Chapter 2: Mechanistic Aspects of COX-2 Expression in Colorectal Neoplasia

Dan A. Dixon[1,*], **Fernando F. Blanco**[1], **Annalisa Bruno**[2], and **Paola Patrignani**[2,*]

[1]Department of Biological Sciences and Center for Colon Cancer Research, University of South Carolina, Columbia, South Carolina

[2]Center of Excellence on Aging (CeSI) and Department of Neuroscience and Imaging, G. d'Annunzio University, School of Medicine, Chieti, Italy

Abstract

The cyclooxygenase-2 (COX-2) enzyme catalyzes the rate-limiting step of prostaglandin formation in pathogenic states and a large amount of evidence has demonstrated constitutive COX-2 expression to be a contributing factor promoting colorectal cancer (CRC). Various genetic, epigenetic, and inflammatory pathways have been identified to be involved in the etiology and development of CRC. Alteration in these pathways can influence COX-2 expression at multiple stages of colon carcinogenesis allowing for elevated prostanoid biosynthesis to occur in the tumor microenvironment. In normal cells, COX-2 expression levels are potently regulated at the post-transcriptional level through various RNA sequence elements present within the mRNA $3'$-untranslated region($3'$UTR). A conserved AU-rich element(ARE) functions to target COX-2 mRNA for rapid decay and translational inhibition through association with various RNA-binding proteins to influence the fate of COX-2 mRNA. Specific microRNAs bind regions within the COX-2 $3'$UTR and control COX-2 expression. In this chapter, we discuss novel insights in the mechanisms of altered posttranscriptional regulation of COX-2 in CRC and how this knowledge may be used to develop novel strategies for cancer prevention and treatment.

2.1 Molecular basis of colorectal adenoma and carcinoma

Colorectal cancer (CRC) is the third most diagnosed cancer in males and the second in females worldwide. The highest incidence rates are found in Australia and New Zealand, Europe, and North America, whereas the lowest rates are found in Africa and South-Central Asia (Jemal et al. 2011). In economically developed countries, death rates for CRC have largely decreased as result of the improved treatment and use of population-based colorectal screening programs. According to a recent randomized clinical trial (RCT) in the United Kingdom, a one-time flexible sigmoidoscopy screening between 55 and 64 years of age reduced CRC incidence by 33% and mortality by 43% (Atkin et al. 2010). However, proximal colon cancers are not effectively prevented by screening using sigmoidoscopy or colonoscopy. It is quite interesting the finding that the use of low-dose aspirin reduces the 20-year risk of developing CRC (by 24%) and the risk of dying from the cancer (by 35%) and that 70% of the reduced risk is associated with fewer cancers in the right section of the colon (proximal colon) (Rothwell et al. 2010).

*Correspondence: Paola Patrignani, Center of Excellence on Aging (CeSI) and Department of Neuroscience and Imaging, G. d'Annunzio University, School of Medicine, Chieti, Italy, Via dei Vestini 31, 66100 Chieti, Italy, Paola Patrignani (ppatrignani@unich.it); Dan A. Dixon, Department of Biological Sciences and Center for Colon Cancer Research, University of South Carolina, Columbia, SC 29208 Dan Dixon (ddixon@biol.sc.edu).

An important limitation in prevention-based strategies of CRC is the lack of biomarkers of early detection and of safe and effective chemopreventive agents. The reduced risk of CRC by low-dose aspirin, if supported by the results of mechanistic studies which are ongoing (see chapter 3), will open the way to primary prevention strategy in CRC with the drug. In fact, the possible enhanced risk of bleeding associated with low-dose aspirin administration could be overcome by the joint reduced risk of vascular events and cancers. Thus, the association of lifestyle changes, population-based colorectal screening programs and the use of chemopreventive agents affecting early phases of tumorigenesis, such as low-dose aspirin (see chapter 3), would lead to a sharp drop in CRC risk within the next 10 years.

Over the last 25 years, important strides have been made in the understanding of the molecular mechanisms associated with the adenoma-carcinoma sequence, which represents the bedrock of our knowledge of the colonic carcinogenesis process (Ahnen 2011). In fact, the study of molecular mechanisms underlying this sequence has had a profound impact on the understanding of the initiation and progression of colon cancer, thus allowing for the development of improved strategies to identify high-risk patients and provide clinical care to patients with colonic polyps and cancer.

2.1.1 Genetic basis of adenoma-carcinoma sequence

Molecular analyses of colorectal adenomas and carcinomas have led to the development of a genetic model of colon carcinogenesis wherein colon tumorigenesis arises from an accumulation of genetic alterations that promote tumor initiation and disease progression. There are at least three distinct molecular pathways to CRC: i) the chromosomal instability (CIN) pathway, which is largely driven by mutational events in oncogenes and tumor suppressor genes; ii) the microsatellite instability pathway, which is driven by mutations in DNA repair genes, and iii) the epigenetic pathway, which is driven in large part by hypermethylation-induced silencing of tumor suppressor genes (Kulendran et al. 2011). Among them, the CIN pathway is the most commonly observed in CRC accounting for up to 80% of cases (Grady and Carethers 2008).

2.1.2 Adenomatous polyposis coli (APC)

Inactivating mutations in the adenomatous polyposis coli (APC) tumor suppressor gene occur early in the adenoma stages of CRC (Kulendran et al. 2011). The absence of functional APC leads to an inappropriately and constitutively activation of the Wnt signaling pathway, thereby promoting CRC tumor initiation (Kulendran et al. 2011; Markowitz and Bertagnolli 2009). Activation of the Wnt pathway leads to the suppression of the phosphorylation of the oncoprotein β-catenin, resulting in its stabilization and nuclear translocation. β-catenin then interacts with T-cell factor/lymphocyte enhancer factor (TCF/LEF) to induce transcription of Wnt target pro-proliferative and anti-apoptotic genes. In normal cells where Wnt signaling is controlled, cytoplasmic β-catenin is phosphorylated by the glycogen synthase kinase 3β (GSK-3β) within a complex containing APC and Axin, resulting in the degradation of β-catenin through the ubiquitin proteasome pathway. APC, as component of this complex, not only contributes to the β-catenin degradation, but also inhibits its nuclear localization, thereby abrogating transcription of Wnt target genes (Buchanan and DuBois 2006).

Germ-line APC mutations give rise to familial adenomatous polyposis (FAP), an autosomal dominantly inherited syndrome in which patients typically develop hundreds to thousands of colorectal adenomatous polyps during their second and third decades of life. Although these are benign tumors, their large numbers virtually guarantee that some will progress to invasive lesions. Somatic mutations and deletions that inactivate both copies of APC are present in most sporadic colorectal adenomas and cancers. In a small subgroup of tumors

with wild-type APC, mutations of β-catenin, that render the protein resistant to its degradation promote constitutive activation of Wnt signalling (Markowitz and Bertagnolli 2009).

Animal models have played an instrumental role in demonstrating the ability of cyclooxygenase (COX)-2 to promote gastrointestinal (GI) tumorigenesis in the context of loss of APC. Genetic studies have demonstrated that deletion of COX-2 in APC$^{Min/+}$ and APC^{Δ716} mouse models of intestinal tumorigenesis results in decreased small intestine and colon tumor formation (Oshima et al. 1996; Chulada et al. 2000). Furthermore, an association between increased nuclear β-catenin levels and COX-2 expression is seen in human and murine colon cancer cells and is related to defects in APC (Mei et al. 1999; Dimberg et al. 2001).

2.1.3 The microsatellite instability pathway in CRC

The evidence that CRC progression occurs as a multistep process characterized by an accumulation of genetic alterations arose from the findings that CRC patients that developed cancer from Lynch syndrome displayed tumors with a different histological profile than those with cases associated with the CIN pathway. Lynch syndrome (also termed hereditary non-polyposis CRC, or HNPCC) is the most common hereditary colon cancer syndrome, accounting for about 3 % of all CRC cases. It is an autosomal dominant disease and it is associated with a risk of CRC of about 70% and an increased risk of a variety of other cancers, such as endometrial, ovarian, gastric, small bowel, and ureter. In Lynch syndrome, CRCs arise from adenomas but the progression of the adenoma-carcinoma sequence in this pathological setting occurs more rapidly than in the CIN pathway. However, CIN tumors have a poorer prognosis as compared to Lynch-associated CRCs. While CIN tumors are characterized by aneuploidy (abnormal number of chromosomes), multiple chromosomal rearrangements, and an accumulation of somatic mutations, Lynch-associated CRCs are typically diploid and are characterized by genetic alterations due to the inactivation of mismatch repair genes (MMR). This type of genetic alteration characterizes a well-identified aspect of genomic instability, named microsatellite instability (MSI) (Ahnen 2011). Among genes identified to be affected by MSI are components of the TGF-β signaling pathway (Markowitz et al. 1995), along with genes regulating cell proliferation, cell cycle, apoptosis and DNA repair (Duval and Hamelin 2002). MSI is found in 15% of CRCs, which are primarily hereditary CRCs, but it is also detected in sporadic cancers. While sporadic colorectal tumors frequently exhibit COX-2 overexpression (Wang D and Dubois 2010b), up to half of MMR-deficient colorectal tumors do not show COX-2 overexpression primarily in patients with sporadic MMR-deficiencies (Castells et al. 2006) in agreement with other observations indicating reduced COX-2 protein expression in CRC with defective MMR (Karnes et al. 1998). These findings support the existence of a novel, epigenetic-based pathway that contributes to CRC disease progression and indicate potential limitations for COX-2 inhibition as chemoprevention strategy in HNPCC due to the lack functional response. However, recent results form the first RCT of aspirin in Lynch syndrome, the Colorectal Adenoma/carcinoma Prevention Programme 2 (CAPP2), strongly support routine use of aspirin for patients with Lynch Syndrome as an adjunct to intensive cancer surveillance (Burn et al. 2011), but further RCTs with a CRC endpoint should be performed to have more definitive answers on aspirin role in the prevention of CRC in a sporadic-risk population.

2.1.4 The epigenetic pathway in CRC

Another mechanism of gene inactivation in CRC occurs *via* epigenetic-based mechanisms mainly due to aberrant DNA methylation through the introduction of a methyl group on carbon 5 of cytosine (5-methylcytosine) by DNA methylases (Kondo and Issa 2004). In the

normal genome, cytosine methylation occurs in repetitive DNA sequences outside of exons, whereas in CRCs there is a global reduction of cytosine methylation and a considerable increase of aberrant cytosine methylation localized to certain gene promoter regions. These aberrant methylations associated with promoter regions can promote epigenetic silencing of genes. The two most commonly affected genes in these sporadic cases and in Lynch Syndrome are MutS homologue (hMSH)1 and hMLH2 (Issa 2004). Consistent with the MSI phenotype, hypermethylation of the COX-2 promoter has been observed in CRC samples, suggesting that the reduced expression of COX-2 may also affect sporadic forms (Toyota et al. 2000) and COX-2 gene silencing *via* hypermethylation was observed in approximately one third of MMR-deficient colorectal tumors (Castells et al. 2006).

2.1.5 COX-2/mPGES-1 pathway

Inflammation is one of the major contributors to the tumor microenvironment. In particular, chronic inflammation acts to initiate tumorigenesis and promote disease progression, and it has long been studied in relation to proinflammatory prostanoids in colon cancer (Wang and DuBois 2008). Clinical studies have shown that the use of nonsteroidal anti-inflammatory drugs (NSAIDs) [both traditional(t)NSAIDs and selective inhibitors of COX-2 (coxibs)], reduces the risk of CRC by 40–50% (Peddareddigari et al. 2011)(for a comprehensive discussion see chapter 4). The most plausible mechanism of the antitumorigenic effects of these drugs is through their inhibitory effect on COX-2-dependent prostanoids(Fig 2.1).

NSAIDs, including aspirin, inhibits COX isozymes (COX-1 and COX-2), which catalyze the rate-limiting step in the metabolic conversion of arachidonic acid (AA) to PGs [i.e. PGE_2, PGD_2, PGF_{2a}, thromboxane $(TX)A_2$ and prostacyclin (PGI_2)] (Vane 1971). Prostanoids are second messengers which can cross the cell membrane, diffuse through the extracellular space, and interact with high-affinity G-protein coupled receptors on the same cell or in neighboring cells. The specific action of the different prostanoids in a particular type of tissue predominantly depends on the cell type-specific expression of their specific receptors and on their biosynthesis (Funk 2001) (Fig 2.1).

COX-2-derived PGE_2 is the most abundant prostanoids found in CRC tissue, and it has been demonstrated to promote colon carcinogenesis along with other malignancies including lung, breast, and head and neck cancer (Wang D and Dubois 2010a). The role of PGE_2 in colon cancer progression arose from studies with the $APC^{Min/+}$ mouse model of intestinal neoplasia. This genetic animal model for FAP maintains inactivating mutations in the APC gene (Moser et al. 1995). Treatment of these animals with PGE_2 promoted a dramatic increase in small and large intestinal tumor burden (Wang D et al. 2004). Moreover, studies in humans revealed that adenoma regression was more effective when PGE_2 tissue levels were profoundly inhibited by treatment with NSAIDs (Giardiello et al. 2004). As aforementioned, activation of the canonical Wnt pathway in the colonic epithelium is a key event in polyp formation, and this event is associated with the up-regulation of several genes involved in tumor development and progression (Buchanan and DuBois 2006). Among them, overexpression of COX-2 plays a central role in intestinal tumorigenesis. In fact, elevated levels of COX-2-derived PGE_2 are associated with i) resistance to apoptosis, through the upregulation of the anti-apoptotic protein Bcl-2 and the induction of nuclear factor-κB (NF-κB) transcriptional activity (Wang D and Dubois 2010a), ii) stimulation of cell proliferation, iii) simulation of cell migration and iii) angiogenesis (Wang D et al. 2004).

The well recognized role of PGE_2 during tumor promotion coupled with findings demonstrating long-term use of NSAIDs may be associated with GI toxicity (Masso Gonzalez et al. 2010) and increased risk of adverse CV events (Garcia Rodriguez et al. 2008; Grosser et al. 2006), provided the rationale for the identification of novel enzymatic

targets within the AA pathway, including the PGE_2 terminal synthases (PGES) (Wang D and DuBois 2008). To date, three different gene products with PGES activity have been identified, encoding microsomal PGES-1 (mPGES-1), mPGES-2 and cytosolic PGES (cPGES), respectively (Kudo and Murakami 2005)(Fig 2.1). mPGES-1 is a member of the MAPEG (membrane-associated proteins involved in eicosanoid and glutathione metabolism) superfamily, showing significant homology with other MAPEG superfamily proteins. mPGES-1 is expressed at minimal levels in most normal tissues, although abundant and constitutive expression is detected in a limited number of organs, such as the lung, kidney, and reproductive organs. mPGES-1 is also induced by cytokines and various growth factors (Samuelsson et al. 2007). The closely related mPGES-2 is expressed constitutively in a variety of human tissues, and unlike mPGES-1, it is not induced by proinflammatory signals. cPGES is expressed in a ubiquitous manner, and is thought to mediate constitutive PGE_2 biosynthesis based on its preferential coupling with COX-1 (Murakami et al. 2000). mPGES-1 is in general functionally coupled with COX-2 and its expression is often concomitantly induced with COX-2 overexpression, thus, contributing to the efficient generation of PGE_2 during inflammation (Jakobsson et al. 1999). However, studies using diverse stimuli provided evidence that COX-2 and mPGES-1 can be independently regulated (Sampey et al. 2005). This observation suggested the possibility that the pharmacological targeting of mPGES-1 may result in the suppression of PGE_2 production (Fig 2.1) by mechanisms that circumvent the CV toxicity associated with inhibition of COX-2 activity by NSAIDs, both traditional and COX-2 selective inhibitors (named coxibs) (Bruno et al. 2010; Wang D and DuBois 2008). On the basis of evidence from cell culture studies, several *in vivo* studies have been performed to address the impact of mPGES-1 targeting on colon tumorigenesis, but the results of these studies are conflicting (Nakanishi et al. 2010). In particular, mPGES-1 knockout mice in a mutant APC background mPGES-1 showed a significant reduction in the number and size of intestinal tumors. Interestingly, mPGES-1 deletion caused a disorganized vascular pattern within primary adenomas, implicating a novel role of PGE_2 in tumor angiogenesis (Nakanishi et al. 2008). In contrast, Elander et al. reported that genetic deletion of mPGES-1 resulted in accelerated intestinal tumorigenesis in APC[Min/+] mice (Elander et al. 2008). Environmental factors (i.e. Helicobacter pylori infection) may represent a possible explanation of these different responses along with genetic differences in the mouse models used. Based on these differences, the role of mPGES-1 in intestinal carcinogenesis should be further elucidated in pre-clinical studies prior to extended development of mPGES-1 inhibitors as novel potential chemopreventive agents (Fig 2.1) (Nakanishi et al. 2010).

A different mechanism of action seems to explain the chemopreventive effect of long-term use of low-dose aspirin (Rothwell et al 2010, Rothwell et al. 2011). In fact, several lines of clinical and experimental evidence support the notion that low-dose aspirin is an efficacious anti-thrombotic agent targeting mainly platelet COX-1 (Patrono et al. 2008; see chapter 3). Thus, it is proposed that the same mechanism plays a role in the antitumorigenic effect of low-dose aspirin. Activated platelets may be involved in the early phase of intestinal transformation through the activation and induction of COX-2 in stromal cells(Patrono et al. 2001); these cells and their released factors may contribute to epithelial COX-2 expression in the intestinal tract, thus causing the increase of cell proliferation and the accumulation of mutations, as a consequence of inhibition of apoptosis (Cao and Prescott 2002; Prescott 2000). According with this scheme of intestinal tumorigenesis, both COX-1 and COX-2 play a role by operating sequentially, with COX-1 acting upstream of COX-2 (see chapter 3). This is supported by experimental results showing that loss of either COX-1 or COX-2 genes blocks intestinal polyposis in mouse models of FAP by about 90% (Chulada et al. 2000). Low-dose aspirin may affect early phase of tumorigenesis by slowing down COX-2 induction in stromal and intestinal epithelial cells, as a consequence of the inhibition of platelet activation. Differently, tNSAIDs and coxibs act, as anticancer agents, in later phases

of the disease by affecting the consequences of COX-2 overexpression and enhanced generation of prostanoids.

Chan et al. have shown that regular aspirin use conferred a significant reduction in the risk of CRC that overexpressed COX-2 (multivariate relative risk, 0.64; 95% confidence interval [CI], 0.52 to 0.78), whereas regular aspirin use had no influence on tumors with weak or absent expression of COX-2 (multivariate relative risk, 0.96; 95% CI, 0.73 to 1.26) (Chan et al. 2007). These findings do not contradict the antiplatelet mechanism of CRC chemoprevention by aspirin but may suggest that the pathway platelet COX-1→stromal/epithelial COX-2 overexpression occurs in the setting of genetic/epigenetic dysregulated mechanisms of COX-2 expression.

2.1.6 Prostaglandin transporters in CRC

Intracellular PGE_2 can cross through the membrane by simple diffusion or *via* a PG efflux transporter such as MRP4, which efficiently transport PGE_2 in an ATP-dependent manner (Reid et al. 2003). Extracellular PGE_2 can be transported into the cell, where it can be inactivated or act *via* nuclear receptors (Zhu et al. 2006). It has been suggested that inactivation of PGE_2 in the tumor microenvironment occurs in two steps. First, the PG transporter (PGT) mediates the carrier-mediated membrane transport of PGs, including PGE_2, $PGF_{2\alpha}$, and PGD_2 from the extracellular milieu to the cytoplasm. Second, 15-hydroxyprostaglandin dehydrogenase (15-PGDH) catabolizes and thus inactivates PGE_2 (Nomura et al. 2004). It has been reported that both the expression and activity of 15-PGDH is repressed in human CRC and $APC^{Min/+}$ mouse adenomas, resulting in decreased catabolism of PGE_2 (Backlund et al. 2005; Myung et al. 2006; Yan et al. 2004). These findings support the role of 15-PGDH as a colon cancer suppressor gene that antagonizes the tumorigenic properties of COX-2-derived PGE_2. Recently, this model used to explain the increased levels of PGE_2 in colorectal neoplasia has been enriched by novel findings showing that other mechanisms of coordinated up- and down-regulation of genes involved in PGE_2 transport may contribute to increase PGE_2 levels in tumor microenvironment. In particular, it has been shown that PGT and MRP4 mRNA levels are inversely regulated in human CRC compared to normal mucosa and in intestinal adenomas from $APC^{Min/+}$ mice, thus suggesting that PGE_2 transport to the cytoplasm, where it can be inactivated by 15-PGDH may be another crucial step in the regulation of PGE_2 levels of in intestinal neoplasia (Holla et al. 2008).

2.1.7 EGFR pathway

Epidermal growth factor receptor (EGFR) mediates another important signaling involving in developing colonic carcinomas. In particular, it has been shown that the early effects of COX-2-derived PGE_2 are in part mediated by the EGFR. The tyrosine kinase EGFR is one of four members of the HER/ErbB growth factor receptor family and its activity is increased in adenomas that occur in $APC^{Min/+}$ mice (Moran et al. 2004). Moreover, the disruption of EGFR signaling through either kinase inhibition or genetic mutation inhibits polyp formation and the growth of established tumors (Roberts et al. 2002).

As reported above, PGE_2 induces increased proliferation, migration, and invasiveness of colorectal carcinoma cells (Wang D et al. 2004). These effects of PGE_2 were dependent upon the activation of the phosphatidylinositol 3-kinase/Akt pathway. PGE_2 also can induce activation of G proteins which classically activate PKA-mediated transcriptional activation *via* an increase in cAMP. This coupled activation of Akt and Gα subunits results in the accumulation of β-catenin of the canonical Wnt pathway in the nucleus, leading to transcriptional activation of target genes. Interstingly, it has been shown that the activation of EGFR pathway by PGE_2 can also lead to the activation of Akt and MAPK (Buchanan and

DuBois 2006). Thus, the trans-activation of EGFR by PGE$_2$ is, at least in part, responsible for subsequent downstream effects including the stimulation of cell migration and invasion by COX-2 dependent PGE$_2$. In light of the CV effects associated with long-term COX-2 inhibitor use and the important role of EGFR signaling in CRC, the inhibition of both these pathways has been proposed as a novel potential therapeutic approach and has inspired several studies in animal models of CRC (Buchanan et al. 2007).

2.1.8 Peroxisome proliferator-activated receptors

Growing evidence has demonstrated that peroxisome proliferator-activated receptor (PPAR)γ serves as a tumor suppressor in CRC. PPARγ is one of the three different isoforms of PPARs (PPARα, PPARβ/δ and PPARγ), which are ligand-activated transcription factors and members of the nuclear hormone receptor superfamily. They are implicated in a variety of physiologic and pathologic processes such as nutrient metabolism, energy homeostasis, inflammation, and cancer.

Evidence that PPARγ has an antitumor effect in CRC comes from several studies *in vitro* showing that PPARγ activation is associated with the inhibition of cell growth. This finding was corroborated in animal studies using a xenograft model of CRC in nude mice and another study using mice carrying an heterozygous deletion of PPARγ, where an increased tendency to develop carcinogen-induced colon cancer compared with wild-type mice was observed. Importantly, the protective role of PPARγ is also supported by the finding that point mutations in the ligand-binding domain of one allele of the PPARγ gene resulted in an inability to bind ligands and control gene regulation. Interestingly, despite numerous lines of evidence supporting the role of PPARγ as tumor suppressor gene in CRC, different groups have reported that administration of PPARγ agonists, such as thiazolidinediones, enhanced colon polyp number in the APC$^{Min/+}$ mice but not in wild type mice. These results suggest that: i) PPARγ activation might be associated to a pro-tumorigenic effect when it occurs after tumor initiation, as in the APC$^{Min/+}$ mice ; ii) a predisposed genetic susceptibility is necessary for the occurrence of this pro-tumorigenic effect. Thus, the assessment of the genetic predisposition may be proven useful to predict the success of PPARγ-targeted therapy.

Although it was well established that PPARγ activation is associated with changes of gene expression translating into the induction of apoptosis, inhibition of cell proliferation, and angiogenesis in colon cancer cells, the molecular mechanisms of CRC suppression by PPARγ are complex and not completely elucidated. To date, the main mechanisms implicated in the antitumor activities of PPARγ ligands include the downregulation of β-catenin, the induction of caveolin-1, caveolin-2 and proline oxidase, and the upregulation of the tumor suppressor p53 (Dai and Wang WH 2010).

2.1.9 Tumor microenvironment in colorectal carcinogenesis

The tumor microenvironment, which essentially consists of tumor-infiltrating cells, vasculature, extracellular matrix (ECM), and other matrix-associated molecules, has an important role in the multistep process characteristic of the adenoma-carcinoma sequence in CRC. The tumor microenvironment is quite distinct from the normal tissue microenvironment and it is composed of a particular phenotype of stromal cells, including: i) tumor-associated macrophages (TAMs), which are activated by microenvironment stimuli to a pro-tumor polarization state (called M2); ii) tumor-associated neutrophils (TANs) with a pro-tumor phenotype (called N2 neutrophils), and cancer-associated fibroblasts (CAFs). In fact, following the initiation of epithelial tumors, reciprocal interactions between transformed epithelial and stromal cells translate into a switch from a normal to a tumor microenvironment which is associated with massive infiltration of immune cells, that have

undergone substantial changes of their functionality. Tumor-infiltrating cells predominantly include TAMs, myeloid-derived suppressor cells (MDSCs), CD4 T-cells, CD8 T-cells, CD4 regulatory T-cells (Tregs), mesenchymal stem cells (MSCs), CAFs, endothelial progenitor cells (EPCs), mast cells, and platelets (Peddareddigari et al. 2011). As aforementioned, various reports demonstrate these cells can contribute to elevated prostanoid biosynthesis in the tumor microenvironment through upregulated COX-2 expression (Singer et al 1998; Wiercinska-Drapalo et al 1999; Prescott 2000; Cao and Prescott 2002; Dixon et al. 2006) indicating these cells are able to support tumor-associated inflammation, angiogenesis, and immune suppression, which in turn promotes tumor growth and metastasis (Peddareddigari et al. 2011).

2.2 COX-2 gene expression in CRC

As reported above, two primary COX enzyme isoforms have been identified to play distinct roles in physiologic and pathologic conditions. COX-1 is constitutively expressed in most cell types, and COX-1-derived PGs are necessary for protection of gastric mucosa and maintenance of vascular tone (Simmons et al. 2004). Whereas COX-2 is normally absent in most cells, this immediate-early response gene is rapidly induced by a variety of pro-inflammatory and growth-associated stimuli, resulting in increased PGE_2 synthesis (Simmons et al. 2004). COX-2 has been identified to play a role in the progression of tumorigenesis, supported by various reports demonstrating that COX-2 levels are increased in premalignant and malignant tumors, and this increase in COX-2 gene expression is associated with decreased cancer patient survival (Wang D and Dubois 2010a).

The relationship between COX-2 expression and CRC was first suggested by studies that demonstrated the efficacy of aspirin, NSAIDs, and coxibs to reduce colon cancer risk and also promote tumor regression in both humans and experimental animal models of colon cancer (Wang D and Dubois 2010b). The molecular basis of these observations indicated that high levels of COX-2 protein were present in both human and animal colorectal tumors, whereas the normal intestinal mucosa has low-to-undetectable COX-2 expression (Wang D and Dubois 2010b). COX-2 is overexpressed in approximately 80% of colorectal adenocarcinomas and has been shown to play roles in invasiveness, apoptotic resistance, and increased tumor angiogenesis (Wang D and Dubois 2010b). This association was further established in genetic studies demonstrating a significant reduction in intestinal polyposis in mice deficient for the COX-2 gene(*PTGS2*) (Oshima et al. 1996). These findings clearly indicate that chronic elevation of COX-2 is pathological and suggest that inhibition of COX-2 *via* pharmacological means or regulation of its expression can limit the development and progression of CRC.

2.2.1 Transcriptional regulation

A variety of evidence gathered from epidemiological, experimental animal, and cellular studies indicate that unregulated COX-2 expression is an important step in CRC and it is generally well accepted that transcriptional activation of COX-2 can occur early during tumorigenesis (Fig 2.2)(Dixon 2003; Wu et al. 2010). Evidence of constitutive activation of the COX-2 promoter occurring in colon cancer cells (Kutchera et al. 1996) suggested that the increased levels of COX-2 mRNA detected in colorectal adenomas and adenocarcinomas (Eberhart et al. 1994; Kutchera et al. 1996) occurs through increased transcription. Within the COX-2 promoter region lies *cis*-acting elements for the binding of several transcriptional factor complexes, such as NF-κB (Kojima et al. 2000; Schmedtje et al. 1997; Singer et al. 1998), C/EBP (Kim and Fischer 1998; Shao et al. 2000), β-catenin/TCF (Araki et al. 2003; Howe et al. 1999; Mei et al. 1999), CREB (Shao et al. 2000; Subbaramaiah et al. 2002a), NFAT (Hernandez et al. 2001), AP-1 (Miller et al. 1998; Subbaramaiah et al. 2002b), PPAR (Meade et al. 1999), and HIFα (Bazan and Lukiw 2002; Kaidi et al. 2006). The control of

COX-2 transcription is a complex regulatory process that requires input from multiple signal transduction pathways (Dixon 2003). Due to the complexity of combined genetic alterations and inflammatory signaling occurring in the tumor microenvironment, identifying a single transcriptional pathway which plays a decisive role in promoting constitutive COX-2 expression in colon cancer has been limiting. Further efforts involving the molecular characterization of individual tumors will aid in identifying the specific cellular defects in these signaling pathways that can promote aberrant COX-2 gene transcription.

2.2.2 Post-transcriptional regulation and the 3′ untranslated region (3′UTR)

An integral method in controlling gene expression is by post-transcriptional mechanisms that regulate mRNA stability and protein translation. The impact of this level of regulation is evident as microarray analysis has detected that 40–50% of the changes in inducible gene expression occurs at the level of mRNA stability (Cheadle et al. 2005). Eukaryotic mRNAs contain two characteristic features that are integral to their function, a 5′ 7-methylguanosine cap and a 3′ poly(A) tail. In mammalian cells, the majority of mRNA decay is initiated by shortening of the poly(A) tail (deadenylation), after which degradation initiating at the 5′ end involves removal of the 5′ cap by decapping enzymes, followed by 5′ to 3′ exonucleolytic decay. Alternatively, the mRNA can be degraded by 3′ to 5′ exonucleolytic degradation through a complex of exonucleases known as the exosome (Garneau et al. 2007). These two decay pathways are not mutually exclusive and there appears to be overlap between the pathways, although the relative contribution of each mechanism is under debate (Garneau et al. 2007; Newbury et al. 2006). Many mRNAs targeted for degradation are localized to processing (P)-bodies, which are small cytoplasmic foci that contain components of both 3′ to 5′ and 5′ to 3′ decay machinery, suggesting these decay pathways converge at P-bodies (Eulalio et al. 2007; Garneau et al. 2007). An alternative fate of mRNAs observed under situations of cellular stress is their trafficking to cytoplasmic stress granules where they are translationally silenced. Current work now indicates a functional interaction between P-bodies and stress granules suggesting that mRNAs destined for decay are sorted at stress granules and delivered to P-bodies for degradation (Anderson and Kedersha 2008).

Messenger RNA regulatory elements that play a critical role in identifying specific transcripts for post-transcriptional regulation typically reside within the 3′ untranslated region (3′UTR) of the mRNA (Garneau et al. 2007). A well-established mechanism of 3′UTR-mediated post-transcriptional regulation occurs through association with various RNA-binding proteins that target select mRNAs containing adenylate- and uridylate (AU)-rich elements (AREs) within their 3′UTR (Garneau et al. 2007). More recently, small non-coding RNAs called microRNAs (miRNAs) have emerged as global mediators of post-transcriptional gene regulation through their ability to control mRNA stability and translation by imperfect base-pairing to the 3′UTR of its target mRNA (Fabian et al. 2010). Currently, it is estimated that nearly one thousand miRNAs function in humans and have been predicted to regulate approximately 60% of all protein-coding genes (Friedman et al. 2009).

Identification of multiple mRNA regulatory elements present within the COX-2 3′UTR was the first evidence suggesting that COX-2 might be regulated at a post-transcriptional level (Dixon 2003). Exon 10 of the human PTGS2 gene contains the entire 3′UTR and within this region are multiple polyadenylation signals with two signals that are primarily used to yield transcripts of ~ 4.6 and 2.8 kb in length (Hall-Pogar et al. 2005). Observed in most cells is the larger 4.6 kb COX-2 transcript that results from processing at a distal canonical (AAUAAA) polyadenylation site. However, a proximal non-canonical polyadenylation signal (AUUAAA) can yield transcripts with shortened 3′UTRs (Hall-Pogar et al. 2005). The significance of this alternative mRNA processing event has been shown in CRC cells as

a polyadenylation variant of COX-2 mRNA lacking the distal region of the 3′UTR was selectively stabilized upon cell growth to confluence (Sawaoka et al. 2003). These findings suggest that COX-2 mRNA can escape rapid decay through alternative polyadenylation site usage resulting in deletion of potential 3′UTR regulatory elements and this phenomenon of shortening of mRNA transcripts appears to be a widespread feature occurring in cancer cells (Mayr and Bartel 2009).

2.2.3 AU-rich elements (AREs) and ARE-binding proteins

A characteristic feature controlling the expression of many inflammatory cytokines, growth factors, and proto-oncogenes is their inherent ability to be targeted for rapid mRNA decay. These cancer-associated gene transcripts are unstable due to the presence of a common *cis*-acting element known as the AU-rich element or ARE (Lopez de Silanes et al. 2007). The importance of this particular RNA element is evident, since estimates ranging from 8% to 16% of human protein coding genes contain an ARE sequence within their 3′UTR (Bakheet et al. 2006; Gruber et al. 2010). The functional ARE is present within the mRNA 3′UTR and is most often composed of multiple copies of an AUUUA sequence motif clustered together (Bakheet et al. 2006). Within the COX-2 3′UTR, a 116 nucleotide region containing a cluster of 6 AUUUA sequence elements located near the stop codon serves as the functional ARE (Dixon et al. 2000). Post-transcriptional regulation has been shown to be dependent upon this ARE since its presence confers rapid decay of a normally stable reporter gene (Dixon et al. 2000). Furthermore, this AU-rich region is highly conserved in both sequence and location among various species of COX-2, implying that ARE function has been evolutionary conserved (Dixon 2003).

Normal cellular growth is associated with rapid decay of ARE-containing mRNAs and targeted mRNA decay is an essential way controlling their pathogenic overexpression. This aspect of COX-2 regulation is observed in non-transformed intestinal epithelial cells where rapid degradation of COX-2 mRNA occurs ($t_{1/2} \sim 13$ min) (Sheng et al. 2000). However, a number of observations have implicated the loss of ARE-mediated post-transcriptional regulation to occur during neoplastic transformation of cells (Lopez de Silanes et al. 2007) and recent findings have demonstrated an enrichment of this sub-set of transcripts to occur during colon tumorigenesis. Gene expression profiling comparing adenomas to late stage adenocarcinomas show a 3- to 4-fold enrichment in ARE-containing genes compared to the genome as a whole and a similar enrichment is observed as early as stage I tumors (Kanies et al. 2008), suggesting loss of ARE-mediated regulation is lost early during tumor development. With regard to COX-2 regulation, similar findings have been observed in human colon carcinoma cells (Dixon et al. 2003; Dixon et al. 2001; Shao et al. 2000; Sheng et al. 2000). As a result of the inability of the COX-2 ARE to function properly in CRC cells, enhanced mRNA stability was detected and increased expression of a reporter gene containing the COX-2 3′UTR was also observed. Based upon the inherent genetic instability of tumor cells, it might be expected that mutations or loss of AREs might occur. However, few naturally occurring mutations in AREs have been described and the ARE region of the PTGS2 gene is intact in healthy individuals as well as in colon tumor cells (Dixon 2003). This implies that loss of ARE function in colon tumors is primarily due to altered ARE recognition by cellular *trans*-acting regulatory RNA binding proteins.

AREs mediate their regulatory function through the association of *trans*-acting RNA binding proteins that display high affinity for AREs. The best studied ARE-binding proteins can promote rapid mRNA decay, mRNA stabilization, or translational silencing (Garneau et al. 2007). Through these mechanisms, ARE-binding proteins exhibit pleiotropic effects on gene expression, since a single ARE-binding protein can bind to multiple mRNAs and binding can occur among different classes of AREs (Barreau et al. 2005; Lopez de Silanes et al. 2007). Various cytoplasmic proteins have been detected to bind AREs and work has focused

on identifying and characterizing COX-2 ARE-binding proteins. To date, 16 different RNA-binding proteins have been reported to bind the COX-2 3′UTR (Young and Dixon 2010). This chapter section will focus on well documented RNA-binding proteins that regulate COX-2 expression and their potential impact on CRC (Fig 2.2).

2.2.3.1 HuR—HuR (Hu antigen R; ELAVL1) is a ubiquitously expressed member of the ELAV (Embryonic-Lethal Abnormal Vision in *Drosophila*) family of RNA-binding proteins (Brennan and Steitz 2001). The human Hu proteins (ubiquitously expressed HuR, and neuronal-specific HuB, HuC, and HuD) were originally discovered as antigens in patients displaying paraneoplastic disorders (Voltz 2002). The cloning and characterization of HuR demonstrated that HuR contains 3 RNA recognition motifs (RRM) with high affinity and specificity for AREs and that cellular HuR overexpression stabilizes ARE-containing transcripts and promotes their translation (Brennan and Steitz 2001).

The ability of HuR to function as an ARE-stability factor appears to be linked to its subcellular localization (Keene 1999). HuR is localized predominantly in the nucleus (>90%) and can shuttle between the nucleus and cytoplasm. It is hypothesized that the ability of HuR to promote mRNA stabilization requires its translocation to the cytoplasm, and overexpression of HuR promotes cytoplasmic localization where it binds target ARE-containing mRNAs and interferes with their rapid decay (Brennan and Steitz 2001; Keene 1999). A variety of cellular signals known to activate MAPK pathways involving p38 and ERK kinases, the PI-3-kinase pathway, and the Wnt signaling pathway, have been shown to trigger cytoplasmic HuR localization and promote ARE-containing mRNA stabilization (Briata et al. 2003; Ming et al. 2001; Yang et al. 2004). Insight into the mechanism of HuR-mediated mRNA stabilization has been advanced with the identification of low-molecular-weight inhibitors for HuR (Meisner et al. 2007). These compounds inhibit HuR cytoplasmic localization by interfering with RNA-binding (Meisner et al. 2007).

HuR has been shown to associate and post-transcriptionally regulate the expression of numerous cancer-associated transcripts bearing AREs of multiple classes (Abdelmohsen and Gorospe 2010; Lopez de Silanes et al. 2005b). Based on its ability to bind the COX-2 ARE, HuR has been identified as a *trans*-acting factor involved in regulating COX-2 expression (Dixon et al. 2001). The enhanced stabilization of COX-2 mRNA observed in colon cancer cells is, in part, due to elevated levels of HuR (Dixon et al. 2001; Young et al. 2009). Recent work evaluating HuR expression in colon cancer demonstrates that HuR is expressed at low levels and is localized to the nucleus in normal tissue, whereas HuR overexpression and cytoplasmic localization was observed in colon adenomas, adenocarcinomas, and metastases; consistent with these observations, overexpression of COX-2 co-localized with elevated HuR (Young et al. 2009). Furthermore, several studies indicate that HuR overexpression and cytoplasmic localization is a marker for elevated COX-2 that is correlated with advancing stages of malignancy and poor clinical outcome (Dixon 2003).

2.2.3.2 CUGBP2—CUGBP2 (CUG triplet repeat–binding protein 2) is an ubiquitously expressed RNA binding protein containing 3 RNA recognition motifs and is a member of the CELF (CUGBP-ETR-3-like factors) family (Mukhopadhyay et al. 2003; Murmu et al. 2004). Work investigating COX-2 regulation in colon cancer cells and intestinal epithelium subjected to ionizing radiation had elucidated a novel role for CUGBP2 in regulating COX-2 expression on a post-transcriptional level (Mukhopadhyay et al. 2003; Murmu et al. 2004). CUGPB2 displays high affinity for the COX-2 ARE and radiation-induced overexpression of CUGBP2 led to COX-2 mRNA stabilization similar to the effects of HuR overexpression (Mukhopadhyay et al. 2003). In contrast to HuR, CUGBP2 repressed COX-2 translation by inhibiting ribosome loading of the COX-2 mRNA (Mukhopadhyay et al. 2003). Given that HuR enhances and CUGBP2 inhibits COX-2 protein expression, indicates that these two

ARE-binding proteins differ in their regulation of COX-2 expression. Although both proteins have similar affinities for the COX-2 ARE, CUGBP2 was effective in competing with HuR for ARE-binding leading to a translational block in COX-2 expression (Sureban et al. 2007). While demonstrating the ability of CUGBP2 to regulate the opposing functions of COX-2 mRNA stabilization and translational repression, these findings suggest a possible role for CUGBP2 in the early stages of tumorigenesis by counteracting the effects of HuR overexpression in order to repress COX-2 protein synthesis.

2.2.3.3 TTP—Tristetraprolin (TTP, ZFP36, TIS11) is a member of a small family of tandem Cys3His zinc finger proteins consisting of TTP, ZFP36L1, and ZFP36L2 (Sanduja et al. 2010). TTP acts on a post-transcriptional level to promote rapid decay of ARE-containing mRNAs by direct ARE binding (Carballo et al. 1998). The binding of TTP to AREs targets the transcript for rapid degradation through association with various decay enzymes (Sanduja et al. 2010). In cells, TTP localizes to P-bodies, which suggests that TTP plays a critical role in ARE-mRNA delivery to cytoplasmic sites of mRNA decay (Franks and Lykke-Andersen 2007; Kedersha et al. 2005). TTP is also a target of phosphorylation by various pathways, and this phosphorylation state likely plays a role in mediating TTP function (Sanduja et al. 2010).

Efforts to characterize the function of TTP have primarily focused on its regulation of inflammatory mediators such as TNF-α and GM-CSF (Carballo et al. 2000; Lai et al. 1999). The physiological consequences of TTP loss is evident, as TTP knock-out mice develop multiple inflammatory syndromes resulting from increased inflammatory factors, including COX-2, due to defects in rapid mRNA turnover (Phillips et al. 2004) and recent work has demonstrated COX-2 to be a target of TTP (Sawaoka et al. 2003; Young et al. 2009). With regard to its role in controlling COX-2 expression in colon cancer, TTP expression is low or not apparent in colon cancer cell lines, adenoma, and adenocarcinoma tissue (Young et al. 2009). This is in contrast to normal tissue where TTP was highest in normal epithelium and predominantly localized to the cytoplasm. Furthermore, adenoviral-mediated delivery of TTP to colon cancer cells resulted in down regulation of COX-2 expression coupled with a dramatic reduction in cell growth and proliferation (Young et al. 2009). These findings indicate that the presence of TTP in normal colon epithelium serves in a protective capacity by controlling expression of various inflammatory mediators including COX-2, whereas the loss of TTP expression in tumors allows for HuR to promote the stabilization and translation of COX-2 mRNA.

2.2.3.4 TIA-1—TIA-1 (T-cell intracellular antigen 1; TIA-1) was originally identified in activated T lymphocytes and is a RNA-binding protein containing 3 RRM motifs with specificity to mRNAs containing short sections of uridylate repeats (Lopez de Silanes et al. 2005a; Tian et al. 1991). Under normal cellular conditions, TIA-1 is predominantly nuclear, and in response to cellular stress, translocates to the cytoplasm where it is associated with untranslated mRNAs in cytoplasmic stress granules, implicating a role in translational regulation (Anderson and Kedersha 2008; Kedersha et al. 2005). TIA-1 has been shown to bind the COX-2 ARE and regulate its expression through translational inhibition without impacting COX-2 mRNA turnover (Dixon et al. 2003). However, in colon cancer cells deficiencies in TIA-1 binding to the COX-2 ARE was observed allowing for increased polysome association with the COX-2 mRNA. *In vivo*, TIA-1 knockout mice maintain elevated levels of COX-2 leading to the development of arthritis (Phillips et al. 2004). These findings implicate TIA-1 as a translational silencer of COX-2 expression, and suggest that loss of TIA-1 function may be a contributing factor promoting enhanced COX-2 levels in cancer and chronic inflammation.

2.2.3.5 RBM3—RBM3 (RNA-binding motif protein 3) is a translational regulatory factor consisting of a single RRM domain and a glycine-rich region (Dresios et al. 2005). Its role in COX-2 regulation was initially identified through its binding to the AU-rich elements in the murine COX-2 3′UTR (Cok and Morrison 2001), and two-hybrid screening analysis identified RBM3 to interact with HuR (Anant et al. 2010). Similar to HuR expression in cancer, RBM3 is significantly upregulated in colorectal tumors and overexpression of RBM3 in fibroblasts promoted cell transformation (Sureban et al. 2008), indicating an oncogenic capacity for this RNA-binding protein.

Consistent with its interaction with HuR, RBM3 is also a nucleocytoplasmic shuttling protein that promotes stabilization of COX-2 mRNA through binding to ARE sequences located in the first 60 nts of the COX-2 3′UTR (Sureban et al. 2008). RBM3 protein also promotes translation of COX-2 mRNA through its 3′UTR (Sureban et al. 2008), however the mechanism underlying this effect and RBM3's partnership with HuR to promote COX-2 mRNA translation remains to be determined. Through its ability to promote mRNA stability and translation of otherwise rapidly degraded transcripts, RBM3 is being recognized as a contributing factor promoting enhanced COX-2 expression during tumorigenesis.

2.2.4 MicroRNAs (miRNAs)

MicroRNAs (miRNAs) are small non-coding RNAs approximately 21–24 nucleotides in length that have emerged as fundamental global regulators of gene expression *via* post-transcriptional mechanisms (Fabian et al. 2010). Within the genome, miRNAs can reside within gene exons or introns and can be transcribed as part of a protein-coding gene or their transcription can be regulated independently (O'Hara et al. 2009). Once transcribed, the primary miRNA transcript (pri-miRNA) is processed in the nucleus into a pre-miRNA stem-loop. The precursor miRNA (pre-miRNA) is actively transported to the cytoplasm where it is processed a second time to form a double-stranded miRNA duplex. One or both strands of the miRNA duplex generate mature miRNAs that can be loaded into the miRNA-induced silencing complex (miRISC) essential to miRNA-mediated gene targeting (Fabian et al. 2010).

MiRNAs regulate gene expression both by influencing translation and by causing degradation of target mRNAs (Fabian et al. 2010) and recent findings have implicated miRNA-mediated mRNA decay to be the predominant mechanism (Guo et al. 2010). Although similar in nature to short interfering RNAs (siRNAs) which bind target mRNA regions with 100% complementary and promote mRNA degradation, miRNAs can bind imperfectly to the 3′UTR of targeted transcripts to attenuate target gene expression (Filipowicz et al. 2008). Based on this, a single miRNA can potentially impact expression of a large number of proteins with varying cellular functions. Due to the substantial amount of control they have over a number of putative mRNA targets, it is of considerable interest how alterations in miRNA expression in cancer can contribute to tumorigenesis. In CRC, differential expression of several miRNAs has been observed and current efforts have demonstrated that specific miRNA loss or overexpression can impact various cellular pathways associated with colon tumorigenesis (O'Hara et al. 2009; Wiemer 2007). Currently, 5 miRNAs have been reported to target COX-2 mRNA and control its expression (Fig 2.2).

2.2.4.1 miR-16—The initial report implicating a role for miRNAs in cancer progression examined miR-16-1 and its function in chronic lymphocytic leukemia (CLL) (Calin et al. 2002). This miRNA is clustered with miR-15a and is located in the most commonly deleted genomic region of individuals affected by CLL who display attenuated expression of miR-16-1 and miR-15a (Calin et al. 2002; Dohner et al. 2000). It was subsequently

determined that loss of miR-16-1 and miR-15a promotes cell survival, as they function to target and repress anti-apoptotic factors BCL-2 and MCL1 (Cimmino et al. 2005; Sanchez-Beato et al. 2003). MiR-16-1 in particular has additionally been shown to play a role in cell cycle maintenance through regulation of several cell cycle regulatory genes (Liu et al. 2008). These results are in agreement with those showing reduced miR-16-1 levels present in the colorectal microRNAome (Cummins et al. 2006).

Based on its RNA sequence, miR-16 displays complementarity to AU-rich regions and it has been demonstrated that miR-16 can target AREs, particularly the AREs of TNF-α, IL-6, IL-8, and COX-2 to alter their mRNA stability (Jing et al. 2005). Interestingly, these same studies determined that miR-16 works in conjunction with TTP to promote decay of ARE-containing mRNAs and this is through interactions between TTP and components of the RISC complex. In CRC cells and tumors, miR-16 levels were observed to be decreased ~2-fold and miR-16 expression in cancer cells attenuated COX-2 expression and PG synthesis (Young et al. 2011). More recent work examining miRNA-mediated regulation of COX-2 demonstrated a functional interaction between HuR and miR-16 in colon cancer cells and this interaction promoted down regulation of miR-16 (Young et al. 2011). Consistent with these observations, studies examining COX-2 regulation in response to diabetic stimuli in leukocytes has identified miR-16 to play a dynamic role in COX-2 regulation under inflammatory conditions (Shanmugam et al. 2008). These findings highlight the significance of this ARE-targeting miRNA in cancer along with demonstrating the ability of miR-16 to regulate COX-2 expression under conditions of chronic inflammation.

2.2.4.2 miR-101 and miR-199—Genomic loss of miR-101 was first detected to occur in 67% of metastatic prostate cancer cells (Varambally et al. 2008). Since this initial report, miR-101 has further been shown to be attenuated in a variety of malignant tissues including colon, hepatocellular, and gastric cancers (Strillacci et al. 2009; Su et al. 2009; Wang HJ et al. 2010). Initial work investigating the role of COX-2 during embryo implantation identified the murine miRNAs miR-101a and miR-199a to post-transcriptionally regulate murine COX-2 and expression of these miRNAs are inversely correlated with uterine COX-2 protein levels (Chakrabarty et al. 2007). Further work in human cells established the ability of miR-101a and miR-199a to target human COX-2 by direct binding to the 3´UTR (Chakrabarty et al. 2007; Hao et al. 2011). In the context of colon cancer, an inverse correlation between human miR-101 and COX-2 expression in colon cancer cell lines and colon tumors has been shown along with the ability of miR-101 to inhibit COX-2 translation when expressed in cells (Strillacci et al. 2009). On these same lines, COX-2 expressing colon cancer cells do not express miR-199 (Chakrabarty et al. 2007), indicating that limited expression of these miRNAs in colon cancer can contribute to observed COX-2 overexpression.

2.2.4.3 miR-143—MiR-143 is located on Chr 5q32 and is thought to originate from the same primary miRNA transcript as miR-145, however some studies have suggested tissue-specific transcriptional regulation may account for their independent regulation (Akao et al. 2007; Cordes et al. 2009; Wu et al. 2011). Expression of miR-143 is attenuated at the adenoma and carcinoma stages of CRC (Michael et al. 2003), and is consistently attenuated in colorectal tumors and cell lines (Akao et al. 2006; Bandres et al. 2006; Slaby et al. 2007). Furthermore, reintroduction of miR-143 into GI cancer cells has been shown to repress abnormal cell growth and proliferation (Akao et al. 2006; Chen et al. 2009; Takagi et al. 2009). More recently, miR-143 has been found to promote COX-2 down regulation in a bladder carcinoma cellular model, resulting in reduced cell proliferation and mobility (Song et al. 2011).

2.2.4.4 miR-542-3p—Various examples of dysregulated miRNA expression have been shown to contribute to the pathogenesis most cancers (Croce 2009) and more recently, genetic polymorphisms in components of miRNA networks and their targets are being recognized as contributing factors in disease etiology (Ryan et al. 2010). Several studies have identified a common single nucleotide polymorphism (SNP) in the COX-2 gene at position 8473 (T8473C; rs5275) that is associated with increased risk and/or NSAID responsiveness in a number of cancers where COX-2 overexpression is a contributing factor(Ali et al. 2005; Campa et al. 2004; Ferguson et al. 2008; Gong et al. 2009; Langsenlehner et al. 2006; Ozhan et al. 2010; Shen et al. 2006; Siezen et al. 2005; Vogel et al. 2008). The T8473C SNP is located in exon 10 which encodes the COX-2 3′UTR suggesting a potential role in post-transcriptional regulation. Recent findings have demonstrated that this SNP lies within a region that targets COX-2 mRNA for degradation by miR-542-3p (Moore et al. 2011). This miRNA was identified to bind transcripts derived from the 8473T allele and promote mRNA decay, whereas the cancer-associated variant 8473C allele interfered with miR-542-3p binding allowing for mRNA stabilization. Colon cancer cells and tissue displayed COX-2 expression levels that were dependent on T8473C allele dosage and allelic-specific expression of COX-2 was observed to be a contributing factor promoting COX-2 overexpression (Moore et al. 2011). These findings provide a novel molecular explanation underlying cancer susceptibility associated with COX-2 T8473C SNP and identify it as a potential marker for identifying cancer patients best served through selective COX-2 inhibition.

2.3 Conclusions

Numerous evidences sustain a key role of COX-2 overexpression in the development of cancers, in particular CRC (Prescott 2000, Cha and Dubois 2007, Harper and Tyson-Capper 2008, Young et al. 2009). A proof of concept of the role of COX-2 in human tumorigenesis is evidenced by the efficacy of selective COX-2 inhibitors (such as celecoxib) to decrease the risk of colorectal adenoma recurrence (Bertagnolli et al. 2006; Steinbach et al. 2000). However the use of selective COX-2 inhibitors seems to be inappropriate due to the interference with CV homeostasis by the coincident inhibition of vascular COX-2-dependent PGI_2 (Grosser et al. 2006). The challenge of the next years will be to improve the management of CRC by using a double approach based on the development of innovative prevention-based strategies and anti-cancer therapies. Although important strides have been made through the understanding of the genetic mechanisms associated with the adenoma-carcinoma sequence, thus allowing for the development of improved strategies to identify patients at high risk to develop CRC, biomarkers of early detection and of safe and effective chemopreventive agents are still lacking.

Furthermore, the reduced capacity of colorectal screening programs in detecting proximal colon cancers represents another important limitation in prevention of CRC. In the future, mechanistic studies should be performed to clarify the molecular pathways of the chemopreventive effects of low-dose aspirin in CRC (Rothwell et al 2010; Rothwell et al. 2011) and the results of these studies may open the way to a new promising strategy for the primary prevention of CRC, based on the use of low-dose aspirin as chemopreventive agent.

On the other hand, the use of selective inhibitors of mPGES-1, which are under development (Samuelsson et al. 2007), may represent a very promising therapeutic approach to overcome the limits of COX-2 inhibitors due to the CV hazard (Grosser et al. 2006). In fact, mPGES-1 inhibitors cause a selective inhibition of PGE_2 by affecting a PGE_2 synthase downstream of COX-2 and thus, they do not affect, or even can augment, the biosynthesis of the vasoprotective PGI_2 (Wang et al. 2008). However, the knowledge that in cancer, COX-2 overexpression is controlled at the post-transcriptional level through changes in its mRNA

stability by miRNAs open the way to innovative strategies for design of therapeutics affecting this pathway involved in colon tumorigenesis (Fig 2.2). In this perspective, the development of tissue-specific delivery system for specific miRNAs is just beginning and it looks promising.

Acknowledgments

Abbreviations

CRC	Colorectal cancer
CV	cardiovascular
CIN	chromosomal instability
APC	adenomatous polyposis coli
FAP	familial adenomatous polyposis
EGF	epidermal growth factor
TGF	transforming growth factor
COX	cyclooxygenase
PG	prostaglandin
(TX)A$_2$	thromboxane
PGI$_2$	prostacyclin
GI	gastrointestinal
NSAIDs	nonsteroidal anti-inflammatory drugs
mPGES	microsomal Prostaglandin E Synthase
15-PGDH	15-hydroxyprostaglandin dehydrogenase
PPAR	peroxisome proliferator-activated receptor
AREs	(AU)-rich elements
miRNAs	microRNAs
HuR	Hu antigen R
TIA-1	T-cell intracellular antigen 1
RBM3	RNA-binding motif protein 3

References

Abdelmohsen K, Gorospe M. Posttranscriptional regulation of cancer traits by HuR. Wiley Interdisciplinary Reviews: RNA. 2010; 1:214–229. [PubMed: 21935886]

Ahnen DJ. The american college of gastroenterology emily couric lecture--the adenoma-carcinoma sequence revisited: Has the era of genetic tailoring finally arrived? Am J Gastroenterol. 2011; 106:190–198. [PubMed: 21266962]

Akao Y, Nakagawa Y, Naoe T. MicroRNAs 143 and 145 are possible common onco-microRNAs in human cancers. Oncol Rep. 2006; 16:845–850. [PubMed: 16969504]

Akao Y, Nakagawa Y, Naoe T. MicroRNA-143 and -145 in colon cancer. DNA Cell Biol. 2007; 26:311–320. [PubMed: 17504027]

Ali IU, Luke BT, Dean M, et al. Allellic variants in regulatory regions of cyclooxygenase-2: Association with advanced colorectal adenoma. Br J Cancer. 2005; 93:953–959. [PubMed: 16205694]

Anant S, Houchen CW, Pawar V, et al. Role of RNA-binding proteins in colorectal carcinogenesis. Curr Colorectal Cancer Rep. 2010; 6:68–73. [PubMed: 20401169]

Anderson P, Kedersha N. Stress granules: The tao of RNA triage. Trends Biochem Sci. 2008; 33:141–150. [PubMed: 18291657]

Araki Y, Okamura S, Hussain SP, et al. Regulation of cyclooxygenase-2 expression by the wnt and ras pathways. Cancer Research. 2003; 63:728–734. [PubMed: 12566320]

Atkin WS, Edwards R, Kralj-Hans I, et al. Once-only flexible sigmoidoscopy screening in prevention of colorectal cancer: A multicentre randomised controlled trial. Lancet. 2010; 375:1624–1633. [PubMed: 20430429]

Backlund MG, Mann JR, Holla VR, et al. 15-hydroxyprostaglandin dehydrogenase is down-regulated in colorectal cancer. J Biol Chem. 2005; 280:3217–3223. [PubMed: 15542609]

Bakheet T, Williams BR, Khabar KS. ARED 3.0: The large and diverse AU-rich transcriptome. Nucleic Acids Res. 2006; 34:D111–114. [PubMed: 16381826]

Bandres E, Cubedo E, Agirre X, et al. Identification by real-time PCR of 13 mature microRNAs differentially expressed in colorectal cancer and non-tumoral tissues. Mol Cancer. 2006; 5:29. [PubMed: 16854228]

Barreau C, Paillard L, Osborne HB. AU-rich elements and associated factors: Are there unifying principles? Nucleic Acids Res. 2005; 33:7138–7150. [PubMed: 16391004]

Bazan NG, Lukiw WJ. Cyclooxygenase-2 and presenilin-1 gene expression induced by interleukin-1beta and amyloid beta 42 peptide is potentiated by hypoxia in primary human neural cells. J Biol Chem. 2002; 277:30359–30367. [PubMed: 12050157]

Bertagnolli MM, Eagle CJ, Zauber AG, et al. Celecoxib for the prevention of sporadic colorectal adenomas. N Engl J Med. 2006; 355:873–884. [PubMed: 16943400]

Brennan CM, Steitz JA. HuR and mRNA stability. Cell Mol Life Sci. 2001; 58:266–277. [PubMed: 11289308]

Briata P, Ilengo C, Corte G, et al. The wnt/beta-catenin-->pitx2 pathway controls the turnover of pitx2 and other unstable mrnas. Mol Cell. 2003; 12:1201–1211. [PubMed: 14636578]

Bruno A, Di Francesco L, Coletta I, et al. Effects of af3442 [n-(9-ethyl-9h-carbazol-3-yl)-2-(trifluoromethyl)benzamide], a novel inhibitor of human microsomal prostaglandin e synthase-1, on prostanoid biosynthesis in human monocytes in vitro. Biochem Pharmacol. 2010; 79:974–981. [PubMed: 19925781]

Buchanan FG, DuBois RN. Connecting COX-2 and wnt in cancer. Cancer Cell. 2006; 9:6–8. [PubMed: 16413466]

Buchanan FG, Holla V, Katkuri S, et al. Targeting cyclooxygenase-2 and the epidermal growth factor receptor for the prevention and treatment of intestinal cancer. Cancer Res. 2007; 67:9380–9388. [PubMed: 17909047]

Burn J, Bishop DT, Chapman PD, et al. A randomized placebo-controlled prevention trial of aspirin and/or resistant starch in young people with familial adenomatous polyposis. Cancer Prev Res. 2011; 4:655–665.

Calin GA, Dumitru CD, Shimizu M, et al. Frequent deletions and down-regulation of micro-RNA genes mir15 and mir16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci U S A. 2002; 99:15524–15529. [PubMed: 12434020]

Campa D, Zienolddiny S, Maggini V, et al. Association of a common polymorphism in the cyclooxygenase 2 gene with risk of non-small cell lung cancer. Carcinogenesis. 2004; 25:229–235. [PubMed: 14604894]

Cao Y, Prescott SM. Many actions of cyclooxygenase-2 in cellular dynamics and in cancer. J Cell Physiol. 2002; 90:279–286. [PubMed: 11857443]

Carballo E, Lai WS, Blackshear PJ. Feedback inhibition of macrophage tumor necrosis factor-a production by tristetraprolin. Science. 1998; 281:1001–1005. [PubMed: 9703499]

Carballo E, Lai WS, Blackshear PJ. Evidence that tristetraprolin is a physiological regulator of granulocyte-macrophage colony-stimulating factor messenger RNA deadenylation and stability. Blood. 2000; 95:1891–1899. [PubMed: 10706852]

Castells A, Paya A, Alenda C, et al. Cyclooxygenase 2 expression in colorectal cancer with DNA mismatch repair deficiency. Clin Cancer Res. 2006; 12:1686–1692. [PubMed: 16551850]

Cha YI, DuBois RN. NSAIDs and cancer prevention: targets downstream of COX-2. Annu Rev Med. 2007; 58:239–252. [PubMed: 17100552]

Chakrabarty A, Tranguch S, Daikoku T, et al. MicroRNA regulation of cyclooxygenase-2 during embryo implantation. Proc Natl Acad Sci U S A. 2007; 104:15144–15149. [PubMed: 17848513]

Chan AT, Ogino S, Fuchs CS. Aspirin and the risk of colorectal cancer in relation to the expression of COX-2. N Engl J Med 2007. 2007; 356:2131–2142.

Cheadle C, Fan J, Cho-Chung YS, et al. Control of gene expression during t cell activation: Alternate regulation of mRNA transcription and mRNA stability. BMC Genomics. 2005; 6:75. [PubMed: 15907206]

Chen X, Guo X, Zhang H, et al. Role of miR-143 targeting Kras in colorectal tumorigenesis. Oncogene. 2009; 28:1385–1392. [PubMed: 19137007]

Chulada PC, Thompson MB, Mahler JF, et al. Genetic disruption of Ptgs-1, as well as Ptgs-2, reduces intestinal tumorigenesis in Min mice. Cancer Res. 2000; 60:4705–4708. [PubMed: 10987272]

Cimmino A, Calin GA, Fabbri M, et al. MiR-15 and miR-16 induce apoptosis by targeting bcl2. Proc Natl Acad Sci U S A. 2005; 102:13944–13949. [PubMed: 16166262]

Cok SJ, Morrison AR. The 3′-untranslated region of murine cyclooxygenase-2 contains multiple regulatory elements that alter message stability and translational efficiency. J Biol Chem. 2001; 276:23179–23185. [PubMed: 11294846]

Cordes KR, Sheehy NT, White MP, et al. MiR-145 and miR-143 regulate smooth muscle cell fate and plasticity. Nature. 2009; 460:705–710. [PubMed: 19578358]

Croce CM. Causes and consequences of microRNA dysregulation in cancer. Nat Rev Genet. 2009; 10:704–714. [PubMed: 19763153]

Cummins JM, He Y, Leary RJ, et al. The colorectal micrornaome. Proc Natl Acad Sci U S A. 2006; 103:3687–3692. [PubMed: 16505370]

Dai Y, Wang WH. Peroxisome proliferator-activated receptor gamma and colorectal cancer. World J Gastrointest Oncol. 2010; 2:159–164. [PubMed: 21160824]

Dimberg J, Hugander A, Sirsjo A, et al. Enhanced expression of cyclooxygenase-2 and nuclear beta-catenin are related to mutations in the APC gene in human colorectal cancer. Anticancer Res. 2001; 21:911–915. [PubMed: 11396184]

Dixon DA. Regulation of COX-2 expression in human cancer. Prog Exp Tumor Res. 2003; 37:52–71. [PubMed: 12795048]

Dixon DA, Balch GC, Kedersha N, et al. Regulation of cyclooxygenase-2 expression by the translational silencer TIA-1. J Exp Med. 2003; 198:475–481. [PubMed: 12885872]

Dixon DA, Kaplan CD, McIntyre TM, et al. Post-transcriptional control of cyclooxygenase-2 gene expression. The role of the 3′-untranslated region. J Biol Chem. 2000; 275:11750–11757. [PubMed: 10766797]

Dixon DA, Tolley ND, King PH, et al. Altered expression of the mRNA stability factor HuR promotes cyclooxygenase-2 expression in colon cancer cells. J Clin Invest. 2001; 108:1657–1665. [PubMed: 11733561]

Dixon DA, Tolley ND, Bemis-Standoli K, et al. Expression of COX-2 in platelet-monocyte interactions occurs via combinatorial regulation involving adhesion and cytokine signaling. J Clin Invest. 2006; 116:2727–2738. [PubMed: 16998585]

Dohner H, Stilgenbauer S, Benner A, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med. 2000; 343:1910–1916. [PubMed: 11136261]

Dresios J, Aschrafi A, Owens GC, et al. Cold stress-induced protein RBM3 binds 60s ribosomal subunits, alters microRNA levels, and enhances global protein synthesis. Proc Natl Acad Sci U S A. 2005; 102:1865–1870. [PubMed: 15684048]

Duval A, Hamelin R. Mutations at coding repeat sequences in mismatch repair-deficient human cancers: Toward a new concept of target genes for instability. Cancer Res. 2002; 62:2447–2454. [PubMed: 11980631]

Eberhart CE, Coffey RJ, Radhika A, et al. Up-regulation of cycloxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology. 1994; 107:1183–1188. [PubMed: 7926468]

Elander N, Ungerback J, Olsson H, et al. Genetic deletion of mPGES-1 accelerates intestinal tumorigenesis in apc(min/+) mice. Biochem Biophys Res Commun. 2008; 372:249–253. [PubMed: 18485889]

Eulalio A, Behm-Ansmant I, Izaurralde E. P bodies: At the crossroads of post-transcriptional pathways. Nat Rev Mol Cell Biol. 2007; 8:9–22. [PubMed: 17183357]

Fabian MR, Sonenberg N, Filipowicz W. Regulation of mRNA translation and stability by microRNAs. Annu Rev Biochem. 2010; 79:351–379. [PubMed: 20533884]

Ferguson HR, Wild CP, Anderson LA, et al. Cyclooxygenase-2 and inducible nitric oxide synthase gene polymorphisms and risk of reflux esophagitis, barrett's esophagus, and esophageal adenocarcinoma. Cancer Epidemiol Biomarkers Prev. 2008; 17:727–731. [PubMed: 18349295]

Filipowicz W, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: Are the answers in sight? Nat Rev Genet. 2008; 9:102–114. [PubMed: 18197166]

Franks TM, Lykke-Andersen J. TTP and BRF proteins nucleate processing body formation to silence mRNAs with AU-rich elements. Genes Dev. 2007; 21:719–735. [PubMed: 17369404]

Friedman RC, Farh KK, Burge CB, et al. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res. 2009; 19:92–105. [PubMed: 18955434]

Funk CD. Prostaglandins and leukotrienes: Advances in eicosanoid biology. Science. 2001; 294:1871–1875. [PubMed: 11729303]

Garcia Rodriguez LA, Tacconelli S, Patrignani P. Role of dose potency in the prediction of risk of myocardial infarction associated with nonsteroidal anti-inflammatory drugs in the general population. J Am Coll Cardiol. 2008; 52:1628–1636. [PubMed: 18992652]

Garneau NL, Wilusz J, Wilusz CJ. The highways and byways of mRNA decay. Nat Rev Mol Cell Biol. 2007; 8:113–126. [PubMed: 17245413]

Giardiello FM, Casero RA Jr, Hamilton SR, et al. Prostanoids, ornithine decarboxylase, and polyamines in primary chemoprevention of familial adenomatous polyposis. Gastroenterology. 2004; 126:425–431. [PubMed: 14762779]

Gong Z, Bostick RM, Xie D, et al. Genetic polymorphisms in the cyclooxygenase-1 and cyclooxygenase-2 genes and risk of colorectal adenoma. Int J Colorectal Dis. 2009; 24:647–654. [PubMed: 19205707]

Grady WM, Carethers JM. Genomic and epigenetic instability in colorectal cancer pathogenesis. Gastroenterology. 2008; 135:1079–1099. [PubMed: 18773902]

Grosser T, Fries S, FitzGerald GA. Biological basis for the cardiovascular consequences of COX-2 inhibition: Therapeutic challenges and opportunities. J Clin Invest. 2006; 116:4–15. [PubMed: 16395396]

Gruber AR, Fallmann J, Kratochvill F, et al. Aresite: A database for the comprehensive investigation of AU-rich elements. Nucleic Acids Res. 2010; 39:D66–69. [PubMed: 21071424]

Guo H, Ingolia NT, Weissman JS, et al. Mammalian microRNAs predominantly act to decrease target mRNA levels. Nature. 2010; 466:835–840. [PubMed: 20703300]

Hall-Pogar T, Zhang H, Tian B, et al. Alternative polyadenylation of cyclooxygenase-2. Nucleic Acids Res. 2005; 33:2565–2579. [PubMed: 15872218]

Hao Y, Gu X, Zhao Y, et al. Enforced expression of miR-101 inhibits prostate cancer cell growth by modulating the COX-2 pathway in vivo. Cancer Prev Res (Phila). 2011; 4:1073–1083. [PubMed: 21430074]

Harper KA, Tyson-Capper AJ. Complexity of COX-2 gene regulation. Biochem Soc Trans. 2008; 36:543–5. [PubMed: 18482003]

Hernandez GL, Volpert OV, Iniguez MA, et al. Selective inhibition of vascular endothelial growth factor-mediated angiogenesis by cyclosporin a: Roles of the nuclear factor of activated t cells and cyclooxygenase 2. J Exp Med. 2001; 193:607–620. [PubMed: 11238591]

Holla VR, Backlund MG, Yang P, et al. Regulation of prostaglandin transporters in colorectal neoplasia. Cancer Prev Res. 2008; 1:93–99.

Howe LR, Subbaramaiah K, Chung WJ, et al. Transcriptional activation of cyclooxygenase-2 in wnt-1-transformed mouse mammary epithelial cells. Cancer Res. 1999; 59:1572–1577. [PubMed: 10197631]

Issa JP. CpG island methylator phenotype in cancer. Nat Rev Cancer. 2004; 4:988–993. [PubMed: 15573120]

Jakobsson PJ, Thoren S, Morgenstern R, et al. Identification of human prostaglandin E synthase: A microsomal, glutathione-dependent, inducible enzyme, constituting a potential novel drug target. Proc Natl Acad Sci U S A. 1999; 96:7220–7225. [PubMed: 10377395]

Jemal A, Bray F, Center MM, et al. Global cancer statistics. CA Cancer J Clin. 2011; 61:69–90. [PubMed: 21296855]

Jiang Y, Kimchi ET, Staveley-O'Carroll KF, et al. Assessment of K-ras mutation: A step toward personalized medicine for patients with colorectal cancer. Cancer. 2009; 115:3609–3617. [PubMed: 19526592]

Jing Q, Huang S, Guth S, et al. Involvement of microRNA in AU-rich element-mediated mRNA instability. Cell. 2005; 120:623–634. [PubMed: 15766526]

Kaidi A, Qualtrough D, Williams AC, et al. Direct transcriptional up-regulation of cyclooxygenase-2 by hypoxia-inducible factor (HIF)-1 promotes colorectal tumor cell survival and enhances HIF-1 transcriptional activity during hypoxia. Cancer Res. 2006; 66:6683–6691. [PubMed: 16818642]

Kanies CL, Smith JJ, Kis C, et al. Oncogenic ras and transforming growth factor-beta synergistically regulate AU-rich element-containing mrnas during epithelial to mesenchymal transition. Mol Cancer Res. 2008; 6:1124–1136. [PubMed: 18644977]

Karnes WE, Shattuck-Brandt R, Burgart LJ, et al. Reduced COX-2 protein in colorectal cancer with defective mismatch repair. Cancer Res. 1998; 58:5473–5477. [PubMed: 9850081]

Kedersha N, Stoecklin G, Ayodele M, et al. Stress granules and processing bodies are dynamically linked sites of mRNP remodeling. J Cell Biol. 2005; 169:871–884. [PubMed: 15967811]

Keene J. Why is Hu where? Shuttling of early-response messenger RNA subsets. Proc Natl Acad Sci USA. 1999; 96:5–7. [PubMed: 9874760]

Kim Y, Fischer SM. Transcriptional regulation of cyclooxygenase-2 in mouse skin carcinoma cells. Regulatory role of ccaat/enhancer-binding proteins in the differential expression of cyclooxygenase-2 in normal and neoplastic tissues. Journal of Biological Chemistry. 1998; 273:27686–27694. [PubMed: 9765305]

Kojima M, Morisaki T, Izuhara K, et al. Lipopolysaccharide increases cyclo-oxygenase-2 expression in a colon carcinoma cell line through nuclear factor-kappa B activation. Oncogene. 2000; 19:1225–1231. [PubMed: 10713711]

Kondo Y, Issa JP. Epigenetic changes in colorectal cancer. Cancer Metastasis Rev. 2004; 23:29–39. [PubMed: 15000147]

Kudo I, Murakami M. Prostaglandin e synthase, a terminal enzyme for prostaglandin E2 biosynthesis. J Biochem Mol Biol. 2005; 38:633–638. [PubMed: 16336776]

Kulendran M, Stebbing JF, Marks CG, et al. Predictive and prognostic factors in colorectal cancer: A personalized approach. Cancers. 2011; 3:1622–1638.

Kutchera W, Jones DA, Matsunami N, et al. Prostaglandin synthase 2 is abnormally expressed in human colon cancer: Evidence for a transcriptional effect. Proc Natl Acad Sci USA. 1996; 93:4816–4820. [PubMed: 8643486]

Lai WS, Carballo E, Strum JR, et al. Evidence that tristetraprolin binds to AU-rich elements and promotes the deadenylation and destabilization of tumor necrosis factor alpha mRNA. Mol Cell Biol. 1999; 19:4311–4323. [PubMed: 10330172]

Langsenlehner U, Yazdani-Biuki B, Eder T, et al. The cyclooxygenase-2 (PTGS2) 8473T>C polymorphism is associated with breast cancer risk. Clin Cancer Res. 2006; 12:1392–1394. [PubMed: 16489098]

Liu Q, Fu H, Sun F, et al. Mir-16 family induces cell cycle arrest by regulating multiple cell cycle genes. Nucleic Acids Res. 2008; 36:5391–5404. [PubMed: 18701644]

Lopez de Silanes I, Galban S, Martindale JL, et al. Identification and functional outcome of mRNAs associated with RNA-binding protein tia-1. Mol Cell Biol. 2005a; 25:9520–9531. [PubMed: 16227602]

Lopez de Silanes I, Lal A, Gorospe M. HuR: Post-transcriptional paths to malignancy. RNA Biol. 2005b; 2:11–13. [PubMed: 17132932]

Lopez de Silanes I, Quesada MP, Esteller M. Aberrant regulation of messenger RNA 3′-untranslated region in human cancer. Cell Oncol. 2007; 29:1–17. [PubMed: 17429137]

Markowitz SD, Bertagnolli MM. Molecular origins of cancer: Molecular basis of colorectal cancer. N Engl J Med. 2009; 361:2449–2460. [PubMed: 20018966]

Markowitz S, Wang J, Myeroff L, et al. Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability. Science. 1995; 268:1336–1338. [PubMed: 7761852]

Masso Gonzalez EL, Patrignani P, Tacconelli S, et al. Variability among nonsteroidal antiinflammatory drugs in risk of upper gastrointestinal bleeding. Arthritis Rheum. 2010; 62:1592–1601. [PubMed: 20178131]

Mayr C, Bartel DP. Widespread shortening of 3′UTRs by alternative cleavage and polyadenylation activates oncogenes in cancer cells. Cell. 2009; 138:673–684. [PubMed: 19703394]

Meade EA, McIntyre TM, Zimmerman GA, et al. Peroxisome proliferators enhance cyclooxygenase-2 expression in epithelial cells. J Biol Chem. 1999; 274:8328–8334. [PubMed: 10075740]

Mei JM, Hord NG, Winterstein DF, et al. Differential expression of prostaglandin endoperoxide H synthase-2 and formation of activated beta-catenin-Lef-1 transcription complex in mouse colonic epithelial cells contrasting in APC. Carcinogenesis. 1999; 20:737–740. [PubMed: 10223208]

Meisner NC, Hintersteiner M, Mueller K, et al. Identification and mechanistic characterization of low-molecular-weight inhibitors for HuR. Nat Chem Biol. 2007; 3:508–515. [PubMed: 17632515]

Michael MZ, SMOC, van Holst Pellekaan NG, et al. Reduced accumulation of specific microRNAs in colorectal neoplasia. Mol Cancer Res. 2003; 1:882–891. [PubMed: 14573789]

Miller C, Zhang M, He Y, et al. Transcriptional induction of cyclooxygenase-2 gene by okadaic acid inhibition of phosphatase activity in human chondrocytes: Co-stimulation of AP-1 and cre nuclear binding proteins. J Cell Biochem. 1998; 69:392–413. [PubMed: 9620167]

Ming XF, Stoecklin G, Lu M, et al. Parallel and independent regulation of interleukin-3 mRNA turnover by phosphatidylinositol 3-kinase and p38 mitogen-activated protein kinase. Mol Cell Biol. 2001; 21:5778–5789. [PubMed: 11486017]

Moore AE, Young LE, Dixon DA. A common single-nucleotide polymorphism in cyclooxygenase-2 disrupts microRNA-mediated regulation. Oncogene in press. 2011

Moran AE, Hunt DH, Javid SH, et al. APC deficiency is associated with increased EGFR activity in the intestinal enterocytes and adenomas of C57Bl/6j-Min/+ mice. J Biol Chem. 2004; 279:43261–43272. [PubMed: 15294912]

Moser AR, Luongo C, Gould KA, et al. APCmin: A mouse model for intestinal and mammary tumorigenesis. Eur J Cancer. 1995; 31A:1061–1064. [PubMed: 7576992]

Mukhopadhyay D, Houchen CW, Kennedy S, et al. Coupled mRNA stabilization and translational silencing of cyclooxygenase-2 by a novel RNA binding protein, CUGBP2. Mol Cell. 2003; 11:113–126. [PubMed: 12535526]

Murakami M, Naraba H, Tanioka T, et al. Regulation of prostaglandin E2 biosynthesis by inducible membrane-associated prostaglandin E2 synthase that acts in concert with cyclooxygenase-2. J Biol Chem. 2000; 275:32783–32792. [PubMed: 10869354]

Murmu N, Jung J, Mukhopadhyay D, et al. Dynamic antagonism between RNA-binding protein cugbp2 and cyclooxygenase-2-mediated prostaglandin E2 in radiation damage. Proc Natl Acad Sci U S A. 2004; 101:13873–13878. [PubMed: 15358864]

Myung SJ, Rerko RM, Yan M, et al. 15-hydroxyprostaglandin dehydrogenase is an in vivo suppressor of colon tumorigenesis. Proc Natl Acad Sci U S A. 2006; 103:12098–12102. [PubMed: 16880406]

Nakanishi M, Gokhale V, Meuillet EJ, et al. mPGES-1 as a target for cancer suppression: A comprehensive invited review "Phospholipase A2 and lipid mediators". Biochimie. 2010; 92:660–664. [PubMed: 20159031]

Nakanishi M, Montrose DC, Clark P, et al. Genetic deletion of mPGES-1 suppresses intestinal tumorigenesis. Cancer Res. 2008; 68:3251–3259. [PubMed: 18451151]

Newbury SF, Muhlemann O, Stoecklin G. Turnover in the alps: An mRNA perspective. Workshops on mechanisms and regulation of mRNA turnover. EMBO Rep. 2006; 7:143–148. [PubMed: 16439997]

Nomura T, Lu R, Pucci ML, et al. The two-step model of prostaglandin signal termination: In vitro reconstitution with the prostaglandin transporter and prostaglandin 15 dehydrogenase. Mol Pharmacol. 2004; 65:973–978. [PubMed: 15044627]

O'Hara SP, Mott JL, Splinter PL, et al. MicroRNAs: Key modulators of posttranscriptional gene expression. Gastroenterology. 2009; 136:17–25. [PubMed: 19049808]

Oshima M, Dinchuk JE, Kargman SL, et al. Suppression of intestinal polyposis in APC 716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). Cell. 1996; 87:803–809. [PubMed: 8945508]

Ozhan G, Yanar TH, Ertekin C, et al. The effect of genetic polymorphisms of cyclooxygenase 2 on acute pancreatitis in Turkey. Pancreas. 2010; 39:371–376. [PubMed: 19820421]

Patrono C, Patrignani P, Garcia Rodriguez LA. Cyclooxygenase-selective inhibition of prostanoid formation: transducing biochemical selectivity into clinical read-outs. J Clin Invest. 2001; 108:7–13. [PubMed: 11435450]

Patrono C, Baigent C, Hirsh J, et al. Antiplatelet drugs: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). Chest. 2008; 133:199S–233S. [PubMed: 18574266]

Peddareddigari VG, Wang D, Dubois RN. The tumor microenvironment in colorectal carcinogenesis. Cancer Microenviron. 2011; 3:149–166. [PubMed: 21209781]

Phillips K, Kedersha N, Shen L, et al. Arthritis suppressor genes TIA-1 and TTP dampen the expression of tumor necrosis factor alpha, cyclooxygenase 2, and inflammatory arthritis. Proc Natl Acad Sci USA. 2004; 101:2011–2016. [PubMed: 14769925]

Prescott SM. Is cyclooxygenase-2 the alpha and the omega in cancer? J Clin Invest. 2000; 105:1511–1513. [PubMed: 10841506]

Reid G, Wielinga P, Zelcer N, et al. The human multidrug resistance protein MRP4 functions as a prostaglandin efflux transporter and is inhibited by nonsteroidal antiinflammatory drugs. Proc Natl Acad Sci U S A. 2003; 100:9244–9249. [PubMed: 12835412]

Roberts RB, Min L, Washington MK, et al. Importance of epidermal growth factor receptor signaling in establishment of adenomas and maintenance of carcinomas during intestinal tumorigenesis. Proc Natl Acad Sci U S A. 2002; 99:1521–1526. [PubMed: 11818567]

Rothwell PM, Fowkes FG, Belch JF, et al. Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data from randomised trials. Lancet. 2011; 377:31–41. [PubMed: 21144578]

Rothwell PM, Wilson M, Elwin CE, et al. Long-term effect of aspirin on colorectal cancer incidence and mortality: 20-year follow-up of five randomised trials. Lancet. 2010; 376:1741–1750. [PubMed: 20970847]

Ryan BM, Robles AI, Harris CC. Genetic variation in microRNA networks: The implications for cancer research. Nat Rev Cancer. 2010; 10:389–402. [PubMed: 20495573]

Sampey AV, Monrad S, Crofford LJ. Microsomal prostaglandin E synthase-1: The inducible synthase for prostaglandin E2. Arthritis Res Ther. 2005; 7:114–117. [PubMed: 15899061]

Samuelsson B, Morgenstern R, Jakobsson PJ. Membrane prostaglandin E synthase-1: A novel therapeutic target. Pharmacol Rev. 2007; 59:207–224. [PubMed: 17878511]

Sanchez-Beato M, Sanchez-Aguilera A, Piris MA. Cell cycle deregulation in B-cell lymphomas. Blood. 2003; 101:1220–1235. [PubMed: 12393483]

Sanduja S, Blanco FF, Dixon DA. The roles of TTP and BRF proteins in regulated mRNA decay. Wiley Interdiscip Rev RNA. 2010; 2:42–57. [PubMed: 21278925]

Sawaoka H, Dixon DA, Oates JA, et al. Tristetrapolin binds to the 3′ untranslated region of cyclooxygenase-2 mRNA: A polyadenylation variant in a cancer cell line lacks the binding site. J Biol Chem. 2003; 278:13928–13935. [PubMed: 12578839]

Schmedtje JF Jr, Ji YS, Liu WL, et al. Hypoxia induces cyclooxygenase-2 via the NF-kappaB p65 transcription factor in human vascular endothelial cells. J Biol Chem. 1997; 272:601–608. [PubMed: 8995303]

Shanmugam N, Reddy MA, Natarajan R. Distinct roles of heterogeneous nuclear ribonuclear protein K and microRNA-16 in cyclooxygenase-2 RNA stability induced by s100b, a ligand of the receptor for advanced glycation end products. J Biol Chem. 2008; 283:36221–36233. [PubMed: 18854308]

Shao J, Sheng H, Inoue H, et al. Regulation of constitutive cyclooxygenase-2 expression in colon carcinoma cells. J Biol Chem. 2000; 275:33951–33956. [PubMed: 10930401]

Shen J, Gammon MD, Terry MB, et al. Genetic polymorphisms in the cyclooxygenase-2 gene, use of nonsteroidal anti-inflammatory drugs, and breast cancer risk. Breast Cancer Res. 2006; 8:R71–80. [PubMed: 17181859]

Sheng H, Shao J, Dixon DA, et al. Transforming growth factor-beta1 enhances Ha-ras-induced expression of cyclooxygenase-2 in intestinal epithelial cells via stabilization of mRNA. J Biol Chem. 2000; 275:6628–6635. [PubMed: 10692471]

Siezen CL, van Leeuwen AI, Kram NR, et al. Colorectal adenoma risk is modified by the interplay between polymorphisms in arachidonic acid pathway genes and fish consumption. Carcinogenesis. 2005; 26:449–457. [PubMed: 15550453]

Simmons DL, Botting RM, Hla T. Cyclooxygenase isozymes: The biology of prostaglandin synthesis and inhibition. Pharmacol Rev. 2004; 56:387–437. [PubMed: 15317910]

Singer, Kawka DW, Schloemann S, et al. Cyclooxygenase 2 is induced in colonic epithelial cells in inflammatory bowel disease. Gastroenterology. 1998; 115:297–306. [PubMed: 9679035]

Slaby O, Svoboda M, Fabian P, et al. Altered expression of miR-21, miR-31, miR-143 and miR-145 is related to clinicopathologic features of colorectal cancer. Oncology. 2007; 72:397–402. [PubMed: 18196926]

Song T, Zhang X, Wang C, et al. Expression of miR-143 reduces growth and migration of human bladder carcinoma cells by targeting cyclooxygenase-2. Asian Pac J Cancer Prev. 2011; 12:929–933. [PubMed: 21790228]

Steinbach G, Lynch PM, Phillips RK, et al. The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. N Engl J Med. 2000; 342:1946–1952. [PubMed: 10874062]

Strillacci A, Griffoni C, Sansone P, et al. Mir-101 downregulation is involved in cyclooxygenase-2 overexpression in human colon cancer cells. Exp Cell Res. 2009; 315:1439–1447. [PubMed: 19133256]

Su H, Yang JR, Xu T, et al. MicroRNA-101, down-regulated in hepatocellular carcinoma, promotes apoptosis and suppresses tumorigenicity. Cancer Res. 2009; 69:1135–1142. [PubMed: 19155302]

Subbaramaiah K, Cole PA, Dannenberg AJ. Retinoids and carnosol suppress cyclooxygenase-2 transcription by creb-binding protein/p300-dependent and -independent mechanisms. Cancer Res. 2002a; 62:2522–2530. [PubMed: 11980644]

Subbaramaiah K, Norton L, Gerald W, et al. Cyclooxygenase-2 is overexpressed in HER-2/neu-positive breast cancer. Evidence for involvement of AP-1 and PEA3. J Biol Chem. 2002b; 277:18649–18657. [PubMed: 11901151]

Sureban SM, Murmu N, Rodriguez P, et al. Functional antagonism between RNA binding proteins HuR and CUGBP2 determines the fate of COX-2 mRNA translation. Gastroenterology. 2007; 132:1055–1065. [PubMed: 17383427]

Sureban SM, Ramalingam S, Natarajan G, et al. Translation regulatory factor RBM3 is a proto-oncogene that prevents mitotic catastrophe. Oncogene. 2008; 27:4544–4556. [PubMed: 18427544]

Takagi T, Iio A, Nakagawa Y, et al. Decreased expression of microRNA-143 and -145 in human gastric cancers. Oncology. 2009; 77:12–21. [PubMed: 19439999]

Tian Q, Streuli M, Saito H, et al. A polyadenylate binding protein localized to the granules of cytolytic lymphocytes induces DNA fragmentation in target cells. Cell. 1991; 67:629–639. [PubMed: 1934064]

Toyota M, Shen L, Ohe-Toyota M, et al. Aberrant methylation of the cyclooxygenase 2 CpG island in colorectal tumors. Cancer Res. 2000; 60:4044–4048. [PubMed: 10945606]

Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nat New Biol. 1971; 231:232–523. [PubMed: 5284360]

Varambally S, Cao Q, Mani RS, et al. Genomic loss of microRNA-101 leads to overexpression of histone methyltransferase EZH2 in cancer. Science. 2008; 322:1695–1699. [PubMed: 19008416]

Vogel U, Christensen J, Wallin H, et al. Polymorphisms in genes involved in the inflammatory response and interaction with NSAID use or smoking in relation to lung cancer risk in a prospective study. Mutat Res. 2008; 639:89–100. [PubMed: 18164040]

Voltz R. Paraneoplastic neurological syndromes: An update on diagnosis, pathogenesis, and therapy. Lancet Neurol. 2002; 1:294–305. [PubMed: 12849427]

Wang D, DuBois RN. Pro-inflammatory prostaglandins and progression of colorectal cancer. Cancer Lett. 2008; 267:197–203. [PubMed: 18406516]

Wang D, Dubois RN. Eicosanoids and cancer. Nat Rev Cancer. 2010a; 10:181–193. [PubMed: 20168319]

Wang D, Dubois RN. The role of COX-2 in intestinal inflammation and colorectal cancer. Oncogene. 2010b; 29:781–788. [PubMed: 19946329]

Wang HJ, Ruan HJ, He XJ, et al. MicroRNA-101 is down-regulated in gastric cancer and involved in cell migration and invasion. Eur J Cancer. 2010; 46:2295–2303. [PubMed: 20712078]

Wang M, Song WL, Cheng Y, et al. Microsomal prostaglandin E synthase-1 inhibition in cardiovascular inflammatory disease. J Intern Med. 2008; 263:500–505. [PubMed: 18410593]

Wang D, Wang H, Shi Q, et al. Prostaglandin E(2) promotes colorectal adenoma growth via transactivation of the nuclear peroxisome proliferator-activated receptor delta. Cancer Cell. 2004; 6:285–295. [PubMed: 15380519]

Wiemer EA. The role of microRNAs in cancer: No small matter. Eur J Cancer. 2007; 43:1529–1544. [PubMed: 17531469]

Wiercinska-Drapalo A, Flisiak R, Prokopowicz D. Effects of ulcerative colitis activity on plasma and mucosal prostaglandin E2 concentration. Prostaglandins Other Lipid Mediat. 1999; 58:159–165. [PubMed: 10560618]

Wu BL, Xu LY, Du ZP, et al. MiRNA profile in esophageal squamous cell carcinoma: Downregulation of miR-143 and miR-145. World J Gastroenterol. 2011; 17:79–88. [PubMed: 21218087]

Wu WK, Sung JJ, Lee CW, et al. Cyclooxygenase-2 in tumorigenesis of gastrointestinal cancers: An update on the molecular mechanisms. Cancer Lett. 2010; 295:7–16. [PubMed: 20381235]

Yan M, Rerko RM, Platzer P, et al. 15-hydroxyprostaglandin dehydrogenase, a COX-2 oncogene antagonist, is a tgf-beta-induced suppressor of human gastrointestinal cancers. Proc Natl Acad Sci U S A. 2004; 101:17468–17473. [PubMed: 15574495]

Yang X, Wang W, Fan J, et al. Prostaglandin a2-mediated stabilization of p21 mRNA through an erk-dependent pathway requiring the RNA-binding protein HuR. J Biol Chem. 2004; 279:49298–49306. [PubMed: 15371446]

Young LE, Dixon DA. Posttranscriptional regulation of cyclooxygenase 2 expression in colorectal cancer. Curr Colorectal Cancer Rep. 2010; 6:60–67. [PubMed: 20577575]

Young LE, Moore AE, Sokol L, et al. The mRNA stability factor HuR inhibits microRNA-16 targeting of cyclooxygenase-2. Mol Cancer Res. 2011 in press.

Young LE, Sanduja S, Bemis-Standoli K, et al. The mRNA binding proteins HuR and tristetraprolin regulate cyclooxygenase 2 expression during colon carcinogenesis. Gastroenterology. 2009; 136:1669–1679. [PubMed: 19208339]

Zhu T, Gobeil F, Vazquez-Tello A, et al. Intracrine signaling through lipid mediators and their cognate nuclear G-protein-coupled receptors: A paradigm based on PGE2, PAF, and LPA1 receptors. Can J Physiol Pharmacol. 2006; 84:377–391. [PubMed: 16902584]
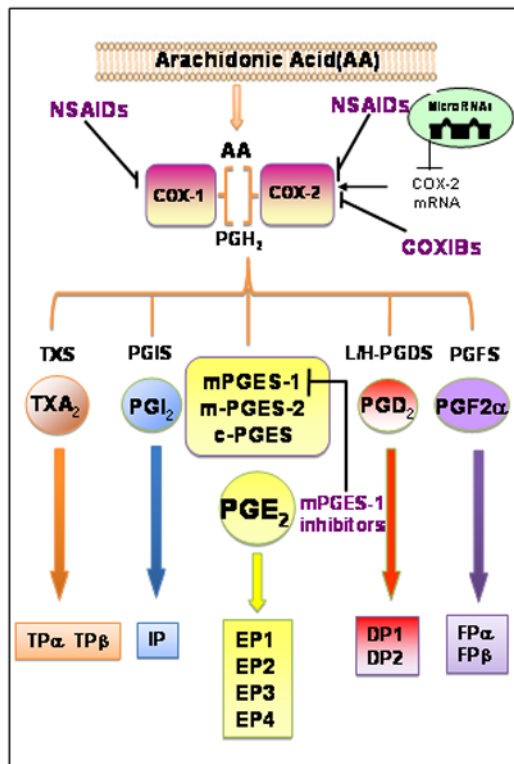
Figure 2.1. COX-2/mPGES-1 pathway
Two primary COX enzyme isoforms (COX-1 and COX-2) catalyze the rate-limiting step of PG formation: the conversion of AA into the intermediate metabolite PGG_2, which is further metabolized by different synthases to generate distinct PGs. The specific action of the different PGs in a particular type of tissue predominantly depends on the cell type-specific expression of their specific receptors and on their biosynthesis. NSAIDs (both traditional and COX-2 selective inhibitors, named coxibs) act by the inhibiting COX-dependent prostanoid generation. Innovative anti-inflammatory and anti-cancer strategies are based on the selective delivery of drugs affecting COX-2 activity or expression to specific tissues or by the use of selective inhibitors of mPGES-1, which by suppressing PGE_2 might augment the protective vascular PGI_2 biosynthesis. In addition, since in CRC COX-2 overexpression is controlled at post-transcriptional level through changes in its mRNA stability by miRNAs, the design of therapeutics affecting this pathway may lead to innovative therapeutic strategies in CRC.AA, arachidonic acid); COX, cyclooxygenase; Prostaglandin G2 (PGG_2); TXA_2 (thromboxane); PGI_2 (prostacyclin); PGD_2 (prostaglandin D_2); $PGF_{2\alpha}$(prostaglandin $F_{2\alpha}$); TXS(TXA_2 synthase); PGIS (PGI_2 synthase); hematopoietic/lipocalin-type PGD synthase; prostaglandin F2a synthase; m(microsomal) or c(cytoplasmatic) PGES (PGE_2 syntase); TP (TXA_2 receptor); IP (PGI_2 receptor); EP1-4 (PGE_2 receptor); DP1-2 (PGD_2 receptors); FP (PGF2a receptors); colorectal cancer (CRC)
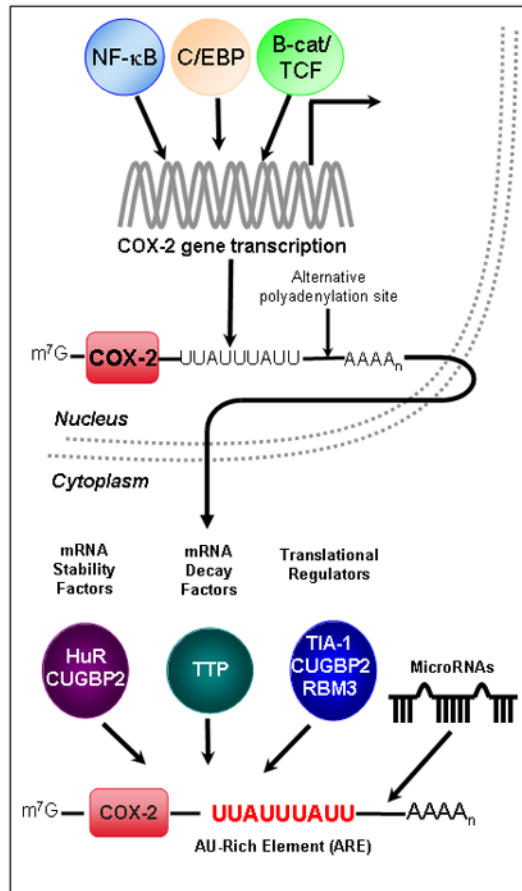
Figure 2.2. Regulation of COX-2 gene expression
Transcription of the COX-2 gene PTGS-2 can be induced by various signaling pathways to activate transcription factors NF-κB, C/EBP, and β-catenin/TCF. The primary transcript undergoes splicing, capping at the 5′ end, and polyadenylation at the 3′ end; the presence of alternative polyadenylation signals may lead to a shortened 3′UTR and subsequent loss of regulatory elements. After trafficking to the cytoplasm, COX-2 mRNA fate is mediated by sequence elements present within its 3′UTR. COX-2 expression is tightly regulated at the post-transcriptional level by RNA-binding proteins that promote mRNA stability (HuR and CUGBP2), mRNA decay (TTP), and translational inhibition (TIA-1, CUGBP2, and RMB3) through their binding of the COX-2 AU-rich element (ARE). Specific microRNAs have been determined to bind the COX-2 3′UTR and control COX-2 expression.