Song *et al.*, 2001). It has been documented that the 3'UTR of cox-2 gene encodes multiple copies of AU rich elements (AREs) motif which when targeted by various trans acting ARE binding factors influence Cox-2 mRNA stability and increase or decrease Cox-2 mRNA expression and enzyme activity level (Appleby *et al.*, 1994; Song *et al.*, 2001). Transcription of Cox-2 is controlled by numerous pathways, which also regulate its expression. It has been hypothesized that Cox-2 is down regulated by APC. Mutations in APC activate wnt signal pathways leading to increased accumulation of β-catenin that binds to TCf-4. Tcf-4 binding element has been identified in cox-2 promoter (Araki *et al.*, 2003). Combined dysregulation of Wnt and Ras pathways increase Cox-2 mRNA production thus implicating their role in Hepatocellular carcinoma (Abou-Shady *et al.*, 1999; Araki *et al.*, 2003).

Expression of Cox-2 is considered to be associated with de-differentiation of adult hepatocytes (Abou-Shady *et al.*, 1999). Cox-2 is rapidly induced in foetal hepatocytes when challenged with pro-inflammatory stimuli like LPS but there is a rapid decline in hepatocytes after birth. It has been suggested that high levels of C/EBP (CCAAT/ enhancer binding proteins) in adult hepatocytes impairs Cox-2 expression when exposed to pro-inflammatory stimuli (Callejas *et al.*, 2000). PPAR" activation in human hepatocellular carcinoma cells results in induction of Cox-2 expression and increased cellular proliferation. The proposed mechanism behind induction is enhanced activity of proximal promoter of cox-2 gene (Glinghammar et al., 2003). HBV infection is a major etiological cause of HCC. Up-regulation of Cox-2 has been reported in HBV mediated hepatocellular carcinoma. A close association has been investigated between HBx and Cox-2 (Cheng *et al.*, 2004a). HBx intensifies metastasis in hepatic tumor cells and is the only HBV protein expressed in hepatocytes during HCC. It is suggested that Cox-2 is major cellular effctor of HBx in HBV associated hepatic tumorogenesis and HBx activates cox-2 gene promoter activity mediated by NF-AT transcription factors thus magnifying tumor cell invasion (Lara-Pezzi *et al.*, 2002). It has been reported that ER stress induced due to expression

of mutant HBV viral surface proteins stimulate Cox-2 expression by activating NF-kB and p38 MAPK pathways (Hung *et al.*, 2004). Also, studies have indicated modification of NF-kB activity through HBx protein of HBV and HCV suggesting its potential role in triggering up activities of many cellular anti-apoptotic genes, perpetuation of inflammation and inhibition of differentiation of antigen presenting cell (APC) leading to chronic hepatatis (Mozer-Lisewska *et al.*, 2006; Waris *et al.*, 2002). Another study showed that there was marked repression in rate of cox-2 gene transcription by wild type p53. Any mutation in p53 or loss of its expression increased cox-2 gene transcription many fold (Subbaramaiah *et al.*, 1999).

3.4. Post-transcriptional Regulation of Cox-2

Post transcriptional regulation plays a significant role in maintaining molecular regulation of cox-2 gene expression. Dysregulation at this level may elevate considerably incidences of tumorigenesis (Dixon, 2004). It has been found that RNA binding proteins HUR and TTP bind to AU rich elements located in 3'UTR of cox-2 gene and regulate its expression (Young *et al.*, 2009). HuR has been reported to be highly crucial for its post transcriptional mRNA stability and its overexpression has shown to augment cox-2 expression (Sengupta *et al.*, 2003; Young *et al.*, 2009). Recently, elevated HuR level has been found in HCC cell lines, emphasizing its probable role in cox-2 associated inflammation and tumor invasion (Sheflin *et al.*, 2001). A key study on CUG triplet repeat RNA- binding protein 2 (CUGBP2) showed that it binds to specific AREs present in first 60 nucleotides of the 3'UTR of cox-2 and plays role in opposing function of imparting stability to cox-2 mRNA coupled with inhibition of its translation (Mukhopadhyay *et al.*, 2003). Genetic make of the tumor cells also regulates Cox-2 expression. Tumor suppressor genes like p53 tend to restrain Cox-2 expression while oncogenes like ras and src stimulate it (Koga *et al.*, 1999).

4. Biological Function of the Cox-2 Pathway and Role in Cancer Prostanoid Synthesis Pathway

Cox-2 regulates key step in prosatnoid (i.e. prostaglandin and thromboxanes) pathway (Fig. 1) (Wang and Dubois, 2006). Various inflammatory mediators implicated in pathological process associated with cancer include prostaglandin, thromboxanes and leukotrienes. They belong to the family of hormonally active, oxygenated C18, C20, C22 fatty acids called eicosanoids derived from polyunsaturated fatty acids (Meric *et al.*, 2006). The precursor molecule for prostanoids is arachidonic acid, which is a 20 carbon unsaturated ω-6 fatty acid, usually esterified at sn-2 position of phospholipids and dispersed throughout the lipid bilayer of the cell membrane

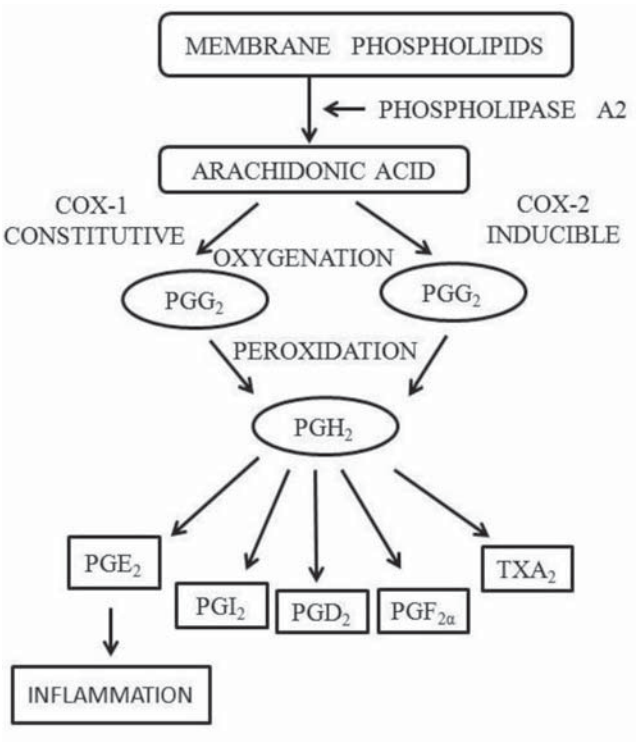

Figure 1: **Phospholipase A_2 (cytosolic or secreted) hydrolyse plasma membrane lipids or lipids derived from diet to generate Arachidonic acid (AA). AA is a 20 carbon unsaturated fatty acid. Cox-1/ Cox-2 catalyse AA to various prostaglandins having several physiological effects. AA is first converted to PGH_2. This conversion is a two-step process. The first step includes introduction of 2 oxygen molecules to AA, to form unstable PGG_2 and second step consists of peroxidation reaction in which PGG_2 is reduced to stable PGH_2, which is a substrate to a number of specific prostaglandin and thromboxane synthases. Prostaglandins are critical molecules, as they regulate various physiological functions. Cox-1 is a constitutive enzyme and is expressed in almost all the tissues of the body. Whereas, the other isoform Cox-2 is induced by several external and internal stimuli and its overexpression mediates abnormal PGE_2 production. Excessive production of PGE_2 leads to chronic inflammation and carcinogenesis**

(Wang *et al.*, 2007). In response to various stimuli like growth factors, hormones and cytokines, arachidonic acid is liberated from membrane and metabolised to various bioactive lipids. This conversion involves three major steps. First is the liberation of arachidonic acid from phospholipids by phospholipase A_2 enzyme (secretory or cytoplasmic). Second is addition of two molecules of oxygen to arachidonic acid forming bicyclic peroxide PGG_2 which is an unstable intermediate. The catalytic site for the next step is located on a different site of the enzyme. Lastly, PGG2 diffuses to the requisite site and here its peroxidation leads to reduction of unstable PGG_2 to stable PGH_2 (Smith, 1992).

Cox-2 and Tumor Progression

Over expression of Cox-2 cause accumulation of pro-inflammatory molecule PGE_2. It acts as a weapon in maintaining tumor survival (Fig. 2). It potentially increases tumor aggressiveness and inhibits apoptosis by various mechanisms. Most evident effect of PGE_2 seen on tumor cells is mediated by synthesis of metastasis promoting matrix metallo-proteinases (MMPs). It has also been documented that production of Cox-2 and PGE_2 modulates replication in hepatitis B virus, cytomegalovirus and gammaherpes virus (Nie and Honn, 2002; Symensma *et al.*, 2003; Tsujii *et al.*, 1998; Zhu *et al.*, 2002). Recent studies emphasise that Cox-2 is stimulated in cancer and this accelerates and intensifies tumor growth, tumor vascularization, angiogenesis, invasion and metastasis (Cheng *et al.*, 2004b; Cianchi *et al.*, 2001; Gallo *et al.*, 2002; Li *et al.*, 2003). In another study, Cox-2 levels were measured and quantified in tumor cytosol. It was found that Cox-2 expression in tumor cytosol potentially influenced tumor division rate, venous invasion, advanced tumor stage and progression. In contrast there was complete lack of association between cytosolic Cox-2 and tumor size (Chi-Man Tang *et al.*, 2005). It is probable that Cox-2 favours phenotypic changes that reduce apoptosis, thereby favouring tumor progression. Cells expressing increased Cox-2 levels elucidated increased adhesion properties to extracellular matrix proteins (ECM) and also mediate in part resistance to apoptosis. Direct relation between Cox-2 and Bcl-2 protein has yet not been established and is still under speculation. Yet it has been found that Cox-2 mediates increased expression of Bcl-2. Interestingly, Cox-2 inhibitors have shown to down regulate Bcl-2 protein expression suppressing tumorigenesis (Tsujii and DuBois, 1995). The correlation between Cox-2 and serine threonine kinase Akt signalling cascade is under investigation. However, their interaction has been hypothesized to have significant implication in angiogenesis (Gately, 2000). In a recent report it has been mentioned, that increased phosphorylation events of Akt and its downstream substrate glycogen synthase kinase-3 beta and pro-apoptotic Bad are observed in Hepatitis C virus expressing cells. These phosphorylation events were sensitive to selective Cox-2 inhibitors (Waris and Siddiqui, 2005).

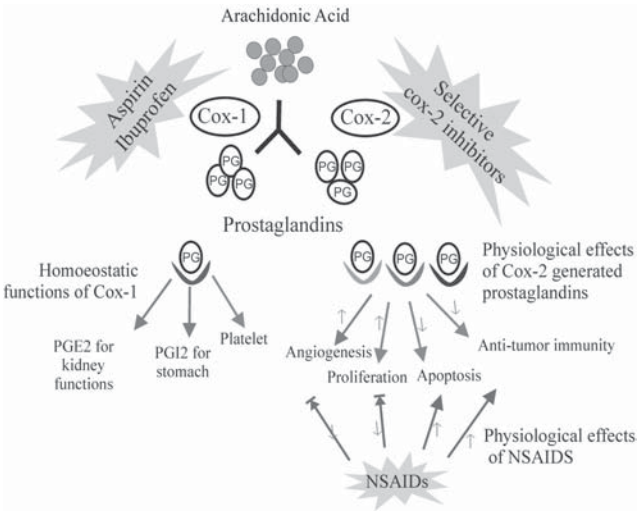

Figure 2: **Arachidonic acid is converted to prostaglandins by action of Cox-1 and Cox-2 enzymes. Cox-1 is important for maintaining homeostatic functions of body like platelet formation for blood, kidney development and its functions, maintenance of gastric mucosa etc. Cox-2 derived prostaglandin PGE_2 is associated with increased inflammation, increased angiogenesis, greater metastatic and proliferative invasion, reduction in apoptosis and formation of immunosuppressive microenvironment. NSAIDs function as cox inhibitors and serve as effective tool against Cox mediated cancer. Aspirin and several other dual acting NSAIDs, which can block both Cox-1 and Cox-2 pathway, have several limitations and side effects associated with them and thus there was a need to develop Cox-2 specific inhibitors. NSAIDs reduce incidences of cancer by increasing apoptosis of tumor tissue, maintaining anti-tumor microenvironment, reducing proliferation and angiogenesis**

Genetic studies using mice model also strongly support the correlation between Cox-2

over-expression and cancer progression. Inhibition of intestinal polyposis has been reported in APCΔ716 mice that were Cox-2 knockouts. Moreover treating APCΔ716 mice with Cox-2 inhibitor further restrained the number and size of polyps (Oshima *et al.*, 1996). In another study on multistage mouse skin model, engineered homozygous deficient for isoforms Cox-1 and Cox-2 showed reduction in skin tumor development by 75% (Tiano *et al.*, 2002). Significant data in literature strongly support that overexpression of Cox-2 accelerates incidences of tumorigenesis in transgenic mice model; and use of chemo-preventive approach to restrain Cox-2 may be a logical therapeutic method to combat Cox-2 mediated cancer (Liu *et al.*, 2001).

Interestingly, in some reports the pattern of Cox-2 expression does not suggest its principle role in tumorigenesis. A recent report on colon cancer has reported that Cox-2 expression is absent in early premalignant lesion human aberrant crypt foci and Cox-2 level seems to increase at adenoma stage, when minimum 45% adenoma are positive (Eberhart *et al.*, 1994). Another fascinating fact advanced by Takeda et al. on polyp of APCΔ716 suggest that Cox-1 expression in stromal cells was basal, present in polyps of any size. The Cox-2 was induced only when the polyp grew bigger than 1mm in diameter (Takeda *et al.*, 2003).

Immunosuppressive Tumor Microenvironment

Cooperative interaction between pro-inflammatory eicosanoids, cytokines, chemokines and carcinoma cells contribute to formation of immunosuppressive tumor microenvironment. PGE_2 functions as immune modulator and plays a crucial role in maintaining microenvironment that favours tumor cell growth and invasion. It has been reported that PGE_2 switches anti-tumor TH_1 microenvironment to TH_2 immunosuppressive microenvironment. It induces down-regulation of TH_1 cytokines like TNFα, IFNγ, IL-2 and IL-12, and upregulates TH_2 cytokines such as IL-4 and IL-10, which have immunosuppressive effect (Huang *et al.*, 1998; Kambayashi *et al.*, 1995; Snijdewint *et al.*, 1993; Stolina *et al.*, 2000). Another interesting finding demonstrated that PGE_2 directly inhibits cytotoxic T cell activity. It has been found that PGE_2 up-regulates CD94/NKG2A heterodimer complex, which is a natural killer receptor. Cross-linking reaction between CD94 and T cells expressing this heterodimer prevents cytotoxic T cell activity (Zeddou *et al.*, 2005). In another study, it has been reported that PGE_2 indirectly eliminates anti-tumor effects of cytotoxic T cells. It inhibits dendritic cell maturation, down-regulates antigen presenting cells and causes abortive activation of naive CD8(+)T cells (Ahmadi *et al.*, 2008).

5. Hepatocellular Carcinoma

Chronic liver infection, when associated with dreaded complications like liver cirrhosis and hepatocellular carcinoma, is followed by liver damage. Multiple factors including infection with HCV, HBV, chemical mutagens like aflatoxin and other environmental or host factor contribute to the development of HCC. However, HCV infection remains one of the major reasons for this disease. It has been documented that HCC consists of many different histological grades of HCC tumor. Mostly, two or more different grades have been observed inside a single tumor. As the size of tumor grows, the foci of less differentiated tumor tissue arises and starts growing inside the pre-existing well differentiated tumor tissue till they replace the original well differentiated HCC tumor (Kenmochi et al., 1987; Sugihara *et al.*, 1992). HCC emerge as well differentiated tumors in early stages and progressively become less differentiated in advance stages (Araki *et al.*, 2003). Recently, HCV mediated HCC has been found to be closely associated with activation and overexpression of Cox-2 gene. Various studies of Cox-2 suggest its possible association with HCC differentiation. It is possible that it plays a pivotal role in early stages of HCC. Its overexpression has been well documented in well-differentiated HCC; however in advanced HCC and in less differentiated HCC, its expression is negligible (Bae *et al.*, 2001; Koga *et al.*, 1999). A recent study reported that Cox-2 expression is significantly influenced by iNOS presence (Rahman *et al.*, 2001). Combined iNOS and Cox-2 expression may modulate prognosis of HCV positive HCC and may be responsible for increased tumor growth and larger tumor size. Together they may also be accountable for modulating angiogenesis in HCC (Rahman *et al.*, 2001).

Cox-2 and De-differentiation of HCC

HCC emerge as well differentiated tumors, and as they advance they progressively become less differentiated (Kenmochi *et al.*, 1987). Tumor de-differentiation has been observed during HCC tumor growth. Liver is supplied with blood through two channels, one is arterial and other is portal (Koga *et al.*, 1999). During pathological conditions, proinflammatory cytokines, notorious reactive chemical species and lipopolysaccharide are present particularly in the portal blood and they victimise hepatocytes. Most of them contribute to induce Cox-2 thus exaggerating the amplification of prostanoids (Belvisi *et al.*, 1997; Feng *et al.*, 1995). Studies have shown that hepatocarcinogenesis comprises of series of sequential changes, which alter haemodynamic status. All these changes are associated with pathological conditions and have been studied using imaging techniques (Winter *et al.*, 1994). It has been speculated that early HCC is mainly characterised by well-differentiated HCC tumor tissue and are bestowed with abundant arterial supply and relatively less pre-existing portal tracts (Sakamoto *et al.*, 1993; Ueda *et al.*, 1992). It has been suggested that Cox-2 inducers attack by portal tracts leading to overexpression of cox-2 gene in early well-differentiated HCC. But on the other side, due to sequential heamodynamic changes, advance HCC are moderately or poorly differentiated and devoid of pre-existing portal tracts. Hence, they are not influenced by Cox-2 inducers (Koga *et al.*, 1999).

Cox-2, Virus Infection and HCC

Both HCV and HBV accelerate Cox-2 expression. HBV mediated HCC involves expression of HBx viral protein. It facilitates Cox-2 overexpression by transactivation of NF-AT transcription factor. A recent study showed that mutation in HBV surface protein caused due to endoplasmic stress activates NF-kB and p38MAPK cascade resulting in up-regulation of Cox-2 (Hung *et al.*, 2004). Furthermore, another report suggested that significant association exist between Cox-2 expression and acute inflammation in adjacent non-tumor liver tissue. Cox-2 mediated inflammation is actively involved in HCC relapse after surgery, imparting shorter disease free survival to patients afflicted with HCC (Kondo *et al.*, 1999). These studies provide new approach and broaden horizon to speculate the mechanism by which Hepatitis viral infection operates through upregulation of Cox-2 and accelerate PG production. They also provide substantial evidence that Cox-2 may prove to be a logical therapeutic target to combat HCC.

Cox-2 and Tumor Angiogenesis

A close link between Cox-2 and angiogenesis has been observed in several human malignancies including HCC. Angiogenesis can be described as budding of new capillaries from the pre-existing vasculature and is the prerequisite for successful establishment of tumor and its growth (Joo *et al.*, 2003). Using pharmacological and genetic methodologies, it has been established that Cox-2 produced prostanoids are responsible for facilitating angiogenesis function in autocrine or paracrine manner (Williams *et al.*, 2000). Potential role played by Cox-2 in angiogenesis and tumor growth becomes more evident when Cox-2 expressing tumor cells were detected to grow larger and showed greater degree of angiogenesis in contrast to tumor cells deficient in Cox-2 expression. In tumor cells expressing Cox-2, it has been elucidated that its growth, aggressiveness and angiogenesis could be repressed by selective Cox-2 inhibitors, but inhibitors failed to inhibit the production of angiogenic proteins in Cox-2 negative tumor cells (Tsujii *et al.*, 1998). Together, these observations support that Cox-2 mediates tumor progression through angiogenesis and neovascularisation. The inhibition of tumor progression in Cox-2 expressing cells by selective Cox-2 inhibitors may pave for a new path in fight against cancer (Sawaoka *et al.*, 1999). Recently, Cox-2 expression in HCC has also been closely related to increased vascularity and sprouting of new capillaries from pre-existing vessels (Rahman *et al.*, 2001). A recent study was designed to explore the association between Cox-2 and angiogenesis and also the significance of the role played by other inflammatory cells such as macrophages, kupffer cells etc. in the progression of primary HCC, using anti-CD-34 antibody. CD34 is commonly used as a marker for the identification and isolation of hematopoietic stem cells (Nielsen and McNagny, 2008). A positive correlation was found between

Cox-2 expression and CD-34. Comparative analysis between early stage HCC and advanced HCC showed decreased level of CD-34 positive cells with dedifferentiation. Cox-2 was reported to be the only independent variable, positively associated with CD-34 in multivariate analysis (Cervello *et al.*, 2005). All these findings support the hypothesis, that inhibiting Cox-2 selectively, by treating with selective Cox-2 inhibitors may be a good strategy in combating HCC associated angiogenesis, thus providing an additional rational approach for treating the deadly disease. However, several safety and efficacy related issues are associated with prolonged usage of Cox-2 selective inhibitors.

Modulation of VEGF

Angiogenic phenotype that can support tumorigenesis results from sequential upregulation of products of angiogenic inducers. It involves both activation of oncogene, production of VEGF, loss of wild type p53 and suppression of thrombospondin, an inhibitor of angiogenesis, which can counteract VEGF. All the above events in step wise manner result in acquisition of pro-angiogenic phenotype in which Cox-2 over-expression has a key role to play (Volpert *et al.*, 1997). Significant correlation exists between Cox-2 expression and VEGF in various human cancers such as endometrium cancer, head and neck cancer (Fujiwaki *et al.*, 2002; Gallo *et al.*, 2001). However, sufficient data is not yet available to prove direct correlation between Cox-2 and VEGF protein expression in HCC. In a recent study it has been shown that VEGF may also function as a biomarker for tumor invasion in HCC as its elevated level was found to mediate venous invasion and metastasis in HCC (Poon *et al.*, 2001). Another study revealed that prostaglandins derived from Cox-2 enhance VEGF protein expression and the use of Cox-2 specific inhibitors down regulates VEGF level (Gallo *et al.*, 2001; Kirkpatrick *et al.*, 2002; Leahy *et al.*, 2000). Studies have also indicated that VEGF expression is considerably very high in differentiated HCC, followed by moderately differentiated HCC and then poorly differentiated HCC. Thus it establishes relation between VEGF expression pattern and histological grade of HCC. It has also been hypothesized that Cox-2 and VEGF genes are down-regulated during de-differentiation, thus expressing low levels of Cox-2 and VEGF in moderately and poorly differentiated HCC. It may be concluded that Cox-2 and VEGF operate in sequential manner and combined together promote HCC in humans (Koga *et al.*, 1999).

Also Cox-2 association with iNOS and VEGF at molecular level has been reported. In mice model for cox-2(-/-) mouse fibroblasts, 94% reduction was found in production of VEGF as against wild type (Williams *et al.*, 2000). Other reports suggest that Cox-2 produced prostaglandins can potentially induce VEGF (Cheng *et al.*, 1998; Majima *et al.*, 2000). Cox-2 and associated prostaglandin are bioactive factors that act in paracrine fashion effecting neighbouring tumor cells (Rak *et al.*, 1996). Prostaglandins exaggerate expression of IL-6, which increases expression of VEGF leading to increase in consequences of angiogenesis (Gruber *et al.*, 2000).

Growth Factors

Well differentiated HCC show high expression level of epidermal growth factor receptor and Transforming growth factor alpha as compared to less differentiated HCC which reveal reduced level of TGFα and EGFR, suggesting high expression level of TGFα and EGFR during early stages of HCC progression (Morimitsu et al., 1995). Recently, it has been found that Cox-2 expression is linked to TGFα (DuBois *et al.*, 1994) and EGFR activation and their expression (Asano *et al.*, 1997). Also elevated TGFα levels are expressed in 82% of human HCC, and it is closely associated with and augments cascade of events during HBV mediated HCC (Hsia *et al.*, 1992). Another study reported that dysregulation of adenomatous polyposis coli (APC) and k-ras genes encourage progression of human HCC. Mutation and consequent loss of function of APC protein upregulates wnt cascade allowing building up of β-catenin, which has strong affinity for T-cell factor (tcf-4). This interaction affects downstream processes. It has been hypothesised that this activation augments Cox-2 expression. In HCC pathogenesis, Cox-2 expression is suppressed by APC and intensified by nuclear accumulation of β-catenin (Araki *et al.*, 2003).

Inflammatory Cells

In addition, tumor tissue also manifests prominent correlation between Cox-2 expression and presence of inflammatory cells like macrophages, kupffer cells, mast cells. As HCC progresses towards advance stages there is a sudden drop in the number of Cox-2 expressing cells and inflammatory cells, suggesting their possible role in early HCC (Cervello *et al.*, 2005). Interestingly, Cox-2 down-regulation with tumor progression seems to be an extraordinarily odd event. A possible answer to this peculiar behaviour, as proposed by Trifan is that Cox-2 overexpression during early HCC possibly causes growth disadvantage. He has proposed that Cox-2 overexpression may bring about cell cycle arrest in various cell types (Trifan *et al.*, 1999).

Cox-2 and tumor Metastasis

A major event observed during solid tumor progression is their ability to invade locally and metastasize to distant organs. We have previously shown that Cox-2 over-expression is also mediated by interaction between human metastasis suppressor protein Nm23-H1 protein and Epstein Barr Virus latent nuclear antigen EBNA3C (Kaul *et al.*, 2006). This suggests the key role of Cox-2 in regulation of metastasis potential of cancer cells. Studies have demonstrated Cox-2 role in modulating the invasive aggressiveness of transformed cancer cells. Tsujji *et al.* (1997) proved experimentally that cells programmed to express Cox-2 constitutively exhibited increased metastatic potential as compared to control cells. Biochemical changes involved in the process include activation of MMP-2 and overexpression of membrane type matrix metalloproteinase 1 (MT-MMP-1). Both these effects were reversed by Cox-2 inhibitor sulindac sulphide. It may be possible that Cox-2 controls MMP activity (Tsujii *et al.*, 1997). MMPs and Cox-2 play critical role in colorectal cancer. Their activation and overexpression has been reported in more than 85% of human colorectal tumor samples. Sufficient data in literature support the proposal that Cox-2 actively participates in MMP-2 production and secretion (Hong *et al.*, 2000; Shattuck-Brandt *et al.*, 1999). The basic underlying mechanism by which Cox-2 modulates MMPs expression has not been fully understood and is under investigation (Dempke *et al.*, 2001). It has also been proposed that combination therapy using selective Cox-2 and MMPs inhibitors may offer therapeutic benefits (Shattuck-Brandt et al., 1999).

Several human malignancies exhibit a fundamental link between Cox-2 expression and tumor metastasis (Murata *et al.*, 1999). It has been reported that colon cancer cells expressing Cox-2 constitutively, acquired increased lymphatic invasion and metastatic potential. These phenotypic changes comprise of activation and overexpression of membrane type metalloprotein-2 (MMP-2). Also it was shown that regular treatment with Cox-2 inhibitors like NSAIDs (non-steroidal anti-inflammatory drugs) potentially altered the metastatic potential of cancer cells (Tsujii *et al.*, 1997). On these grounds, it was explored that there could be strong possibility of close link between prostanoid activation and secretion of MMPs, which were reported to mediate migratory capacity and adhesion properties in human hepatoma cell lines (Mayoral *et al.*, 2005). There is compelling evidence that showed Cox-2 produced PGE_2 can induce expression and activation of MMP-2 in HCC tumor cells (Mayoral *et al.*, 2005). Human hepatoma cell lines also exhibited link with integrins in mediating cell invasion (Mayoral *et al.*, 2005). Integrins belong to the family of heterodimeric cell adhesion receptor. Their function is to mediate cell movement on matrix molecules and regulate expression of matrix degrading enzymes called as MMPs, thereby playing a key role in cell invasion. Dysregulation of integrin's is closely linked to increased metastatic potential (Ivaska and Heino, 2000). Recent studies have demonstrated that integrin $\alpha5$ is repressed (Yao *et al.*, 1997) and $\alpha8$, $\beta1$, $\beta7$ and $\beta8$ are upregulated in aggressive HCC phenotype (Liu *et al.*, 2002). Another recent study on integrins demonstrated that αV integrin have affinity for vitronectin which is produced by liver. The interaction between them mediates early stage liver metastasis (Kikkawa *et al.*, 2002). The significant role played by Cox-2 and PGE_2 pathway in tumor metastasis is further strengthened by the fact that treatment with aspirin and Cox-2 selective inhibitor NS398 inhibits Hepatocyte Growth Factor (HGF)

induced invasiveness of human hepatoma cells (Abiru *et al.*, 2002).

6. Cox-2 inhibition as a Potential Therapeutic Strategy

6.1. Non-steroidal anti-inflammatory Drugs (NSAIDs)

Cox-2 is an inducible enzyme and is not expressed constitutively. Rather it is overexpressed in neoplasm and malignant tissue. Studies have shown substantial increase in Cox-2 expression in human HCC cell lines including HuH7 (Kern *et al.*, 2002), but the mechanism involved remains a mystery (Kern *et al.*, 2004). Clinical trials using specific Cox-2 inhibitors suggest that it may prove to be a viable molecular target in cancer treatment (Dang *et al.*, 2002). The data suggest that Cox-2 inhibitors might serve as an effective therapeutic tool to combat HCC (Cheng *et al.*, 2002). It has been found that NSAIDs and selective Cox-2 inhibitors block Cox-2 prostanoid pathway followed by reduction in production of inflammatory mediators, thus reducing inflammation (Jachak, 2007). NSAIDs act in three favourable ways: analgesic, anti-pyretic and anti-inflammatory. They have a wide spectrum with varying degree of inhibitory capability against Cox-1 and Cox-2. NSAIDs having moderate selectivity for Cox-1 include ketorolac, fluriprofen, ketoprofen etc. Inhibitors having dual specificity i.e. against both Cox-1 and Cox-2 include indomethacin, aspirin and ibuprofen. But recently, it has been found that selective inhibition of Cox-2 is more promising strategy in fighting tumor progression. Drugs that fall under this category include celecoxib, rofecoxib, valdecoxib, etoricoxib, and lumiracoxib. Recently limitations associated with extensive use of Cox-2 inhibitors have been found. They are highly toxic and can potentially cause gastrointestinal bleeding and myocardial infarction (Jachak, 2007). Strong evidences in literature suggest that aspirin and other NSAIDs inhibit tumor progression by inducing apoptosis (Qiao *et al.*, 1998). Aspirin blocks Cox-2 enzymatic activity by acetylation of Ser-530 in Cox-1 and Ser-516 in Cox-2. Most of the other NSAIDs block Cox cascade by competing with arachidonic acid for active site (Williams *et al.*, 1999).

6.2. Other Inhibitors

Separate study on hepatoma cell lines show that selective Cox-2 inhibitor NS-398 and Sulindac (NSAID analogue) potentially suppress growth of tumor by reducing metastasis and proliferation and by inducing apoptosis (Bae *et al.*, 2001; Hu, 2002). Another report suggests that in HCC tumor cells, NS398 and UOI26 (MEK inhibitor) act synergistically to accelerate apoptosis, thus exhibiting anti tumor activity (Schmidt *et al.*, 2003). Also Cox-2 is regarded as determinant of differentiation level in HCC during tumor growth, therefore inhibition of Cox-2 by NS-398 may suppress HCC (Bae *et al.*, 2001). In a recent study on hepatoma cell lines it was found that Cox-2 inhibition using specific inhibitor meloxicam and Sulindac decreased solid tumor formation. Thus Cox-2 inhibitors may have substantial preventive and therapeutic potential in treating HCV (Kern *et al.*, 2002). Another report suggest that Cox-2 selective inhibitor NS-398 and indomethacin, which inhibits both Cox isoforms repress invasion and metastatic potential of tumor cells by down-regulating expression of VEGF and MMP-2 (Li *et al.*, 2002). Recent study on Celecoxib which is a selective Cox-2 inhibitor, and SC560, which is a selective Cox-1 inhibitor, showed that both induced G0/G1 cell cycle arrest by reducing expression of cyclinA, cyclinB and cyclin dependent kinase I. It was further assessed that Celecoxib functions in Cox-2 dependent and independent pathway and are not limited to tumors expressing Cox-2 (Grosch *et al.*, 2001). In HCC cell lines, Celecoxib has been shown to arrest cell cycle by down regulating cell cycle protein cyclin D1 (Chi-Man Tang *et al.*, 2005). For long term treatment of HCC, it is thought that use of selective Cox-2 inhibitors reduce the consequences of undesirable side effects as they specifically down regulate pro-inflammatory prostaglandins (PGE_2) (Kern *et al.*, 2004).

6.3. Adverse Effects of Cox-2 Inhibition

Benefits of using NSAIDs as therapeutic tool in reducing incidences of tumorigenesis have been extensively shown in preclinical and clinical studies. Yet the adverse gastrointestinal and cardiovascular side effects remain. Although precise data showing the incidences of side effects

of prolonged use of NSAIDs is unavailable, yet it is estimated that upto 4% of the patients per year suffer catastrophic complications (Bjorkman, 1999). Safety and efficacy are the two prohibitive limitations that NSAIDs used today cannot overcome (Rigas and Kashfi, 2005). The discovery of potential role played by Cox-2 selective inhibitors such as NSAIDs in fighting cancer opens new horizon in treating cancer. However, the inevitable side effects associated with their prolonged use is a matter of concern (Rigas and Kashfi, 2005). Therefore more work need to be done to study the downstream cascade of PGs, as PGE_2 is found in huge quantities in tumor microenvironment (Cha and DuBois, 2007).

6.4. Cox-2 and Multidrug Resistance

Multidrug resistance in cancer cells is a big hurdle in successful chemotherapy. Substantial body of evidences suggest that overexpression of Cox-2 may also lead to enhanced level of MDR1 gene expression and its downstream product, the P-glycoprotein. In tumor tissue P-gp has been implicated as major cause of MDR and it acts as multidrug efflux pump. A causal link between Cox-2 and P-gp has been established and it is also predicted that use of Cox-2 inhibitors NS398 may check Cox-2 mediated MDR-1 over-expression (Patel *et al.*, 2002; Sorokin, 2004). Thus the idea of selective Cox-2 inhibition might reinforce tumor suppressive action of conventional chemotherapy by opposing P-gp expression. A recent study on human liver cancer cell lines indicated that MDR-1 is linked with overexpression of angiogenic phenotype including cox-2 gene (Fantappie *et al.*, 2002).

7. Problems with Cox-2 inhibition: Activation of Alternative Pathway and Generating Cancer?

A little shift from focus of Cox-2 as central mediator is required as there is accumulated data conferring role of eicosanoids in cancer progression. A well designed system of talented enzymes referred to as 'terminal enzymes' include phospholipases, Coxs and Loxs as they metabolise poly unsaturated fatty acids to end products forming biologically active eiconsanoids (Rigas and Kashfi, 2005). Lox products have also drawn attention as some have pro-tumorigenic properties while others have anti-tumorigenic activities (Shureiqi and Lippman, 2001). The terminal enzymes act in peculiar manner and play individual role in cancer. A recent report on murine model for pulmonary cancer suggested that over-expression of prostacyclin synthase results in exaggerated pulmonary PGI_2 production and prevents murine lung cancer. These finding broaden our horizon for new therapeutical approach that may include manipulation of PG metabolism downstream from Cox in lung cancer prevention rather than focussing on Cox-2 inhibition alone (Keith *et al.*, 2002). Literature reveals that inhibition of Cox-2 using selective inhibitors further complicates the situation by channelizing its substrate fatty acid to non-cox cascade resulting in production of pro-tumorigenic end product. Also, Cox-2 inhibition could switch arachidonic acid to Lox pathway, thus repressing apoptosis and thinning chances of cancer prevention (Rigas and Kashfi, 2005). Another interesting finding on human lung cancer showed that oral administration of celeoxib accelerated leukotriene B4 level in lung microenvironment under physiological conditions, though there is no functional significance of this effect (Mao *et al.*, 2004).

8. Summary

Cox-2 metabolise production of eicosanoids. Their downstream products contribute to various physiological processes including inflammation, immune and development function. Extensive literature evidences have shown that Cox-1 is housekeeping in function while Cox-2 is inducible. It is activated by various inflammatory, chemical, mutagenic and physiological stimuli and acts in pro-inflammatory manner. Sufficient genetic and pharmacologic evidences implicate its crucial role in neoplasia and it is also now clear that Cox-2 plays a crucial role in tumor progression. Cox-2 overexpression is associated with maintaining tumor microenvironment and has crucial implication for angiogenesis. Cox-2 operates in multifactorial fashion. It not only promotes production of pro-angiogenic proteins but also results in production of PGE_2, PGI_2 and TXA_2 that are directly linked to cancer development; not to forget its key role in tumor

enhancement, survival and metastatic aggression by upregulating several anti-apoptotic proteins and several signalling cascades. Cox-2 contribution at several points in angiogenic and inflammatory cascade makes it an ideal target to fight cancer. Cox-2 selective inhibition has been reported as a successful tool in suppressing angiogenesis and metastasis. Thus, pharmacological suppression of Cox-2 represents a bright future as therapeutic tool for treatment of various malignancies. Yet the extent to which conventional NSAIDs are involved in prevention of various malignancies is confined. Although selective pharmacological repression of Cox-2 may present a bright future as therapeutic tool for prevention of various malignancies, yet multiple safety and efficacy issues associated with regular use of NSAIDs limits their role. There is a compelling need to shift the focus from selective Cox-2 inhibition alone, and device strategy to identify agents or combinations of molecular targets offering high efficacy and minimal toxic side effects. A new era in cancer therapy has already begun, involving not only generation of mechanistic insight but also taking them to clinical evaluation and trials.

9. Future Prospects

Discovery of Cox-2 upregulation and overexpression in various cancers has provided a major turning point and strong stimulus in last few years to unfold several mechanisms related to cancer pathogenesis thus narrowing the focus on combating cancer. The conventional concept of dominant role of Cox-2 in preventing cancer has critical complications. These subtle issues necessitate reappraisal in devising strategies that may include multi-pathway suppression as chemo-preventive measure. Further development of dual inhibitors such as of Lox and Cox pathway like Licofelone may pave next milestone in the fight against cancer (Rigas and Kashfi, 2005).

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Abbreviations

HCC, hepatocellular carcinoma; Cox-2, cyclooxygenase-2; HCV, Hepatitis C Virus; HBV, Hepatitis B Virus; EGFR, epidermal growth factor receptor; TGFá, transforming growth factor alpha; NSAIDs, non-steroidal anti-inflammatory drugs; PGE_2, prostaglandins; MDR1, multi drug resistance; P-gp, P-glycoprotein.

References

[1] Abiru, S., Nakao, K., Ichikawa, T., Migita, K., Shigeno, M., Sakamoto, M., Ishikawa, H., Hamasaki, K., Nakata, K., and Eguchi, K. (2002), Aspirin and NS-398 inhibit hepatocyte growth factor-induced invasiveness of human hepatoma cells. *Hepatology* **35**, 1117-1124.

[2] Abou-Shady, M., Baer, H. U., Friess, H., Zimmermann, A., and Buchler, M. W. (1999), Molecular aspects of hepatocellular carcinoma. *Swiss Surg* **5**, 102-106.

[3] Ahmadi, M., Emery, D. C., and Morgan, D. J. (2008), 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* **68**, 7520-7529.

[4] Appleby, S. B., Ristimaki, A., Neilson, K., Narko, K., and Hla, T. (1994), Structure of the human cyclo-oxygenase-2 gene. *Biochem J* **302** *(Pt 3)*, 723-727.

[5] Araki, Y., Okamura, S., Hussain, S. P., Nagashima, M., He, P., Shiseki, M., Miura, K., and Harris, C. C. (2003), Regulation of cyclooxygenase-2 expression by the Wnt and ras pathways. *Cancer Res* **63**, 728-734.

[6] Asano, K., Nakamura, H., Lilly, C.M., Klagsbrun, M., and Drazen, J. M. (1997), Interferon gamma induces prostaglandin G/H synthase-2 through an autocrine loop via the epidermal growth factor receptor in human bronchial epithelial cells. *J Clin Invest* **99**, 1057-1063.

[7] Bae, S. H., Jung, E. S., Park, Y. M., Kim, B. S., Kim, B. K., Kim, D. G., and Ryu, W. S. (2001), Expression of cyclooxygenase-2 (COX-2) in hepatocellular carcinoma and growth inhibition of hepatoma cell lines by a COX-2 inhibitor, NS-398. *Clin Cancer Res* **7**, 1410-1418.

[8] Bakhle, Y. S., and Botting, R. M. (1996), Cyclooxygenase-2 and its regulation in inflammation. *Mediators Inflamm* **5**, 305-323.

[9] Belvisi, M. G., Saunders, M. A., Haddad el, B., Hirst, S. J., Yacoub, M. H., Barnes, P. J., and Mitchell, J. A. (1997), Induction of cyclo-oxygenase-2 by cytokines in human cultured airway smooth muscle cells: novel inflammatory role of this cell type. *Br J. Pharmacol* **120**, 910-916.

[10] Bjorkman, D. J. (1999), Current status of nonsteroidal anti-inflammatory drug (NSAID) use in the United States: risk factors and frequency of complications. *Am J. Med*. **107**, 3S-8S; discussion 8S-10S.

[11] Callejas, N. A., Bosca, L., Williams, C. S., Du, B. R., and Martin-Sanz, P. (2000), Regulation of cyclooxygenase 2 expression in hepatocytes by CCAAT/enhancer-binding proteins. *Gastroenterology* **119**, 493-501.

[12] Cervello, M., Foderaa, D., Florena, A. M., Soresi, M., Tripodo, C., D'Alessandro, N., and Montalto, G. (2005). Correlation between expression of cyclooxygenase-2 and the presence of inflammatory cells in human primary hepatocellular carcinoma: possible role in tumor promotion and angiogenesis. *World J Gastroenterol* **11**, 4638-4643.

[13] Cervello, M., and Montalto, G. (2006), Cyclooxygenases in hepatocellular carcinoma. *World J Gastroenterol* **12**, 5113-5121.

[14] Cha, Y. I., and DuBois, R. N. (2007), NSAIDs and cancer prevention: targets downstream of COX-2. *Annu Rev Med* **58**, 239-252.

[15] Cheng, A. S., Chan, H. L., Leung, W. K., To, K. F., Go, M. Y., Chan, J. Y., Liew, C. T., and Sung, J. J. (2004a), Expression of HBx and COX-2 in chronic hepatitis B, cirrhosis and hepatocellular carcinoma: implication of HBx in upregulation of COX-2. *Mod Pathol* **17**, 1169-1179.

[16] Cheng, A. S., Chan, H. L., To, K. F., Leung, W. K., Chan, K. K., Liew, C. T., and Sung, J. J. (2004b), Cyclooxygenase-2 pathway correlates with vascular endothelial growth factor expression and tumor angiogenesis in hepatitis B virus-associated hepatocellular carcinoma. *Int. J. Oncol* **24**, 853-860.

[17] Cheng, J., Imanishi, H., Amuro, Y., and Hada, T. (2002), NS-398, a selective cyclooxygenase 2 inhibitor, inhibited cell growth and induced cell cycle arrest in human hepatocellular carcinoma cell lines. *Int. J. Cancer* **99**, 755-761.

[18] Cheng, T., Cao, W., Wen, R., Steinberg, R.H., and LaVail, M. M. (1998), Prostaglandin E2 induces vascular endothelial growth factor and basic fibroblast growth factor mRNA expression in cultured rat Muller cells. *Invest Ophthalmol Vis Sci* **39**, 581-591.

[19] Chi-Man Tang, T., Tung-Ping Poon, R., and Fan, S. T. (2005), The significance of cyclooxygenase-2 expression in human hepatocellular carcinoma. *Biomed Pharmacother* **59** *Suppl 2*, S311-316.

[20] Choi, J., Lee, K. J., Zheng, Y., Yamaga, A. K., Lai, M. M., and Ou, J. H. (2004), Reactive oxygen species suppress hepatitis C virus RNA replication in human hepatoma cells. *Hepatology* **39**, 81-89.

[21] Cianchi, F., Cortesini, C., Bechi, P., Fantappie, O., Messerini, L., Vannacci, A., Sardi, I., Baroni, G., Boddi, V., Mazzanti, R., *et al.* (2001), Up-regulation of cyclooxygenase 2 gene expression correlates with tumor angiogenesis in human colorectal cancer. *Gastroenterology* **121**, 1339-1347.

[22] Costa, C., Soares, R., Reis-Filho, J. S., Leitao, D., Amendoeira, I., and Schmitt, F. C. (2002), Cyclo-oxygenase 2 expression is associated with angiogenesis and lymph node metastasis in human breast cancer. *J. Clin Pathol* **55**, 429-434.

[23] Coussens, L. M., and Werb, Z. (2002), Inflammation and cancer. *Nature* **420**, 860-867.

[24] Dang, C. T., Shapiro, C. L., and Hudis, C. A. (2002), Potential role of selective COX-2 inhibitors in cancer management. Oncology (Williston Park) **16**, 30-36.

[25] Dempke, W., Rie, C., Grothey, A., and Schmoll, H.J. (2001). Cyclooxygenase-2: a novel target for cancer chemotherapy? *J. Cancer Res Clin Oncol* **127**, 411-417.

[26] Deng, W.G., Zhu, Y., and Wu, K. K. (2003), Up-regulation of p300 binding and p50 acetylation in tumor necrosis factor-alpha-induced cyclooxygenase-2 promoter activation. *J Biol Chem* **278**, 4770-4777.

[27] DeWitt, D. L., and Smith, W. L. (1988), Primary structure of prostaglandin G/H synthase from sheep vesicular gland determined from the complementary DNA sequence. *Proc Natl Acad Sci U S A* **85**, 1412-1416.

[28] Dixon, D. A. (2004), Dysregulated post-transcriptional control of COX-2 gene expression in cancer. *Curr Pharm Des* **10**, 635-646.

[29] DuBois, R. N., Awad, J., Morrow, J., Roberts, L. J., 2nd, and Bishop, P. R. (1994), Regulation of eicosanoid production and mitogenesis in rat intestinal epithelial cells by transforming growth factor-alpha and phorbol ester. *J Clin Invest* **93**, 493-498.

[30] Eberhart, C. E., Coffey, R. J., Radhika, A., Giardiello, F.M., Ferrenbach, S., and DuBois, R. N. (1994), Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. *Gastroenterology* **107**, 1183-1188.

[31] Fantappie, O., Masini, E., Sardi, I., Raimondi, L., Bani, D., Solazzo, M., Vannacci, A., and Mazzanti, R. (2002), The MDR phenotype is associated with the expression of COX-2 and iNOS in a human hepatocellular carcinoma cell line. *Hepatology* **35**, 843-852.

[32] Feng, L., Xia, Y., Garcia, G.E., Hwang, D., and Wilson, C. B. (1995), Involvement of reactive oxygen intermediates in cyclooxygenase-2 expression induced by interleukin-1, tumor necrosis factor-alpha, and lipopolysaccharide. *J Clin Invest* **95**, 1669-1675.

[33] Fujiwaki, R., Iida, K., Kanasaki, H., Ozaki, T., Hata, K., and Miyazaki, K. (2002), Cyclooxygenase-2 expression in endometrial cancer: correlation with microvessel count and expression of vascular endothelial growth factor and thymidine phosphorylase. *Hum Pathol* **33**, 213-219.

[34] Funk, C. D., Funk, L. B., Kennedy, M. E., Pong, A. S., and Fitzgerald, G. A. (1991), Human platelet/erythroleukemia cell prostaglandin G/H synthase: cDNA cloning, expression, and gene chromosomal assignment. *FASEB J* **5**, 2304-2312.

[35] Gallo, O., Franchi, A., Magnelli, L., Sardi, I., Vannacci, A., Boddi, V., Chiarugi, V., and Masini, E. (2001), Cyclooxygenase-2 pathway correlates with VEGF expression in head and neck cancer. Implications for tumor angiogenesis and metastasis. *Neoplasia* **3**, 53-61.

[36] Gallo, O., Masini, E., Bianchi, B., Bruschini, L., Paglierani, M., and Franchi, A. (2002), Prognostic significance of cyclooxygenase-2 pathway and angiogenesis in head and neck squamous cell carcinoma. *Hum Pathol* **33**, 708-714.

[37] Gately, S. (2000), The contributions of cyclooxygenase-2 to tumor angiogenesis. *Cancer Metastasis Rev* **19**, 19-27.

[38] Gierse, J. K., McDonald, J. J., Hauser, S. D., Rangwala, S. H., Koboldt, C. M., and Seibert, K. (1996), A single amino acid difference between cyclooxygenase-1 (COX-1) and -2 (COX-2) reverses the selectivity of COX-2 specific inhibitors. *J Biol Chem* **271**, 15810-15814.

[39] Glinghammar, B., Skogsberg, J., Hamsten, A., and Ehrenborg, E. (2003), PPARdelta activation induces COX-2 gene expression and cell proliferation in human hepatocellular carcinoma cells. *Biochem Biophys Res Commun* **308**, 361-368.

[40] Grosch, S., Tegeder, I., Niederberger, E., Brautigam, L., and Geisslinger, G. (2001), COX-2 independent induction of cell cycle arrest and apoptosis in colon cancer cells by the selective COX-2 inhibitor celecoxib. *FASEB J* **15**, 2742-2744.

[41] Gruber, R., Nothegger, G., Ho, G.M., Willheim, M., and Peterlik, M. (2000), Differential stimulation by PGE(2) and calcemic hormones of IL-6 in stromal/osteoblastic cells. *Biochem Biophys Res Commun* **270**, 1080-1085.

[42] Hong, B. K., Kwon, H. M., Lee, B. K., Kim, D., Kim, I. J., Kang, S. M., Jang, Y., Cho, S. H., Kim, H. K., Jang, B. C., *et al.* (2000), Coexpression of cyclooxygenase-2 and matrix metalloproteinases in human aortic atherosclerotic lesions. *Yonsei Med J* **41**, 82-88.

[43] Hsia, C. C., Axiotis, C. A., Di Bisceglie, A. M., and Tabor, E. (1992), Transforming growth factor-alpha in human hepatocellular carcinoma and coexpression with hepatitis B surface antigen in adjacent liver. *Cancer* **70**, 1049-1056.

[44] Hu, K. Q. (2002), Rationale and feasibility of chemoprovention of hepatocellular carcinoma by cyclooxygenase-2 inhibitors. *J Lab Clin Med* **139**, 234-243.

[45] Huang, M., Stolina, M., Sharma, S., Mao, J. T., Zhu, L., Miller, P. W., Wollman, J., Herschman, H., and Dubinett, S. M. (1998), Non-small cell lung cancer cyclooxygenase-2-dependent regulation of cytokine balance in lymphocytes and macrophages: up-regulation of interleukin 10 and down-regulation of interleukin 12 production. *Cancer Res* **58**, 1208-1216.

[46] Hung, J. H., Su, I. J., Lei, H. Y., Wang, H. C., Lin, W. C., Chang, W. T., Huang, W., Chang, W. C., Chang, Y. S., Chen, C. C., *et al.* (2004), Endoplasmic reticulum stress stimulates the expression of cyclooxygenase-2 through activation of NF-kappaB and pp38 mitogen-activated protein kinase. *J Biol Chem* **279**, 46384-46392.

[47] Ivaska, J., and Heino, J. (2000), Adhesion receptors and cell invasion: mechanisms of integrin-guided degradation of extracellular matrix. *Cell Mol Life Sci* **57**, 16-24.

[48] Jachak, S. M. (2007), PGE synthase inhibitors as an alternative to COX-2 inhibitors. *Curr Opin Investig Drugs* **8**, 411-415.

[49] Jones, D. A., Carlton, D. P., McIntyre, T. M., Zimmerman, G. A., and Prescott, S. M. (1993), Molecular cloning of human prostaglandin endoperoxide synthase type II and demonstration of expression in response to cytokines. *J Biol Chem* **268**, 9049-9054.

[50] Joo, Y. E., Rew, J. S., Seo, Y. H., Choi, S. K., Kim, Y. J., Park, C. S., and Kim, S. J. (2003), Cyclooxygenase-2 overexpression correlates with vascular endothelial growth factor expression and tumor angiogenesis in gastric cancer. *J Clin Gastroenterol* **37**, 28-33.

[51] Kambayashi, T., Alexander, H.R., Fong, M., and Strassmann, G. (1995), Potential involvement of IL-10 in suppressing tumor-associated macrophages. Colon-26-derived prostaglandin E2 inhibits TNF-alpha release via a mechanism involving IL-10. *J Immunol* **154**, 3383-3390.

[52] Kang, Y. J., Mbonye, U. R., DeLong, C. J., Wada, M., and Smith, W. L. (2007), Regulation of intracellular cyclooxygenase levels by gene transcription and protein degradation. *Prog Lipid Res* **46**, 108-125.

[53] Kaul, R., Verma, S. C., Murakami, M., Lan, K., Choudhuri, T., and Robertson, E. S. (2006), Epstein-Barr virus protein can upregulate cyclo-oxygenase-2 expression through association with the suppressor of metastasis Nm23-H1. *J Virol* **80**, 1321-1331.

[54] Keith, R. L., Miller, Y. E., Hoshikawa, Y., Moore, M. D., Gesell, T. L., Gao, B., Malkinson, A. M., Golpon, H. A., Nemenoff, R. A., and Geraci, M. W. (2002), Manipulation of pulmonary prostacyclin synthase expression prevents murine lung cancer. *Cancer Res* **62**, 734-740.

[55] Kenmochi, K., Sugihara, S., and Kojiro, M. (1987), Relationship of histologic grade of hepatocellular carcinoma (HCC) to tumor size, and demonstration of tumor cells of multiple different grades in single small *HCC. Liver* **7**, 18-26.

[56] Kern, M. A., Schoneweiss, M. M., Sahi, D., Bahlo, M., Haugg, A. M., Kasper, H. U., Dienes, H. P., Kaferstein, H., Breuhahn, K., and Schirmacher, P. (2004), Cyclooxygenase-2 inhibitors suppress the growth of human hepatocellular carcinoma implants in nude mice. *Carcinogenesis* **25**, 1193-1199.

[57] Kern, M. A., Schubert, D., Sahi, D., Schoneweiss, M. M., Moll, I., Haugg, A. M., Dienes, H. P., Breuhahn, K., and Schirmacher, P. (2002), Proapoptotic and antiproliferative potential of selective cyclooxygenase-2 inhibitors in human liver tumor cells. *Hepatology* **36**, 885-894.

[58] Kikkawa, H., Kaihou, M., Horaguchi, N., Uchida, T., Imafuku, H., Takiguchi, A., Yamazaki, Y., Koike, C., Kuruto, R., Kakiuchi, T., *et al.* (2002), Role of integrin alpha(v)beta3 in the early phase of liver metastasis: PET and IVM analyses. *Clin Exp Metastasis* **19**, 717-725.

[59] Kirkpatrick, K., Ogunkolade, W., Elkak, A., Bustin, S., Jenkins, P., Ghilchik, M., and Mokbel, K. (2002), The mRNA expression of cyclo-oxygenase-2 (COX-2) and vascular endothelial growth factor (VEGF) in human breast cancer. *Curr Med Res Opin* **18**, 237-241.

[60] Koga, H., Sakisaka, S., Ohishi, M., Kawaguchi, T., Taniguchi, E., Sasatomi, K., Harada, M., Kusaba, T., Tanaka, M., Kimura, R., *et al.* (1999). Expression of cyclooxygenase-2 in human hepatocellular carcinoma: relevance to tumor dedifferentiation. *Hepatology* **29**, 688-696.

[61] Kondo, M., Yamamoto, H., Nagano, H., Okami, J., Ito, Y., Shimizu, J., Eguchi, H., Miyamoto, A., Dono, K., Umeshita, K., *et al.* (1999), Increased expression of COX-2 in nontumor liver tissue is associated with shorter disease-free survival in patients with hepatocellular carcinoma. *Clin Cancer Res* **5**, 4005-4012.

[62] Kraemer, S. A., Meade, E. A., and DeWitt, D. L. (1992), Prostaglandin endoperoxide synthase gene structure: identification of the transcriptional start site and 5'-flanking regulatory sequences. *Arch Biochem Biophys* **293**, 391-400.

[63] Kulinsky, V. I. (2007), Biochemical aspects of inflammation. *Biochemistry (Mosc)* **72**, 595-607.

[64] Lara-Pezzi, E., Gomez-Gaviro, M. V., Galvez, B. G., Mira, E., Iniguez, M. A., Fresno, M., Martinez, A. C., Arroyo, A. G., and Lopez-Cabrera, M. (2002), The hepatitis B virus X protein promotes tumor cell invasion by inducing membrane-type matrix metalloproteinase-1 and cyclooxygenase-2 expression. *J Clin Invest* **110**, 1831-1838.

[65] Leahy, K. M., Koki, A. T., and Masferrer, J. L. (2000), Role of cyclooxygenases in angiogenesis. *Curr Med Chem* **7**, 1163-1170.

[66] Leng, J., Han, C., Demetris, A.J., Michalopoulos, G.K., and Wu, T. (2003), Cyclooxygenase-2 promotes hepatocellular carcinoma cell growth through Akt activation: evidence for Akt inhibition in celecoxib-induced apoptosis. *Hepatology* **38**, 756-768.

[67] Li, G., Yang, T., and Yan, J. (2002), Cyclooxygenase-2 increased the angiogenic and metastatic potential of tumor cells. *Biochem Biophys Res Commun* **299**, 886-890.

[68] Li, H. X., Chang, X. M., Song, Z. J., and He, S. X. (2003), Correlation between expression of cyclooxygenase-2 and angiogenesis in human gastric adenocarcinoma. *World J Gastroenterol* **9**, 674-677.

[69] Liu, C. H., Chang, S. H., Narko, K., Trifan, O. C., Wu, M. T., Smith, E., Haudenschild, C., Lane, T. F., and Hla, T. (2001), Overexpression of cyclooxygenase-2 is sufficient to induce tumorigenesis in transgenic mice. *J Biol Chem* **276**, 18563-18569.

[70] Liu, L. X., Jiang, H. C., Liu, Z. H., Zhou, J., Zhang, W. H., Zhu, A. L., Wang, X. Q., and Wu, M. (2002), Integrin gene expression profiles of human hepatocellular carcinoma. *World J Gastroenterol* **8**, 631-637.

[71] Majima, M., Hayashi, I., Muramatsu, M., Katada, J., Yamashina, S., and Katori, M. (2000), Cyclo-oxygenase-2 enhances basic fibroblast growth factor-induced angiogenesis through induction of vascular endothelial growth factor in rat sponge implants. *Br J Pharmacol* **130**, 641-649.

[72] Mao, J.T., Tsu, I.H., Dubinett, S.M., Adams, B., Sarafian, T., Baratelli, F., Roth, M.D., and Serio, K.J. (2004), Modulation of pulmonary leukotriene B4 production by cyclooxygenase-2 inhibitors and lipopolysaccharide. *Clin Cancer Res* **10**, 6872-6878.

[73] Mayoral, R., Fernandez-Martinez, A., Bosca, L., and Martin-Sanz, P. (2005), Prostaglandin E2 promotes migration and adhesion in hepatocellular carcinoma cells. *Carcinogenesis* **26**, 753-761.

[74] Meric, J.B., Rottey, S., Olaussen, K., Soria, J.C., Khayat, D., Rixe, O., and Spano, J.P. (2006), Cyclooxygenase-2 as a target for anticancer drug development. *Crit Rev Oncol Hematol* **59**, 51-64.

[75] Merlie, J.P., Fagan, D., Mudd, J., and Needleman, P. (1988). Isolation and characterization of the complementary DNA for sheep seminal vesicle prostaglandin endoperoxide synthase (cyclooxygenase). *J Biol Chem* **263**, 3550-3553.

[76] Morimitsu, Y., Hsia, C.C., Kojiro, M., and Tabor, E. (1995), Nodules of less-differentiated tumor within or adjacent to hepatocellular carcinoma: relative expression of transforming growth factor-alpha and its receptor in the different areas of tumor. *Hum Pathol* **26**, 1126-1132.

[77] Mozer-Lisewska, I., Kaczmarek, M., and Zeromski, J. (2006). [The role of NF-kappaB transcription factor in chronic viral hepatitis C and B]. *Postepy Biochem* **52**, 56-61.

[78] Mukhopadhyay, D., Houchen, C.W., Kennedy, S., Dieckgraefe, B.K., and Anant, S. (2003), Coupled mRNA stabilization and translational silencing of cyclooxygenase-2 by a novel RNA binding protein, *CUGBP2. Mol Cell* **11**, 113-126.

[79] Murata, H., Kawano, S., Tsuji, S., Tsuji, M., Sawaoka, H., Kimura, Y., Shiozaki, H., and Hori, M. (1999), Cyclooxygenase-2 overexpression enhances lymphatic invasion and metastasis in human gastric carcinoma. *Am J Gastroenterol* **94**, 451-455.

[80] Nie, D., and Honn, K.V. (2002). Cyclooxygenase, lipoxygenase and tumor angiogenesis. *Cell Mol Life Sci* **59**, 799-807.

[81] Nielsen, J.S., and McNagny, K.M. (2008), Novel functions of the CD34 family. *J Cell Sci* **121**, 3683-3692.

[82] Oshima, M., Dinchuk, J.E., Kargman, S.L., Oshima, H., Hancock, B., Kwong, E., Trzaskos, J.M., Evans, J.F., and Taketo, M.M. (1996). Suppression of intestinal polyposis in Apc delta716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). *Cell* **87**, 803-809.

[83] Patel, V.A., Dunn, M.J., and Sorokin, A. (2002). Regulation of MDR-1 (P-glycoprotein) by cyclooxygenase-2. *J Biol Chem* **277**, 38915-38920.

[84] Poon, R.T., Ng, I.O., Lau, C., Zhu, L.X., Yu, W.C., Lo, C.M., Fan, S.T., and Wong, J. (2001), Serum vascular endothelial growth factor predicts venous invasion in hepatocellular carcinoma: a prospective study. *Ann Surg* **233**, 227-235.

[85] Qiao, L., Hanif, R., Sphicas, E., Shiff, S.J., and Rigas, B. (1998), Effect of aspirin on induction of apoptosis in HT-29 human colon adenocarcinoma cells. *Biochem Pharmacol* **55**, 53-64.

[86] Rahman, M.A., Dhar, D.K., Yamaguchi, E., Maruyama, S., Sato, T., Hayashi, H., Ono, T., Yamanoi, A., Kohno, H., and Nagasue, N. (2001), Coexpression of inducible nitric oxide synthase and COX-2 in hepatocellular carcinoma and surrounding liver: possible

involvement of COX-2 in the angiogenesis of hepatitis C virus-positive cases. *Clin Cancer Res* **7**, 1325-1332.

[87] Rak, J., Filmus, J., and Kerbel, R. S. (1996), Reciprocal paracrine interactions between tumour cells and endothelial cells: the 'angiogenesis progression' hypothesis. *Eur J Cancer* **32A**, 2438-2450.

[88] Rigas, B., and Kashfi, K. (2005), Cancer prevention: a new era beyond cyclooxygenase-2. *J Pharmacol Exp Ther* **314**, 1-8.

[89] Sakamoto, M., Ino, Y., Fujii, T., and Hirohashi, S. (1993), Phenotype changes in tumor vessels associated with the progression of hepatocellular carcinoma. *Jpn J Clin Oncol* **23**, 98-104.

[90] Sawaoka, H., Tsuji, S., Tsujii, M., Gunawan, E. S., Sasaki, Y., Kawano, S., and Hori, M. (1999), Cyclooxygenase inhibitors suppress angiogenesis and reduce tumor growth in vivo. Lab Invest **79**, 1469-1477.

[91] Schmidt, C.M., Wang, Y., and Wiesenauer, C. (2003), Novel combination of cyclooxygenase-2 and MEK inhibitors in human hepatocellular carcinoma provides a synergistic increase in apoptosis. *J. Gastrointest Surg* **7**, 1024-1033.

[92] Sengupta, S., Jang, B.C., Wu, M.T., Paik, J.H., Furneaux, H., and Hla, T. (2003). The RNA-binding protein HuR regulates the expression of cyclooxygenase-2. *J Biol Chem* **278**, 25227-25233.

[93] Shattuck-Brandt, R. L., Lamps, L. W., Heppner Goss, K. J., DuBois, R. N., and Matrisian, L. M. (1999), Differential expression of matrilysin and cyclooxygenase-2 in intestinal and colorectal neoplasms. *Mol Carcinog* **24**, 177-187.

[94] Sheflin, L. G., Zhang, W., and Spaulding, S. W. (2001), Androgen regulates the level and subcellular distribution of the AU-rich ribonucleic acid-binding protein HuR both in vitro and in vivo. *Endocrinology* **142**, 2361-2368.

[95] Shureiqi, I., and Lippman, S.M. (2001), Lipoxygenase modulation to reverse carcinogenesis. *Cancer Res* **61**, 6307-6312.

[96] Smith, W.L. (1992), Prostanoid biosynthesis and mechanisms of action. *Am J Physiol* **263**, F181-191.

[97] Smith, W.L., DeWitt, D.L., Kraemer, S.A., Andrews, M.J., Hla, T., Maciag, T., and Shimokawa, T. (1990), Structure-function relationships in sheep, mouse, and human prostaglandin endoperoxide G/H synthases. Adv Prostaglandin Thromboxane Leukot Res **20**, 14-21.

[98] Snijdewint, F.G., Kalinski, P., Wierenga, E.A., Bos, J.D., and Kapsenberg, M.L. (1993), Prostaglandin E2 differentially modulates cytokine secretion profiles of human T helper lymphocytes. *J Immunol* **150**, 5321-5329.

[99] Sobolewski, C., Cerella, C., Dicato, M., Ghibelli, L., and Diederich, M. The role of cyclooxygenase-2 in cell proliferation and cell death in human malignancies. *Int J Cell Biol* **2010**, 215158.

[100] Song, S.H., Jong, H.S., Choi, H.H., Inoue, H., Tanabe, T., Kim, N.K., and Bang, Y.J. (2001), Transcriptional silencing of Cyclooxygenase-2 by hyper-methylation of the 5' CpG island in human gastric carcinoma cells. *Cancer Res* **61**, 4628-4635.

[101] Sorokin, A. (2004), Cyclooxygenase-2: potential role in regulation of drug efflux and multidrug resistance phenotype. *Curr Pharm Des* **10**, 647-657.

[102] Stolina, M., Sharma, S., Lin, Y., Dohadwala, M., Gardner, B., Luo, J., Zhu, L., Kronenberg, M., Miller, P.W., Portanova, J., *et al.* (2000), Specific inhibition of cyclooxygenase 2 restores antitumor reactivity by altering the balance of IL-10 and IL-12 synthesis. *J Immunol* **164**, 361-370.

[103] Subbaramaiah, K., Altorki, N., Chung, W.J., Mestre, J.R., Sampat, A., and Dannenberg, A.J. (1999), Inhibition of cyclooxygenase-2 gene expression by p53. *J Biol Chem* **274**, 10911-10915.

[104] Sugihara, S., Nakashima, O., Kojiro, M., Majima, Y., Tanaka, M., and Tanikawa, K. (1992), The morphologic transition in hepatocellular carcinoma. A comparison of the individual histologic features disclosed by ultrasound-guided fine-needle biopsy with those of autopsy. *Cancer* **70**, 1488-1492.

[105] Symensma, T. L., Martinez-Guzman, D., Jia, Q., Bortz, E., Wu, T.T., Rudra-Ganguly, N., Cole, S., Herschman, H., and Sun, R. (2003), COX-2 induction during murine gammaherpesvirus 68 infection leads to enhancement of viral gene expression. *J Virol* **77**, 12753-12763.

[106] Takeda, H., Sonoshita, M., Oshima, H., Sugihara, K., Chulada, P. C., Langenbach, R., Oshima, M., and Taketo, M. M. (2003), Cooperation of cyclooxygenase 1 and cyclooxygenase 2 in intestinal polyposis. *Cancer Res* **63**, 4872-4877.

[107] Tang, H., and Grise, H. (2009), Cellular and molecular biology of HCV infection and hepatitis. *Clin Sci (Lond)* **117**, 49-65.

[108] Tang, T.C., Poon, R.T., Lau, C.P., Xie, D., and Fan, S.T. (2005). Tumor cyclooxygenase-2 levels correlate with tumor invasiveness in human hepatocellular carcinoma. *World J Gastroenterol* **11**, 1896-1902.

[109] Tiano, H. F., Loftin, C. D., Akunda, J., Lee, C. A., Spalding, J., Sessoms, A., Dunson, D. B., Rogan, E. G., Morham, S. G., Smart, R. C., *et al.* (2002), Deficiency of either cyclooxygenase (COX)-1 or COX-2 alters epidermal differentiation and reduces mouse skin tumorigenesis. *Cancer Res* **62**, 3395-3401.

[110] Trifan, O. C., Smith, R. M., Thompson, B. D., and Hla, T. (1999), Overexpression of cyclooxygenase-2 induces cell cycle arrest. Evidence for a prostaglandin-independent mechanism. *J. Biol Chem*. **274**, 34141-34147.

[111] Trujillo-Murillo, K., Alvarez-Martinez, O., Garza-Rodriguez, L., Martinez-Rodriguez, H., Bosques-Padilla, F., Ramos-Jimenez, J., Barrera-Saldana, H., Rincon-Sanchez, A. R., and Rivas-Estilla, A. M. (2007), Additive effect of ethanol and HCV subgenomic replicon expression on COX-2 protein levels and activity. *J Viral Hepat* **14**, 608-617.

[112] Tsatsanis, C., Androulidaki, A., Venihaki, M., and Margioris, A. N. (2006), Signalling networks

regulating cyclooxygenase-2. *Int J Biochem Cell Biol* **38**, 1654-1661.

[113] Tsujii, M., and DuBois, R. N. (1995), Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. *Cell* **83**, 493-501.

[114] Tsujii, M., Kawano, S., and DuBois, R. N. (1997), Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. Proc Natl Acad Sci U S A **94**, 3336-3340.

[115] Tsujii, M., Kawano, S., Tsuji, S., Sawaoka, H., Hori, M., and DuBois, R. N. (1998), Cyclooxygenase regulates angiogenesis induced by colon cancer cells. *Cell* **93**, 705-716.

[116] Ueda, K., Terada, T., Nakanuma, Y., and Matsui, O. (1992), Vascular supply in adenomatous hyperplasia of the liver and hepatocellular carcinoma: a morphometric study. *Hum Pathol* **23**, 619-626.

[117] Volpert, O. V., Dameron, K. M., and Bouck, N. (1997), Sequential development of an angiogenic phenotype by human fibroblasts progressing to tumorigenicity. *Oncogene* **14**, 1495-1502.

[118] Wang, D., and Dubois, R. N. (2006), Prostaglandins and cancer. *Gut* **55**, 115-122.

[119] Wang, M. T., Honn, K. V., and Nie, D. (2007), Cyclooxygenases, prostanoids, and tumor progression. *Cancer Metastasis Rev* **26**, 525-534.

[120] Waris, G., and Siddiqui, A. (2005), Hepatitis C virus stimulates the expression of cyclooxygenase-2 via oxidative stress: role of prostaglandin E2 in RNA replication. *J Virol* **79**, 9725-9734.

[121] Waris, G., Tardif, K.D., and Siddiqui, A. (2002). Endoplasmic reticulum (ER) stress: hepatitis C virus induces an ER-nucleus signal transduction pathway and activates NF-kappaB and STAT-3. *Biochem Pharmacol* **64**, 1425-1430.

[122] Williams, C. S., Mann, M., and DuBois, R. N. (1999), The role of cyclooxygenases in inflammation, cancer, and development. *Oncogene* **18**, 7908-7916.

[123] Williams, C. S., Tsujii, M., Reese, J., Dey, S. K., and DuBois, R. N. (2000), Host cyclooxygenase-2 modulates carcinoma growth. *J Clin Invest* **105**, 1589-1594.

[124] Winter, T. C., 3rd, Takayasu, K., Muramatsu, Y., Furukawa, H., Wakao, F., Koga, H., Sakamoto, M., Hirohashi, S., and Freeny, P. C. (1994), Early advanced hepatocellular carcinoma: evaluation of CT and MR appearance with pathologic correlation. *Radiology* **192**, 379-387.

[125] Wolff, H., Saukkonen, K., Anttila, S., Karjalainen, A., Vainio, H., and Ristimaki, A. (1998), Expression of cyclooxygenase-2 in human lung carcinoma. *Cancer Res* **58**, 4997-5001.

[126] Xu, X. C. (2002), COX-2 inhibitors in cancer treatment and prevention, a recent development. *Anticancer Drugs* **13**, 127-137.

[127] Xu, Z., Choi, J., Yen, T. S., Lu, W., Strohecker, A., Govindarajan, S., Chien, D., Selby, M. J., and Ou, J. (2001), Synthesis of a novel hepatitis C virus protein by ribosomal frameshift. *EMBO J* **20**, 3840-3848.

[128] Xue, K. X. (2005), [Molecular genetic and epigenetic mechanisms of hepatocarcinogenesis]. *Ai Zheng* **24**, 757-768.

[129] Yao, M., Zhou, X. D., Zha, X. L., Shi, D. R., Fu, J., He, J. Y., Lu, H. F., and Tang, Z. Y. (1997), Expression of the integrin alpha5 subunit and its mediated cell adhesion in hepatocellular carcinoma. *J Cancer Res Clin Oncol* **123**, 435-440.

[130] Yip-Schneider, M. T., Barnard, D. S., Billings, S. D., Cheng, L., Heilman, D. K., Lin, A., Marshall, S. J., Crowell, P. L., Marshall, M. S., and Sweeney, C. J. (2000), Cyclooxygenase-2 expression in human pancreatic adenocarcinomas. *Carcinogenesis* **21**, 139-146.

[131] Young, L. E., Sanduja, S., Bemis-Standoli, K., Pena, E. A., Price, R. L., and Dixon, D. A. (2009), The mRNA binding proteins HuR and tristetraprolin regulate cyclooxygenase 2 expression during colon carcinogenesis. *Gastroenterology* **136**, 1669-1679.

[132] Zeddou, M., Greimers, R., de Valensart, N., Nayjib, B., Tasken, K., Boniver, J., Moutschen, M., and Rahmouni, S. (2005), Prostaglandin E2 induces the expression of functional inhibitory CD94/NKG2A receptors in human CD8+ T lymphocytes by a cAMP-dependent protein kinase A type I pathway. *Biochem Pharmacol* **70**, 714-724.

[133] Zhu, H., Cong, J. P., Yu, D., Bresnahan, W. A., and Shenk, T. E. (2002), Inhibition of cyclooxygenase 2 blocks human cytomegalovirus replication. *Proc Natl Acad Sci U S A* **99**, 3932-3937.